Evaluation of Antibacterial Potential of Phytochemicals from *Nerium oleander* and Molecular Docking Analysis

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A thesis submitted in partial fulfillment of the requirement for the degree of
Master of science in Industrial Biotechnology

By

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III

Dedicated to my beloved Parents and Siblings

for their love, endless support & encouragement

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MARYAM AYYAZ

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List of Abbreviations

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
FTIR	Fourier transform infrared
GC-MS	Gas Chromatography-Mass Spectrometry
SBVS	Structure-based virtual screening
VHTS	Virtual High Throughput Screening
MD	Molecular dynamics
ADMET	Chemical absorption, distribution, metabolism, excretion, and toxicity
AST	Antimicrobial susceptibility testing
MDR	Multidrug resistance
CNS	Central nervous system
HPLC	High-performance liquid chromatography
C. neoformans	*Cryptococcus neoformans*
S. cerevisiae	*Saccharomyces cerevisiae*
C. albicians	*Candida albicans*
AuNPs	gold-conjugated nanoparticles
EGFR	epidermal growth factor receptor
NO	*N. oleander*
SBDD	Structure-Based Drug Design
CCl4	Carbon tetrachloride
DHQS	3-dehydroquinate synthase
MRSA	Methicillin-resistant Staphylococcus aureus
H2SO4	Sulfuric Acid

CFU	Colony forming unit
ZOI	Zone of inhibition
DMSO	Dimethyl sulfoxide
RCSB	Research Collaboratory for Structural Bioinformatics
VDW	Van der Waals
NCBI	National Centre for Biotechnology Information
PDB	Protein Databank
PASS	Prediction of Activity the Spectra for Substances
GHS	Globally Harmonized System
MIC	Minimum inhibitory concentration
RMSF	Root Mean Square Fluctuation
RMSD	Root mean Square Deviation

Abstract

Plants possess a rich abundance of bioactive chemicals and nutrients. Plants include a wide array of phytochemicals that possess the capacity to alleviate many disorders. *Nerium oleander*, an underutilized herb, possesses substantial potential in the treatment of several human and animal illnesses. Different extracts were prepared and FTIR analysis of the extracts was done that exhibits distinct functional groups that will be observed by the application of FTIR analysis. GCMS analysis was performed for the identification of bioactive compounds. In *Nerium oleander* distinct bioactive compounds were determined by the utilization of gas chromatography-mass spectrometry (GC-MS) analysis. Antibacterial susceptibility testing (AST) was done to check the antibacterial activity of the extract. The distinct zones of inhibition were observed against *Escherichia coli, Klebsiella pneumonia, Bacillus subtillis, Staphylococcus aureus, Enterobacter mori, and Acinetobacter baumannii*. By measuring the inhibition zone of the different bacteria, the antibacterial activities of different extracts were estimated. AutoDock vina software was used for docking. The docking analysis of the compounds will be done to unveil the possible antibacterial properties of the main compounds. The target proteins 1xal, 7p2m, 8iwl, 6f86, and 3zgf with selected bioactive compounds, binding energies will be calculated. The lesser the binding energy, the greater will be the affinity b/w protein and inhibitor. ADMET analysis and Toxicity analysis was done using the Swiss ADME and PROTOX II server. Molecular docking simulation was performed by Desmond Schrodinger tool at 100 ns. MD simulation of the selected compounds shows stability of the protein with ligand. This study will represent a pioneering effort in documenting the interactions between bioactive components of *Nerium oleander* extract and bacterial proteins, as per the existing knowledge. By utilizing *Nerium Oleander* as a foundational resource, this research holds the potential to yield innovative pharmaceuticals for the treatment of diverse medical conditions.

Chapter 1: Introduction

1. Introduction

Antibiotics are of the highest priority in combatting bacterial illnesses; yet, the emergence of antibacterial resistance has significantly disrupted the healthcare and drugs sectors, leading to accelerated socio-economic repercussions. It has been projected that the phenomenon of multidrug resistance is anticipated to contribute to a staggering increase of 10 million fatalities annually by the year 2050.(Mickymaray, 2019). The screening of plants and the process of separating phytochemicals, and conducting clinical studies on medicinal plants, has made significant progress over time, leading to the discovery of valuable insights into the ancient practices of herbal treatments. Bacterial or oxidative stress-related illnesses can be effectively treated with traditional medicine. (Mickymaray & Alturaiki, 2018).

Extensive investigations have been conducted on natural chemicals in the search of novel drug discoveries. Throughout history, the human species has consistently exhibited a profound interest in natural molecules derived from many sources such as pre-biotic substances, microorganisms, plants, and animals. Various components derived from diverse plant sections possess bioactive properties that contribute to their efficacy in combating diseases. These components include tannins, glycosides, alkaloids, steroids, volatile oils, phenols, fixed oils, resins, terpenoids, and flavonoids. Phenolic phytochemicals derived from plants are of significant importance due to their antibacterial properties (Houlihan, Conlin, & Chee-Sanford, 2019). Antimicrobial drugs are known to degrade the protein constituents of the cellular wall, thereby interfering with the functioning of enzymes as well as the replication processes of DNA and RNA. This study presents an overview of plants along with their corresponding phytochemicals that exhibit antimicrobial properties and explores the diverse applications of these compounds.

Over the course of billions of years, nature has undergone extensive evolutionary processes, resulting in an extensive variety of biodiversity. Plants hold significant importance due to the presence of over 250,000 plant species and their corresponding metabolites. These plants have a comparable chemical composition essential for cellular function (primary metabolites) (Süntar, 2020). A natural compound is described as a molecule which is chemically synthesized by living things, typically possessing medicinal

properties that may have therapeutic potential for a range of disorders. There are two types of methods by which organic substances can be collected, one is chemical and the other is semi-synthesis. These compounds are frequently employed as initial stages in the process of drug finding, from which analogues are made to enhance their clarity, efficacy, potency, and safety.

Plants are not only a source of basic nutrients for human consumption, but they also contain compounds which are active biologically, and have the potential to be used in therapeutic applications. As a result, plants offer a wide array of health advantages and the possibility of treating a variety of ailments. (R. H. Liu, 2003). Plants exhibit a huge variety of important components, including phytochemicals, medicines, lipids, flavors, and aromas, which make them significant resources in several industries encompassing food, medicine, and cosmetics.(Velmurugan & Anand, 2017). In the world at large, infectious diseases play a significant role in determining death rates and constitute an immense barrier to the area of public health. Infections resulting from the presence of harmful microorganisms pose a substantial threat to human health. In developing countries like Pakistan, the healthcare system primarily depends on traditional cures and folk medicines. The use of botanical remedies in traditional healing methods can be predominantly ascribed to the existence of phytochemical constituents. The utilization of traditional medicine is prevalent in local contexts, particularly in rural areas (Hikaambo et al., 2022). Plants which are highly used in medicines contain a large variety of compounds which are active biologically contain anticancer, antibacterial, anti-allergic, and antioxidant properties. The need for new antimicrobial therapies that are both secure and effective has emerged because of the increased levels of drug resistance, the undesirable effects linked to antibiotics.

During 1970s, there was a significant focus on conducting rigorous scientific investigations aimed at revealing the chemical makeup of plants and devising methodologies to assess their biological activity. During the 1980s and 1990s, this research held significant significance in the context of establishing regulatory frameworks concerning homeopathic medicines in the USA. It can be observed that around 80% of the global population derives therapeutic benefits from plants in the context of disease treatment. This rate might be high

in developing nations. The utilization of botanical resources for medicinal reasons is deemed advantageous due to the cost-effectiveness and availability of plant materials in these regions, as compared to synthetic pharmaceuticals. In contrast, a significant proportion, approximately one-third, of population residing in both the US and European countries engage in the utilization of herbal treatments with the purpose of promoting their health. The current body of knowledge indicates that there are over 70,000 plant species that are utilized for medicinal purposes. However, it is worth noting that a mere 15% of the total plant species cultivated globally have undergone scientific scrutiny to explore their potential medical applications. Notwithstanding this relatively modest percentage, it is noteworthy that a significant proportion, specifically 25%, of traditional pharmaceuticals employed in contemporary medical practice are derived from botanical sources. This observation implies that additional research is warranted regarding the potential utilization of natural sources for medicinal applications.

Nerium oleander is classified within the taxonomic family Apocyanaceae (Ayouaz, Arab, Mouhoubi, & Madani, 2023). *Nerium oleander* is a tiny perennial plant or compactly branched arboreal species, often attaining a height ranging from 2 to 5 meters (9). The branches of the oleander plant exhibit flexibility and are characterized by a smooth pale light gray to green bark that releases a milky sap when pierced. Every stem node is characterized by the presence of two or three narrow, elliptical leaves that possess smooth edges and are attached to short petioles (Garima & Amla, 2010; Sinha & Biswas, 2016). The arrangement of the leaves is either opposite or whorled, with a length ranging from 5 to 20 cm. The leaves are characterized by their durability, exhibiting pinnate secondary veins that are many, well-spaced, and possess a dark green coloration on the upper surface and a lighter green hue on the lower surface. These flowers possess a diameter of around 5 cm and are characterized by the presence of five petals. The coloration of these petals varies over a spectrum that includes shades such as red, light purple, carmine, deep and mild pink, purple, copper, orange, white, yellow, and bicolored. The flowers in question have olfactory properties, however their aroma is not universally present, and their blooming period spans from the month of June to September.(Farooqui & Tyagi, 2018). The plant is geographically dispersed across various regions, including Asia (Nepal, Turkey, Iran, Jordan, United Arab Emirates, India, Pakistan, Iraq, Afghanistan, Lebanon, Cyprus, China,

Palestine, Syria), Africa (Tunisia, Niger, Libya, Algeria, Mauritania, Morocco), Europe (Spain, Albania, Greece, France, Italy, Croatia, Malta, Portugal). Furthermore, it was cultivated extensively in these regions (Farooqui & Tyagi, 2018). *Nerium Oleander* is a prevalent plant species in the desert area of North Africa. The occurrence of this species is prevalent in Algeria, particularly in areas characterized by sandy and rocks.(Garima & Amla, 2010)

Nerium oleander, has a huge variety of phytochemicals and have antioxidant, anticancer, antibacterial, and anti-diabetic properties (Ali, El-Ella, & Nasr, 2010).). *Nerium oleander* has a wide range of phytochemicals and exhibits anti-allergic, anti-cancerous, anti-bacterial, and anti-diabetic properties (Boswell, Dorweiler, Erbs, & Caplan, 2013). Due to its high phytochemical content, *Nerium oleander* has a diverse array of biological and pharmacological effects. The compounds present in the sample include flavonoids, steroids, tannins, sugars, alkaloids, and cardiac glycosides are abundant in leaves (Al-Snafi, 2020). Cinnamic acid, which makes up a large portion of the polyphenols in the leaf, is present in less concentrations than epicatechin, catechin, and chlorogenic acid. The main polysaccharides found in the leaves, in addition to phenolic substances, include galacturonic acid, galactose, arabinose, and rhamnose (Sinha & Biswas, 2016). The flowers of *N. oleander* exhibit a substantial presence of phenol, tannin, flavonoid, coumarin, alkaloid, and triterpene compounds. (Bakir Çilesizoğlu, Yalçin, Çavuşoğlu, & Sipahi Kuloğlu, 2022).

FTIR spectroscopy is another powerful analytical tool for identifying organic, polymeric, and, in some cases, inorganic materials. It can identify chemical compounds based on their characteristic absorption of infrared light, allowing researchers to identify functional groups and the chemical bonds within a sample. FTIR spectroscopy is widely used in fields such as chemistry, biology, physics, and materials science. It is particularly useful in the analysis of polymers, natural compounds, & biomolecules (Stuart, 2004).

GC-MS is a hybrid method that combines the separation prowess of Gas Chromatography (GC) with the compound detection capabilities of Mass Spectrometry (MS). In this approach, GC is employed to partition the chemical mixture into its constituent elements, and MS is utilized to ascertain and measure these compounds. This method proves highly

valuable for scrutinizing intricate mixtures, pinpointing unfamiliar substances, and tracking impurities. (Konappa et al., 2020).

Structure-based. virtual screening.is an essential element in the process of molecular docking. (Sohraby, Bagheri, & Aryapour, 2019). Docking is employed in molecular modelling, which facilitates the investigation of the binding and interaction patterns between macromolecules and small molecule compounds. This methodology relies on the utilization of software such as AutoDock Vina (Trott & Olson, 2010) . Small molecule chemicals, commonly referred to as ligands, attach with the binding pockets present in proteins structures and enzymes, which effect on their structures and activities. The utilization of molecular docking techniques enables the screening of a collection of small molecule compounds, facilitating the identification of those that possess the ability to bind within a designated pocket. The non-covalent interaction energies, often known as "binding affinity," can be estimated or predicted using certain mathematical methods called scoring functions (J. Liu & Wang, 2015). Scoring functions are firstly employed to differentiate between accurate and inaccurate conformations, as well as to priorities different ligands based on their binding affinities (Huang, Grinter, & Zou, 2010). Non-covalent or non-bonded interactions comprise electrostatic forces, & Vander Waals forces The energy associated with these interactions is determined by considering the relative positions and distances between each molecule of the phytochemical and the atoms of the protein molecules. (Wong et al., 2022).

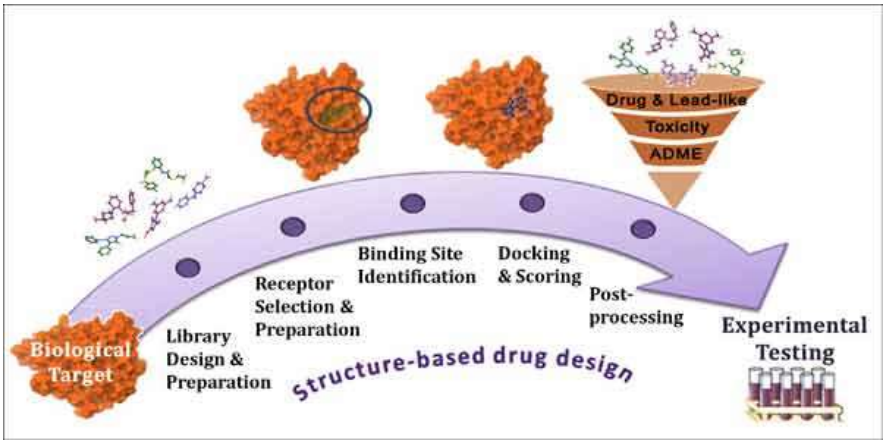


Figure 1.1 Structure based drug designing strategy

Molecular dynamics simulation was first introduced in 1970 (McCammon, Gelin, & Karplus, 1977). The utilization of simulations allows for the comprehension of the natural flexibility attributes exhibited by biomolecules and their interaction with one another. Molecular dynamics (MD) simulations have demonstrated a high degree of accuracy, as evidenced by the consistent integration of results between simulations and experimental investigations conducted by researchers worldwide. Molecular dynamics simulation becomes essential with the Virtual High Throughput Screening (VHTS) method and the subsequent identification of the hit compounds. It is possible for the particles to exhibit their intrinsic properties in a highly natural environment in a molecular dynamic simulation. The simulation box encompasses a majority of the prevailing environmental parameters, including temperature, pressure, and the presence of solvent molecules. Through the application of these specified circumstances, the residues, and certain areas of the protein exhibit movement, thereby elucidating the underlying mechanisms of inhibition or activation. The employed methodology has a high level of practicality in effectively distinguishing between false positives and true positives (Kollman et al., 2000).

Dug-likeness and ADMET analysis employ computation to rapidly and economically discover compounds with biological action.(Bhat, Mir, Sheikh, Alkanani, & Mir, 2022). Computational models of prediction are of greatest significance in the development of in-silico estimations pertaining to pharmacological, pharmacokinetic, and toxicological consequences. The mentioned expects play a crucial role in providing valuable insights for decision-making processes, ultimately leading to advancements in technology and the development of pharmaceuticals. Molecular docking is an economically viable and physically fast methodology employed in the advancement and assessment of pharmaceutical molecules. This research provides useful knowledge into the interactions between pharmaceutical substances and receptors, allowing for the prediction of the binding ability of drug models to specific target proteins. In recent years, the field of computer-aided drug discovery has experienced notable progress, characterized by the introduction of sophisticated tools that facilitate the investigation of Phyto-ligands sourced from diverse plants, aiming to identify their capacity for drug development (Loza-Mejía, Salazar, & Sánchez-Tejeda, 2018). The utilization of computational prediction models has great importance in informing the choice of methodologies for technical and

pharmacological investigations. Additionally, these models have been utilized to predict the in silico behavior of pharmacokinetics, pharmacology, and toxicology. (Mir et al., 2022).

Bacteria are posing serious concerns to human health and are causing diseases. Many drugs and antibiotics are available to control the disease. Regardless of the successful therapies there is still several downsides which include their poor bioavailability, large dosage, unfavorable adverse effects, poor therapeutic outcomes, patients developing greater medication resistance, and non-specific targeting. These pathogens have developed resistance against the available antibiotics and have serious side effects. The current approach will determine the antibacterial activity of natural compounds of *N. oleander to* combat antimicrobial resistance and to devise better treatment options against the bacteria the have developed resistance against the current treatment therapies.

Aims and Objectives

The study aims to identify the natural compound the has the capacity to act as a drug to combat the prevailing antimicrobial resistance.

To achieve these aims, following are the objectives:

- Preparation of extract and check its antibacterial activity through AST technique.
- Identify the phytochemicals of extract through GCMS and FTIR.
- Search these compounds from database for virtual screening of ligands.
- Study the interaction of antimicrobial compounds with selected proteins through molecular docking.
- Use of molecular dynamic modelling techniques to investigate the interaction between the target molecule and phytochemical.

Chapter 2: Review of Literature

2. Review of Literature

The phenomenon of antibiotic resistance in bacteria has given rise to the issue of chronic diseases. The global concern regarding the growth and transmission of multidrug-resistant (MDR) bacteria is of considerable significance, as it leads to heightened morbidity and mortality rates linked with infectious diseases. Additionally, these measures provide challenges in the management of a diverse patient population, encompassing individuals in critical care, surgical patients, organ transplant recipients, and those undergoing cancer therapy. (Michael, Dominey-Howes, & Labbate, 2014). The 2017 study issued by the World Health Organization's Global Antimicrobial Surveillance System underscores the substantial apprehension surrounding the worldwide incidence of antibiotic resistance. (Control & Prevention, 2019). The management of antibiotic-resistant illnesses makes significant financial costs, costing about $50,000 per person. It adds up to about $20 billion every year for society. The problem gets worse because we use antibiotics a lot, and sometimes we don't use them correctly. Plus, there aren't many new antibiotics being developed to replace the old ones that don't work anymore. All of this makes antibiotic resistance a big threat to public health.

At the moment, antibiotics are the main way we try to treat both regular bacterial infections and infections caused by biofilms. These medicines target processes that are important for bacteria to grow and stay alive. These processes include building and maintaining the cell wall and cell membrane, as well as making DNA, RNA, and vital proteins. Over time, bacteria have developed ways to defend themselves against these attack molecules, which is why some antibiotics don't work so well anymore (Aminov, 2010). To get rid of bacteria that are resistant to many drugs, doctors might need to use a lot of antibiotics or try the few 'last-resort' antibiotics we have left (Ventola, 2015). When bacteria form biofilms, it becomes even harder to treat infections because these biofilms create a shield that makes the bacteria resistant to drugs. To deal with this, doctors might have to physically remove the biofilm, like scraping it off, and use high doses of antibiotics at the same time (Arciola, Campoccia, & Montanaro, 2018; Wu, Cheng, & Cheng, 2019). These treatments can take a long time, cost a lot of money, and sometimes lead to side effects. And even with all this effort, the outcome is still uncertain.

2.1. Antibiotic Resistance Mechanisms

2.1.1. Cell membrane and Cell wall disruption

The cell envelope, which constitutes the outer layer of bacteria, has evolved as a defense mechanism against antimicrobial agents that possess the ability to eradicate these microorganisms. Teichoic acids can be found within the cellular wall structure of Gram-positive bacteria, while Gram-negative bacteria exhibit lipopolysaccharide within their outer membrane. The presence of phosphate groups on these entities confers a negative charge to the bacterial surface. This poses a challenge for hydrophobic medications to traverse bacterial membranes due to their aversion to water. The efficacy of medications against these bacteria is limited. (Wu et al., 2019).

2.1.2. Resistance gene transfer and target modification

Bacterial transmission and horizontal gene transfer facilitate the dissemination of resistance genes among bacterial populations (Van Acker, Van Dijck, & Coenye, 2014). The activation of resistance genes has the potential to induce chemical modifications in antibiotics or their targets, so limiting their mode of action and providing a protective effect. (Pelgrift & Friedman, 2013).

2.1.3. Deactivating enzymes

Resistant bacteria have the ability to produce extracellular and/or intracellular enzymes that can enzymatically degrade or hinder the binding of antibiotics. Some bacteria have special enzymes that they can use to change or break down drugs. These enzymes are usually found in or around the bacteria's surface, and they are very picky. They can choose to target and deactivate specific drugs. One way these enzymes work is by using water to break the drug apart, and another way is by changing the drug's structure. This is a big way that bacteria become resistant to natural antibiotics that are supposed to kill them. In most cases, the bacteria that become resistant inherit these special drug-resistance genes from other bacteria. Plasmids are frequently utilized as carriers for these genes, which are typically found on little genetic fragments. Pathogenic bacteria, the ones that make us sick,

likely get these resistance genes from a pool of genes that exist in other types of bacteria, including the ones that make antibiotics (Yoneyama & Katsumata, 2006).

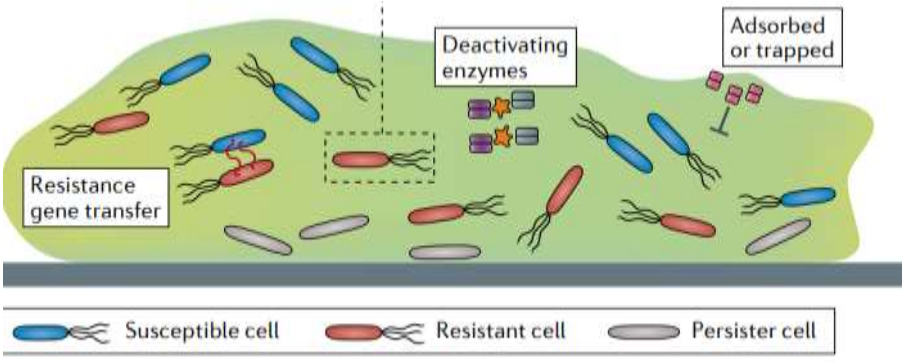


Figure 2.1: Mechanism of resistance by deactivating enzymes

2.1.4. Efflux pumps

Efflux pumps, a common feature observed in antibiotic-resistant bacterial cells, exhibit an upregulation phenomenon where they actively facilitate the transportation of antimicrobial drugs out of bacterial cells. (Makabenta et al., 2021). Bacteria have ways to defend themselves against antibiotics. They have special pumps that can push these drugs out of their cells. These pumps are like doors that let the antibiotics escape from the bacteria. One of the contributing factors to the inherent resilience of Gram-negative bacteria against antibiotic treatment is their structural characteristics. Some bacteria go even further to protect themselves. For example, when antibiotics try to stop the bacteria's protein-making process, the bacteria can make proteins that shield the target of the antibiotics. This makes it harder for the antibiotics to do their job, like tetracycline. These defense mechanisms can make antibiotics less effective, especially when the bacteria have pumps that can push out many kinds of antibiotics. Bacteria like Enterobacteriaceae, Pseudomonas aeruginosa, and Staphylococcus aureus have been found to have these pumps (Abebe, Tegegne, & Tibebu, 2016).

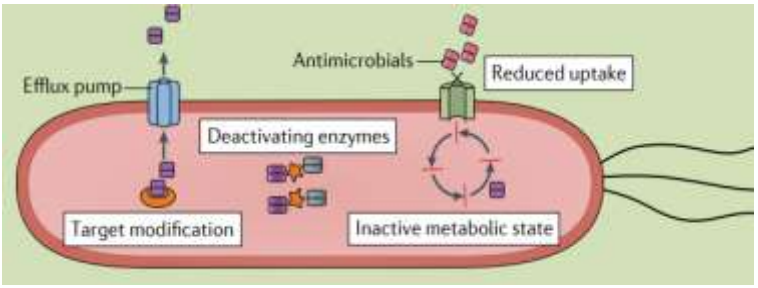


Figure 2.2: Mechanism of resistance by Efflux pump

2.1.5. Antibiotic penetration Limitation by Extracellular polymeric substances

The protective gooey stuff around bacterial biofilms makes it hard for some antibiotics, like aminoglycosides, to get in because they push against each other. Even for other types of antibiotics that can get inside biofilms, it's still tricky because the food and waste inside the biofilm create a maze that weakens their germ-killing power (Del Pozo & Patel, 2007; Tseng et al., 2013).

2.1.6. Utilization of plants to combat antimicrobial resistance.

Plants that have healing properties have been important in basic healthcare. They also provide a valuable source of new and useful substances that can be used to make medicines. Illnesses caused by harmful germs are a big problem for public health and lead to many deaths all over the world. Infections caused by these tiny harmful creatures are a major worry for human health. We are seeing more cases where the microbes that cause these infections are becoming resistant to the drugs we use, and the drugs we have now can sometimes have bad side effects. Also, some old infections that we thought were gone are coming back. So, we really need new medicines that can fight these infections and are safe and effective (Bhat et al., 2022). Plants aren't just important for giving us food, they also contain special natural compounds that can be good for our health and help treat diseases. These compounds can be used in things like food, medicine, and cosmetics. In countries like India, many people rely on traditional medicines made from plants because they have these helpful compounds. In a place called northeastern India, lots of people use traditional medicine, probably because there are many different plants there and because of how people live. These special compounds in plants can do things like fight germs, stop cancer

from growing, reduce swelling, and act as antioxidants. When we make medicines from plants, we usually use extracts from the plants, which have a mix of these compounds. Some plants have a lot of these useful compounds, but we haven't studied all of them yet. To find new chemicals from plants and make sure they're good quality, we need to have good ways to test them.

2.1.7. Ribosomes Protection

The ribosomal protection mechanism is a common way bacteria defend themselves against drugs. This defense is used by bacteria (gram-negative) and (gram-positive), to resist drugs like tetracyclines. When bacteria change the place where the drug is supposed to work, it's often because of changes in a specific gene in their DNA. This gene change happens naturally, and the bacteria that survive when the drug is around are the ones that have this gene change. The important protective proteins in this process are called TetO and TetM, and they're the most well-known ones (Chopra & Roberts, 2001).

2.2. Classes of phytochemicals

2.2.1. Alkaloids

Alkaloids are a big group of chemicals found in nature, mostly made up of ammonia compounds. They're like building blocks created from amino acids, and they often have different parts attached to them instead of hydrogen atoms. Many alkaloids also contain oxygen. Alkaloids are basic in nature, meaning they have properties like chemicals. This basicity can be strong or weak, depending on how the molecule is built and where the different parts are located.(Nahar & Sarker, 2019)

Many alkaloids, like atropine, are solid substances, while some are liquids made up of hydrogen, nitrogen and carbon, Alkaloids can dissolve well in alcohol, but they don't dissolve much in water. However, their salt forms can usually dissolve in water. When you taste alkaloid solutions, they often have a strong bitter taste. These nitrogen-containing compounds serve as a defense mechanism for plants, helping to protect them from plant-eating animals and diseases. People have found various uses for alkaloids, including in medicines, such as stimulants, painkillers, and even poisons, due to their powerful effects

on living organisms. In the natural world, you'll find alkaloids in high amounts in plant seeds and roots, often mixed with other compounds like vegetable acids. Some alkaloids are used in medicine as anesthetics (to numb pain) and as stimulants for the central nervous system (CNS), which affects how our brains and nerves work (Madziga, Sanni, & Sandabe, 2010).

2.2.2. Glycosides

Glycosides, in simple terms, are compounds formed when sugars, including complex sugars, combine with various organic compounds that have hydroxyl groups (sometimes thiol groups). This combination involves the participation of a specific part of the sugar molecule. Glycosides are characterized as optically clear and crystalline compounds composed mostly of carbon, hydrogen, and oxygen, with the potential inclusion of nitrogen and sulfur. These substances exhibit solubility in aqueous solutions and are commonly present in the cellular sap of several plant species. Glycosides are composed of two distinct components, namely a sugar moiety, typically glucose, and a non-sugar moiety referred to as either aglycone (Doughari, 2012; Kar, 2007). Phenol, Alcohol, and glycerol are like the building blocks in a special kind of compound called glycosides. These compounds don't have a strong taste and can be easily taken apart into their parts with the help of special substances or acids. Scientists put glycosides into groups based on the sugar they're made from, what they're made of, or what they do in the body. Glycosides are typically found in plants belonging to the Genitiaceae botanical family. Despite lacking molecular relatedness, these compounds consistently exhibit an obvious bitter taste. When you taste something bitter, it sends signals to your brain to produce more saliva and stomach juices. Chemically, these bitter compounds have something called a lactone group, which can be diterpene lactones. Some bitter compounds are used to make things tight, like tannic acid, while others help fight tiny organisms, or even slow down certain body processes. Several examples of glycosides may be identified, such as cardiac glycosides, which exert their effects on the heart. Additionally, anthracene glycosides are known to assist with bowel movements and address skin disorders. Chalcone glycoside, on the other hand, finds application in cancer treatment. Furthermore, glycosides such amarogentin, gentiopicrin, andrographolide, ailanthone, and polygalin can also be mentioned. (Doughari, 2012).

Extracts from certain plants with cyanogenic glycosides are added to medicines to make them taste better. Amygdalin, one of these compounds, has been used to treat cancer by releasing a substance that can kill cancer cells in the stomach. It's also used in some cough medicines. But if you eat too much of these cyanogenic glycosides, it can be very dangerous and even deadly. Some foods that have these compounds can make you sick and hurt your stomach if they're not handled and prepared correctly (Doughari, 2012).

2.2.3. Flavonoids

Flavonoids are an unique class of polyphenolic compounds that are widely distributed throughout various plant species. In terms of their structure, they contain multiple benzene rings (which are certain kinds of chemical structures made up of carbon atoms) and are part of a group of aromatic compounds known as C15. Many studies have shown that they can act as antioxidants, helping to get rid of harmful molecules called free radicals (Doughari, 2012). Over four thousand flavonoids have been identified, with certain members of this class contributing to the pigmentation of numerous plant species. Approximately 70% of plant species include the compounds quercetin, kaempferol, and quercitrin. Moreover, it is worth noting that there are other subclasses of flavonoids, namely flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones, catechin, and leucoanthocyanidins.

2.2.4. Phenolics

Phenolics are natural chemicals found in many plants that give fruits their colorful appearance. These substances are created in plants from a molecule called phenylalanine, with the help of an enzyme called phenylalanine ammonia lyase. Phenolics serve several important purposes in plants, with one of their main roles being to protect the plant from diseases and plant-eating animals. Interestingly, they can also be useful in fighting infections in humans (Cheynier, 2012). There are several distinct classifications of phenolic compounds, which include phenolic acids and flavonoid polyphenolics, such as flavanones, flavones, xanthones, and catechins. Additionally, there exist polyphenols that are not classified as flavonoids. Among these compounds, caffeic acid is widely distributed and frequently encountered in many plant species. Chlorogenic acid, another type of

phenolic, can sometimes cause skin allergies in people. Phenolics are like natural antioxidants, which means they can help protect our bodies. They are often used in supplements and can be found in foods like apples, green tea, and red wine. These substances are believed to have the potential to fight cancer and may reduce the risk of heart problems. They can also have anti-inflammatory effects (Kar, 2007).

2.2.5. Saponins

Saponins are compounds found in plants, and they get their name from a plant called Saponaria vaccaria, which was once used as soap because it contains a lot of saponins. These saponins behave like soap in water by producing foam. When saponins are broken down, they produce something called sapogenin. Most saponins have sugar attached at a specific spot, usually because the sapogenin has a hydroxyl group there (Desai, Desai, & Kaur, 2009). They dissolve in water and alcohol but not in things like benzene. Interestingly, saponins have some therapeutic uses. They've been shown to have effects on cholesterol levels and can even help fight cancer. They're also important for the activity of cardiac glycosides, which affect the heart. There are two main classifications of steroidal sapogenin: diosgenin and hecogenin. These chemicals are employed in the manufacturing of therapeutic sex hormones. One example is the derivation of progesterone from diosgenin. The primary source of diosgenin, the precursor for progesterone synthesis, was formerly derived from plants indigenous to Mexico. However, in recent times, the predominant supplier of diosgenin has shifted to China (Desai et al., 2009).

2.2.6. Tannins

Tannins are frequently present in several botanical groups and are distinguished by their phenolic composition.. These substances have the ability to undergo dissolution in both water and alcohol, and can be found within many parts of plants, including roots, bark, stems, and outer layers. Tannins possess a distinctive capacity to induce tanning processes, resulting in the transformation of materials into leather. (Hassanpour, MaheriSis, & Eshratkhah, 2011). The substance's acidity can be linked to the existence of phenolic or carboxylic functional groups. Tannins possess the capacity to form chemical interactions with proteins, carbohydrates, gelatin, and alkaloids. Tannins can be categorized into two

distinct classifications, namely hydrolysable tannins, and condensed tannins. Hydrolysable tannins, when subjected to hydrolysis, generate chemicals such as gallic acid and ellagic acid. The nomenclature of these compounds is determined by the specific acid they generate, resulting in their classification as either gallotannins or ellagitannins. When subjected to heat, pyrogallol can also undergo a chemical reaction to produce pyrogallic acid. Several hydrolysable tannins can be identified, such as theaflavins (which are commonly present in tea), daidezein, genistein, and glycitein. Plants possessing a high concentration of tannins are used in the treatment of many diseases. In Ayurveda, tannin-rich plants are used in formulations to treat conditions like leucorrhoea, rhinorrhea, and diarrhea (Okuda & Ito, 2011).

2.2.7. Terpenes

Terpenes encompass a wide array of naturally occurring chemicals that can be detected in various substances such as essential oils, resins, and oleoresins. They are flammable and made up of carbon and hydrogen. Terpenoids are a subgroup, and they can be categorized based of carbon atoms they contain mono-, di-, tri-, and sesquiterpenoids. Diterpenes, which have twenty carbon atoms, are often considered resins (Doughari, 2012). Taxol, an antitumor drug, is an example of a diterpene. Sesquiterpenes, containing 15 carbon atoms, are also found in many essential oils. When applied externally or consumed, they can be irritating to the skin or gastrointestinal tract. (Silvestre & Gandini, 2008). Some sesquiterpenes, like palasonin from Butea monosperma, have anthelmintic (worm-expelling) properties and can affect glucose levels in certain parasites.

2.2.8. Anthraquinones

These compounds are derived from anthracene and belong to the phenolic and glycosidic compound groups. They can be oxidized in various ways, resulting in substances like anthrones and anthranols. Several derivatives, such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin, and emodin, have a common feature of possessing two hydroxyl groups located at positions C-1 and C-8. (Doughari, 2012).

2.2.9. Essential oils

These are fragrant and easily evaporate liquids obtained from plants and sometimes animals. They're also called volatile or ethereal oils. They give plants their distinct smells and are used to enhance the aroma of spices. These oils can come from different plant parts like leaves, stems, flowers, and roots (Bassolé & Juliani, 2012). A single essential oil can have over 200 different chemical components, with trace elements responsible for their unique scent and flavor. To get essential oils, you can use methods like steam distillation, expression, or extraction. Steam distillation involves boiling plant parts and collecting the vapor, which turns into oil. Expression, or extrusion, is done by squeezing or pricking plant parts, like citrus fruit, to release the oil. Some examples of essential oils are bitter almond oil, black mustard oil, and Geum urbanum oil, which contains eugenol. (Adorjan & Buchbauer, 2010).

2.2.10. Steroids

Steroids, also called 'cardiac glycoside,' are natural compounds found in plants. They have been used for different purposes, like making arrow poisons or medicines for the heart. When injected into a person or an animal, these steroids, which are like special types of steroids, have a very strong and specific effect on the heart muscle (Kumavath et al., 2021). Another type of steroid, known as anabolic steroids, can help the body keep more nitrogen, which is important for people with bone problems like osteoporosis and for sick animals. However, it's crucial to use steroidal glycosides with care because a small amount can provide the right kind of boost for a sick heart, but too much can be harmful, even causing death. (Doughari, 2012).

2.3. Antioxidant properties of Plants

Antioxidants are like protective helpers in our body. They stop or slow down the damage that can happen to our cells. There are two types of antioxidants: natural ones that come from plants and synthetic ones made in labs. That's why scientists are trying to find safer antioxidants from natural sources, like plants. Many plants have these special substances called phytochemicals, which work as antioxidants. Several plants commonly used in food practices include tamarind, cardamom, lemon grass, and galangal basil. These plants has the ability to promote human health by combating cellular damage inside our physiological

systems. (Javanmardi, Stushnoff, Locke, & Vivanco, 2003). Food going bad because of bacteria or fungi is a big problem that costs a lot of money for food companies and communities all around the world. Also, the spread of harmful germs in food is a serious health issue. People are now more aware that synthetic preservatives can be bad for health, so they want to use safe, natural preservatives instead. Many of these natural preservatives can either stop food from spoiling or protect it from harmful germs (Baharlouei, Sharifi-Sirchi, & Bonjar, 2010; Negi, Chauhan, Sadia, Rohinishree, & Ramteke, 2005)

2.4. Phytochemistry of *Nerium Oleander*

In the oleander plant, different parts contain various types of natural compounds with medicinal properties. The substances that have been mentioned encompass phenolics, tannin, terpenoid, alkaloids, saponin, and anthraquinone. Prior research has indicated that leaves exhibit the most significant concentrations of saponin, riboflavin, thiamine, and tannins, whilst roots are characterized by heightened quantities of alkaloids and ascorbic acid. The main objective of the in vitro study was to assess the antioxidative and free-radical scavenging properties of oleander. The findings of the study revealed that the root exhibited the highest concentration of total phenolics, but the leaves exhibited the highest concentration of flavonoids. Furthermore, a particular extract derived from oleander leaves has demonstrated considerable promise in the management of diabetes. This extract has several advantageous components, including p-coumaric acid, jasmonic acid, gallic acid, vanillic acid, 4-hydroxybenzoic acid, and rutin. Additional investigation into oleander has revealed the existence of several phytochemicals, encompassing fatty acids and sterol compounds, that provide a spectrum of advantageous effects on human health. Furthermore, it has been discovered that oleander extracts possess ellagic acid, methyl gallate, catechin, and reserpine, in addition to their hepatoprotective activities. The utilization of high-performance liquid chromatography (HPLC) facilitated the identification of many chemical compounds present in oleander leaves, including cinnamic acid, chlorogenic acid, rutin, catechin, epicatechin, and quercetin, which are recognised for their antioxidant characteristics. In addition, it has been shown that oleander possesses catechin and epicatechin, compounds that are commonly linked to a range of advantageous effects on human health. The oleander plant is known to contain a range of fatty acids, such

as lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and arachidic acid. Sterol compounds originating from cholesterol were detected in oleander, with each molecule providing unique health benefits. The investigation of oleander extracts possessing immunomodulatory properties resulted in the detection of many chemical compounds, such as phytol, catechol, apocynin, syringol, isopseudocumenol, p-vinylguaiacol, vanillin, syringic acid, γ-sitosterol, stigmasterol, α/β-amyrin, campesterol, and additional unidentified constituents. (Dey, 2020). Pectic polysaccharides, which consist mostly of galactomeric acid, have been identified in the leaves of the Nerium oleander plant. Furthermore, it is worth noting that these leaves are rich in sugars, namely rhamnose, arabinose, and galactose. In this study, a team of researchers has successfully identified four novel cardenolide monoglycosides, three pregnanes that were previously unknown, and several compounds with intricate nomenclatures such as "21-hydroxypregna-4,6-diene-3,12,20-trione," "20R-hydroxy pregna-4,6-diene,3,12-dione," and "16β,17β–epoxy-12β hydroxy pregna-4,6-diene-3,20-dione" within the examined botanical specimen. Moreover, the implementation of isolation protocols has resulted in the discovery of two supplementary compounds known as "nericomaric" and "isoneriu-coumaric acids" derived from the foliage of the Nerium oleander botanical species. (Namian, Talebi, Germi, & Shabani, 2013). The study demonstrated that this particular plant harbours significant chemicals possessing therapeutic potential. A range of chemical compounds, such as carbohydrates, proteins, alkaloids, flavonoids, terpenoids, cardiac glycosides, tannins, and saponins, were identified within the plant's foliage.(Hase et al., 2016)

2.5. Pharmacological properties of *Nerium oleander*

Nerium oleander has pharmacological properties. It has antioxidants, antimicrobial, neuro protective, Antioxidant, anti-cancerous, anti-inflammatory and chemosensitizer properties.

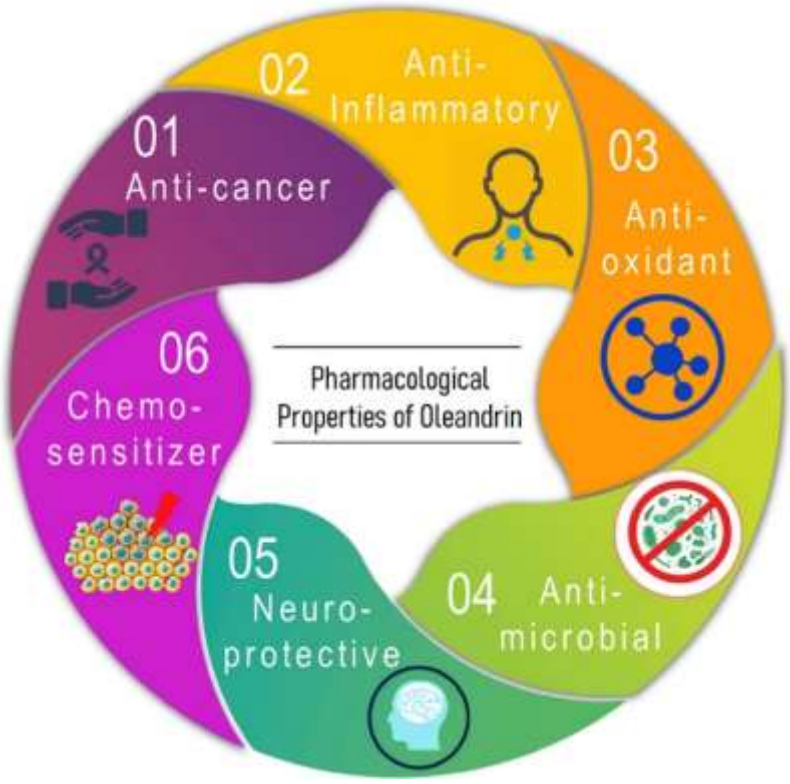


Figure 2.3: Pharmacological properties of Oleander

2.6. Antimicrobial activities of *Nerium oleander*

A research investigation was conducted utilizing the roots of *Nerium oleander*, which contained a novel cardenolide known as tetraenolide. This compound exhibited both antibacterial properties and cardiac activities similar to digoxin. (Huq, Jabbar, Rashid, & Hasan, 1999)**.** Another investigation demonstrated the efficacy of the ethanolic extract (Hussain & Gorsi, 2004).

This study aims to assess the antibacterial efficacy of an ethanolic extract through the utilization of the agar well diffusion technique, while also analyzing the dimensions of the inhibition zones. The presented extracts exhibited a diverse range of action against gram-positive bacteria and fungi. The extracts derived from *Nerium indicum* exhibit a reduction in microbial growth, indicating their potential micro biostatic properties. The acquired results are promising, as the methanolic, chloroform, and hexane extracts have demonstrated significant antibacterial efficacy. (Malik et al., 2015).

2.7. Antifungal properties of *Nerium Oleander*

The leaf and stem extracts from *Nerium oleander* have the ability to fight off certain harmful microorganisms like *C. neoformance, S. cerevisiae, and C. albicans*. Among these extracts, the one made with methanol is the most effective at stopping the growth of these fungi. The chloroform and ethanol extracts also work, but the petroleum ether extract doesn't seem to have any effect. When it comes to the stem extract, it works best against *C. neoformance,* followed by *S. cerevisiae,* and then *C. albicans* (Bameta, Kumari, & Upadhyaya, 2017).

Different parts of the plant had varying effects on the three fungi. For Macrophomina phaseolina, the roots of the plant were the most effective in slowing down the fungus. The chloroform root extract was the best, reducing the fungus's growth the most, followed by the acetone root extract. For Sclerotium rolfsii, the chloroform leaf extract worked the best, reducing the size of the fungal colony the most. The shoots of the plant had the most significant effect, especially the acetone shoot extract, which reduced the size of the fungal colony the most, followed by the chloroform and ethanol shoot extracts. We also talked about how these extracts from Nerium oleander could potentially be used to control fungus species (Siddiqui, Bokhari, & Perveen, 2016)

2.8. Larvicidal activity

The natural extracts from *Nerium oleander* leaves were tested to see if they can kill the larvae of *Thaumetopoea wilkinsoni*, a type of insect. Different concentrations of the plant extract (ranging from 62.5 ppm to 8000 ppm) were used and observed the larvae's mortality at different time points. It was found that the extract made from the plant's leaves was most effective in killing the larvae. This means that at these concentrations, about half of the larvae died (Semiz, 2017).

The study was conducted to create natural insecticides from plant materials, specifically from *Nerium oleander L*. leaves, to find new ways of controlling diseases spread by insects like Aedes aegypti. Metallic nanoparticles were made from gold & silver nanoparticles using these leaves and tested their safety and effectiveness in killing Aedes aegypti mosquito larvae (Al-Hakimi, Abdulghani, Alhag, Aroua, & Mahyoub, 2022). In another

study flower extracts from the Nerium oleander plant was tested on Culex quinquefasciatus larvae to see if it would kill them. It was found that the flower extract mixed with hexane was better at killing the larvae than the one mixed with water.(Raveen, Kamakshi, Deepa, Arivoli, & Tennyson, 2014)

2.9. Antidiabetic activity

In a recent study *Nerium oleander* flower extract was used which led to several positive changes in the bodies of diabetic rats. It lowered their blood sugar levels and a marker called HbA1c, while increasing their insulin and C-peptide levels. *Nerium oleander* flower extract also helped improve the health of their livers and their cholesterol levels in the blood. It even stopped some harmful processes like lipid peroxidation and made sure that the liver's antioxidant enzymes were working properly. Moreover, *Nerium oleander* flower extract had beneficial effects on the immune system and nervous system in the liver of rats that are diabetic. The results showed that liver tissues of these rats under a microscope had significant damage in the diabetic group. However, in the group that received 225 $mgkg^{-1}$ of Nerium oleander flower extract, some of this damage was reduced. The activity of a specific gene called SLC2A2 in the liver was also checked. In diabetic rats, this gene was less active compared to healthy rats. But when we treated the diabetic rats with 25 mg/kg of NFE, the gene became more active (Battal et al., 2023).

It is found that when they gave diabetic rats a single dose of *Nerium indicum* plant extract (300.00 mg per kg of body weight), it significantly lowered their blood sugar levels. The ethanolic extract took about three hours to show this effect, while the chloroform extract worked after just one hour. However, the water extract didn't lower blood sugar levels in the rats. They also tested how different extracts affected glucose tolerance in normal rats. After giving them glucose, the blood sugar levels in these rats rose quickly but then went down. A medicine called Glibenclamide helped control their blood sugar levels better than the control group. Among the *Nerium oleander* extracts, the chloroform extract worked best in improving glucose tolerance, and the water extract worked the least. They also found that diabetic rats treated with *Nerium oleander* chloroform and ethanolic extracts gained more weight and had lower blood sugar levels. (Sikarwar, Patil, Kokate, Sharma, & Bhat, 2009)

2.10. Anticancer activity

The stem bark extract of *Nerium oleander*, commonly known as Karabi, was employed by the researchers to isolate phytochemicals. These phytochemicals were then used to synthesize gold-conjugated nanoparticles in an environmentally benign manner. A comprehensive investigation was conducted to analyze the eco-friendly production of gold nanoparticles, employing various methodologies such as surface plasmon resonance spectroscopy, high-resolution transmission electron microscopy, X-ray diffraction analysis, and dynamic light scattering. A thorough mechanism was devised to elucidate the intricate mechanisms behind the synthesis of gold-conjugated nanoparticles (AuNPs). In order to assess their potential, a research investigation was undertaken to examine the anti-cancer characteristics of stabilized gold nanoparticles (AuNPs) in relation to the MCF-7 breast cancer cell line.. The findings exhibited a notable efficacy in specifically triggering programmed cell death (apoptosis) in cancerous cells. Furthermore, we demonstrated the practicality of utilizing stabilized gold nanoparticles (AuNPs) as a catalyst in diverse applications. (Barai et al., 2018).

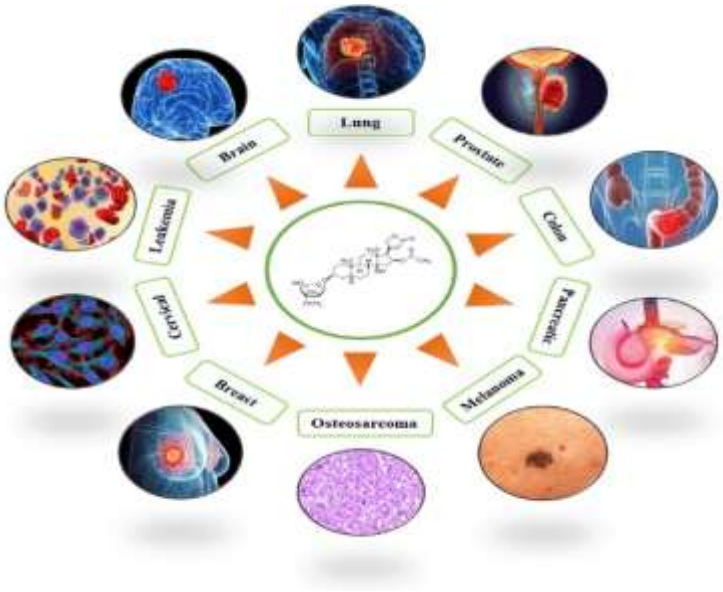


Figure 2.4: N. oleander anticancer activity in the body organs

A study was conducted, revealing that an extract derived from the leaves of *Nerium oleander* has the ability to stop the proliferation of HeLa cervical cell lines. when cultured in a laboratory setting. Additionally, it inhibits the mobility of these cells. Furthermore, it

decelerates a particular stage of the cellular division process. (Mohapatra, Biswal, Dandapat, & Debata, 2021).

2.11. Hepatoprotective activity

In a research study, scientists tested how effective a flower extract (made with methanol) was at protecting the liver in rats that were exposed to a harmful chemical called CCl4. They found that when rats were given flower extract (MENO-F) for 7 days before being exposed to CCl4, their livers were better protected. In the rats that only got CCl4 (Group II), their liver health was not good. Their blood the blood tests revealed elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and total bilirubin, which are signs of liver damage. However, the rats who were administered varying doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the floral extract (referred to as MENO-F) for a duration of one week before to exposure to CCl4 (classified as Groups IV, V, and VI), exhibited significantly improved liver conditions. Their blood tests showed that the flower extract helped to protect their livers from the damage caused by CCl4. There was also a group of rats that received a standard liver-protective medicine called Silymarin (Group III). This medicine also helped to bring their liver function back to normal levels (Singhal & Gupta, 2012).

2.12. Wound healing activity

The wound healing activity in rats is significantly improved by *N. oleander* extract, likely because of its antimicrobial and antioxidant properties. Aloe vera enhances collagen levels, which in turn strengthens and maintain the tissue structure. This plays a crucial role in maintaining balance within the body and promoting epithelialization. (Rout, Kar, & Maharana, 2014). In a different study, the use of an extract from Aloe vera mixed with *Nerium oleander* showed notable improvements in skin healing. This treatment not only significantly reduced tissue damage and inflammation but also promoted the movement of fibroblast cells. These fibroblasts play a crucial role in producing various substances necessary for wound healing, such as fibronectin, proteoglycan, hyaluronan, and collagens. The enhanced antioxidant and anti-inflammatory properties, along with improved DNA repair capabilities and observed tissue changes, suggest that the utilization of an extract derived from Nerium oleander, which is based on Aloe vera, exhibits potential as a novel

strategy for the management of burn wounds within the realm of cosmetics. This study provides scientific support for future research in this area (Akgun, Aydemir, Ozkan, Yuksel, & Sardas, 2017).

2.13. Cardioprotective Effect

In a study, researchers looked at a substance called the hydroethanolic extract of *N. oleander* (ENO), which comes from the flower. It was found that ENO was better at getting rid of these free radicals compared to its parts. The researchers also wanted to see if ENO could protect the heart. They tested this on rats by giving them ENO in different amounts and a medicine called propranolol. Then, they gave the rats a substance called isoproterenol to harm their hearts. They looked at certain markers in the rats' blood to see if their hearts were damaged. What they found was that when the rats were given ENO or propranolol for two weeks before the harmful substance, their hearts were better protected. The markers in their blood didn't show as much damage. They also looked at how well the rats' bodies were able to defend against damage, especially in the heart. They found that ENO and propranolol helped the rats' bodies do a better job at protecting against this damage. In simple terms, this study suggests that the extract from Nerium oleander Linn flowers might be good for the heart by improving the body's defense system against damage.

2.14. In silico Drug designing and virtual screening

Considerable advancements have been made in the field of pharmaceutical research by employing computer-based methods. These technologies have enabled the process of screening prospective drugs generated from bioactive chemicals found in medicinal plants. Plant-based medicines are frequently employed in the management of chronic diseases and infectious conditions. These therapeutic treatments are frequently obtained from unprocessed extracts that contain a wide array of different phytochemical compounds.(Ralte, Khiangte, Thangjam, Kumar, & Singh, 2022).

In the past, the process of drug discovery was not primarily focused on identifying the molecular target associated with a certain ailment. Instead, the identification of effective treatments was often serendipitous, lacking the systematic and sophisticated approach seen in current drug design practices. However, throughout the mid-1980s, significant progress

was made in the fields of structural biology and bioinformatics, enabling the rational design of drugs based on the structure of three dimensional target protein. (Nantasenamat, Isarankura-Na-Ayudhya, & Prachayasittikul, 2010). During the early stages of development, Structure-Based Drug Design (SBDD) was not regarded as a crucial aspect in the field of drug design. However, recent developments have substantially enhanced these methodologies, making them virtually vital across all phases of drug design procedures. Structure-based drug design (SBDD) is a comprehensive approach that incorporates many approaches, such as molecular docking and molecular dynamics modelling, along with other tools that largely emphasize the interaction between a molecular target, particularly the target protein, and small molecule medicines. The tools aid in the understanding of the role of each individual component and their potential application in improving the efficacy of a specific pharmaceutical compound. (Wang et al., 2016). An investigation was conducted and subsequently published in the Proceedings of the National Academy of Sciences (PNAS) in 2014. This work utilised molecular dynamics simulations to gain insight into the effects of a restricted set of mutations in BCR-ABL kinase proteins on the emergence of substantial drug resistance. Moreover, this study successfully showcased the effectiveness and accuracy of the used procedures and approaches. Currently, the prevailing strategy for the discovery and refinement of potential drug candidates in the field of drug design primarily relies on structure-based methods, with a specific focus on virtual high-throughput screening (VHTS).

Virtual high-throughput screening (VHTS) is a computational methodology employed within the context of structure-based drug development. The procedure involves the utilization of approaches such as molecular docking and scoring functions to examine extensive collections of small molecule compounds. The primary aim of Very High Throughput Screening (VHTS) is to discern a potential effector through the analysis of the biological structure of the target (Shoichet, 2004).

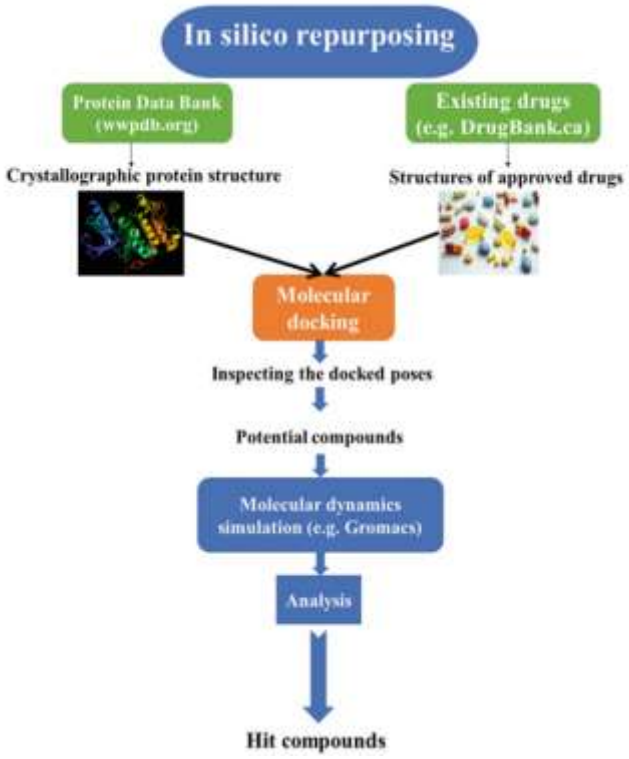


Figure 2.5: An overview of the steps for in silico drug designing by molecular dynamics modelling and virtual screening

2.15. Bacterial proteins

Different bacterial proteins have been selected. These proteins have distinct roles in the bacterial life cycle and are necessary for the survival of bacteria. Previously these proteins have been used as an antibacterial target.

2.15.1. (PDB ID: 6F86)

Crystal Structure of *E. coli* GyraseB for supercoiling of chromosomal DNA (Narramore, Stevenson, Maxwell, Lawson, & Fishwick, 2019).

2.15.2. (PDB ID: 8IWL)

Acinetobacter baumannii protein YdjH, a sugar kinase, remains uncharacterized. The bacterial sugar kinase is a pivotal enzyme involved in the efficient breakdown of sugars in bacteria, playing a critical role in their survival and proliferation. Hence, the focus of antibacterial medication research lies mostly on this particular enzyme family, whereby

YdjH has a notable preference for phosphorylating higher-order monosaccharides possessing a carboxylate terminal. The expression of sugar kinases exhibits a wide range of specificities and functions, hence posing a significant challenge in determining their specificities within this family. The current investigation examines the crystal structure of YdjH, which is produced from Acinetobacter baumannii and will be referred to as YdjH throughout this study known for its remarkable antibiotic resistance and classification as a superbug (Lee, Kim, Ha, & Park, 2023).

2.15.3. (PDB ID: 1XAL)

The subject of discussion is the crystal structure of Staphylococcus aureus 3-dehydroquinate synthase (DHQS). The enzyme Dehydroquinate synthase (DHQS) shows potential as a viable candidate for the development of novel antimicrobial drugs with broad-spectrum activity, effectively targeting both bacteria and lower eukaryotes. (Nichols et al., 2004).

2.15.4. (PDB ID: 7P2M)

The protein *E. coli* GyrB24 is known to have the ability to bind to DNA. The GyrB24 protein possesses a specific binding site where ligands can connect, hence serving as a target location for the antibacterial activity exhibited by the chemicals. (Cotman et al., 2023).

2.15.5. (PDB ID: 3ZG5)

It is a penicillin binding protein of the MRSA *S. aureus*. This protein is involved in the broad-spectrum antibiotic resistance and is resistant to beta lactams class. By binding the ligand to its target site we can develop the antibacterial drug to combat the resistance.

Chapter 3: Materials and Methods

3. Materials and Methods

3.1. Plant material collection

N. oleander leaves, flowers and stems were collected from Islamabad in September 2022. The plant was identified by Dr. Qasim Hayat at the National University of Sciences and Technology Department of Plant Biotechnology.

3.2. Washing of plant material

The methodology for processing the plant material involves a thorough cleansing of the leaves, flowers, and stems. Initially, these plant parts are carefully washed under water to wash off any contaminants such as dust, dirt, or impurities. Once they have been adequately rinsed, the cleaned plant materials are spread out evenly on a sheet of paper to facilitate a rapid drying process. This step is crucial to ensure that the plant components are completely dry before further processing or extraction, as any residual moisture can affect the quality and composition of the final extract. The use of appropriate drying techniques enables the preservation of the botanical material's integrity and the sustenance of the dependability of our research outcomes.

3.3. Drying of the plant material

Plant material was subjected to a thorough drying process using a hot air oven. This crucial step spanned over a duration of 8 hours, during which the plant material was carefully exposed to controlled, elevated temperatures. The primary objective of this drying phase was to eliminate any residual moisture present within the plant components. This meticulous drying ensured that the leaves, flowers, and stems were rendered free from moisture, a critical condition for subsequent processing. Once all moisture was effectively removed, the dried plant material was ready for further preparation and analysis.

3.4. Grinding of plant material

To create a plant extract for our research purposes, we will need to grind the plant's leaves, stems, and flowers into a fine powder. This process is essential to maximize the surface area and access the valuable compounds contained within these plant parts. Once ground into a fine powder, we can proceed with the extraction process, allowing us to isolate and study the various constituents of the plant.

3.5. Extracts preparation

In the present study, a wide variety of plant components, such as stems, flowers, and leaves, were employed. The selection of ethanol and methanol as extraction solvents was determined based on their respective polarity indexes. A quantity of 10 grammes of plant material, which had been dried and finely powdered, was utilized for the extraction process. The extraction was carried out by employing a solvent-to-sample ratio of 1:10, resulting in a total volume of 100 ml. The provided specimen was meticulously conserved and subjected to extraction by being placed within an incubator rotatory shaker operating at a controlled temperature of 25 °C and a rotational speed of 200 revolutions per minute for a duration of 48 hours. Subsequently, the extract underwent filtration using Whatman filter paper no. 42. The inclusion of this step was crucial in preserving the inherent antibacterial properties of the extract and achieving an optimal extraction process. The extracts acquired through the utilization of various solvents were afterwards concentrated utilizing a rotatory evaporator operating under reduced pressure. Following this, the resultant residue was diluted in order to generate a stock solution possessing a concentration of 10 mg/ml. The identified samples were discovered to possess a wide array of phytochemicals, encompassing alkaloids, saponins, flavonoids, tannins, and terpenoids. In order to ensure their long-term preservation, the extracts were placed in falcon tubes, which were thereafter protected with aluminum foil and maintained at a temperature of 4 °C.

3.6. Antimicrobial activity

The antibacterial activity of *N. oleander* extract against six bacterial strains, namely *Bacillus subtillis, Escherichia coli, Staphylococcus aureus, Enterobacter mori, Acinetobacter baumannii, and Klebsiella pneumoniae,* was assessed using the agar well diffusion method. A positive control was cefotaxime. The Antibiotic Inhibition Scale was used to calculate each bacterium's zone of inhibition. Calculations were made and the antibacterial activity was described.

3.6.1. Preparation of McFarland standard

A McFarland standard with a concentration of 0.5 was created by mixing 9.9 ml of a 1% H2SO4 solution in distilled water with 0.05 ml of a 1% BaCl2 solution in distilled water. This standard was utilized for the purpose of quantifying bacterial density. The mixture was stored in a tightly closed container and utilized as necessary for the purpose of comparing bacterial cultures.

3.6.2. Bacterial strains and culture preparation

The microbes were taken from virology Lab. For antibacterial activity, six bacterium strains were investigated (*Bacillus subtillis, E. coli, S. aureus, Enterobacter mori, A. baumannii & K. pneumoniae)*

In order to enhance growth within the incubator, the temperature of all the cultures was maintained at 37 °C. The produced aliquots for each experiment were defrosted, subculture in broth, and grown to stationary phase before being processed for additional experiments.

3.7. Minimum inhibitory concentration (MIC)

The antimicrobial efficacy of the extracts was evaluated utilizing Mueller Hinton agar medium. Bacterial strains, namely Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Enterobacter mori, Acinetobacter baumannii, and Klebsiella pneumoniae, were acquired from the virology laboratory. A bacterial suspension with a concentration of 106 colony-forming units per milliliter (CFU/mL) was uniformly distributed onto solid plates by use of a sterile brush saturated with the inoculum. Multiple wells were generated on the agar plates using a cork borer measuring 6 mm in diameter. Each well was subsequently filled with extracts at concentrations of 25mg/ml, 50mg/ml, and 100mg/ml. Subsequently, the plates were subjected to incubation at a temperature of 37 °C for a duration of 24 hours, while ensuring that the lids remained securely in place. This facilitated the diffusion of the samples into the surrounding media. Following an incubation period lasting overnight, the plates were examined to determine the existence of a zone of inhibition (ZI). Subsequently, the diameter of the ZI was quantified using a calibrated scale. Multiple samples of each were analysed. The positive control in this study consisted of the administration of cefotaxime, while the negative control was the use of DMSO.

3.8. FT-IR analysis

Identification of functional groups included in the *N. oleander* extract was conducted by the utilization of Fourier transformed infrared spectroscopy, as per the guidelines provided by the manufacturer. The spectroscopic analysis encompassed a frequency range of 400 to 4000 cm^{-1}

3.9. GC/MS analysis

3.9.1. Sample preparation

A 100 µl aliquot of a methanolic plant extract was mixed with 1 ml of methanol solvent. The resulting solution underwent thorough agitation using a vortex stirrer for 10 seconds, followed by filtration through a 0.2-micron membrane filter. The resulting clear extract was then utilized for gas chromatography-mass spectrometry (GC-MS) analysis.

The chemical identification procedure involved the utilization of gas chromatography-mass spectrometry (GC-MS) employing a capillary column. The experimental setup was originally adjusted to a temperature of 40 °C and subsequently subjected to a linear temperature increase of 10 °C per minute until it reached a final temperature of 110 °C. Following this, the temperature was sustained at this magnitude for a duration of three minutes. Subsequently, the temperature saw an increase at a pace of 10 °C per minute until it reached a value of 210 °C. Following this, a period of 3 minutes was upheld at the aforementioned temperature. Following this, a further increase in temperature at a rate of 8 °C per minute was applied until reaching the critical point of 250 °C, at which juncture it was sustained for a period of 8 minutes. Following this, the temperature was maintained at a constant level of 250 °C for an extra period of 3 minutes. At the conclusion of the designated time frame, the oven temperature was elevated to 280 °C, demonstrating a gradual increase of 10°C per minute, and sustained at this magnitude for a duration of 10 minutes. The temperature of the injection port was maintained at a regulated level of 250 °C, while the flow rate of Helium gas was adjusted to a value of 1.5 ml/min. The ionization voltage was determined to have a value of 70 electron volts (eV). The samples were injected using a split mode ratio of 10:1. The mass spectral scan was performed within the specified range of 45–350 (m/z). The commencement of the mass spectrometry (MS)

analysis was established to be 3 minutes, while the conclusion of the analysis transpired after 75 minutes, encompassing a solvent cut period of 3 minutes. The analysis and comparison of the acquired spectra of volatile chemicals, obtained using gas chromatography-mass spectrometry (GC-MS), were performed using the NIST 17 (National Institute of Standards and Technology) online library Version 2.3.

3.10. Molecular docking analysis

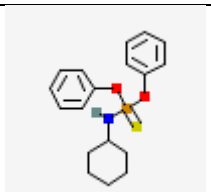
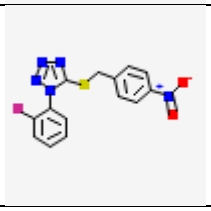
In our study, we utilized molecular docking, a computational methodology, to ascertain the most favorable spatial configuration of a ligand and a protein. The docking procedure entailed the application of AutoDock Vina software to assess the ligands and target proteins. During the docking approach, many conformations for the ligand were created, and subsequently, a final energy refinement was performed for the ligand pose. The docking score for the optimal pose of each bioactive chemical was computed for the target proteins being studied. In the preliminary stage, all compounds that were identified using GC-MS analysis were included in the evaluation of their potential antibacterial activity through the utilization of molecular docking studies. Our study specifically concentrated on the extraction of bioactive compounds from N. oleander, which were subsequently selected for molecular docking investigation. To facilitate this analysis, three-dimensional (3D) structures of these phytocompounds were retrieved from the PubChem database in SDF file format (https://pubchem.ncbi.nlm.nih.gov/). For accurate docking conformation studies, we optimized the 3D structures of these phytocompounds. The study employed structure-based molecular docking techniques to examine the interactions between the phytocompounds and bacterial target proteins. Target proteins, namely 1xal, 7p2m, 8iwl, 6f86, and 3zgf, along with their respective bioactive chemicals, were chosen for this study. The binding energies were computed using the BIOVIA Discovery Studio (version 2021) software and AutoDock Vina, which was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org). To ensure standardized procedures, both ligands and target proteins were synthesized based on established industry protocols for protein and ligand production. These synthesized structures were subsequently uploaded into AutoDock Vina for further analysis. The evaluation of binding affinity, the binding interactions of each ligand, and the docked data

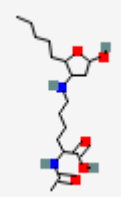
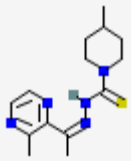
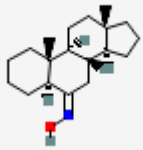
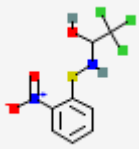
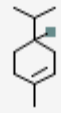
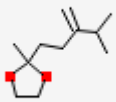
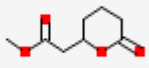
was conducted using Discovery Studio Visualizer. The optimal binding conformation of phytocompounds with target proteins was ascertained by evaluating the conformations created and selecting the one with the lowest overall binding energy. The AutoDock program was employed to assess the relative strengths of binding interactions by examining the ideal binding conformation of the identified phytocompound. This analysis included the determination of various energy values such as binding energy, ligand efficiency, inhibition constant, and the combined effect of Van der Waals and hydrogen bonding. In addition, the application of PyMol 3D visualization software facilitated the visualization of complex interactions between the most potent phytocompound and its binding sites within bacterial target proteins.

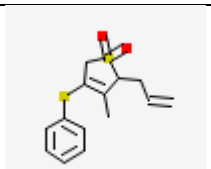
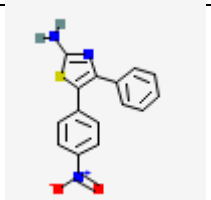
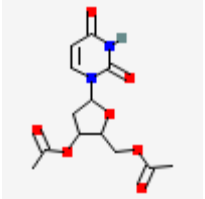
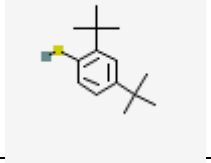
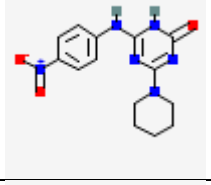
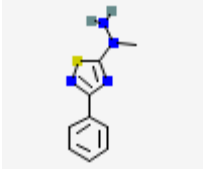
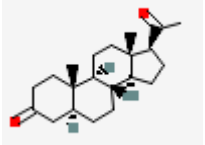
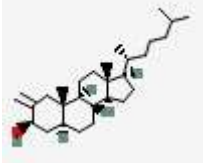
3.10.1. Preparation of ligand

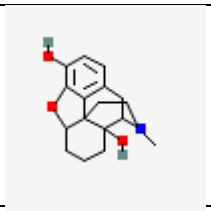
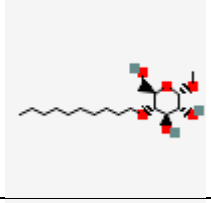
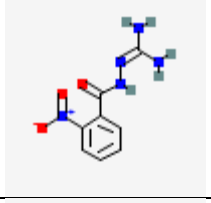
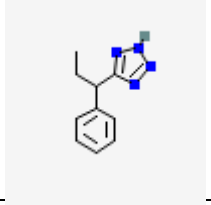
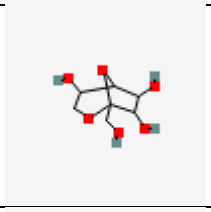
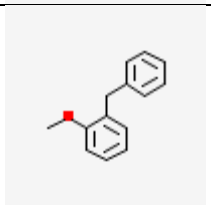
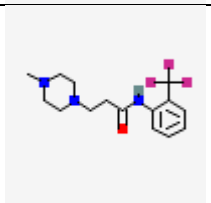
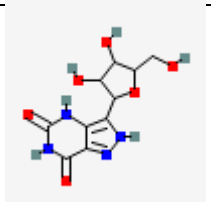
The selection of chemicals for the docking analysis was based on Lipinski's rule of five. The chemical structures of the chosen bioactive compounds were obtained from the PubChem compound database, which is maintained by the National Centre for Biotechnology Information (NCBI) and may be accessed at http://pubchem.ncbi.nlm.nih.gov/. The phytochemicals were obtained from PubChem in SDF files and afterwards transformed into PDB format using Biovia Discovery Studio v2021. Before the docking process, the ligands were subjected to charge fixing and polar hydrogen addition, and subsequently saved in the PDBQT format. Comprehensive data pertaining to physicochemical properties, molecular formulas, and three-dimensional structures of the selected phytochemicals has been furnished.

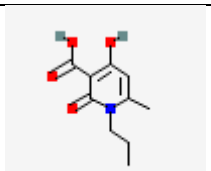
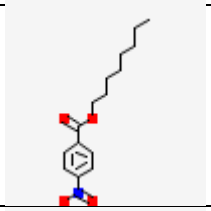
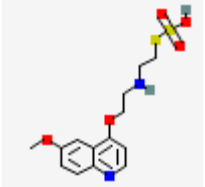
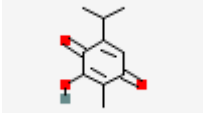
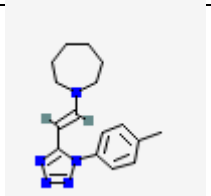
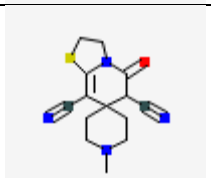
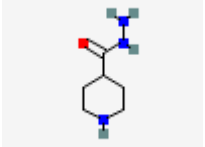
Table 3.1: Molecular weight, molecular formula, PubChem ID and structure of the selected ligand

Compound	PubChem CID	Molecular Formula	Molecular Weight	Structures
O,O-Diphenyl N-cyclohexyl.phosphoramidothioate	544513	C18H22NO2PS	347.4 g/mol	
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl).-1H-tetrazole	704644	C14H10FN5O2S	331.33 g/mol	

1,2-Epoxy-5,9-.cyclododecadiene	5357430	C12H 18O	178.27 g/mol	
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5,-N-[5-hydroxy-2-pentylcyclopentyl)-	541722	C17H32N2O5	344.4 g/mol	
4-Methyl-N'-[(Z)-1-(3-methyl-2-pyrazinyl)ethylidene]-1-piperidinecarbothiohydrazide	5989884	C14H21N5S	291.42 g/mol	
Androstan-6-one, oxime, (5alpha)-	20845286	C19H31NO	289.5 g/mol	
2,2,2- Trichloro-1-(2-nitrophenylthioamino) ethanol	555272	C8H7Cl3N2O 3S	317.6 g/mol	
Cyclohexene, 1-methyl-4-(1-methylethyl)	21671	C10H18	138.25 g/mol	
2-Methyl-2- (4-methyl-3-methylidenepentyl)- 1,3-dioxolane	559996	C11H20O2	184.27 g/mol	
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester	5 35548	C8H12O4	172.18 g/mol	

3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	6420230	C14H16O2S2	280.4 g/mol	
5-(4-Nitrophenyl)-4-phenyl-1,3-thiazol-2-amine	626715	C15H11N3O2S	297.3 g/mol	
Uridine, 2'-deoxy-, 3',5'-diacetate	556908	C13H16N2O7	312.27 g/mol	
2,4-Di-tert-butylthiophenol	519681	C14H22S	222.39 g/mol	
4-[(4-Nitrophenyl)amino]-6-(piperidin-1-yl)-1,3,5-triazin-2-ol	135461552	C14H16N6O3	316.32 g/mol	
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	609752	C9H10N4S	206.27 g/mol	
5alpha-Pregnane-3,20-dione	92810	C21H32O2	316.5 g/mol	
5alpha-Cholestan-3beta-ol, 2-methylene	22213932	C28H48O	400.7 g/mol	

Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5alpha)-	542107	C17H21NO3	287.35 g/mol	
Methyl 4-O-decylhexopyranoside	57384728	C17H34O6	334.4 g/mol	
[(2-Nitrophenyl)formohydrazido]methanimidamide	46786287	C8H9N5O3	223.19 g/mol	
5-(1-Phenyl-propyl)-1H-tetrazole	6422822	C10H12N4	188.23 g/mol	
2,7-Anhydro-l-galacto-heptulofuranose	552320	C7H12O6	192.17 g/mol	
1-Benzyl-2-methoxybenzene	607699	C14H14O	198.26 g/mol	
3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-phenyl)-propionamide	535882	C15H20F3N3O	315.33 g/mol	
3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	294874	C10H12N4O6	284.23 g/mol	

1,2-Dihydro-4-hydroxy-6-methyl-2-oxo-1-propyl-3-pyridinecarboxylic acid	54678655	C10H13NO4	211.21 g/mol	
Benzoic acid, 4-nitro-, octyl ester	80997	C15H21NO4	279.33 g/mol	
S-2-[2-[6-Methoxy-4-quinolyloxy]ethylamino]ethyl thiosulfate	548560	C14H18N2O5 S2	358.4 g/mol	
2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-	549873	C10H12O3	180.20 g/mol	
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	5362879	C16H21N5	283.37 g/mol	
2H-Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-5-oxo-7,4'-spiro-(1-methylpiperidine)-	535725	C14H16N4OS	288.37 g/mol	
Piperidine-4-carbohydrazide	456704	C6H13N3O	143.19 g/mol	

3.10.2. Target protein retrieval

Crystal Structure of *Escherichia coli* GyraseB (PDB ID: 6F86) for supercoiling of chromosomal DNA, as well as being involved in decatenation of newly synthesized chrosomal. *E. coli* GyrB24 (PDB ID: 7P2M) necessary for DNA replication. Crystal structure of *S. aureus* 3-dehydroquinate synthase (PDB ID: 1XAL) necessary for

carbohydrates metabolism. S aureus hydrolase enzyme (PDB ID: 3ZG5) necessary for the cleavage of large molecules into small fragments and recycling. *A. baumannii* uncharacterized sugar kinase protein (PDB ID: 8IWL) necessary for the glucose utilization for carbon source. The protein structures were obtained from the RCSB Protein Data Bank. The three-dimensional X-ray crystallographic structure of the proteins was acquired from the Protein Data Bank (PDB) located at https://www.rcsb.org/.

3.10.3. Target protein preparation

The three-dimensional crystal structure of a particular bacterial protein was obtained from the RCSB database in the file format of the Protein Data Bank (PDB). Following that, the acquired data was imported into Discovery Studio software with the intention of eliminating the initial ligands and other small molecules that could have been attached to the protein structures. Before performing docking analyses, the 3D protein structures underwent a refining procedure. The process encompassed the elimination of superfluous ions, ligands (if any), and water molecules from the chosen proteins. Furthermore, the receptors underwent alterations through the inclusion of polar hydrogen atoms and Kollman charges. Subsequently, the revised structures were saved in PDBQT format in order to optimize the efficiency of the docking process.

3.11. Pass analysis

Prediction of activity Spectra for Substances (PASS) analysis program uses the structure-activity relationship (SAR) to make predictions about the biological properties, pharmacological features, drug-like properties, possible side effects, and how less-studied phytoconstituents work. To do PASS analysis for this work, several online and offline tools were used, which are described below.

3.12. Lipinski's rule of five

Lipinski's rule of five shows the molecular features of drugs, taking into account important pharmacokinetic factors such as absorption, metabolism, distribution, and elimination. The previously mentioned guideline proves to be advantageous in the process of designing and developing pharmaceutical substances. The anticipated drug-likeness of the

phytochemicals derived from N. oleander, which were examined in this study, was assessed based on the Lipinski rule of five (http://www.swissadme.ch/. The Molsoft web tool (available at "https://molsoft.com/mprop/") was utilized for the computation of drug-likeness scores for phytochemical compounds.

3.13. Druglikeness

Druglikeness of the compounds under investigation was carried out using Molsoft, a valuable resource and platform for predicting druglikeness (https://molsoft.com/mprop/). By using this software, we were able to gain valuable insights into the druglikeness of the compounds, contributing to the overall understanding and selection of promising candidates for our research endeavors. This critical assessment added a layer of rigor to our study, ensuring that the compounds chosen for further investigation met the requisite criteria for potential drug development.

3.14. Toxicity potential study

The toxicity risk assessment offers initial data regarding potential negative impacts of phytoconstituents that could be employed in the exploration and advancement of novel pharmaceuticals. Using the Protox-II server (https://tox-new.charite.de/protox_II/) and OSIRIS Data Warrior V5.2.1 software, we investigated druglikeness and drug toxicity risk features such as druglikeness, tumorigenicity, carcinogenicity, immunotoxicity, hepatotoxicity, mutagenicity, reproductive effects, and irritating effects. The compounds that have received limited research attention have also undergone evaluation for their classification as drug toxicants and estimate of their LD50 value. The LD50 values of toxic dosages are frequently expressed in milligrams per kilogram of an individual's body weight. The metric denoting the dosage level at which 50% of test subjects succumb to the effects of a substance is commonly referred to as the median lethal dosage, abbreviated as LD50. Toxicology courses are structured in accordance with the Globally Harmonized System (GHS) for the classification and labelling of substances.

3.15. Pharmacokinetic property prediction

To gain insights into the pharmacokinetic characteristics of all the phytoconstituents under investigation in this study, we employed the web based SwissADME software, accessible

at http://www.swissadme.ch/. SwissADME is a valuable online tool specifically designed for the comprehensive analysis of important pharmacokinetic properties of chemical substances. This online tool analyzes the important pharmacokinetic properties of a substance, such as its distribution, absorption in the gastrointestinal tract (GI), metabolism as a substrate for P-glycoprotein (P-gp), inhibition of cytochrome P450 enzymes including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, and lipophilicity for absorption across the plasma membrane.

3.16. Molecular dynamic simulations

The molecule with the strongest binding attraction was chosen to be the basis for our docking calculations. Desmond Schrodinger v3.8, a powerful computer program, was used to do these estimates. We used the NPT ensemble for our research and kept the temperature at 300 kelvin and the pressure at 1 bar during the nanosecond-long simulations. To make sure our models were accurate, we used the Ewald method to figure out the electrostatic charges. We took 10 ns-long snapshots of the track to get important data points. We used the modelling interaction tool that comes with the Desmond package to learn more about how ligands and proteins interact with each other. We also checked how stable the ligand-protein complex was by looking at its Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF). This thorough method gave our study a solid base and let us investigate molecular interactions and complex stability in detail.

Chapter 4: Results

4. Results

4.1. Extract Preparation

Six different Plant extracts were prepared in methanol and ethanol solvents. It includes the extracts in Methanol leaves, Methanol flowers and Methanol stems. The others three extracts are in ethanol solvents, and it includes extract of ethanol leaves, ethanol stems and ethanol Flowers. The amount of extract obtained was reduced to $1/10^{th}$ of the initial amount of the plant material used.

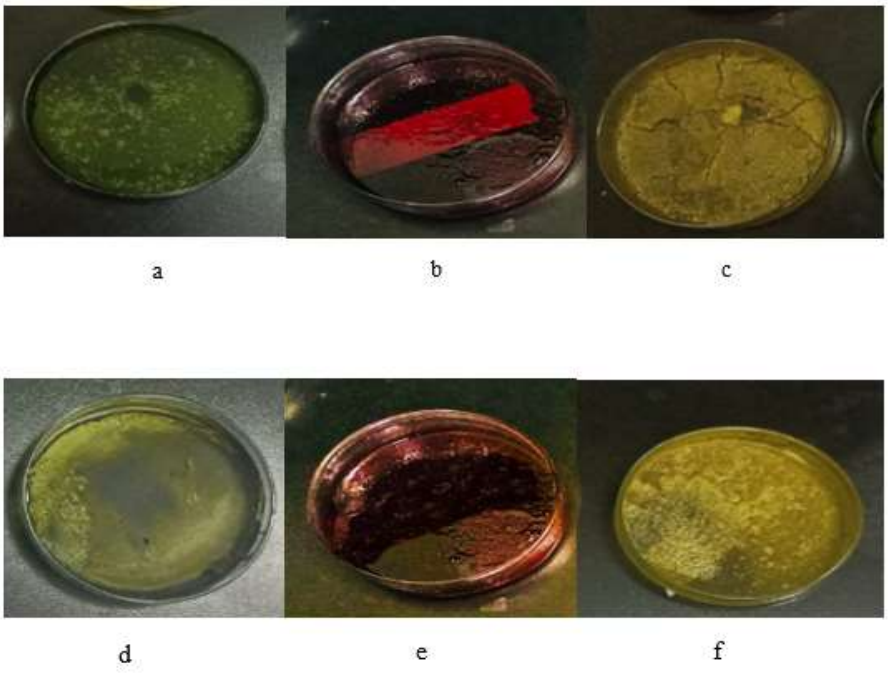


Figure 4.1: a) Leaves in Ethanol, b) Flowers in Ethanol, c) Stems in Ethanol, d) Leaves in Methanol, e) Flowers in Methanol, and f) Stem in Methanol

4.2. FTIR analysis

FTIR spectroscopy was employed to identify the functional groups present in the bioactive constituents of N. oleander. The compounds mentioned include alkyl halides, glycogen, carboxylic acids, phenols, primary and secondary amines, esters, ethers, aromatics, lipids, triglycerides, nitro compounds, and aliphatic amines.

4.3. Methanolic and Ethanolic Extracts FTIR

FTIR spectroscopy played a pivotal role in our study to characterize the functional groups within the bioactive constituents extracted from *N. oleander*. The methanolic Extracts FTIR spectrum of leaves, flowers and stems is shown in the figure. This spectrum shows that the different components of plants contain the same compounds and different compounds which is represented by their functional groups and show the transmittance in their respective range. Many functional groups were clearly visible in the FTIR spectrum. These included hydroxyl (O–H), aldehyde (C–H), alkenes (C=C), carboxyl (C=O), nitrogen-containing groups (N–O), alkanes (C–C), aromatic primary amines (C–N), amines (N–H), alkynes (C≡C), phenols, carboxylic acids, glycogen, aliphatic amines, primary and secondary amines, esters, ethers, aromatics, lipids, triglycerides, and nitro compounds. These different functional groups are important parts of many secondary metabolites that can be found in N. oleander, such as alkaloids, flavonoids, terpenoids, polyphenols, and tannins. The in-depth FTIR study taught us a lot about the bioactive compounds in the plant extract, including their chemical make-up and how complicated they are.

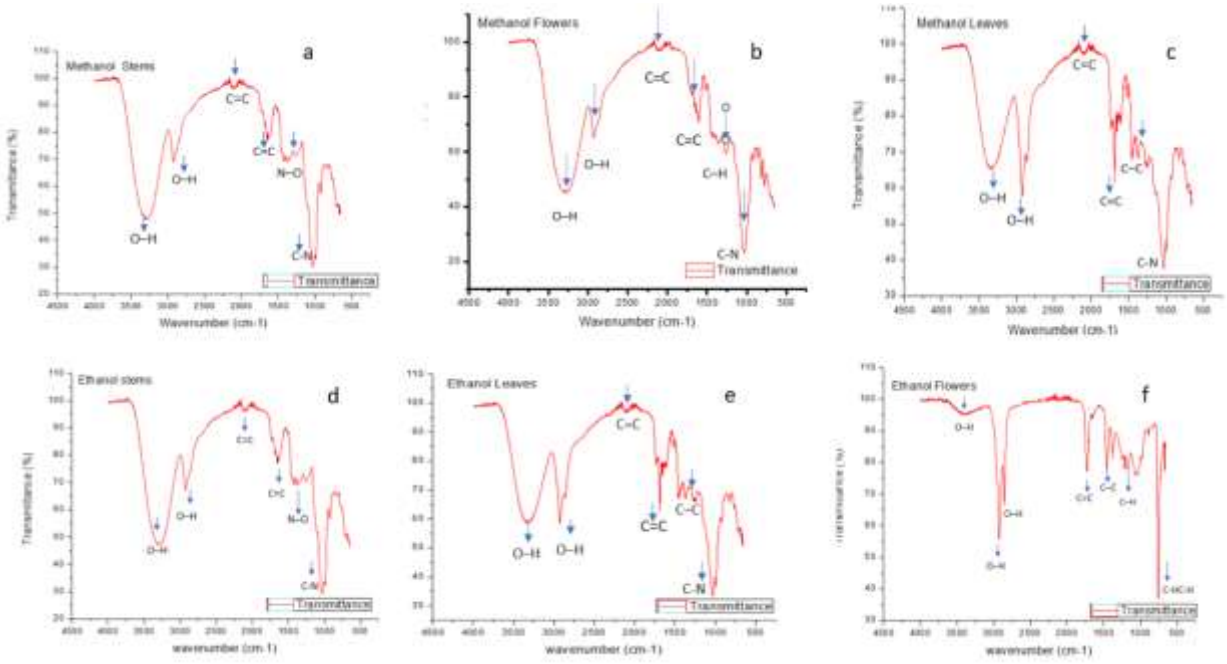


Figure 4.2: FTIR Analysis of a) Leaves in Ethanol, b) Flowers in Ethanol, c) Stems in Ethanol, d) Leaves in Methanol, e) Flowers in Methanol, and f) Stem in Methanol

4.4. GC-MS analysis

The examination of chemical composition and content within extracts provides valuable insights into the diverse biological potentials inherent in different extracts originating from medicinal plants. In our study, we conducted plant metabolic profiling of *Nerium Oleander* using GC-MS analysis, which unveiled the presence of a range of bioactive phytochemicals in both methanolic and ethanolic extracts obtained from its leaves, stems, and flowers. The (GC-MS) analysis was an integral part of our predetermined investigation. During the analysis, numerous peaks were detected in all plant components, each of which represented distinct bioactive compounds. To find these compounds, their peak retention times, molecular weights, and molecular formulas were compared to reference compounds in the NIST library.

4.4.1. GCMS-Analysis of Methanolic Leaves extract

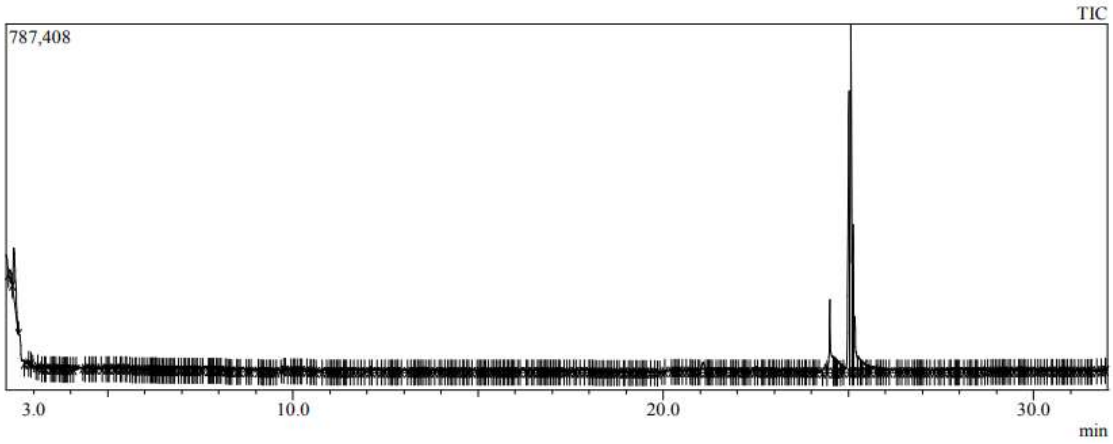


Figure 4.3: GCMS spectrum of Methanolic extract of leaves

Table 4.1: Compounds identified by the GCMS analysis of the methanolic leaves extract

Area	Area%	Name
1958145	13.76	Heptanoic acid, methyl ester
1516955	10.66	Heptanoic acid, methyl ester
453340	3.19	2-Chloroethyl methyl sulfoxide
453020	3.18	Uridine, 2'-deoxy-, 3',5'-diacetate
429442	3.02	1,3- Dioxolane, 2-methyl-2-.(4-methyl-
371912	2.61	Cyclohexane, 1-methyl-4-(1-methylethyl)-,
139066	0.98	8-Thiabicyclo[3.2.1]octane
121698	0.86	Tetradecanoic acid, 12-methyl-, methyl

118502	0.83	4,4-Dimethyl-cyclohex-2-en-1-ol
111666	0.78	Tetrazole, 1-(1,3-dioxolan-4-ylmethyl)-
108235	0.76	Oxirane, decyl-
88263	0.62	5-Decen-1-ol, acetate, (E)-
80604	0.57	2,6-Diamino-4-hexenoic acid
78513	0.55	(Z)-1-Chloro-2-(methylsulfonyl)ethylene
75548	0.53	2-Oxotetrahydropyranyl-6-acetic
73812	0.52	3-Methyl-4-(phenylthio)-2-prop-
72818	0.51	2-Thiazolamine, 5-[(4-nitrophenyl)

4.4.2. GCMS-Analysis of Methanolic Flowers extract

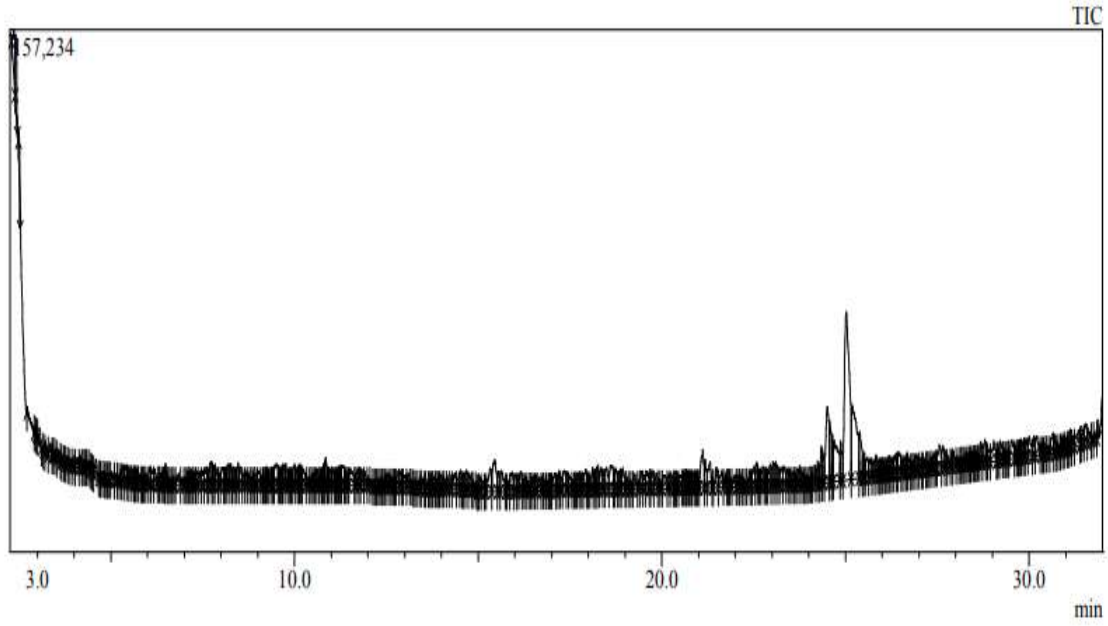


Figure 4.4: GCMS spectrum of Methanolic extract of flowers

Table 4.2: Compounds identified by the GCMS analysis of the methanolic flower extract

Area	**Area%**	**Name**
471476	6.65	Methyl stearate
209635	2.96	Kauran-18-oic acid, 16-hydroxy-, (4.alpha.)-
134995	1.9	6-Octadecenoic acid, methyl ester, (Z)-
105280	1.49	4,5,9-Trihydroxy-dodeca-1,11-diene
65846	0.93	1,4-.Dioxaspiro [4.5]decane -6- carboxylic acid, dimethyl.amide.
65517	0.92	4,4-Bis(dichloro.fluoro.methyl)-1,2- oxathietane-.2,2-dioxide.
64693	0.91	6-Ethyl-2,3.-dimethyl-tetrahydro-.thiopyran-4-ol…
63757	0.9	Hexadecanoic acid, methyl ester

52749	0.74	Hexane, 2,2,3-trimethyl-
49518	0.7	2-Heptene, 5-methyl-
46674	0.66	Trimethylphenylgermanium
46773	0.66	4-Cyclopentene-1,3-diol, trans-
41526	0.59	O,O-Diphenyl N-cyclohexylphosphoramidothioate
41942	0.59	Acetic acid, chloro-, 2-butoxyethyl ester
38717	0.55	1,2-Epoxy-5,9-cyclododecadiene
37560	0.53	1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole
35235	0.5	cis-2-Methyl-7-octadecene
35485	0.5	Cyclohexanone, 3-methyl-, (2,4-dinitrophenyl) hydrazone

4.4.3. GCMS-Analysis of Methanolic stems extract

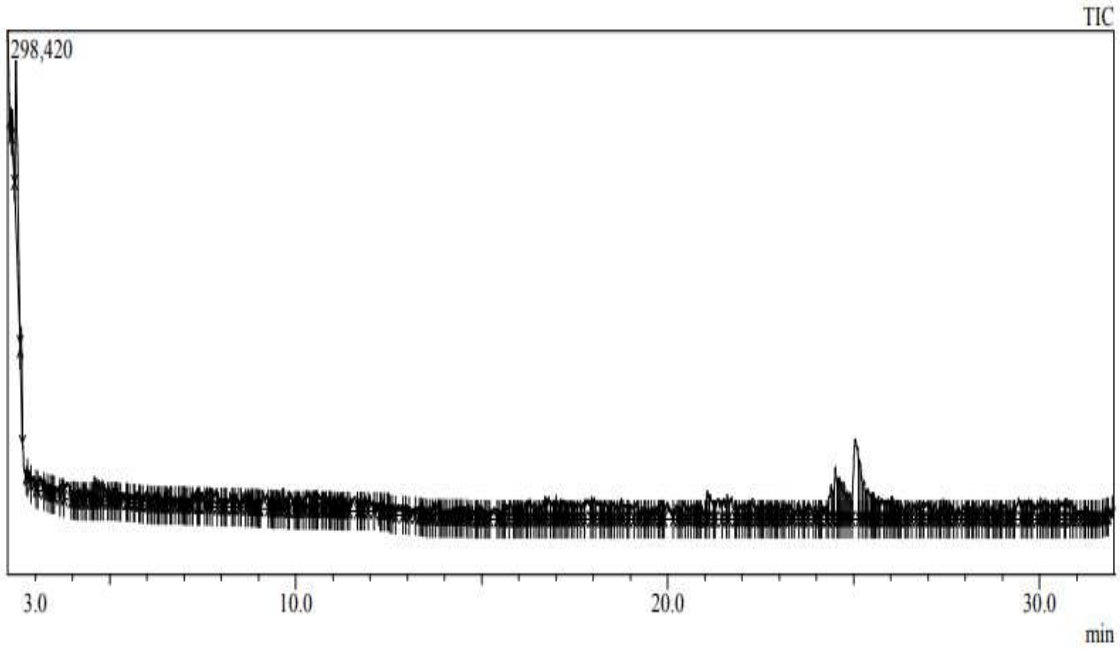


Figure 4.5: GCMS spectrum of Methanolic extract of stems

Table 4.3: Compounds identified by the GCMS analysis of the methanolic stems extract

Area	**Area%**	**Name**
390041	3.46	2-Chloroethyl methyl sulfoxide
374888	3.32	Decanoic acid, methyl ester
154936	1.37	3,3-Dichloropivalic acid
123337	1.09	4-Decenoic acid, methyl ester, Z-
98051	0.87	2-Methoxy-diphenylmethane

91365	0.81	Methyl phosphochlorofluoridate
91365	0.81	5,10-Dioxatricyclo[7.1.0.0(4,6)]decane
84140	0.75	3-Pyridine.carboxylic.acid,.1,2-dihydro-4-hydroxy-6-methyl-
83459	0.74	Cholest.-5-en-3-ol,. (3.alpha.)-, TMS derivative
83543	0.74	5-Butyl-1,3-oxathiolan-2-one
82341	0.73	Dimethylthexylsilyl chloride
82663	0.73	.alpha.-D-Glucopyranoside, methyl-4-O-decyl-
80430	0.71	Cyclopentaneundecanoic acid, methyl ester
78212	0.69	8,11-Octadecadiynoic acid, methyl ester
73026	0.65	[(2-Nitrophenyl)formohydrazido]methanimidamide
72616	0.64	5-Butyl-1,3-oxathiolan-2-one
66758	0.59	Nonanenitrile
65832	0.58	L-Homoserine, O-propyl-
63985	0.57	3-Imidazoline-3-oxide-1-oxyl,5,5-dimethyl 2,2pentamethylene-
64694	0.57	2,7-Anhydro-l-galacto-heptulofuranose
60555	0.54	1-N-(7-Azido-[1,2,3,4]tetrazolo[1,5-a][1,3,5]triazin-
60697	0.54	3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-
60293	0.53	3-..beta.-d-Ribo.furanosylpyrazolo[4,3-d].pyrimidin-5,7-4H,6H-
57803	0.51	3-(Methylthio)pent-4-yn-1-ol
58032	0.51	Benzoic acid, 4-nitro-, octyl ester
56833	0.5	Ethanol, 2-[(2-phenylcyclohexyl)oxy]-
56899	0.5	dl-3-Methyl-dl-glutamic acid
56099	0.5	S-2-.[2-[6-Methoxy.-4-quinolyloxy]ethyl amino]ethyl thiosulfate.

4.4.4. GCMS analysis of Ethanol Leaves Extract

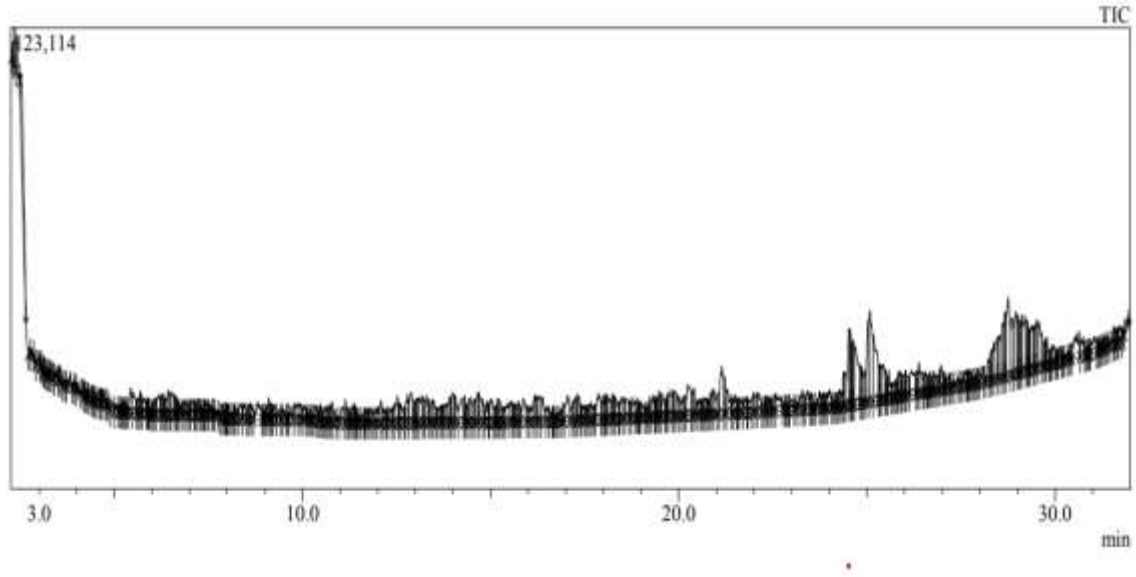


Figure 4.6: GCMS spectrum of Ethanolic extract of leave

Table 4.4: Compounds identified by the GCMS analysis of the ethanolic leaves extract

Area	**Area%**	**Name**
127219	1.67	Tris(2,4-di-tert-butylphenyl) phosphate
125213	1.65	Propanoic acid, decyl ester
123512	1.63	Cholestan.-3-ol, .2-methylene-,. (3.beta.,5.alpha.)-….
114536	1.51	Ethane, .1,2-dichloro-1,1,2-.trifluoro-..
111589	1.47	Diethyl pyrocarbonate
93879	1.24	3.beta.,4.beta.-Bis(trimethylsiloxy)cholest-
85422	1.12	Heptadecanoic acid, 9-methyl-, methyl ester
82998	1.09	Hexadecanoic acid, methyl ester
82234	1.08	7-Oxononanoic acid, TMS derivative
70595	0.93	4-(4-Nitroanilino)-6-piperidino.-1,2,3-triazin-
69881	0.92	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester
63765	0.84	(3,5-Dimethyl-1H-pyrazol-1-ylmethyl)-thiazol.-
61220	0.81	Gorgost-5-en-3-ol, (3.beta.)-
59269	0.78	3-Bromo-2-[(3-bromothien-2-yl)thio]methyl]
58450	0.77	Acetamide, N-methyl -N-[4-[3-fluoro-1-pyrrolidyl]-2-butynyl]-..
57291	0.75	Tetraacetyl-d-xylonic nitrile
56845	0.75	5.alpha.-Dihydroprogesterone
56294	0.74	Methyl 10,11-tetradecadienoate
55167	0.73	2-Acetyl-5-bromothiophene
54968	0.72	1,3:2,5:4,6-Trimethylene-d-glycero-d-mannoheptitol
52369	0.69	.alpha.-Phenethyl cyanide, 2-methoxy-6-nitro-
48241	0.63	4-Indanpropionic acid, 3a.alpha.,4.beta.,5,6,7,7a-
47285	0.62	
47213	0.62	2,4-Di-tert-butylthiophenol
45654	0.6	
45063	0.59	Carbonic acid, propyl undec-10-enyl ester

44648	0.59	5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole
40834	0.54	Morphinan -3,14-diol,4,5-epoxy-17- methyl- (5.alpha.)-
40023	0.53	9H-Purine-9-propanoic acid, 6-hydroxy-
39360	0.52	3-Dimethylsilyloxypentadecane
38793	0.51	2-Hydroxy-2,3-dimethylsuccinic acid
38019	0.5	Lup-20(29)-ene-3,21,28.-triol,.28-acetate, (3.beta.,21.beta.)-

4.4.5. GCMS analysis of Ethanol Flowers Extract

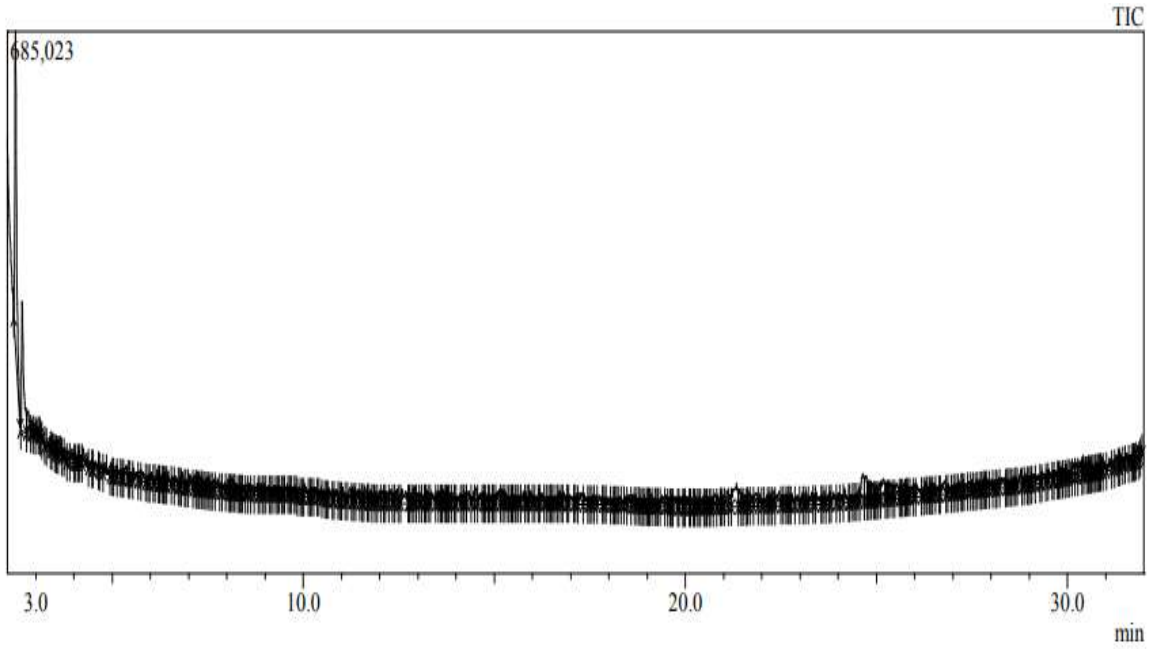


Figure 4.7: GCMS spectrum of Ethanolic extract of flowers

Table 4.5: Compounds identified by the GCMS analysis of the ethanolic flowers extract

Area	**Area%**	**Name**
1316709	6.96	Acetic acid, mercapto-, methyl ester
624489	3.3	Oxirane, [(hexyloxy)methyl]-
227329	1.2	l- Felinine
194982	1.03	Cyclo propane butanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)
155329	0.82	4-Methyl-2-mercaptopyridine-1-oxide
145953	0.77	Dodecane, 1- (ethylthio)-
123113	0.65	2(3H)- Naphthalenone, 4, 4a, 5,6,7,8-hexa hydro-1-methoxy-
105949	0.56	1-Cyclohexylethanol, n-propyl ether
104791	0.55	Cyclopropane -1- carbohydrazide, 2-phenyl -N2-(3-methylcyclohexylideno)-
104060	0.55	4-Cyclopentene-1,3-diol, trans-
103755	0.55	Androstan-6-one, oxime, (5.alpha.)-
103274	0.55	2,2,2-Trichloro-1-(2-nitro phenyl thioamino)ethanol

4.4.6. GCMS analysis of Ethanol stem Extract

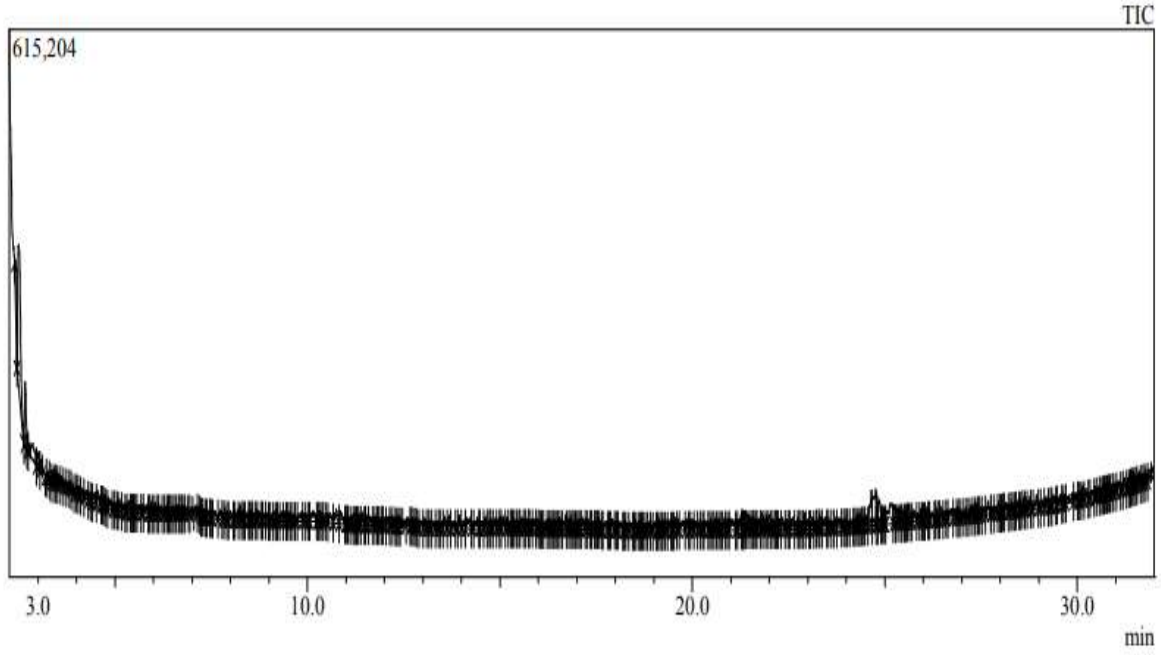


Figure 4.8: GCMS spectrum of Ethanolic extract of stems

Table 4.6: Compounds identified by the GCMS analysis of the ethanolic stems extract

Area	Area%	Name
741727	5.11	Methyl 2-hydroxyethyl sulfoxide
213106	1.47	Card -20 (22)-enolide, 3- [(2,6-dideoxy-4-O.-.beta.-D-gluco pyranosyl-
210516	1.45	Lup- 20(29)-ene-3,21,28-triol, 28-acetate, (3.beta.,21.beta.)-
203307	1.4	Cycloheptane, 1,4-dimethoxy-, cis-
160350	1.1	Bicyclo [4.1.0]heptan-2 -ol, 3,7,7 -trimethyl-, (1.alpha.,2.alpha.,
120614	0.83	4-Piperidinecarboxylic acid hydrazide
103678	0.71	Tricyclo [4.3.1.1(2,5)]undec-3-en-10-ol, stereoisomer
95389	0.66	2H-Thiazolo [3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7- tetrahydro-
86500	0.6	1,6-Octadiene, 8-methoxy-
81322	0.56	S-Methyl methanethiosulphonate
79960	0.55	Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-
73990	0.51	2 -(1-Methylthiodecyl)-5-(3-acetoxy-1-methylthio)thiane
73385	0.51	2,5-Cyclohexadiene- 1,4-dione , 3-hydroxy-2-methyl-5-(1-methylethyl)-

4.5. Antibacterial activity

The current research aims to examine the antibacterial efficacy of the leaves, flowers, and stems of N. oleander. The findings indicate that the methanol extracts derived from N.

oleander show antibacterial properties against the bacterial strain used in the experiment. The measured diameter of the zones of inhibition varied between 5 and 20 mm. The largest inhibition zone, measuring 20 mm, was recorded in *A. baumannii*, followed by *K. pneumoniae* with a zone of 17 mm. B. subtilis exhibited an inhibition zone of 16 mm, while *E. mori*, *E. coli*, and *S. aureus* had inhibition zones measuring 13 mm, 11.5 mm, and 10 mm, respectively.

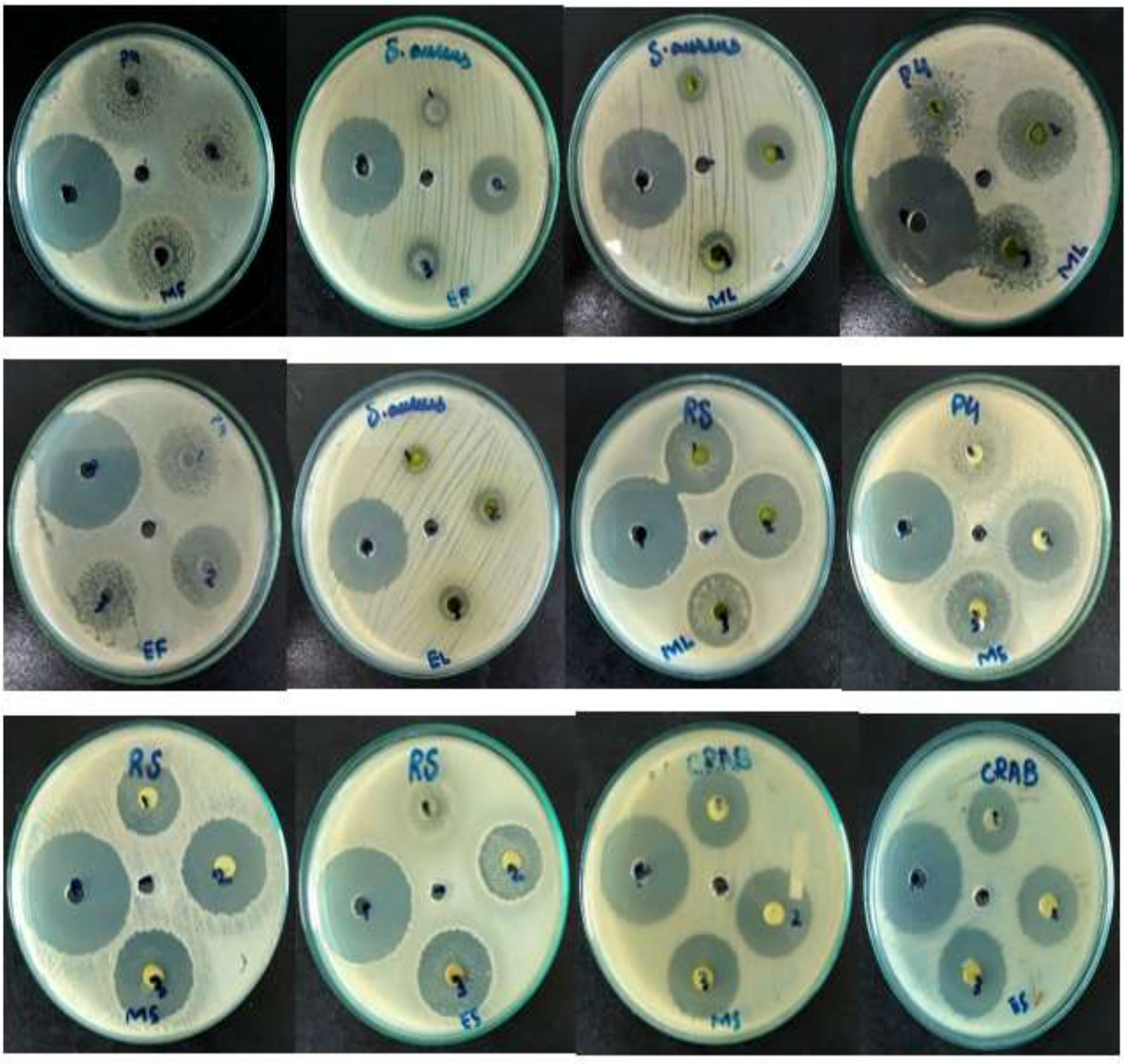


Figure 4.9: Antibacterial activity of plant extract against the bacteria

Table 4.7: Methanolic extract of leaves, flowers and stems representing the zone of inhibition against bacterial strains

Methanol Extract		**Zone of inhibition (mm)**					
Sr No	Plant part used	*A. baumannii*	*K. pneumoniae*	*B. subtilis*	*E. mori*	*E. coli*	*S. aureus*
1	Leaves	20	17	16	13	11.5	10
2	Flowers	16	9	12.5	12	9	8.75
3	Stems	13.75	12.75	14.25	12	11.5	11
Control							
Cefotaxime		22	19	19	18	13	14

Table 4.8: Ethanolic extract of leaves, flowers and stems representing the zone of inhibition against bacterial strains

Ethanol Extract		**Zone of inhibition (mm)**					
Sr No	Plant part used	*A. baumannii*	*K. pneumoniae*	*B. subtilis*	*E. mori*	*E. coli*	*S. aureus*
1	Leaves	13	11	13.5	10	10	8
2	Flowers	13.5	9.5	14	14	10	9
3	Stems	14	11.5	12.5	15	10.5	12.5
Control							
Cefotaxime		22	19	19	18	13	14

The quantitative assessment revealed that the leaves exhibited significant inhibitory effects against all bacterial strains that were examined.

4.6. Calculation of Minimum inhibitory concentration (MIC)

The extracts gave MIC values between 25 and 100 mg/ml, which is the lowest amount needed to stop an enzyme from working. We checked how well the leaves killed germs by

finding the percentage inhibition. For A. baumannii, K. pneumoniae, B. subtilis, E. mori, E. coli, and S. aureus, the minimum inhibitory concentration (MIC) was found to be 25 mg/ml, 50 mg/ml, and 100 mg/ml, in that order. The blocking zones that were seen were 20 mm, 17 mm, 16 mm, 13 mm, 11.5 mm, and 10 mm in size.

4.6.1. MIC of methanolic and ethanolic leaves extract

The presented graphs depict the zone of inhibition observed at concentrations of 25, 50, and 100 mg/ml. The methanolic extract of leaves exhibited the highest level of inhibition against A. baumannii, while demonstrating the lowest level of inhibition against B. subtillis. The ethanolic extract of leaves exhibited the highest level of inhibition against E. mori, while displaying the lowest level of inhibition against K. pneumonia.

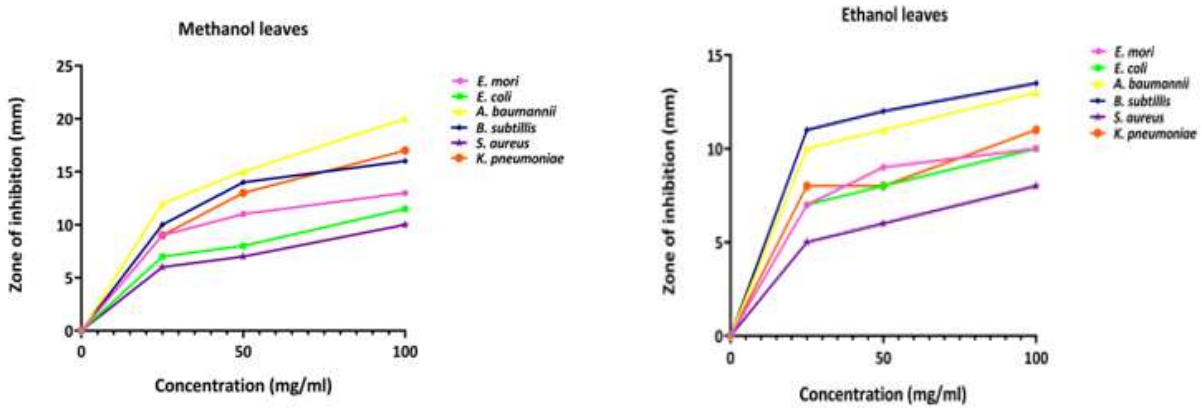


Figure 4.10 (a): MIC of methanolic and ethanolic Leaves extract for the tested bacterial strains

4.6.2. MIC of methanolic and ethanolic flowers extract

The graphs represent the zone of inhibition measured at 25, 50 and 100 mg/ml concentration of flower extract. Methanolic and ethanolic flowers extract shows the maximum zone of inhibition against the *E.mori* and minimum zone of inhibition against *K.pneumonia.*

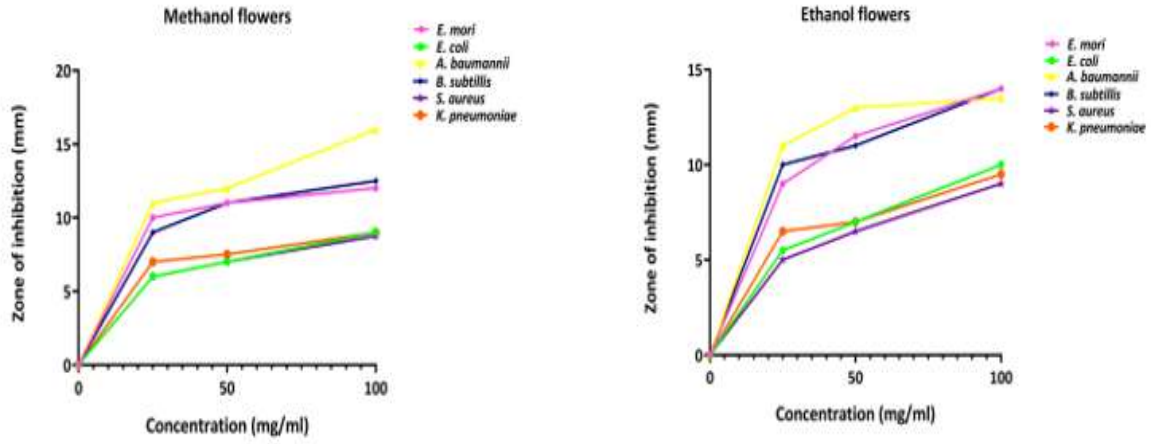


Figure 4. 10 (b): MIC of the different plant extracts is shown above in the graph for the tested bacterial strains

4.6.3. MIC of methanolic and ethanolic stems extract

The presented graphs depict the zone of inhibition observed at concentrations of 25, 50, and 100 mg/ml. The methanolic stems extract exhibited the highest level of inhibition against B. subtillis, while demonstrating the lowest level of inhibition against S. aureus. The ethanolic stems extract exhibited the highest level of inhibition against E. mori and the lowest level against K. pneumonia.

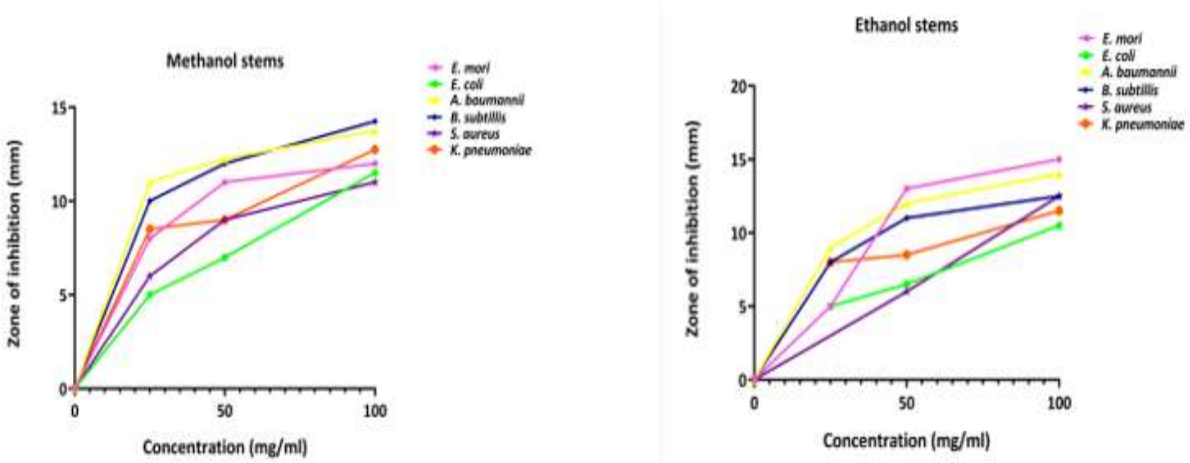


Figure 4.10 (c): MIC of the different plant extracts is shown above in the graph for the tested bacterial strains

4.7. Lipinski's rule of five

The physicochemical characteristics of the phytoconstituents of N. oleander were assessed using PASS analysis, in accordance with Lipinski's rule of five. Among the thirty-four phytoconstituents that were analysed, four of them exhibited a solitary Lipinski violation. These four phytoconstituents are Androstan-6-one, oxime, (5.alpha.)-, Cyclohexane, 1-methyl-4-(1-methylethyl)-, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha..)-, and 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-. Surprisingly, the remaining thirty phytocompounds displayed no instances of non-compliance. According to Lipinski's rule of five, an ideal lead compound should not have more than one violation. Therefore, all thirty compounds included in this study adhere to the specified requirements.

Table 4.9: PASS analysis of the phytochemical from N. oleander

ligand	MW	TPSA ≤140 Å²	Number of rotatable bonds ≤10	H-bond acceptor (NON)≤10	H bond donar (NOHNH) ≤5	Molar refractivity	Lipinski Rule
O,O-Diphenyl N-cyclohexylphosphoramidothioate	347.41 g/mol	72.39 Å²	6	3	1	99.34	yes,o violation
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole	331.32 g/mol	114.72 Å²	5	6	0	84.14	yes,o violation
1,2-Epoxy-5,9-cyclododecadiene	178.27 g/mol	12.53 Å²	0	1	0	55.71	yes,o violation
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5-	344.45 g/mol	107.89 Å²	13	6	4	91.54	yes, o violation
4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-	291.42 g/mol	85.50 Å²	4	3	1	89.39	yes, 0 violation
Androstan-6-one, oxime, (5.alpha.)-	289.46 g/mol	32.59 Å²	0	2	1	88.89	**Yes; 1 violation**
2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol	317.58 g/mol	103.38 Å²	5	4	2	70.18	Yes; 0 violation

Cyclohexane, 1-methyl-4-(1-methylethyl)-,	138.25 g/mol	0.00 Å²	1	0	0	47.6	**Yes; 1 violation**
1,3- Dioxolane,. 2-methyl-2-(4-methyl-	278.41 g/mol	43.76 Å²	5	2	0	81.05	Yes; 0 violation
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester	172.18 g/mol	52.60 Å²	3	4	0	41.03	Yes; 0 violation
3-Methyl-4-(phenylthio)-2-prop-	280.41 g/mol	67.82 Å²	4	2	0	77.71	Yes; 0 violation
2-Thiazolamine, 5-[(4-nitrophenyl)	297.33 g/mol	112.97 Å²	3	3	1	86.21	Yes; 0 violation
Uridine, 2'-deoxy-, 3',5'-diacetate	440.19 g/mol	111.24 Å²	6	7	1	91.31	Yes; 0 violation
1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol	290.27 g/mol	81.68 Å²	3	8	0	61.25	Yes; 0 violation
2,4-Di-tert-butylthiophenol	222.39 g/mol	38.80 Å²	2	0	0	72.23	yes;o violation
4-(4-Nitroanilino)-6-.piperidino-1,2,3-triazin-.	316.32 g/mol	119.73 Å²	4	5	2	90.47	Yes; 0 violation
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	206.27 g/mol	83.28 Å²	2	3	1	57.45	Yes; 0 violation
5.alpha.-Dihydroprogesterone	316.48 g/mol	34.14 Å²	1	2	0	94.49	Yes; 0 violation
Cholestan -3-ol, 2-methylene-,.(3.beta.,5.alpha.)-	400.68 g/mol	20.23 Å²	5	1	1	128.42	**Yes; 1 violation**
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-,. (5.alpha.)-	287.35 g/mol	52.93 Å²	0	4	2	82.78	Yes; 0 violation
.alpha.-D-Glucopyranoside, methyl-4-O-decyl-	334.45 g/mol	88.38 Å²	12	6	3	88.46	Yes; 0 violation
[(2-Nitrophenyl)formohydrazido]methanimidamide	223.19 g/mol	139.32 Å²	4	4	3	57.36	Yes; 0 violation
5-(1-Phenyl-propyl)-1H-tetrazole	188.23 g/mol	54.46 Å²	3	3	1	53.24	Yes; 0 violation
2,7-Anhydro-l-galacto-heptulofuranose	192.17 g/mol	99.38 Å²	1	6	4	38.39	Yes; 0 violation
2-Methoxy-diphenylmethane	198.26 g/mol	9.23 Å²	3	1	0	62.39	Yes; 0 violation

3-(4-Methyl-piperazin.-1-yl)-N-(2-trifluoromethyl-	315.33 g/mol	35.58 Å²	6	6	1	86.11	Yes; 0 violation
3-.beta.-d-Ribofuranosyl.pyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	284.23 g/mol	164.32 Å²	2	7	6	63.99	**Yes; 1 violation**
3-Pyridine.carboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-	211.21 g/mol	79.53 Å²	3	4	2	55.53	Yes; 0 violation
Benzoic acid, 4-nitro-, octyl ester	279.33 g/mol	72.12 Å²	10	4	0	80.19	Yes; 0 violation
S-2-[2-[6-Methoxy-4-quinolyloxy]ethylamino]ethyl thiosulfate	358.43 g/mol	131.43 Å²	9	7	2	90.08	Yes; 0 violation
2,5-.Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-	180.20 g/mol	54.37 Å²	1	3	1	49.09	Yes; 0 violation
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	283.37 g/mol	46.84 Å²	3	3	0	87.75	Yes; 0 violation
2H-Thiazolo.[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-.	288.37 g/mol	96.43 Å²	0	4	0	83.25	Yes; 0 violation
4-Piperidinecarboxylic acid hydrazide	143.19 g/mol	67.15 Å²	2	3	3	41.27	Yes; 0 violation

4.8. Pharmacokinetic property prediction

The ADMET study conducted on lesser-studied phytoconstituents indicated the presence of 1,2-Epoxy-5,9-cyclododecadiene, Androstan-6-one, oxime, (5.alpha.)-, Cyclohexane, 1-methyl-4-(1-methylethyl)-, and 1,3-Dioxolane, 2-methyl-2-(4-methyl-. The compound referred to is methyl ester of 2-oxotetrahydropyranyl-6-acetic acid. The compound denoted as 3-Methyl-4-(phenylthio) isThe compound -2-prop is a chemical substance that is of interest in academic research. The compound referred to as 2,4-Di-tert-butylthiophenol, often known as 5. alpha., is of interest. The chemical compounds mentioned include dihydroprogesterone, morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-, and 5-(1-phenyl-propyl).The compounds mentioned include -1H-tetrazole, 2-Methoxy-diphenylmethane, 3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-, Benzoic acid, 4-

nitro-, octyl ester. The compounds 2,5-Cyclohexadiene-1,4-dione and 3-hydroxy-2-methyl-5-(1-methylethyl) have the ability to traverse the blood-brain barrier, whereas other specific Phyto ligands have demonstrated favorable outcomes. The compounds mentioned in the text include 4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-, 4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-, Morphinan-3,14The compound referred to as methyl-4-O-decyl-D-glucopyranoside and 2,7-Anhydro-l-galacto-heptulofuranose is of interest. The compound referred to as 3-(4-Methyl-piperazin-1-The compound in question is N-(2-trifluoromethyl-, S-2-[2-[6-Methoxy-Ethyl thiosulfate is a chemical compound. The results indicated that certain phytoconstituents exhibited positive characteristics as substrates for permeability glycoprotein (P-gp), but the remaining phytoconstituents demonstrated negative characteristics. The findings of this study indicate that the phytoconstituents, as non-Pgp substrates, have the ability to remain in the cells for a longer duration, resulting in enhanced pharmacokinetic efficacy. In order to achieve continuous plasma concentrations and improved bioavailability, it was anticipated that the compounds being investigated would demonstrate inhibitory effects on the five major classes of cytochrome P450 enzymes, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The acquired results have provided confirmation of the inhibition of CYP1A2 by O,O-Diphenyl N-cyclohexylphosphoramidothioate,1-(2-Fluoro-phenyl).The compound -5-(4-nitro-benzylsulfanyl) is ofThe compounds mentioned include -1H-tetrazole, 2,2,2-Trichloro-1-(2 nitrophenylthioamino)ethanol, 1,3-Dioxolane, 2-methyl-2-(4-methyl-), 2-Thiazolamine,The compounds -3-phenyl-1,2,4-thiadiazole, 2-Methoxy-diphenylmethane, and Benzoic acid are being discussed. The inhibition of CYP2D6 by 1, 4-nitro-, octyl ester.The chemical compounds mentioned in the text include 3-Dioxolane, 2-methyl-2-(4-methyl-, 2-Thiazolamine, 5-[(4-nitrophenyl), Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-, 2-Methoxy-diphenylmethane, and 3-(4-MethylThe compound N-(2-trifluoromethyl) and the enzyme CYP3A4. The chemical compounds referred to are O,O-Diphenyl N-cyclohexylphosphoramidothioate, 3-Methyl-4-(phenylthio)-2-prop-, 2-Thiazolamine, and The inhibition of CYP2C19 by O,O-Diphenyl N-cyclohexylphosphoramidothioate, 1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl) is being investigated.The compound referred to as -1H-tetrazole is a chemical entity that possesses The compound referred to as 4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-

, Androstan-6-one, oxime, (5.alpha.) is of interest.The chemical compounds mentioned are 2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol, 1,3-Dioxolane, 2-methyl-2-(4-methyl-, -Methyl-4-(phenylthio)-2-prop-, 2-Thiazolamine,5 The objective of this study was to investigate the inhibitory effects of O,O-Diphenyl N-cyclohexylphosphoramidothioate,1-(2-Fluoro-phenyl) on the enzyme CYP2The compound -5-(4-nitro-benzylsulfanyl)-1H-tetrazole, also known asThe compound -5,9-cyclododecadiene is a chemical substance that Androstan is a chemical compound that belongs to the class of organic compounds known as steroidsThe compounds mentioned are -6-one, oxime, (5.alpha.)-, 2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol, 1,3-Dioxolane, 2-methyl-2-(4-methyl- , 3-Methyl-4-(phenylthio)-2-prop-, and 2-Thiazolamine, 5-[(4-nitrophenyl). The remaining chemicals exhibited no inhibitory activity against cytochrome P450. The consensus lipophilicity value (log P o/w) was computed in order to evaluate the lipophilic properties of the phytoconstituents. The analysis indicated that a significant proportion of these compounds demonstrate a tendency to dissolve in lipids. All substances, with the exception of Cyclohexane, 1-methyl-4-(1-methylethyl)-, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, [(2-Nitrophenyl) formohydrazido] methanimidamide, and 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H, exhibited high human intestine absorption (gastrointestinal absorption).

Table 4.10: Pharmacokinetic properties of the phytochemical from N. oleander

ligand	CYP 1A2 Inhibitor	CYP2 C19 Inhibitor	CYP 2C9 Inhibitor	CYP 2D6 Inhibitor	CYP 3A4 Inhibitor	P-gp Substrate	BBB permanent	Consensus log Po/w	GI Absorption
O,O-Diphenyl N-cyclohexylphosphoramidothioate	yes	yes	yes	No	yes	No	No	4.71	High
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole	yes	yes	yes	No	No	No	No	2.52	High
1,2-Epoxy-5,9-cyclododecadiene	No	No	yes	No	No	No	Yes	2.92	High
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5-	No	No	No	No	No	Yes	No	1.18	High
4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-	No	Yes	No	No	No	No	No	2	High

Androstan-6-one, oxime, (5.alpha.)-	No	Yes	Yes	No	No	No	yes	4.66	High
2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol	Yes	Yes	Yes	No	No	No	No	1.88	High
Cyclohexane, 1-methyl-4-(1-methylethyl)-,	No	No	No	No	No	No	Yes	3.37	Low
1,3-Dioxolane, 2-methyl-2-(4-methyl-	yes	yes	yes	yes	No	No	Yes	3.99	High
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester	No	No	No	No	No	No	Yes	0.96	High
3-Methyl-4-(65henylthiol)-2-prop-	No	yes	yes	No	yes	No	Yes	3.2	High
2-Thiazolamine, 5-[(4-nitrophenyl)	Yes	Yes	Yes	yes	Yes	No	No	2.67	High
Uridine, 2'-deoxy-, 3',5'-diacetate	No	No	No	No	No	No	No	0.28	High
1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol	No	No	No	No	No	No	No	-0.04	High
2,4-Di-tert-butylthiophenol	No	No	No	No	No	No	yes	4.61	High
4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-	No	No	No	No	No	yes	No	0.95	High
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	Yes	No	No	No	No	No	No	1.52	High
5.alpha.-Dihydroprogesterone	No	No	No	No	No	No	yes	4.17	High
Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	No	No	No	No	No	No	No	7.03	Low
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	No	No	No	Yes	No	yes	yes	1.92	High
.alpha.-D-Glucopyranoside, methyl-4-O-decyl-	No	No	No	No	No	Yes	No	2.15	High
[(2-Nitrophenyl)formohydrazido] methanimidamide	No	No	No	No	No	No	No	-0.85	Low
5-(1-Phenyl-propyl)-1H-tetrazole	No	No	No	No	No	No	Yes	1.93	High
2,7-Anhydro-l-galacto-heptulofuranose	No	No	No	No	No	Yes	No	-1.75	Low
2-Methoxy-diphenylmethane	Yes	yes	No	yes	No	No	Yes	3.55	High
3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-	No	No	No	Yes	No	Yes	Yes	2.19	High
3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	No	No	No	No	No	No	No	-1.78	Low
3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-	No	No	No	No	No	No	No	1.16	High
Benzoic acid, 4-nitro-, octyl ester	Yes	yes	No	No	No	No	Yes	3.52	High
S-2-[2-[6-Methoxy-4-quinolyloxy]ethylamino]ethyl thiosulfate	No	No	No	No	No	Yes	No	0.57	High
2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-	No	No	No	No	No	No	Yes	1.36	High

Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	No	Yes	Yes	No	No	No	Yes	2.86	High
2H-Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-	No	No	No	No	No	Yes	No	0.36	High
4-Piperidinecarboxylic acid hydrazide	No	No	No	No	No	No	No	-0.5	High

4.9. Toxicity analysis

Assessment of expected drug-likeness and toxicity potential of selected phytochemicals by data warrior and protox-II server revealed that O,O-Diphenyl N-cyclohexyl phosphoramidothioate, Cyclohexane, 1-methyl-4-(1-methylethyl)-, 1,3-Dioxolane, 2-methyl-2-(4-methyl-, 3-Methyl-4-(phenylthio)-2-prop-, Uridine, 2'-deoxy-, 3',5'-diacetate, 2,4-Di-tert-butylthiophenol, 3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-,3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-, 3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-, 2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-, were non-toxic. 4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-, 5.alpha.-Dihydroprogesterone, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, alpha.-D-Glucopyranoside, methyl-4-O-decyl-, S-2-[2-[6-Methoxy-4-quinolyloxy]ethylamino]ethyl thiosulfate, were appeared to have projected immunotoxin effects. Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5- , 2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol, 5.alpha.-Dihydroprogesterone, 2,7-Anhydro-l-galacto-heptulofuranose, and 2H-Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-toxic effects on the reproductive system.1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole, 2-Thiazolamine,5-[(4-nitrophenyl),5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole, Nitrophenyl)formohydrazido]methanimidamide,5-(1-Methylhydrazino)-3-phenyl-1,2,4 thiadiazol, have shown slightly toxic effect on liver and have shown mildly hepatotoxicity. whereas Androstan-6-one, oxime, (5. alpha.)- , Benzoic acid, 4-nitro-, octyl ester also have slight immunotoxicity. Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5- , 2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol, and 5.alpha.-Dihydroprogesterone are highly mutagenic in nature. Of all the ligand no one is irritant. 1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole, 1,2-Epoxy-5,9-cyclododecadiene, Androstan-6-one, oxime, (5.alpha.)-, 1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol, 4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-, 5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole, and 2-Methoxy-diphenylmethane iare

slightly carcinogenic in nature. 1,2-Epoxy-5,9-cyclododecadiene, 2,2,2-Trichloro-1-(2-nitrophenylthioamino) ethanol and 4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin- are mildly tumorigenic. None of the Phyto ligand show cytotoxicity, Androgen Receptor Ligand Binding Domain (AR-LBD) and Estrogen Receptor Ligand Binding Domain (ER-LBD) effectThe anticipated toxicity risk evaluation of N. oleander's phytoconstituents is summarized in the table provided. To be considered a promising drug candidate, a compound should ideally possess a druglikeness score that approaches or equals 1. If the drug score is zero or falls into the negative range, it indicates that the compound being studied is not a suitable candidate for drug development. 4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-, Uridine, 2'-deoxy-, 3',5'-diacetate, 4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-, 5.alpha.-Dihydroprogesterone, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-,3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-,3-.beta.-d, Ribofuranosylpyrazolo [4,3-d]pyrimidin-5,7-4H,6H- have shown positive scores for drug-likeness. The table displays computational predictions for LD50 values and toxicity classifications. Additionally, Table provides comprehensive information regarding drug similarity and the potential assessment of toxicity for the examined phytochemicals.

Table 4.11: Druglikeness and toxicity analysis of the phytochemicals from N. oleander

ligand	Druglikeness	Toxicity Class	LD 50 mg/kg
O,O-Diphenyl N-cyclohexylphosphoramidothioate	-0.67	3	270
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole	-1.2	4	1000
1,2-Epoxy-5,9-cyclododecadiene	-1.27	5	5000
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5-	-0.28	4	1600
4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-	**0.17**	1	3
Androstan-6-one, oxime, (5.alpha.)-	-0.09	4	2000
2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol	-1.04	5	4470

Cyclohexane, 1-methyl-4-(1-methylethyl)-,	-1.24	5	5000
1,3-Dioxolane, 2-methyl-2-(4-methyl-	-0.40	4	1145
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester	-1.01	5	2991
3-Methyl-4-(phenylthio)-2-prop-	-0.51	4	1440
2-Thiazolamine, 5-[(4-nitrophenyl)	-0.64	4	330
Uridine, 2'-deoxy-, 3',5'-diacetate	**0.59**	4	750
1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol	-1.31	5	5000
2,4-Di-tert-butylthiophenol	-1.24	5	3100
4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-	**0.35**	5	4000
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	-0.5	4	2000
5.alpha.-Dihydroprogesterone	**0.22**	4	775
Cholestan.-3-ol, 2-.methylene-, (3.beta.,5.alpha.)-..	**0.08**	5	5000
Morphinan-3.,14-diol, 4,5-epoxy.-17-methyl-, (5.alpha.)-	**0.75**	4	402
.alpha.-D-Glucopyranoside, methyl-4-O-decyl-	-0.6	4	2000
[(2-Nitrophenyl)formohydrazido]methanimidamide	-0.54	4	6000
5-(1-Phenyl-propyl)-1H-tetrazole	**0.2**	4	400
2,7-Anhydro-l-galacto-heptulofuranose	-1.07	6	23000
2-Methoxy-diphenylmethane	-0.74	5	3550
3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-	**1.01**	4	1210
3-.beta.-d- Ribofuranosyl.pyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	**0.52**	4	300
3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-	-0.34	4	572
Benzoic acid, 4-nitro-, octyl ester	-0.55	5	3250
S-2-[2-[6-Methoxy-4-quinolyloxy].ethylamino]ethyl thiosulfate	0.45	4	900
2,5-Cyclohexadiene-1,4-dione,.3-hydroxy-2-methyl-5-(1-methylethyl)-	-0.68	5	2800
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	-0.7	4	500
2H-Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-	0.85	4	1600
4-Piperidinecarboxylic acid hydrazide	-1.04	4	1610

Class I: Deadly if swallowed (LD50 ≤ 5); Class II: Very poisonous if swallowed (5 < LD50 ≤ 50). Class III: Moderately harmful if swallowed (50 < LD50 ≤ 300); Class IV: Slightly harmful if swallowed (300 < LD50 ≤ 2000). Class V: Could be harmful if swallowed (2000 < LD50 ≤ 5000); Class VI: Not harmful (LD50 > 5000)."

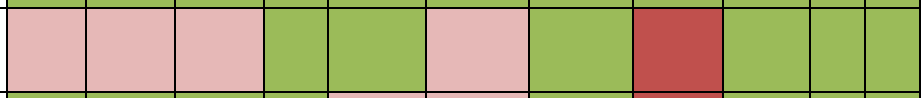
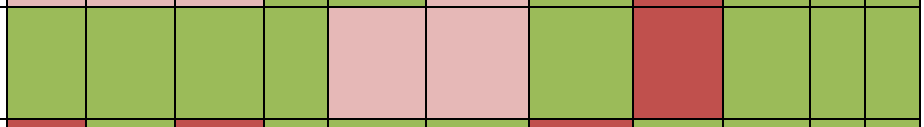
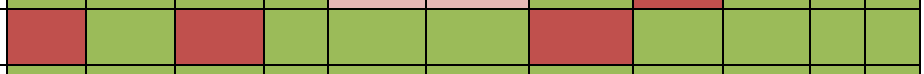
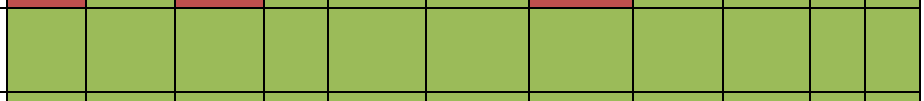
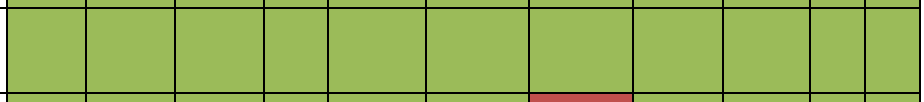
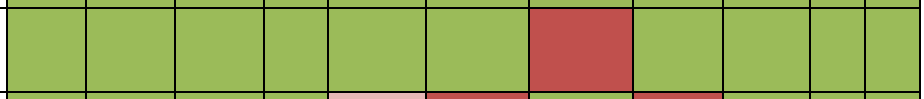
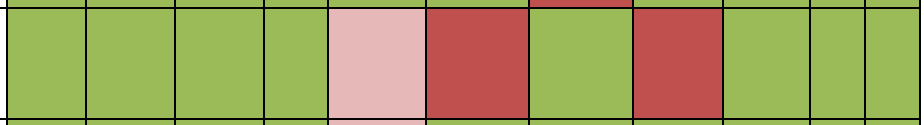
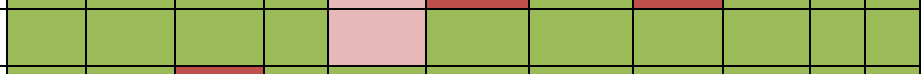
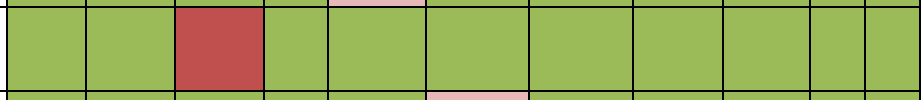
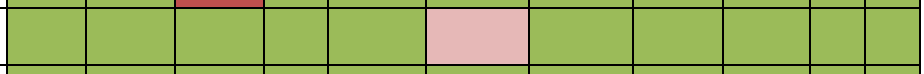
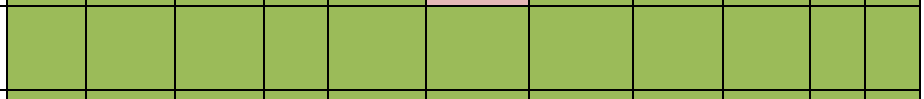
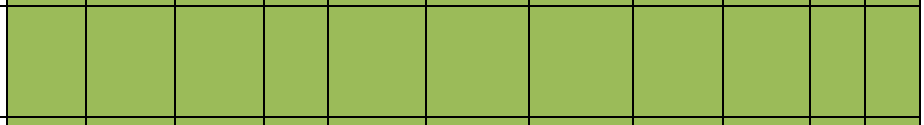
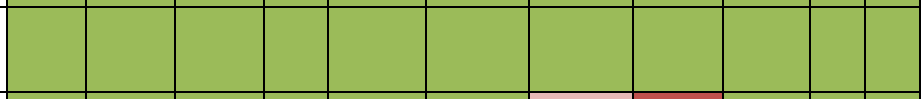
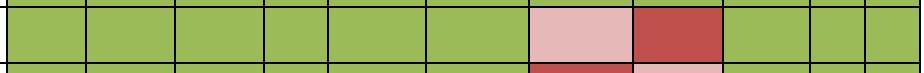
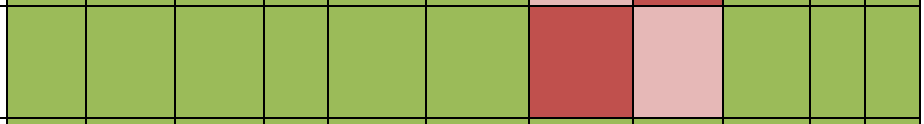
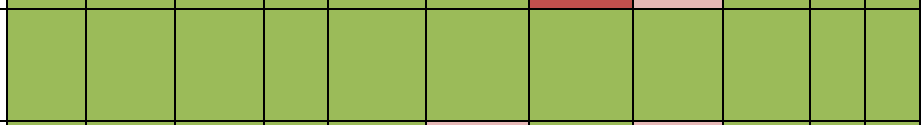
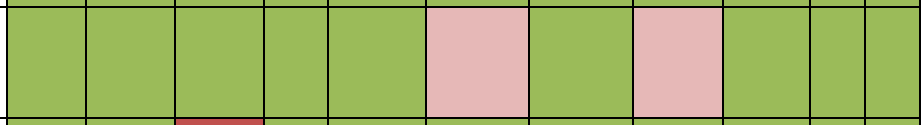
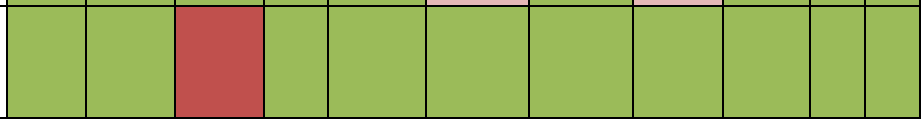
4.10. Toxicity Risk assessment

Toxicity of different phytochemicals was performed using the different software and the results are shown in the table.

Table 4.12: Predicted toxicity risk assessment of phytochemical of N. oleander

	Nontoxic
	Less toxic
	Highly toxic

ligand	Mutagenic	Tumorigenic	reproductive effective	Irritant	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	(AR-LBD)	(ER-LBD)
O,O-Diphenyl N-cyclohexylphosphoramidothioate											
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole											
1,2-Epoxy-5,9-cyclododecadiene											
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5-											
4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-											
Androstan-6-one, oxime, (5.alpha.)-											
2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol											
Cyclohexane, 1-methyl-4-(1-methylethyl)-,											
1,3-Dioxolane, 2-methyl-2-(4-methyl-											
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester											
3-Methyl-4-(phenylthio)-2-prop-											
2-Thiazolamine, 5-[(4-nitrophenyl)											
Uridine, 2'-deoxy-, 3',5'-diacetate											
1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol											

2,4-Di-tert-butylthiophenol	
4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-	
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	
5.alpha.-Dihydroprogesterone	
Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	
.alpha.-D-Glucopyranoside, methyl-4-O-decyl-	
[(2-Nitrophenyl)formohydrazido]methanimidamide	
5-(1-Phenyl-propyl)-1H-tetrazole	
2,7-Anhydro-l-galacto-heptulofuranose	
2-Methoxy-diphenylmethane	
3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-	
3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	
3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-	
Benzoic acid, 4-nitro-, octyl ester	
S-2-[2-[6-Methoxy-4-quinolyloxy]ethylamino]ethyl thiosulfate	
2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-	
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	
2H-Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile, 3,5,6,7-tetrahydro-	

4-Piperidinecarboxylic acid hydrazide	

4.11. Docking Analysis

We utilized AutoDock Vina v1.5.6 for the docking process, where we paired target proteins with phytochemicals obtained from N. oleander plants. During the docking, the Phyto ligands exhibited diverse interactions within the binding pockets, resulting in various binding affinities. The phytochemicals displayed a range of binding energies for the target proteins, spanning from -4.8 kcal mol^{-1} to -8.9 kcal $mol^{-1.}$ Notably, 5.alpha.-Dihydroprogesterone demonstrated the most robust binding affinity, characterized by the lowest binding energy recorded, which was -8.9 kcal mol^{-1} .

Table 4.13: Docking interaction and the calculation of binding energies with target proteins

Ligand	Binding energy in kcal mol−1 with protein **(6f86)**	Binding energy in kcal mol−1 with protein **(3zg5)**	Binding energy in kcal mol−1 with protein **(1xal)**	Binding energy in kcal mol−1 with protein **(8iwl)**	Binding energy in kcal mol−1 with protein **(7p2m)**
O,O-Diphenyl N-cyclohexylphosphoramidothioate	-6.2	-7.1	-7.4	-6.7	-6.5
1-(2-Fluoro-phenyl)-5-(4-nitro-benzylsulfanyl)-1H-tetrazole	-7.6	-7.7	-7.6	-8.4	-7.7
1,2-Epoxy-5,9-cyclododecadiene	-5.4	-6.3	-6	-6.7	-6.5
Pentane-1,5-diamine, 1-N-acetyl-1-hydroxycarbonyl-5-N-[5-	-6	-6.1	-6.6	-6.8	-5.9
4-Methylpiperidine-1-thiocarboxylic acid hydrazide, 2-[1-[3-	-5.3	-6.3	-6.8	-6.3	-6.7
Androstan-6-one, oxime, (5.alpha.)-	-7	-9.1	-7.9	-8	-7.4
2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol	-5.2	-6.1	-6.2	-6.6	-5.9
Cyclohexane, 1-methyl-4-(1-methylethyl)-,	-5	-5.5	-5.3	-6.2	-5.7
1,3-Dioxolane, 2-methyl-2-(4-methyl-	-4.8	-5.7	-5.1	-5.7	-5.3
2-Oxotetrahydropyranyl-6-acetic acid, methyl ester	-5.6	-5.8	-5	-6.1	-5.7
3-Methyl-4-(phenylthio)-2-prop-	-5.7	-6.6	-6.3	-7.1	-6.7
2-Thiazolamine, 5-[(4-nitrophenyl)	-7.1	-7.1	-7.1	-6.8	-6.7
Uridine, 2'-deoxy-, 3',5'-diacetate	-6.7	-6.8	-6.3	-6.8	-6.4
1,32,54,6-Trimethylene-d-glycero-d-mannoheptitol	-6.3	-6.9	-6.7	-6.7	-6.6
2,4-Di-tert-butylthiophenol	-5	-6	-5.6	-6	-6.2
4-(4-Nitroanilino)-6-piperidino-1,2,3-triazin-	-7.1	-7.5	-8.6	-8.6	-8.3
5-(1-Methylhydrazino)-3-phenyl-1,2,4-thiadiazole	-6.2	-6.5	-6.4	-6.9	-6.6
5.alpha.-.Dihydroprogesterone..	-7	**-8.9**	-7.8	**-8.5**	-7.2
Cholestan-3-ol, .2-methylene-, (3.beta.,5.alpha.)-	-7.2	-6.5	**-7.9**	-7.7	-7.1

Morphinan-3,.14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	**-7.4**	-7.7	**-8.5**	-7.2	**-8**
.alpha.-D-Glucopyranoside, methyl-4-O-decyl-.	-5.6	-5.6	-6.3	-6.3	-5.5
[(2-Nitrophenyl) formohydrazido]methanimidamide	-5.9	-6.4	-6.5	-6.9	-7
5-(1-Phenyl-propyl)-1H-tetrazole	**-8**	-7.6	-7.7	-8.3	-8.4
2,7-Anhydro-l-galacto-heptulofuranose	-5.4	-5.5	-5.9	-6.4	-5.5
2-Methoxy-diphenylmethane	-5.2	-6.4	-6	-7.3	-7.1
3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-	-6	-6.3	-7.6	-7.9	-6.8
3-.beta.-d- Ribo furanosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	-7.2	-7.5	-7.3	**-8.4**	**-8.1**
3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-	-5.8	-6.7	-5.7	-6.3	-5.9
Benzoic acid, 4-nitro-, octyl ester	-4.8	-6.7	-5.8	-6.5	-6.1
S-2- [2-[6-Methoxy-4-..quinolyloxy]ethylamino]ethyl thiosulfate	-5.8	-5.6	-6.5	-6.8	-5.3
2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)-	-5.6	-6.9	-6.1	-6.9	-6.3
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	-6.4	**-7.3**	-7.5	-7.6	-7
2H- Thiazolo[3,2-a]pyridine-6,8-dicarbonitrile,. 3,5,6,7- tetrahydro-	-6.4	-6.5	-6.5	-7.4	-6.8
4-Piperidinecarboxylic acid hydrazide	-5.4	-5.8	-5.3	-5.5	-5.6

4.11.1. Docking analysis of selected Phyto ligands from N. oleander with protein (PDB ID 6F86)

On the basis of the different analysis we have selected 2 phytochemicals with highest binding energy with the bacterial protein. Crystal Structure of *E. coli* GyraseB (PDB ID: 6F86) for supercoiling of chromosomal DNA. *E. coli* GyrB24 was selected. On the basis of selection criteria we selected 5-(1-Phenyl-propyl)-1H-tetrazole, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-

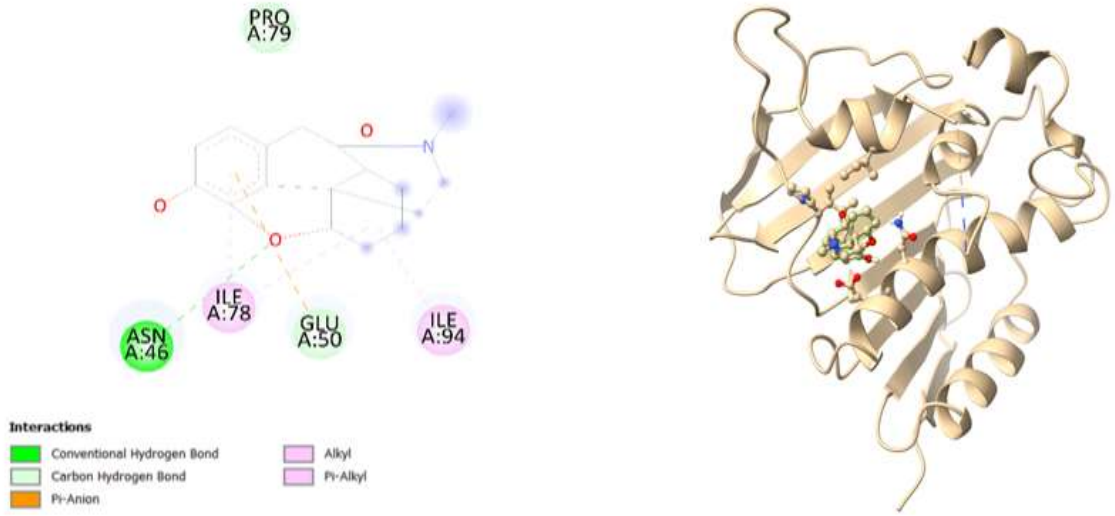


Figure 4.11: Docking pose of Morphinan -3,14-diol, 4,5-epoxy-17- methyl-, (5. alpha.)-. with the selected target protein 6F86 represented as 2D and 3D structure

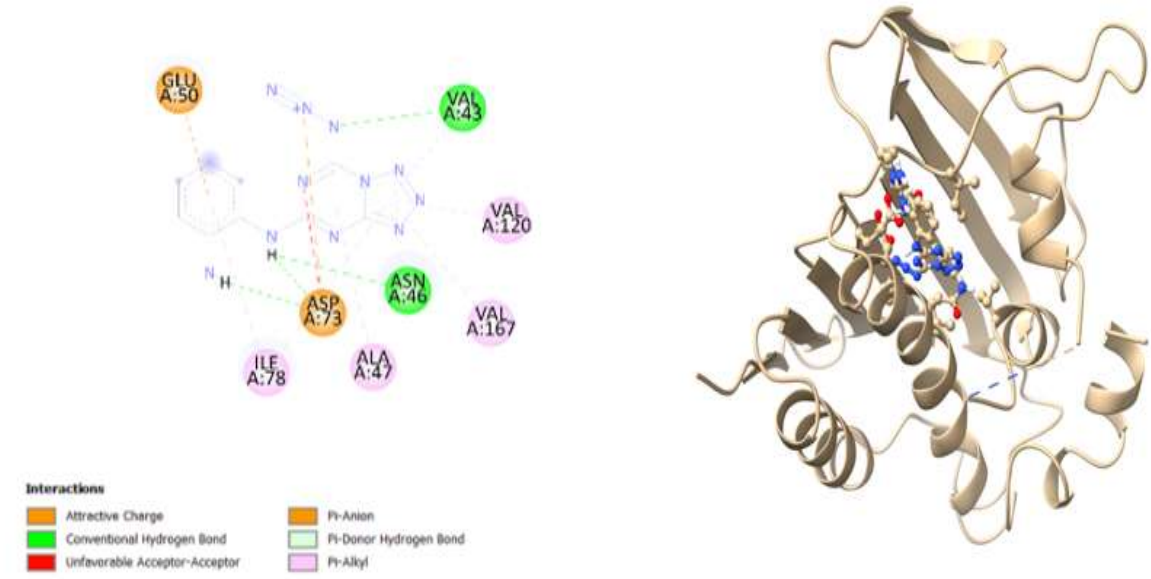


Figure 4.12: Docking pose of 5-(1-Phenyl-propyl)-1H- tetrazole with the selected target protein 6F86 represented as 2D and 3D structure

4.11.2. Docking analysis of selected Phyto ligands from N. oleander with protein (PDB ID 3ZG5)

Based on the different analysis, we have selected 2 phytochemicals with highest binding energy with the target *S. aureus* hydrolase enzyme **(PDB ID: 3ZG5)** necessary for the cleavage of large molecules into small fragments and recycling was selected. Based on selection criteria, we selected 5. alpha. -Dihydroprogesterone, and Tetrazole, 5-[2-(1-perhydroazepinyl) ethenyl]-1-(4-methylphenyl)-

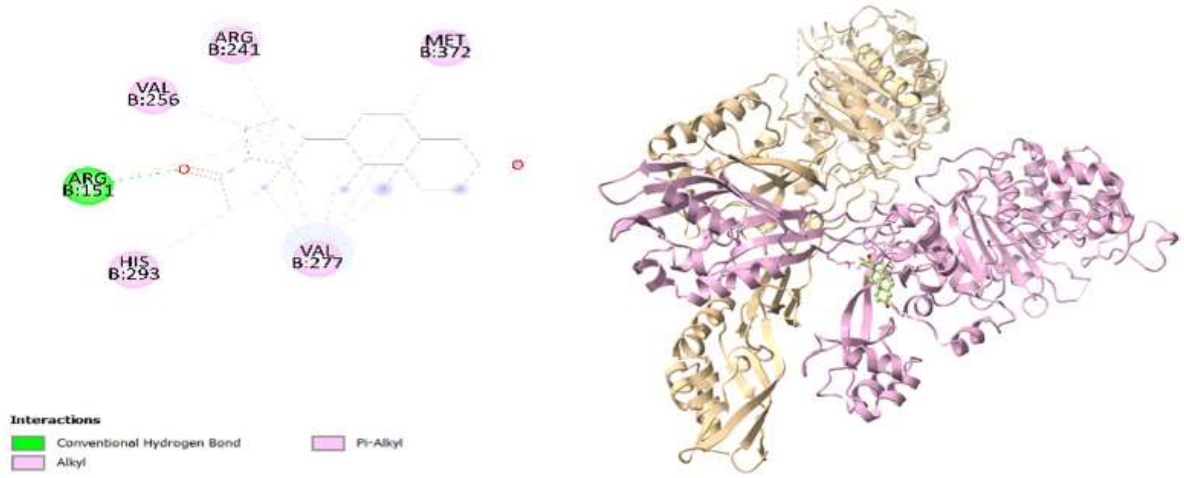


Figure 4.13: Docking pose of 5. alpha. -Dihydroprogesterone with the selected target protein 3ZG5 represented as 2D and 3D structure

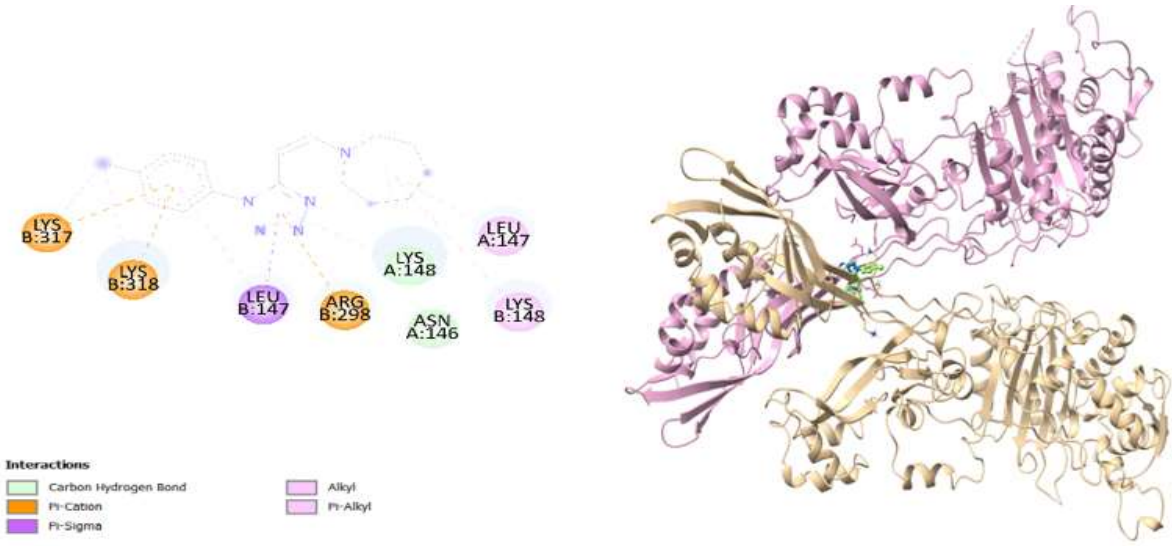


Figure 4.14: Docking pose of Tetrazole, 5-[2-(1-perhydroazepinyl) ethenyl]-1-(4-methylphenyl. with the selected target protein 3ZG5 represented as 2D and 3D structure

4.11.3. Docking analysis of selected Phyto ligands from N. oleander with protein (PDB ID 1XAL)

Based on the different analysis, we have selected 2 phytochemicals with highest binding energy with the target Crystal structure of S. aureus 3-dehydroquinate synthase (PDB ID: 1XAL) necessary for carbohydrates metabolism was selected. On the basis of selection criteria we selected Cholestan-3-ol, 2-methylene-, (3.beta., 5.alpha.)-, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-..

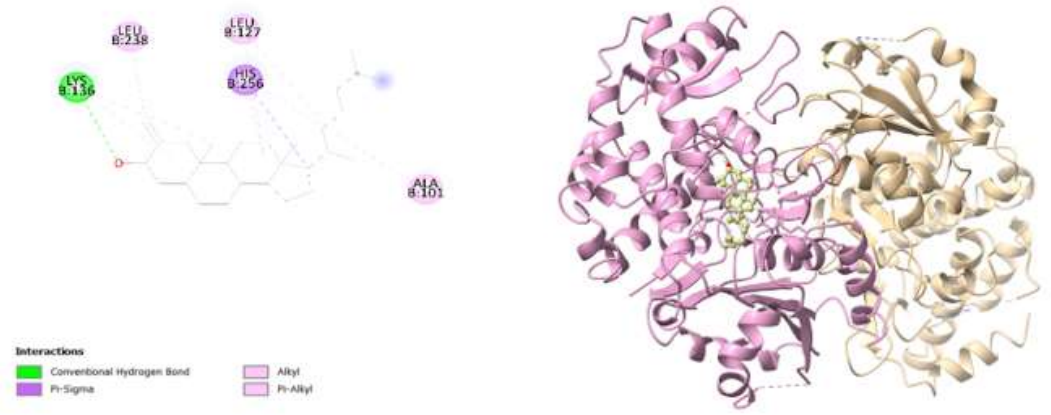


Figure 4.15: Docking pose of Cholestan-3-ol , 2-methylene-, (3.beta.,5.alpha.)- with the selected target protein 1XAL represented as 2D and 3D structure

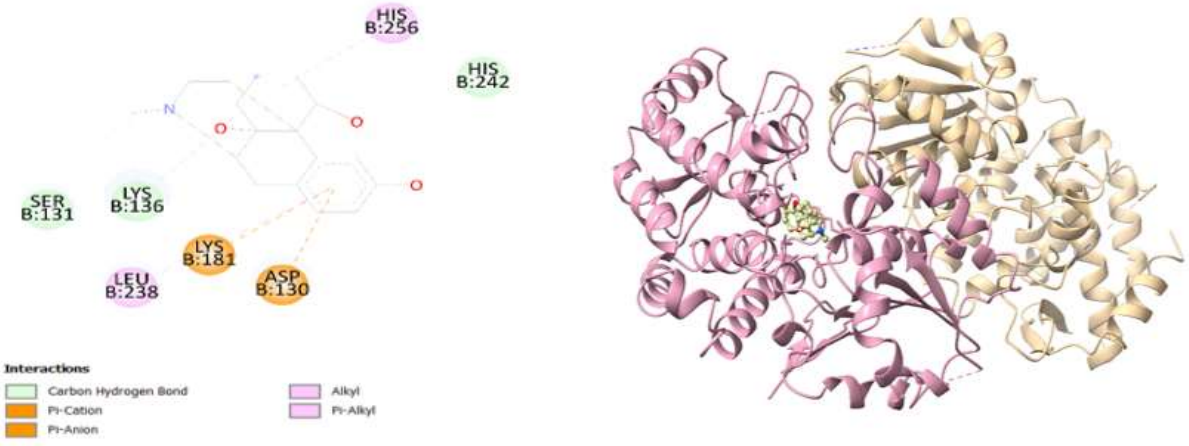


Figure 4.16: Docking pose of Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)- with the selected target protein 1XAL represented as 2D and 3D structure

4.11.4. Docking analysis of selected Phyto ligands from N. oleander with protein (PDB ID 8IWL)

On the basis of the different analysis we have selected 2 phytochemicals with highest binding energy with the target A. baumannii uncharacterized sugar kinase protein (PDB ID: 8IWL) necessary for the glucose utilization for carbon source was selected. On the basis of different analysis we selected 5.alpha.-Dihydroprogesterone, 3-.beta. -d-Ribofuranosyl pyrazolo[4,3-d]pyrimidin-5,7-4H,6H-.

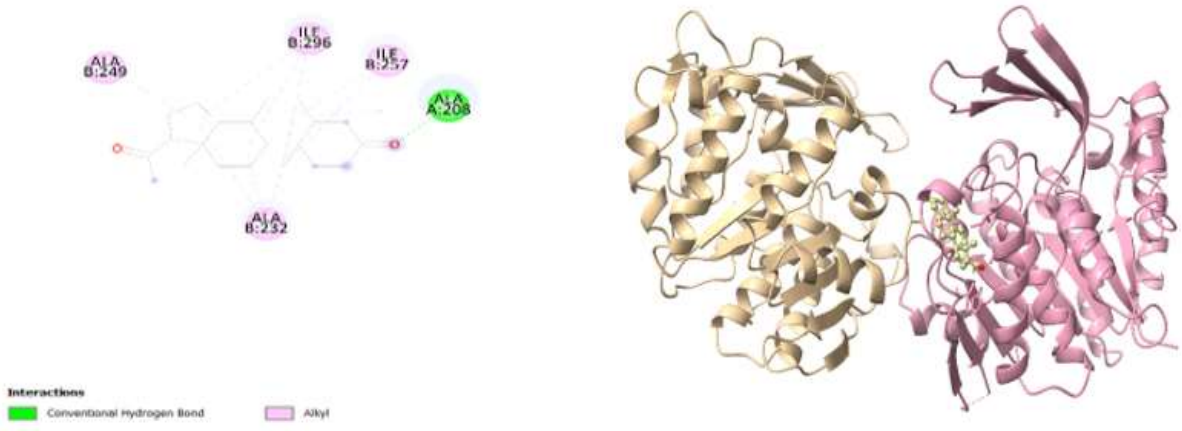


Figure 4.17: Docking pose of 5.alpha.-Dihydroprogesterone with the selected target protein 8IWL represented as 2D and 3D structure

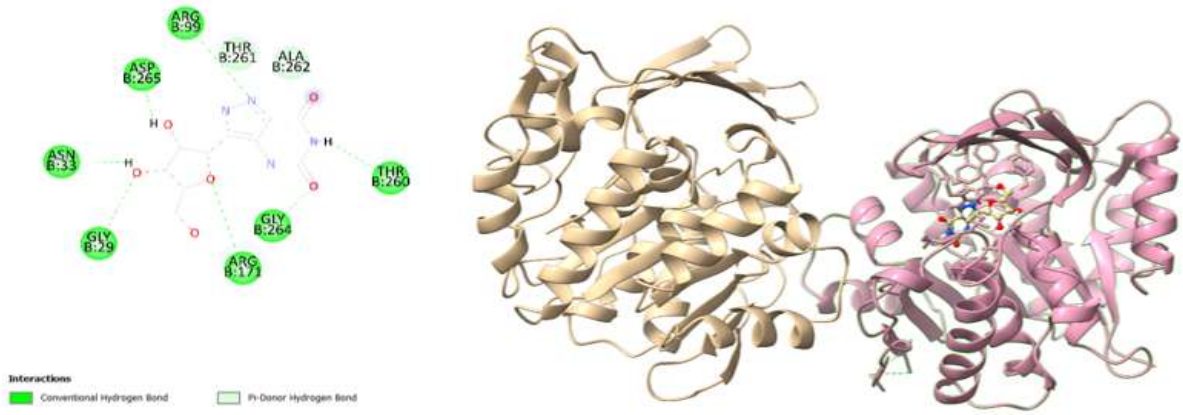


Figure 4.18: Docking pose of 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H- with the selected target protein 8IWL represented as 2D and 3D structure

4.11.5. Docking analysis of selected Phyto ligands from N. oleander with protein (PDB ID 7P2M)

On the basis of the different analysis we have selected 2 phytochemicals with highest binding energy with the target E. coli GyrB24 (PDB ID: 7P2M) necessary for DNA replication was selected. On the basis of different analysis we selected Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-, 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-

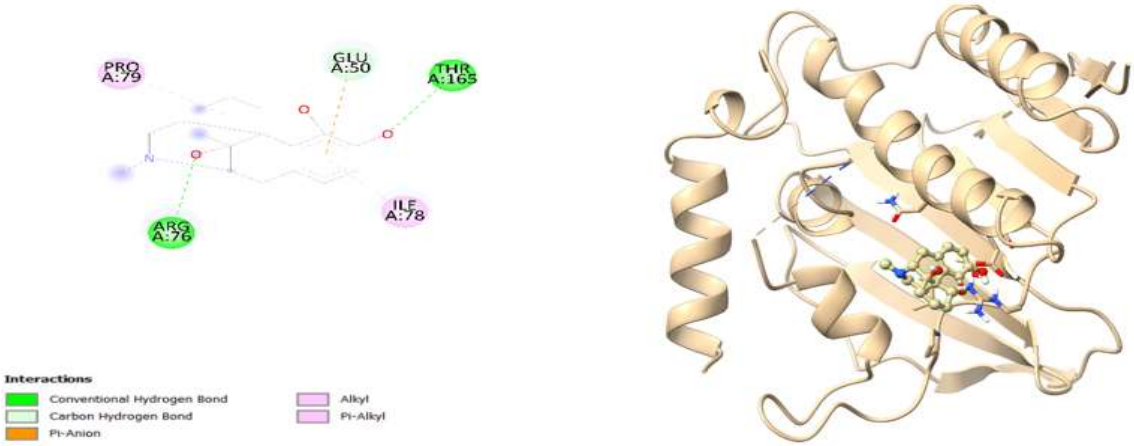


Figure 4.19: Docking pose of Morphinan-3,14-diol, 4,5-epoxy-17-methyl- with the selected target protein 7P2M represented as 2D and 3D structure

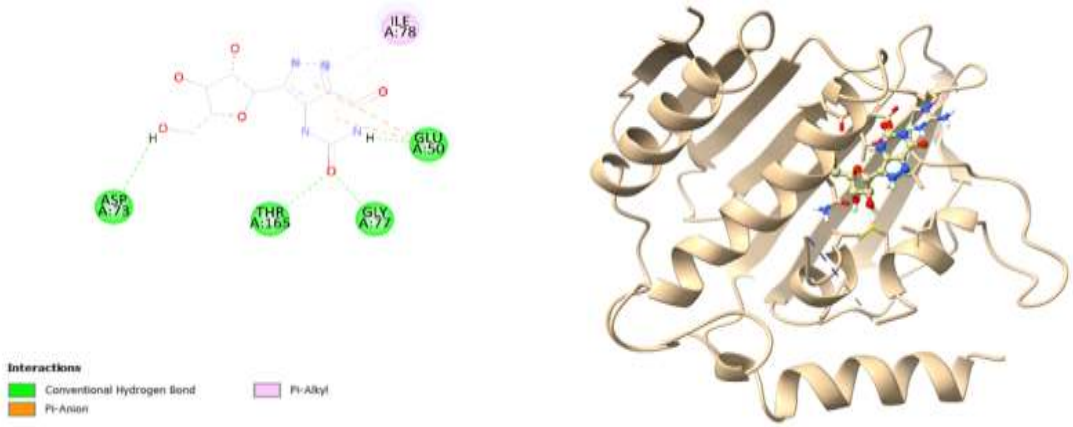


Figure 4.20: 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H- with the selected target protein 7P2M represented as 2D and 3D structure

Table 4.14: Docking interaction and the interacting amino acids residues

Phytochemicals	**protein**	**Binding Energy k cal mol^{-1}**	**Interacting Amino acids**
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	6F86	-7.4	Pro79, Asn46 ,Ile78, Glu50, Ile94
5-(1-Phenyl-propyl)-1H-tetrazole	6F86	-8	Glu50, Val43, Ile78, Asp73, Ala47, Asn46, Val167, Val120
5.alpha.-Dihydroprogesterone	3ZG5	-8.9	Arg151, val256, Arg241, Met372, His293, Val277
Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-	3ZG5	-7.3	Lys371, Lys318, Leu147, Arg298, Lys148, Asn146, Leu147, Lys148
Cholestan -3-ol, 2-methylene-, (3.beta.,5.alpha.)-	1XAL	-7.9	Lys136, Leu238, Leu127, His256, Ala101
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	1XAL	-8.5	Ser131, Lys136, Leu238, Lys181, Asp130, His256, His242
5.alpha.-Dihydroprogesterone	8IWL	-8.5	Ala249, Ala232, Ile296, Ile257, Ala208
3-.beta.-d-Ribofuranosyl. pyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	8IWL	-8.4	Asn33, Asp265, Arg99, Thr261, Ala262, Thr260, Gly264, Arg171, Gly29
Morphinan.-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	7P2M	-8	Pro79, Glu50, Thr165, Ile78, Arg76
3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	7P2m	-8.1	Asp73, Thr165, Gly77, Glu50, Ile78

The analysis of GC-MS data revealed the presence of numerous bioactive compounds within N. oleander extracts. These phytochemicals were further investigated for their potential activities against bacterial target proteins. Using the AutoDock Vina program, we conducted docking studies of these phytochemicals to determine their binding affinities with the target proteins. Among the 35 phytochemicals assessed, we selected the best-performing ligand, based on ADME screening results, considering parameters such as toxicity, absorption, and molecular weight. Subsequently, we employed AutoDock Vina to perform docking experiments between the selected ligand and five microbial drug target proteins. The optimal docking complex was determined by evaluating their respective binding energy scores. The visualisation of the interactions between the ligand and target protein was conducted using Biovia Discovery Studio. This involved the creation of 2D-

interaction diagrams that depict the bonds established between the ligand and target protein. The results of our study are consistent with previous research, which suggests that protein-ligand interaction is more durable when lower binding energy scores are seen. Based on the analysis of binding energies, the docking outcomes for Morphinan are as follows.The compound (5.alpha.)-3,14-diol, 4,5-epoxy-17-methyl- was reacted with 1XAL and 5.alpha.The compound dihydroprogesterone, when combined with 3ZG5 and 8IWL, had the most favourable binding energy.. The highest number of interacting residues was observed in the interaction of 8IWL with 3-.beta.-d Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-, with nine interacting amino acid residues, including Asn33, Asp265, Arg99, Thr261, Ala262, Thr260, Gly264, Arg171, and Gly29. 5.alpha.-Dihydroprogesterone, which displayed the highest binding affinity with the protein 3ZG5, interacted with six amino acid residues: Arg151, Val256, Arg241, Met372, His293, and Val277. In contrast, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-, which exhibited the least binding affinity with the protein 6F86, interacted with only five amino acid residues: Pro79, Asn46, Ile78, Glu50, and Ile94. Details of these docking interactions between selected phytochemicals from N. oleander and the target proteins are presented in the table, which also showcases the binding affinity of the selected compound against antibacterial proteins.

4.12. Molecular dynamic simulation

The selection of the most effective compound was determined by evaluating its binding affinity and transformation properties, which were afterwards used for conducting molecular dynamic simulations. These simulations aimed to assess the compound's stability in relation to the protein under investigation. The simulation investigations utilised the Desmond Schrodinger suite programme to examine receptor complexes. Subsequently, the root mean square deviation (RMSD) of the protein demonstrated a consistent course throughout the 100 nanosecond dynamic run. The results obtained from a simulation lasting 100 nanoseconds demonstrate notable transformations and consistent conformations when evaluating the root-mean-square deviation (RMSD) between the protein and ligand. In the context of dynamic experiments, it was shown that the root mean square deviation (RMSD) exhibited a fluctuating trajectory up to a duration of 30 nanoseconds. Furthermore, there

were comparatively fewer variations observed between the conformations of the ligand and the protein. In general, the root mean square deviation (RMSD) of both the receptor and ligand was observed to be steady, as depicted in the accompanying figures. Furthermore, an analysis was conducted on the amino acid interactions within our complexes, including the identification of any distinctive hydrogen bonds. The optimal docking complex was determined by evaluating their respective binding energy scores. The visualization of the interactions between the ligand and target protein was conducted using Biovia Discovery Studio. This involved the creation of 2D-interaction diagrams that depict the bonds established between the ligand and target protein. The results of our study are consistent with previous research, which suggests that protein-ligand interaction is more durable when lower binding energy scores are seen. Based on the analysis of binding energies, the docking outcomes for Morphinan are as follows.The compound 3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)- was reacted with 1XAL and 5.alpha.The compound dihydroprogesterone, when interacting with the molecules 3ZG5 and 8IWL, demonstrated the most favorable binding energy.

Table 4.15: Selected ligands and target protein

Ligand	**Abbreviation**	**Target Protein**
Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-	MP	7P2M
Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-	CL	1XAL
3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-	RP	8IWL

4.12.1. Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5. alpha)- with target protein (PDB ID 7P2M)

4.12.1.1. Root mean square deviation

In the figure, it can be observed that the root mean square deviation (RMSD) of the ligand, derived using a least square fit, exhibits some variations within the initial 10 nanoseconds (ns) of the simulation. Subsequently, the RMSD remains relatively steady until the 100 ns mark.

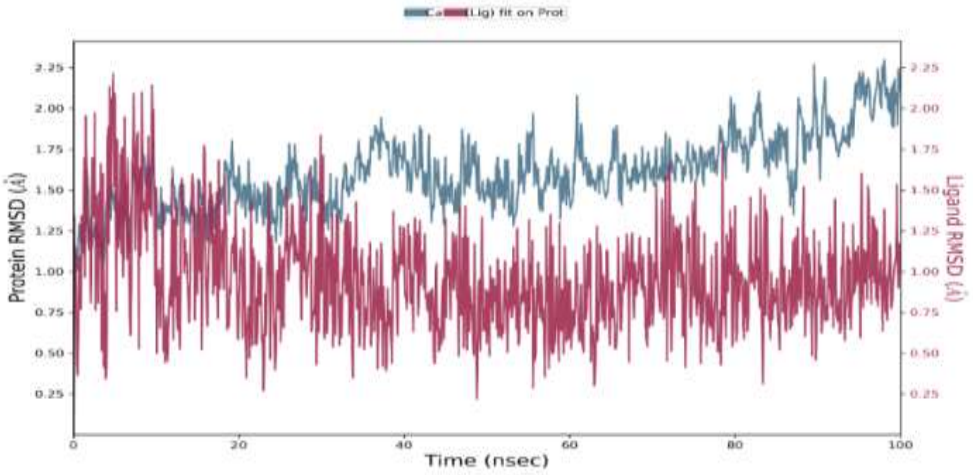


Figure 4.21: RMSD value of MP with target protein 7P2m

4.12.1.2. Root mean square fluctuation

The analysis of the internal mobility and variations of the residues was conducted through the calculation of the Root Mean Square Fluctuation (RMSF). Increased variations were detected in the residues within the range of 60 to 75.

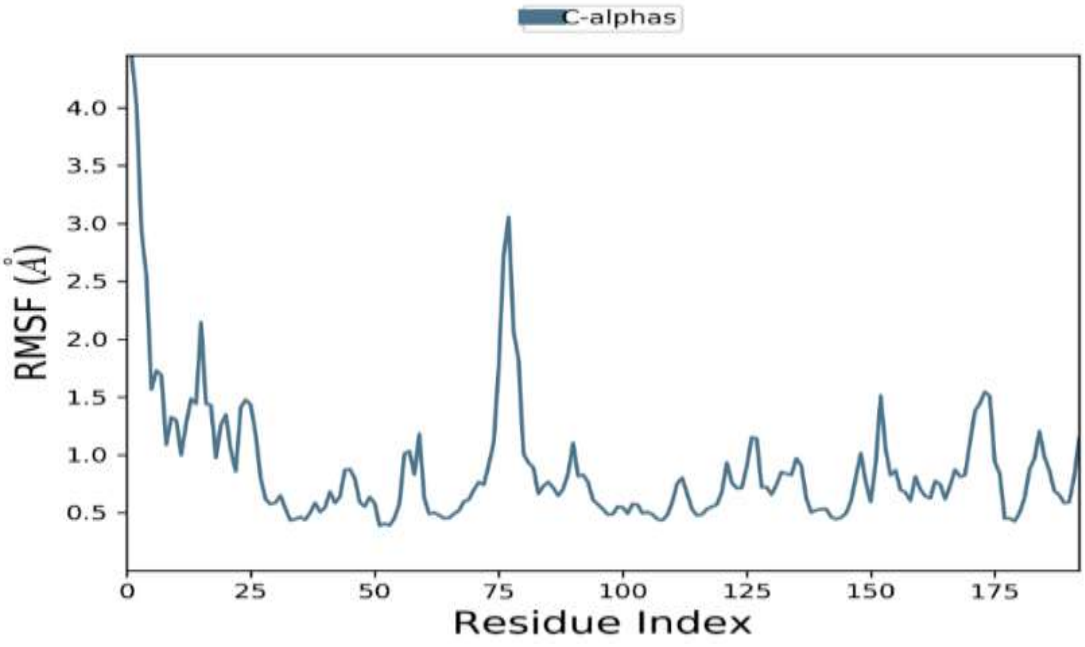


Figure 4.22: RMSF value of MP with target protein 7P2m

4.12.1.3. Protein ligand interacting amino acid

The ligand establishes water bridges, participates in hydrogen bonding, and engages in hydrophobic interactions with the amino acid residue. During the simulation, the amino acid residues of the protein established hydrogen bond connections with the ligands, hence facilitating protein-ligand interactions.

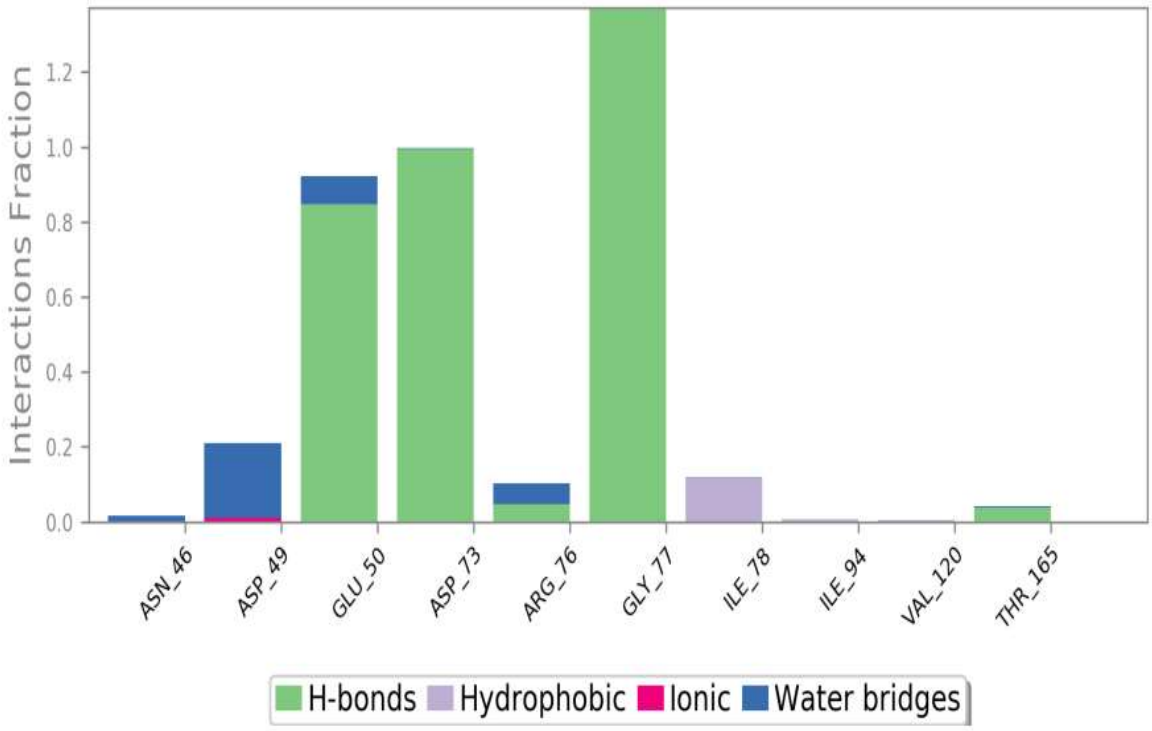


Figure 4.23: Protein- ligand interacting amino acid residue of MP with target protein 7P2m

4.12.2. Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)- with target protein (PDB ID 1XAL

4.12.2.1. Root mean square deviation

In the figure, it can be observed that the root mean square deviation (RMSD) of the ligand, derived using a least square fit, was stable initially and exhibits some variations within 40 to 50 nanoseconds (ns) of the simulation. And then stable again until the 100 ns mark.

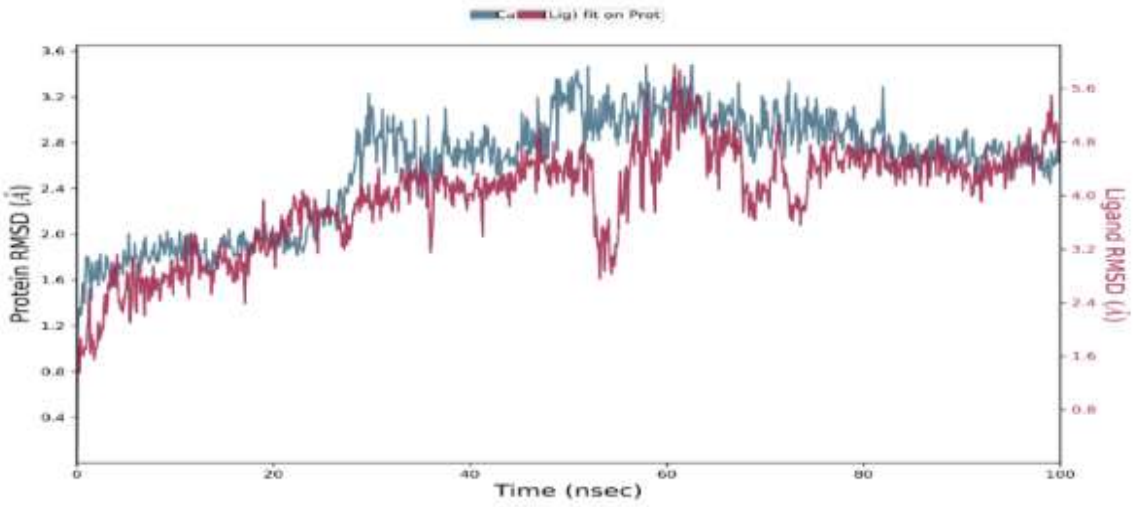


Figure 4.24: RMSD value of CL with target protein 1XAL

4.12.2.2. Root Mean Square Fluctuation

The analysis of the internal mobility and variations of the residues was conducted through the calculation of the Root Mean Square Fluctuation (RMSF). Increased variations were detected in the residues within the range of 300-350.

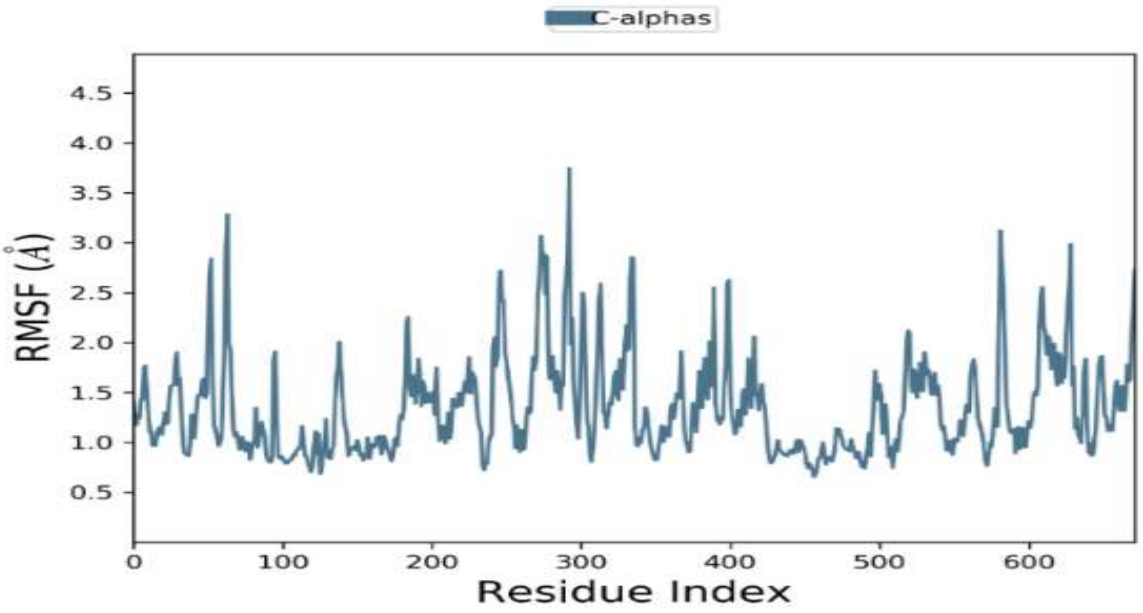


Figure 4.25: RMSF value of CL with target protein 1XAL

4.12.2.3. Protein ligand interacting amino acid

The ligand establishes water bridges, participates in hydrogen bonding, and engages in hydrophobic interactions with the amino acid residue. During the course of the simulation, the amino acid residues inside the protein established hydrogen bond connections with the ligands, thereby facilitating protein-ligand interactions.

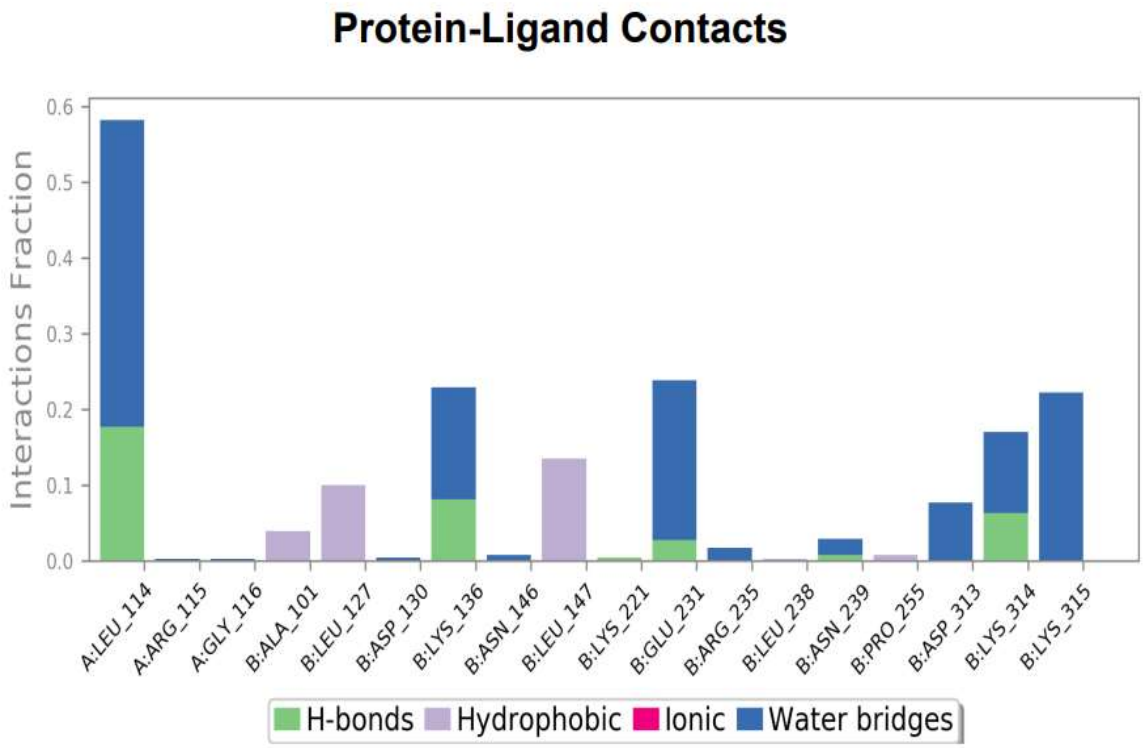


Figure 4.26: Protein- ligand interacting amino acid residue of CL with target protein 1XAL

4.12.3. 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-with target protein (PDB ID 8IWL)

4.12.3.1. Root mean square deviation

In the figure, it can be observed that the root mean square deviation (RMSD) of the ligand, derived using a least square fit, exhibits some variations within the initial 10 nanoseconds (ns) of the simulation. Subsequently, the RMSD remains relatively steady until the 100 ns mark

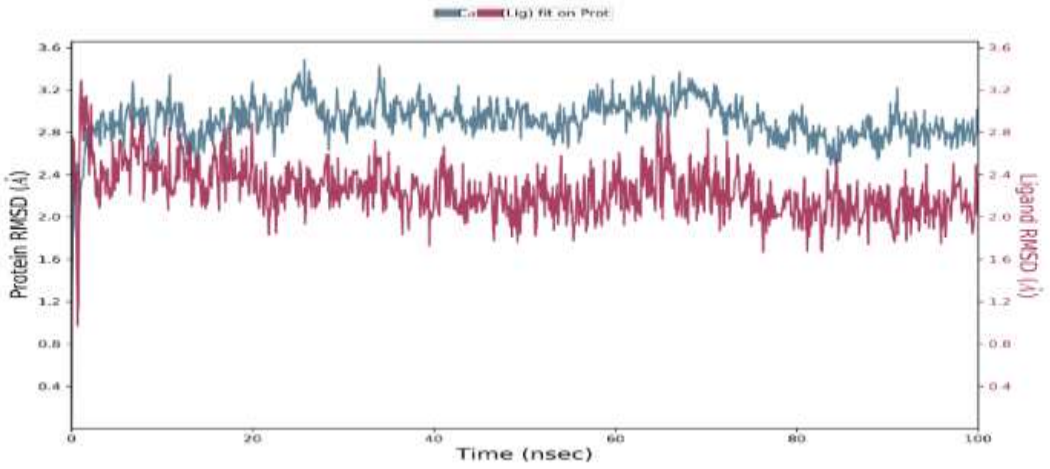


Figure 4.27: RMSD value of RP with target protein 8IWL

4.12.3.2. Root mean square fluctuation

The analysis of the internal mobility and variations of the residues was conducted through the calculation of the Root Mean Square Fluctuation (RMSF). Increased variations were detected in the residues within the range of 300-350 and 500-550.

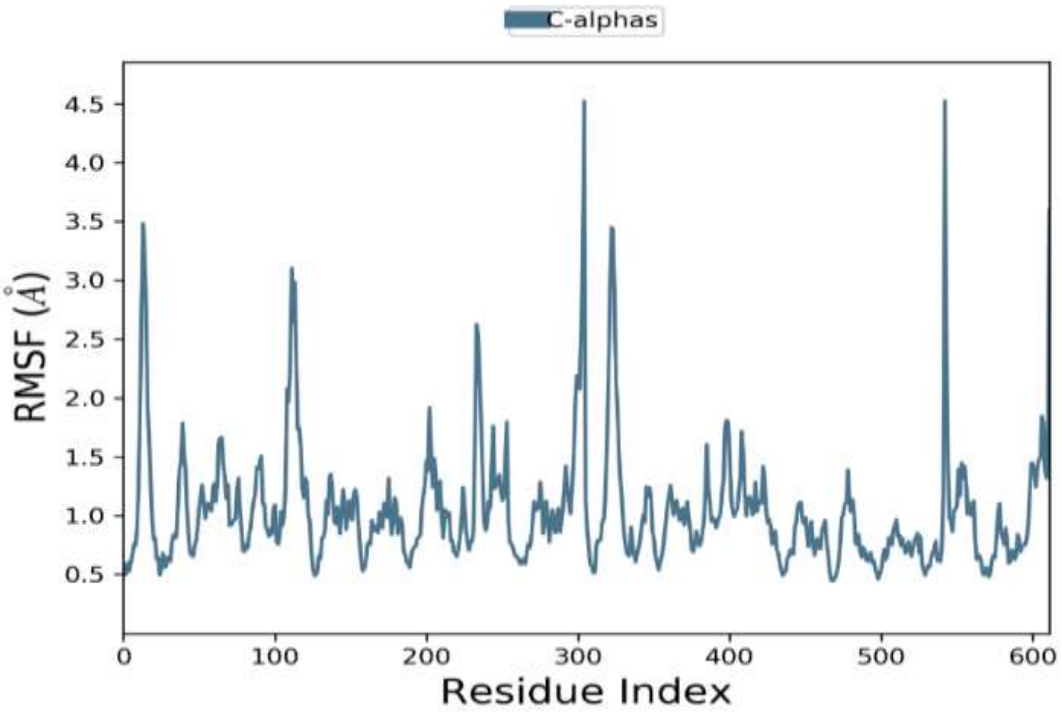


Figure 4.28: RMSF value of RP with target protein 8IWL

4.12.3.3. Protein ligand interacting amino acid

The ligand forms water bridges, engages in hydrogen bonding, and forms hydrophobic interactions with the amino acid residue. Throughout the simulation, the amino acid residues of the protein formed hydrogen bond connections with the ligands, establishing protein-ligand interactions.

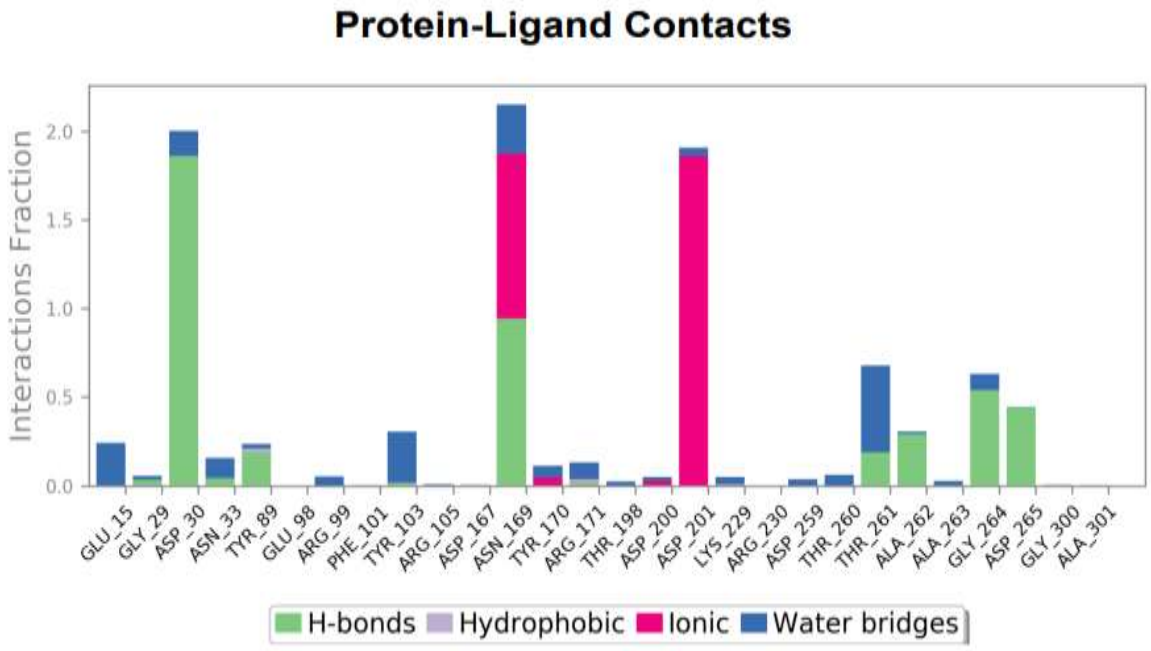


Figure 4.29: Protein- ligand interacting amino acid residue of RP with target protein 8IWL

Chapter 5: Discussion

5. Discussion

The use and overuse of antibiotics around the world has led to a situation where bacteria are becoming resistant to available drugs (Mancuso, Midiri, Gerace, & Biondo, 2021). Back in the 1950s and early 1960s, there was a widespread issue with the bacteria and they becoming resistant to a common antibiotic called Penicillin. (Morrison & Zembower, 2020). About 20% of infected people die because of this resistance. (Algammal et al., 2020). Bacteria are able to pick up resistance genes from other bacteria and even bacteria from different species by horizontal gene transfer and causing resistance to available drugs. (Abushaheen et al., 2020). Bacteria also became resistant to many antibiotics because they collected a lot of resistance genes over time. Despite all the progress we've made in understanding bacteria and how to treat diseases caused by them, we still face problems with bacteria becoming resistant to our antibiotics. Plus, new dangerous types of bacteria are appearing. So, scientists are now looking into using advanced technology to study plants and find new compounds that can fight these stubborn bacteria and help protect public health (Huffman, 2001).

People have been using plants for a very long time to treat infections, and scientific studies have shown that many plants have medicinal properties. Even today, in many countries, people use plants to treat various illnesses. In fact, a significant number of new medicines created in recent years to fight infectious diseases, like cancer, are based on natural substances found in plants. Traditional plant-based medicines are especially important in many developing countries, where they help prevent and treat diseases (Jain, Khatana, & Vijayvergia, 2019; Mintah et al., 2019).

This study aimed to investigate the antibiotic resistance of bacterial strains towards commercially available drugs and check the antibacterial properties of the *N. oleander* plant extract against the *E. coli, S. aureus, K. pneumonia, B. subtillis, E. mori, and A. baumannii*. The FTIR and GCMS analysis of the N. oleander plant showed that both its leaves stems and flowers contain saponins, oils, flavonoids, alkaloids, starch, and fatty acids, resins, betacyanin, terpenoids, and coumarins. These chemicals have medicinal properties, and plants with such substances have therapeutic potential (Hase, Deshmukh,

Pokharkar, Gaje, & Phatanagre, 2017). Different solvents have different properties like methanol, ethanol, acetone, and water to extract the different compounds from the plant material. Extracts from the leaves, flowers and stems against different types of bacteria, showed different zone of inhibition. The best results for stopping bacteria came from methanolic leaf extracts of N. oleander. It's interesting to note that the type of solvents used to extract these components from the plant can affect their activity against bacteria. In fact, some studies have shown that using special organic solvents is even more effective against bacteria compared to using just water (Jamal et al., 2012). The presence of phytochemicals in Nerium oleander has been known to affect Gram-positive bacteria by causing cell wall proteins to clump, disrupting microtubules (which are part of the bacterial cell's structural framework), and binding to certain protein receptors that ultimately lead to the death of the bacterial cell. This is believed to be responsible for the plant's ability to combat bacteria. The methanolic extract of leaves shows the maximum zone of inhibition against the tested bacteria. The diameter of the zones of inhibition ranged from 5 to 20 mm, inhibition zone was observed in *A. baumannii* (20 mm), followed by *K. pneumoniae* (17mm), *B. subtilis* (16 mm), *E. mori* (13mm), *E. coli* (11.5) *and S. aureus* (10mm) respectively (Dassanayake, Khoo, & An, 2021; Dey & Chaudhuri, 2014).

The application of bioinformatics tools and methodologies is widely established in the field of drug discovery, with a particular emphasis on in-silico based drug design. This approach plays a pivotal role in modern drug discovery. Our current study is centered on employing structural-based drug design to pinpoint a potential therapeutic compound for a specific ailment (Hazarika & Gupta, 2020; López-López, Barrientos-Salcedo, Prieto-Martínez, & Medina-Franco, 2020). To better understand how these bioactive chemicals from *N. oleander* work, molecular docking and simulations studies have been performed *(in-silico* studies) to see how they interact with specific proteins. The activity of different Ligands was checked by targeting the bacterial proteins 1Xal, 7P2m, 8IWl, 3ZG5, and 6F86 which is important for bacterial cell wall formation the DNA replication, glucose utilization and carbohydrate metabolism which is necessary for survival of bacteria (Chakravarty, Ray, & Talapatra, 2019; Shidiki & Vyas, 2022).

The effectiveness of these interactions is based on various types of bonds, including hydrogen bonding, amide-π interactions, π-π interactions, and more (Ramalingam, Selvi, & Jayaseelan, 2019). SwissADME and toxicity analysis indicated that the bioactive compounds from N. oleander have the potential to be developed into drugs and are likely safe for use (Barbhuyan et al., 2023; Gurumallappa et al., 2021). The effectiveness of these interactions is based on various types of bonds, including hydrogen bonding, amide-π interactions, π-π interactions.

Subsequent to the docking process, computational molecular dynamics simulations was performed to look deeper into the interactions between ligand and various amino acids. Notably, a significant portion of these amino acids are engaged in interactions through the binding forces of hydrogen bonding and Vander Waals forces. Interestingly, when we looked into the docking results, we identified a correlated binding affinity with specific targeted receptors. In this study, three compounds, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)- (-8.0kcal/mol) with bacterial protein (PDB ID 7P2M), 3-.beta.-d Ribofuranosyl pyrazolo[4,3-d]pyrimidin-5,7-4H,6H-(-8.4 kcal/mol), with bacterial protein (PDB ID 8IWL), and Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.) (-7.9 kcal/mol) with bacterial protein (PDB ID 1XAL), demonstrated a stronger binding affinity with the compounds than commonly used antibiotics. Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5. alpha.)- formed hydrogen and alkyl bonds with an amino acid residue Pro79, Glu50, Thr165, Ile78, Arg76 while Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-formed conventional hydrogen bonds and alkyl bonds with amino acids Lys136, Leu238, Leu127, His256, Ala101. The third compound 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H- forms hydrogen and alky bonds with the amino acid residues Asn33, Asp265, Arg99, Thr261, Ala262, Thr260, Gly264, Arg171, Gly29 (Mirzaei, Hajali, & Pourhadi, 2022).

SwissADME and toxicity analysis indicated that the bioactive compounds from *N. oleander* have the potential to be developed into drugs and are likely safe for use (Gurumallappa et al., 2021). Out of thirty-five compounds tested twelve were found to be non-toxic. These compounds include. O,O-Diphenyl N-cyclohexylphosphoramidothioate, Cyclohexane, 1-methyl-4-(1-methylethyl)-, 1,3-Dioxolane, 2-methyl-2-(4-methyl-, 2-

Oxotetrahydropyranyl-6-acetic acid, methyl ester, 3-Methyl-4-(phenylthio)-2-prop-, Uridine, 2'-deoxy-, 3',5'-diacetate, 2,4-Di-tert-butylthiophenol, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-, 3-(4-Methyl-piperazin-1-yl)-N-(2-trifluoromethyl-,3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-, 3-Pyridinecarboxylic acid, 1,2-dihydro-4-hydroxy-6-methyl-, 2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-2-methyl-5-(1-methylethyl)- Out of these twelve compounds three compounds (Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.)-. , Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-,were selected with best binding energies for molecular dynamic simulation (Alzain et al., 2022).

The molecular dynamics simulation spanned 100 nanoseconds, which was closely monitored and interpreted the root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) values. The progressive increase in RMSD readings over time provided insights into the protein's propensity for frequent structural deviations from its native conformation and showed the stability of complex. Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-with target protein PDBID 1XAL showed the highest stability of the complex. Our analysis of the elevated root-mean-square fluctuation (RMSF) uncovered the presence of robust hydrogen bonding interactions. These interactions were predominantly observed with polar amino acids.

Conclusion

This study pinpointed a select group of potential phytochemicals that exhibit promising inhibitory effects against bacterial proteins. These findings pave the way for future exploration through in vitro and animal testing, formulation development, and drug synthesis. The identified phytoconstituents hold substantial potential as antibacterial agents, given their anticipated bioavailability, drug-like characteristics, and demonstrated safety with no mutagenic effects. Our investigation centered on uncovering various bioactive compounds within extracts from N. oleander's leaves, flowers, and stems using GC–MS analysis. These compounds underlie the plant's diverse therapeutic and pharmacological properties. Furthermore, we have presented compelling evidence supporting the antimicrobial activity of N. oleander extracts, shedding light on their interactions with antimicrobial proteins. We elucidated the roles of these proteins, including E. coli GyraseB (PDB ID: 6F86) involved in DNA supercoiling, E. coli GyrB24 (PDB ID: 7P2M) essential for DNA replication, S. aureus 3-dehydroquinate synthase (PDB ID: 1XAL) vital for carbohydrate metabolism, S. aureus hydrolase enzyme (PDB ID: 3ZG5) responsible for breaking down large molecules, and A. baumannii uncharacterized sugar kinase protein (PDB ID: 8IWL) playing a role in glucose utilization as a carbon source. Among all the bacterial proteins, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-, and 3-.beta.-d-Ribofuranosylpyrazolo[4,3-d]pyrimidin-5,7-4H,6H-, along with Morphinan-3,14-diol, 4,5-epoxy-17-methyl-, (5.alpha.), exhibited the most favorable docking scores against the target proteins i.e 7P2M, 1XAL, and 8IWL. Subsequently, dynamic simulation experiments were performed to evaluate the stability, root mean square deviation (RMSD), and root mean square fluctuation (RMSF) values associated with protein-ligand interactions. These chemicals exhibit potential for the advancement of efficacious pharmaceuticals targeting pathogenic bacteria and diverse disorders. Furthermore, the conducted ADMET (absorption, distribution, metabolism, excretion, and toxicity) experiments have demonstrated that the investigated compounds possess advantageous drug-like characteristics. This positions them as highly prospective contenders for the creation of microbial drugs. Additional research is required to further the field of broad-spectrum drug discovery, which should include investigations into bioactivity, toxicity profiling, and clinical studies.

Future Prospects

Further investigation could focus on isolating and characterizing specific bioactive compounds from the plant extracts. This can involve techniques such as chromatography and spectroscopy. Identifying the active compounds responsible for antibacterial activity is crucial for drug development. Moving beyond in vitro antibacterial assays, conducting in vivo studies in animal models is the next step. These studies can provide insights into the compounds' efficacy, bio-distribution, and potential side effects. If the results from preclinical studies are promising, the compounds can advance to clinical trials. These trials evaluate the safety and efficacy of the compounds in humans. The achievement of positive outcomes in clinical trials has the potential to facilitate the advancement of novel antibiotics or antimicrobial medicines. Understanding how these compounds interact with bacteria at the molecular level can help combat evolving drug resistance. Continue to employ computational methods to design and optimize new drug candidates. This can include virtual screening of compound libraries and predicting their binding affinities with bacterial targets. If you discover novel drug candidates, consider patenting them and exploring opportunities for commercialization through partnerships with pharmaceutical companies or establishing a startup.

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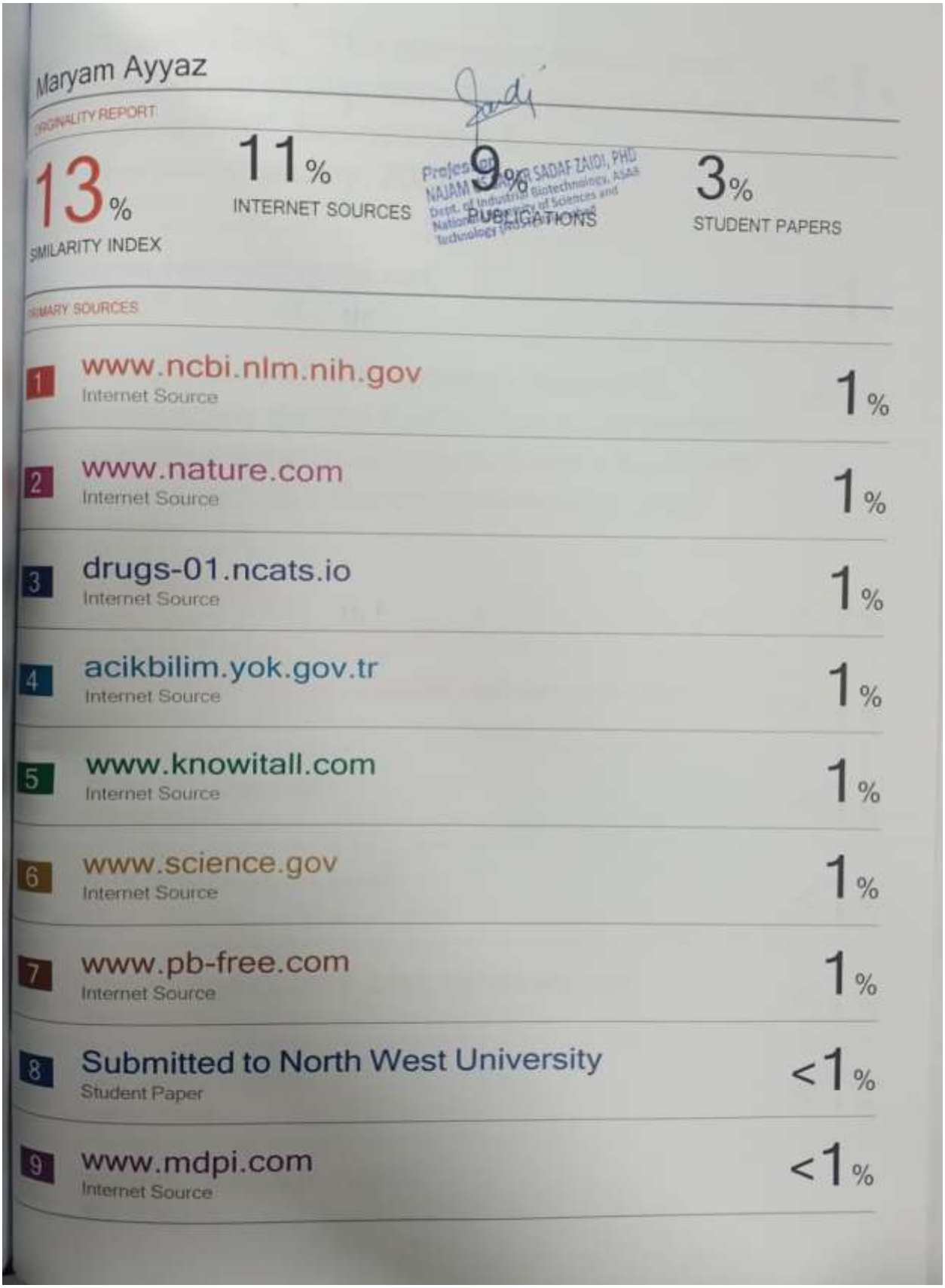

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