In silico and *in vitro* antimicrobial evaluation of methanol extract of *Artocarpus heterophyllus* leaves against gram-positive and gram-negative bacteria

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Approval

A project paper **"*In silico* and *in vitro* antimicrobial evaluation of methanol extract of *Artocarpus heterophyllus* leaves against gram-positive and gram-negative bacteria"** submitted to the Department of Pharmacy, Faculty of Allied Health Science, Daffodil International University has been accepted as satisfactory for partial fulfillment of the requirement for the degree of the Masters of Pharmacy and approved as to its style and contents.

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Declaration

I at this moment declare that the thesis work title **"*In silico* and *in vitro* antimicrobial evaluation of methanol extract of *Artocarpus heterophyllus* leaves against gram-positive and gram-negative bacteria"** requires the complete the degree Masters of Pharmacy program under the Faculty of Allied Health Science at Daffodil International University.

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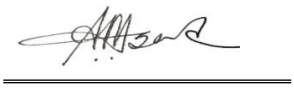

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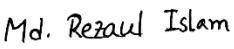

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Abstract

Antibiotic resistance threatens global health, necessitating a search for new antibiotic drugs. *Artocarpus heterophyllus* has therapeutic potential in many cultures. The purpose of this study evaluation of antimicrobial activities of leaf compounds based on in silico and in vitro approaches. The study evaluated the phytochemical screening test of *Artocarpus heterophyllus* leaves, focusing on its antibacterial activity against gram-positive and gram-negative bacteria through in vitro and silico analysis. The antibacterial activity was assessed in the laboratory where Ciprofloxacin was used as the standard. We used computational tools to predict the interaction between bioactive compounds and major bacterial proteins Peptidoglycan glycosyltransferase, involving molecular docking studies. We selected 10 compounds from a literature review and analyzed their PASS prediction data for further confirmation. The zone of inhibition on gram-positive and gram-negative bacteria was determined by the disk diffusion method using two concentration samples, with maximum diameters of 11mm and minimum diameters of 6mm for 250µg/disc and 15mm for 10mm for 500µg/disc. The binding affinity was determined through molecular docking, while the stability analysis and calculation were performed using the Quantitative Structure-Activity Relationship (QSAR) study. The molecular weight and bioavailability of a drug were determined through analysis of pharmacokinetics data and the Lipinski Rule. Ligands 01, 05, and 07 contain low molecular weight and the highest binding affinity to bacterial protein Peptidoglycan glycosyltransferase. In the same way, these ligands have more stability and no hepatotoxicity. These results provide a new opportunity for the advancement of pharmaceutical research and further experimental studies need to be performed on the *Artocarpus heterophyllus* leaf compounds and Peptidoglycan glycosyltransferase.

Keywords: *Artocarpus heterophyllus,* anti-microbial activity, molecular docking, binding affinity, QSAR, ADMET

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CHAPTER- 01

Introduction

1. General Introduction

The jackfruit tree, scientifically named *Artocarpus heterophyllus* Lam and classified under the Moraceae family, is an indigenous tree to the Western Ghats of India. Fruits are nutritionally valuable and serve as a significant supply of carbohydrates, proteins, fats, minerals, and vitamins. Heartwood is an exceptionally resilient timber that is utilized in the fabrication of household furnishings. Jackfruit, a fruit with various therapeutic properties, is used in traditional medical practices to treat various health conditions. Preclinical studies show it has hypoglycemic, antifungal, antibacterial, anti-inflammatory, antioxidant, and wound-healing properties. Clinical trials show it has hypoglycemic properties in both healthy individuals and those with diabetes [1]. Artocarpus, a genus comprising 60 arboreal and shrubby species, holds great importance in the tropical regions of Bangladesh, India, Brazil, Africa, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, Sri Lanka, and Nepal. *A. heterophyllous*, commonly referred to as jackfruit, is grown in the southern regions of China and possesses therapeutic properties that include treating asthma, reducing diarrhea, and stimulating lactation. The plant's wood and bark include prenylated steroids, triterpenoids, stilbenoids, and flavonoids, which possess various biological functions such as antimalarial properties, anti-inflammatory effects, antioxidative properties, cytotoxicity, and inhibition of 5α-reductase [2]. Gram-positive bacteria cell walls consist of an intricate combination of glycopolymers and proteins. The structure comprises a strong peptidoglycan sacculus that envelops the cytoplasmic membrane and is adorned with proteins, polysaccharides, and teichoic acids. The bacterial cytoskeleton is crucial for maintaining cell form and stability throughout division and growth and serves as contact amidst bacteria with its surroundings. Lactic acid bacteria (LAB) have long been utilized for food fermentation and are increasingly being investigated for their possible health advantages. It is increasingly acknowledged that comprehending the arrangement, makeup, and characteristics of LAB cell walls is an essential aspect of advancing technology and health-related purposes by utilizing these microbes [3]. Gram-negative cell walls are powerful, tough, and elastic, able to withstand extreme pHs, temperatures, and turgor pressure. They can increase the area of their surfaces multiple times above their usual size, making them a remarkable structure that has stood the test of time, contributing to their success as a life-form [4]. Molecular docking is an approach of computer modeling used to study the formation of complexes resulting from the interaction of two or more molecules. It utilizes the binding characteristics of the ligand and target molecules

to forecast the three-dimensional configuration of adducts. Molecular docking generates a variety of potential candidate structures, which are then organized and evaluated using a scoring system within the molecular docking software. Docking simulations utilize total energy calculations to estimate the most optimized docked conformer. This paper presents an analysis of the many computational features of molecular docking, focusing on its techniques and kinds [5]. Molecular docking is a crucial method used in Computer-aided development of drugs and structural molecular biology. Ligand-protein docking aims to forecast a ligand's primary binding configuration(s) using a protein that has a well-established 3D arrangement. Efficient docking techniques effectively explore high-dimensional environments and employ a scoring formula that accurately prioritizes potential dockings. Docking enables the execution of virtual screening on extensive collections of compounds, allowing for the prioritization of outcomes and the formulation of structural hypotheses regarding the inhibition of the target by the ligands. This process is highly important in lead optimization. Establishing the input structures for the docking process is crucial to the docking process itself, and the interpretation of the outcomes of stochastic search methods can occasionally be ambiguous [6]. Molecular docking technology investigates the interaction of tiny molecules within the binding region of a target protein. Molecular docking is being utilized more frequently in drug development as the number of experimentally confirmed protein structures utilizing nuclear magnetic resonance (NMR) spectroscopy or X -ray crystallography continues to grow. Proteins that lack known structures can now be docked against homology-modeled targets. Docking techniques can be used to calculate the compound's potential for use as a medication and its ability to specifically target a specific molecule, which is useful for additional lead optimization operations. Molecular docking programs utilize a search technique to recursively analyze the configuration of the ligand till it reaches convergence to the minimal energy. Ultimately, a scoring system called ΔG [U total in kcal/mol] is utilized to prioritize the candidate postures based on the combined electrostatic and van der Waals energies. The underlying determinants of these particular relationships in systems of biology are focused on the complementary nature of the form and electrostatic properties of the surfaces of binding site and the substrate or ligand [7].

1.1. Phyto-constituents of the leaves of *Artocarpus heterophyllus*

The *Artocarpus heterophyllus* plant includes a variety of chemical compounds, including flavones such as artocarpanone, cycloartinone, norartocarpetin, artocarpetin, oxydihydroartocarpesin, isoartocarpin, artocarpesin, cyloartocarpin, artocarpin, cynomacurin, dihydromorin, and morin. The heartwood, when analyzed, contains 1.7% albumin, 0.7% lipids, 38.0% glucosides, 6.7% moisture, and 59.0% cellulose. The plant furthermore includes ellagic acid, fatty acids, sucrose, and important amino acids such as tryptophan, threonine, methionine, lysine, leucine, histidine, cystine, and arginine. The outer protective covering of the primary stem includes two recently discovered flavone pigments, namely cycloheterophyllin and also betullic acid. The presence of triterpenic compounds such as cycloartenone and cycloartenyl acetate has been documented. The phenolic chemical heterophylol, which has a unique structure, was isolated from Artocarpus heterophyllus [8].

1.2. Herbal Medicine

Explores the therapeutic importance of the jackfruit, also referred to as the "poor man's food" in Bangladesh, as well as its key components and applications. The fruit is consumed by rural inhabitants and is used as sustenance, fodder, and furniture wood. Jackfruit has antibacterial, antidiabetic, antioxidant, anti-inflammatory, and anthelmintic effects, and its seeds are rich in vitamins and minerals. Jackfruit is abundant in, minerals, vitamins, carbs, dietary fiber, and carboxylic acids. The seeds contain significant amounts of manganese, magnesium, potassium, calcium, iron, and lectins, making it a suitable source of nourishment for rural populations. The objective of this research was to examine the medicinal significance and health benefits of jackfruit and its seeds, particularly focusing on their applications in the food industry [9].

CHAPTER- 02

Plant Profile

1.3 Plant Review

1.3.1. Plant

Artocarpus heterophyllus is an indigenous species found in the Western Ghats of India, Malaysia, and other parts of Africa, Asia, Pacific Islands, Puerto Rico, Australia, Brazil, Florida, and the Caribbean. The tree is a perennial plant that reaches a height of 10-15 meters. It is indigenous to woods that maintain their green foliage throughout the year, namely at elevations ranging from 450 to 1,200 meters. The plant has a linear and coarse stem, with bark that may be either green or black in color, broad obovate leaves, and a sweet yellow sheath around its seeds [10].

Fig. 1: Plant

1.3.2. *Artocarpus heterophyllus*

Artocarpus heterophyllus, a Moraceae plant, is a common Indian diet staple and has medicinal properties mentioned in Ayurveda. It contains compounds like cycloartinone, artocarpin, cynomacurin, dihydromorin, and morin, useful in snake bites, skin diseases, wounds, boils, and fever [10].

1.3.3. Naming

Naming of this plant is *Artocarpus integrifolia* L.f., *Artocarpus integer* auct, *Artocarpus integrifolius* auct. [10].

1.3.4. Synonyms

- *Artocarpus maxima* Blanco
- *Artocarpus heterophylla* Lam.
- *Artocarpus brasiliensis* Gomez.[10].

1.3.5. Taxonomical classification

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Tracheobionta -- vascular plants

Division: Magnoliophyta -- angiosperms, flowering plants, phanérogames

Class: Magnoliopsida -- dicots, dicotylédones, dicotyledons

Subclass: Hamamelidae

Order: Urticales

Family: Moraceae -- mulberries

Genus: Artocarpus – breadfruit

Species: *Artocarpus heterophyllus* Lam

-Scientific classification *Artocarpus heterophyllus* [10].

1.3.6. Origin and cultivation

Jackfruit, a fruit native to Southeast Asia and the cultivation of this plant dates back to ancient times and it has been established in several areas of the tropical regions, such as India, Philippines, Thailand, Indonesia, Malaysia, Sri Lanka, China, and the Myanmar. It is particularly important in Fiji, where Indian populations are prevalent [10]. The study conducted a village-based survey to assess jackfruit diversity across three habitats: forest or fallow lands, public lands, and homesteads. The findings showed that the cultivation of this fruit in homesteads resulted in a favorable pressure of selection for desired market characteristics. Nevertheless, the percentage of fruits deemed good on forest/fallow areas and public lands was 23.7% and 18.7% respectively. The study indicatees that the "wild" Bangladeshi jackfruit germplasm has significant genetic variability for cultivation, and Collecting samples from various ecosystems along a continuum may help detect domestication pressures [11].

1.3.7. Distribution

The incursion of extraterrestrial organisms is a highly significant determinant impacting planetary biodiversity, resulting in the standardization and depletion of species on a global scale. Jackfruit is a highly noticeable invasive plant species in Brazil. It conducted a study on the invasion of jackfruit within Ilha Grande State Park, situated on the Ilha Grande island in the Atlantic Forest. Our assessment involved examining the distribution and density of jackfruit along 23 paths spanning a total distance of 90 kilometers. Verification of existence or nonexistence was conducted in 857 plots. The data revealed that across Ilha Grande jackfruit is widely distributed, with notable variations in plant density observed both along and between different routes. This fruit was recorded as the prevailing tree species on several pathways. Effective oversight by park administrators is vital to guarantee the preservation of indigenous biodiversity. A selection key is available for managing jackfruit based on factors such as slope, age class, and density [12]. The study isolated free sugars and fatty acids from jackfruit components using gas-liquid chromatography. Sucrose, glucose, and fructose were predominant,

except for bark. Primary fatty acids found were arachidic, linoleic, stearic, oleic, palmitic, lauric, myristic, and capric acids [13].

1.3.8. Description

This explores the phytochemistry and pharmacognosy of *Artocarpus heterophyllus*, a plant rich in secondary metabolites promoting health. The plant's bark, roots, leaves, and fruit have therapeutic properties, including hypoglycemic, antineoplastic, antifungal, anticarcinogenic, antibacterial, anti-inflammatory, and antioxidant effects. This suggests that *A. heterophyllus* has the potential as an advanced anthelminthic agent [14].

1.3.9. Traditional uses

This study provides an in-depth analysis of Artocarpus, a plant known for its chemical components, pharmacological and biological researches. Artocarpus species have a high concentration of phenolic chemicals, which includs Jacalin, arylbenzofurons, stilbenoids, and flavonoids. These compounds have been found to have many functions, including cytotoxicity, tyrosinase inhibitory, antiarthritic, antiplatelet, antifungal, antiviral, antitubercular, and antibacterial. The review emphasizes the importance of strong interdisciplinary programs regarding the potential advancement of Artocarpus as a pharmaceutical product [15].

Common names, uses and geographical distribution of some *Artocarpus* species.

	Synonyms	Common names	Uses	Origin and geographical distribution
1. *Artocarpus altilis* (Parkinson) Fosberg	*Artocarpus camansi* Blanco *Artocarpus communis* J.R. & G. Forst. *Artocarpus incisa* *Artocarpus incisus* (Thunb.) L.f.	Breadfruit	Fruit pulp tonic for liver, leaves to treat liver cirrhosis, hypertension and diabetes.	Native to Pacific and Tropical Asia, Indonesia, Papua New Guinea
2. *Artocarpus chama* Buch.-Ham.	*Artocarpus chaplasha* Roxb. *Artocarpus melinoxylus* Gagnep	Chaplasha	–	India, Burma
3. *Artocarpus chempeden* Spreng.	–	Chempedak	Seeds in diarrhea, roots in malaria fever.	South-East Asia, Indonesia
4. *Artocarpus elasticus* Reinw. Ex Blume	–	–	Bark in inflammation and female contraception, latex in dysentery, leaves to treat tuberculosis.	South-East Asia, West Malaysia
5. *Artocarpus gomezianus* Wall. Ex Trecul.	*Artocarpus pomiformis* Teijsm & Binn	Tampang burung	–	Western part of Indonesia
6. *Artocarpus heterophyllus* Lam.	*Artocarpus brasiliensis* Gomez *Artocarpus integra* *Artocarpus integrifolia* auct. *Artocarpus jaca* Lam. *Artocarpus maxima* Blanco *Artocarpus philippensis* Lamk	Jackfruit	Fruits edible, roots in diarrhea and fever, leaves as antisyphilic and vermifuge, ulcers and wound healing, leaves and stem barks used to treat anemia, asthma, dermatitis, diarrhoea, cough.	Native to Western Ghats India. Introduced in South-East Asian region
7. *Artocarpus hirsutus* Lam.	*Artocarpus hirsuta* Lam.	–	–	South India
8. *A integer* (Thunb.) Merr.	*Artocarpus champeden* (Lour.) Stokes *Artocarpus integrifolius* L.f. *Artocarpus polyphema* Persoon *Polyphema champeden* Lour. *Radermachia integra* Thunb.	–	Fruits edible.	Burma, Peninsular Thialand, Peninsular Malaysia, Sumatra, Borneo, Sulawesi lingga, Archipelago
9. *Artocarpus lacucha* Buch.-Ham.	*Artocarpus lakoocha* Roxb.	Monkeyjack, Lakoocha	Bark chewed like betel nut used to treat skin ailments.	Native to humid sub. Himalayan Regions of India, South China, South-East Asia
10. *Artocarpus lowii* King	–	Miku	Sap used as an ointment and as cooking oil.	Rare species in Malaysia
11. *Artocarpus nobilis* Thw.	–	–	Seeds and young fruits edible.	Endemic to Sri Lanka
12. *Artocarpus odoratissimus* Blanco.	–	Marang, Terap	Fruits edible.	Borneo, Philippines
13. *Artocarpus rotunda* (Hout) Panzer	–	–	–	South-East Asia, Indonesia

Fig. 2: Traditional uses

1.3.10. Role the pharmaceutical industries

Jackfruit, a fruit with high nutritional value, is underutilized due to waste and lack of post-harvest technology. It contains essential nutrients like protein, calcium, iron, and vitamins. However, there is a gap in marketing and value-added food products, which could provide additional income and food security [16].

CHAPTER- 03

Purpose of the study

1.3.11. Purpose of the study

A medicinal plant, *Artocarpus heterophyllus*, is a key component in Bangladesh's primary health care system. With over 500 medicinal plants, over 300 are commonly used in traditional medicine. The plant's chemical contents and biological activity have been studied globally, but the *Artocarpus heterophyllus* plant in Bangladesh has been the focus of research. The primary goal is to explore the potential of creating novel therapeutic candidates from *Artocarpus heterophyllus* for treating various ailments, as it has a long history of indigenous medicinal herb systems and is considered an important component of the country's primary health care system. According to traditional uses in Bangladesh, *Artocarpus heterophyllus* has therapeutic activity; for recognition and justification of the therapeutic activity of the active compounds of this plant we investigate the following studies – Preparation of the crude methanolic extract, Analysis of plant compounds, and Pharmacological screening: Antimicrobial test (in vitro), Computational study: • Ligand pass prediction • Lipinski rule data for ligands • ADMET data analysis for ligands • Protein-ligand molecular docking • Analysis of binding affinity between protein and ligands • NMR research of ligands • QSAR analysis and calculation.

- Antibacterial activity
- Molecular docking
- ADMET data analysis

CHAPTER- 04

Literature Review

2. Literature Review

The antibacterial activity, antioxidant properties, and phenolic content of the *Artocarpus heterophyllus* leaves were assessed against foodborne pathogens. The extract and fractions shown substantial antioxidant effects in various in vitro systems, with the total extract showing strong antiradical activity. The Folin–Ciocalteau process was applied to ascertain the total phenol content's association between antibacterial and antioxidant properties. The MICs varied from 221.9 μg/mL for the ethyl acetate fraction to 488.1 μg/mL for the whole extract [17]. Multidrug-resistant bacteria, such as Staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa,* pose global concerns about the failure to cure certain infectious diseases. MRSA is the most prevalent resistant bacterium, causing high nosocomial infections. Gram-negative bacteria, like *Pseudomonas aeruginosa*, are also experiencing drug resistance due to mutations in target enzymes. Developing new antibiotics with new targets and modes of action is urgently needed, but time and cost factors are crucial [18]. The study evaluated the antibacterial effects of flavonoid artocarpin from A. heterophyllus in opposition to cariogenic pathogens, including *S. mutans*. Artocarpin showed strong antibacterial activity against *S. mutans* and confirmed that it has a wide range of antibacterial effects towards Gram-positive and Gram-negative bacteria. It also raised the antibacterial efficacy of some medications, like norfloxacin, ampicillin, and tetracycline, against MRSA when used in combination. Some bacterial species, like *S. mutans*, exhibit resistance to some antibiotics because of low permeability in their cell walls and cytoplasmic leakage. Overexpression of the efflux pump can lower antibiotic buildup in these cells. The study focuses on the antibacterial action of artocarpin, which alters membrane cell structure. Bacteriolysis assays were employed to ascertain the impact of artocarpin on membrane cell damage and cytoplasmic leakage. Membrane integrity is crucial for controlling physiological activities and cytoplasmic hemostasis in bacteria. This study found that artocarpin has strong antibacterial activity against *S. mutans* and significantly alters membrane permeability, leading to cell lysis. Flavonoid compounds possess diverse pharmacological characteristics, which includes antibacterial effects. Even so, it's not clear how these actions work. Flavonoid interactions with membrane cells and their lipophilicity may also be significant variables in their biological activity. Previous studies have shown that flavonoid compounds can disrupt membrane permeability, decreasing outer membrane permeability. The structure-activity

relationship suggests that substitutions of isoprenyl, 3-methyl-1-butenyl, and free hydroxyl groups are required for artocarpin's antibacterial activity [19]. During the last two years, substantial progress has been achieved in studying the fundamental structure of bacterial cell envelope glycoconjugate biosynthesis. This includes the study of several components such as capsules, lipopolysaccharides, peptidoglycan, cellulose, and teichoic acid. The current cryo-electron and crystal microscopy protein structures engaged in various steps of glycosyltransferase in the cytoplasm, cellular export of molecules, the polymerization of glycans in the periplasm, and The translocation of lipid-linked glycoconjugates across the inner membrane have provided insights into the mechanisms of cell envelope glycoconjugate synthesis [20]. Molecular docking is an approach of computer-based simulation used to predict the most favorable alignment of one molecule for binding (such as a ligand) to another (such as a receptor) when they combine to create a stable complex. The preferred alignment of molecules that are bound together can be used to anticipate the energy characteristics, like binding stability, strength, and free energy, of the complexes. The utilization of the scoring function in molecular docking enables the accomplishment of this task. Currently, molecular docking is often employed to forecast the alignment of binding of small molecules (potential medicinal agents) to their biomolecular targets (like nucleic acid, carbohydrate, and protein) to determine their preliminary binding properties. This gives fundamental data for the rational design of drugs (structure-based drug development) for the creation of novel medications that have improved effectiveness and greater specificity [5]. Virtual screening of small molecules databases identifies potential inhibitors against targets. Molecular docking is a computer simulation procedure using search algorithms to predict receptor-ligand complex conformations. This discusses search algorithms and programs, and discusses optimizing virtual screening with fast docking algorithms [21].

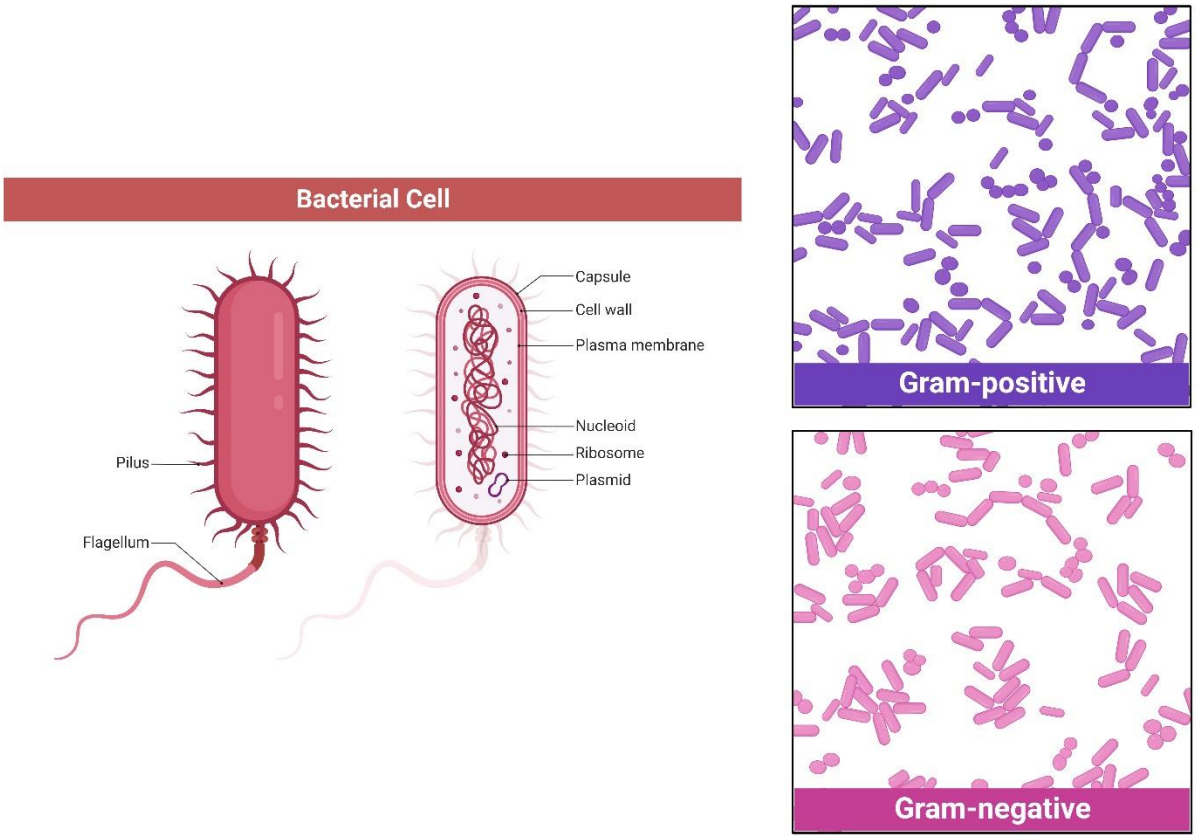


Fig. 3: Bacterial cell structure

CHAPTER- 05

Material and Method

Phytochemical Screening

3. Introduction of plant

Jackfruit, also known as *Artocarpus heterophyllus*, is native to India, Malaysia, and other parts of Africa, Asia, and the Caribbean. It is used in Indonesian conventional folk medicine for treating asthma, skin disease, abscesses, dysentery, diarrhea, ulcers, stomachaches, malarial fever, and inflammation. Standardization helps establish consistent potency and control bioactive chemical constituents in medicinal plants. A study was conducted to ascertain pharmacognostic variables and investigate conventional assertions. for the plant's anti-diabetic effect in rats. The *Artocarpus heterophyllus* (Table 1) leaves extract was investigated in rats [22].

3.1. Experimental Plant

We select this plant for our thesis. The name of this plant is *Artocarpus heterophyllus.*

Table 1: Plant introduction

Plant Name	**Family**	**Plant part used**
Artocarpus heterophyllus	Moraceae	Leaves

3.2. Material of phytochemical evaluation *Artocarpus heterophyllus*

3.2.1. Collection of plant material

Artocarpus heterophyllus was gathered from the Madaripur region of Dhaka, Bangladesh, for this research works.

Fig. 4: Collected Plant

3.2.2. Drying and grinding

The harvested plant pieces were carefully isolated from any non-desirable elements or whole plants. After being cut into little fragments, they were left to air dry for several weeks. By using an appropriate grinder, the plant fragments were pulverized into a rough powdered form. Prior to analysis, the granulate was kept in a tightly closed container in a cool, dim, and arid environment.

Fig. 5: Dry plant leaves

3.2.3. Methanolic extract preparation

Initially, a pure glass container with a level bottom was extracted, followed by the placement of approximately 500 g of powdered leaves in distinct, untainted glass containers with level bottoms. Subsequently, the powder was immersed in 1500 ml of methanol and introduced into the container. Subsequently, the container was hermetically sealed and stored for a duration of 2 weeks, with periodic agitation and stirring. White cotton was employed to segregate the loose fragments of the leaves from the mixture. Subsequently, the liquid component was subjected to triple filtration using white cotton. The solution was further purified by passing it through filter paper. In order to obtain the required raw extract, the liquid mixture is placed on a rotary evaporator to separate the solvent.

Fig. 6: Extraction preparation (DIU Lab)

3.2.4. Evaporation of Methanol from the extract

Metabolic extract was evaporated by the rotary evaporator and plant extract were collect.

Fig. 7: Rotary Evaporator (DIU Lab)

4. Phytochemical evaluation

The below reagents were utilized for the various chemical group tests.

4.1. Reagents used for the different chemical group test

For the various chemical group test, the following reagents were utilized.

i) Mayer's reagent: A solution containing 1.36 grams of mercuric iodide, consisting of sixty milliliters of water, was combined with a solution having five grams of potassium iodide in twenty milliliters of water.

ii) Dragendroff's Reagent: Dissolve 1.7 grams of basic bismuth nitrate and 20 grams of tartaric acid in 80 ml of water. The solution was amalgamated with a 16 gram potassium iodide and 40 ml water solution.

iii) Fehling's solution A: In a mixture of 0.50 ml sulfuric acid and enough water to make 500 ml, 34.64 gm copper sulphate was dissolved.

iv) Fehling's solution B: 500 mL was made by dissolving 176 gm sodium potassium tartarate and 77 gm sodium hydroxide in enough water. At the time of usage, an equal volume of the aforesaid solution was combined.

v) Benedicts Reagent: The volume was raised up to 100 ml with water after10 gm anhydrous sodium carbonate, 1.73 gm sodium citrate, and 1.73 gm cupric sulphate were dissolved in water [55].

4.2. Tests procedure for identifying different chemical groups

To distinguish between various chemical groups, the experiments listed below were performed.

4.2.1. Test for alkaloids

a) Mayer's test: A test tube was filled with a 0.2 ml dilute hydrochloric acid and 2 ml extract solution. The Mayers reagent was then added in 1 ml increments. The existence of alkaloids was determined by a yellow-colored precipitation.

Fig. 8: Mayer's Test Result (DIU Lab)

b) Dragendroff's test: The test tube was filled with a 0.2 ml of hydrochloric acid and 2 ml extract solution that had been diluted. Subsequently, 1 ml of Dragendroff's reagent was introduced. The detection of alkaloids has been confirmed through the production of an orange-brown precipitate.

Fig. 9: Dragendroff's Test Result (DIU lab)

4.2.2. Test for carbohydrates: Each of the extracts were filtered after diluted in five milliliters of distilled water. The presence of carbohydrates was determined using the filtrates.

a) Benedict'sTest: Benedict's reagent was used to treat the filtrates, which were then gently heated. The existence of reducing sugars is specified by an orange red precipitation.

Fig. 10: Benedict's Test Result (DIU lab)

b) Fehling's Test: The filtrates underwent hydrolysis in diluted hydrochloric acid, were subsequently neutralized with an alkali, and then subjected to heating in Fehling's A and B solutions. The existence of reducing sugars can be observed by the production of red color precipitation.

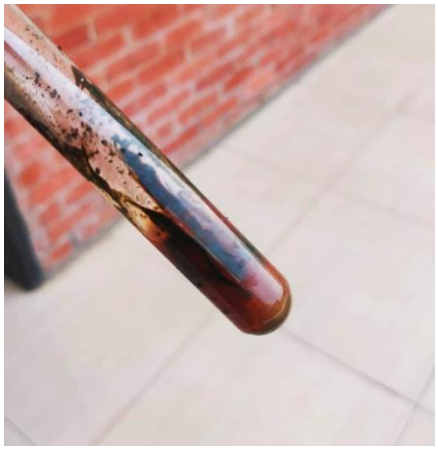


Fig. 11: Fehling's Test Result (DIU lab)

4.2.3. Test for Steroids

Sulphuric acid test: 1 ml chloroform extract solution was obtained, and 1 ml sulphuric acid was added. The presence of steroid is shown by the color red.

Fig. 12: Steroids Test Result (DIU Lab)

4.2.4. Test for gums: A 5 ml sample of the extract solution was obtained and subsequently treated with sulphuric acid and Molish reagent. At the interface of two liquids the formation of a red-violet ring indicates the existence of gums and carbohydrates.

Fig. 13: Gums Test Result (DIU lab)

4.2.5. Test for Flavonoids: A minute quantity of an alcoholic extract of the botanical substance was subjected to a few droplets of concentrated hydrochloric acid. Flavonoids are detected by the rapid emergence of a red hue.

Fig. 14: Flavonoids Test Result (DIU Lab)

4.2.6. Test for Saponins: The extract was diluted into twenty milliliters of distilled water to one milliliter of the solution then stirred for 15 minutes using a graduated cylinder. Saponnins can be detected by a one-centimeter layer of foam.

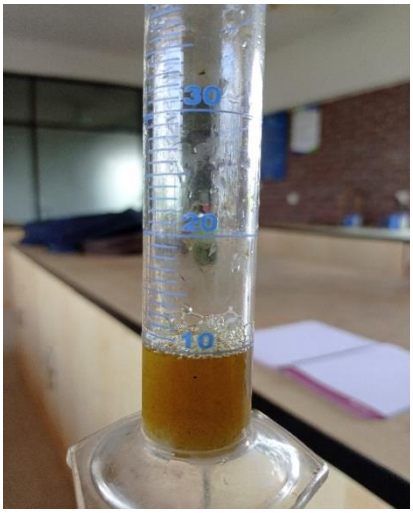


Fig. 15: Saponins Test Result (DIU lab)

4.2.7. Tests for Glycosides

• A little quantity of an alcoholic extract was obtained from either fresh or dried plant material, and then mixed with one milliliter of water. Subsequently, a small amount of sodium hydroxide solution was added. The yellow tint was considered to be indicative of the existence of glycosides.

• A minute quantity of an alcoholic extract was derived from the vegetative matter then combined with the Fehling solution in a preparation of alcohol and water. The red brick precipitate was believed to be indicative of the existence of glycosides.

• Some extract was dissolved in a mixture of alcohol and water, and then heated with a small amount of dilute sulfuric acid. The resulting solution was then neutralized with solution of sodium hydroxide and subsequently boiled with the Fehling solution. The red brick precipitate was believed to be indicative of the presence of glycosides [23].

Fig. 16: Glycosides Test Result (DIU Lab)

In vitro Study

5. ANTIBACTARIAL TEST

Antimicrobial activity refers to the ability of chemicals to hinder microbial growth. The utilization of medicinal plants and their extracts to treat localized and particular ailments in both humans and animals is an ancient practice that dates back to time immemorial. Various researchers have conducted surveys to find anti-microbial substances. These surveys have consistently shown that marine algae and higher plants contain a wide range of anti-microbial compounds. Bioassay-directed fractionation methodology has greatly accelerated advancements in the advancement of antimicrobial drugs derived from higher plants. The molecular structures of the higher plants separated chemicals possess anti-microbial activities vary. These compounds include anthraquinones, flavonoids, alkaloids, essential oils, and others. Plants' in vitro antibacterial activity may be measured by measuring microbe growth in solvent fractions or plant extracts. There are three methods to achieve this (diffusion, dilution, and bioautographic methods), and the results might be affected by various factors like temperature of incubation, pH, composition of culture medium, inocula volume, and the extraction procedure.

5.1. Principle

This method involves dissolving the specified quantity of the test samples in certain quantities of solvent to create solutions with known concentrations (µg / ml). Next, sterile discs of Matricel filter paper are saturated with a precise quantity of test chemicals via a micropipette, and subsequently dried. The positive and negative controls consist of standard antibiotic disks and disks that have been treated with the solvent used to dissolve the samples, which are then absorbed and dried. After employing a sterile transfer loop, the discs are subsequently positioned in petri dishes of 120 mm in diameter. These dishes contain an appropriate agar medium inoculated with the test organisms for the purpose of conducting antimicrobial screening. In order to facilitate optimal dispersion, the plates are kept at a temperature of 40 degrees Celsius. The test material permeates the surrounding medium from the discs. In order to promote the

growth of microorganisms, the plates are incubated at 37 °C for a period of 12 to 18 hours. If the test substance has antimicrobial activity, it will prevent the growth of microorganisms, resulting in the formation of a clearly defined "zone of inhibition." The test agent's antibacterial activity is evaluated through calculating the inhibition zone diameter in millimeters. The tests are replicated three times, and the mean measurement is documented [24].

5.2. Materials and methods

5.2.1. Test material

Methanol crude extract.

5.2.2. Microorganisms used for the activity test

The test included both gram-positive and gram-negative strains of bacteria. The strains of bacteria used for the experiment are enumerated in **Table 2.**

Table 2: List of bacteria. We took the 2 gram-positive and 2 gram-negative bacteria for antibacterial test.

Bacteria Strains	**Gram (+)**	**Gram (-)**
	Bacillus megaterium	*Pseudomonas aeruginosa*
	Staphylococcus aureas	*Escherichia coli*

5.2.3. Culture media

Culture medium refers to a combination of essential nutrients employed in laboratory study to facilitate the proliferation and expansion of a crop, which consists of a population of microorganisms. Bacterial nutritional requirements exhibit significant variation, with certain bacteria necessitating a medium consisting predominantly of inorganic substances, while others necessitate a medium containing both inorganic and organic chemicals such as coenzymes, or vitamins, pyrimidines or purines, sugars, amino acids. Every biological species necessitates specific physical circumstances for its growth, together with particular nutrients. The following media are commonly utilized to illustrate antibiotic activity and to cultivate test organisms.

a) Agar powder 2gm/100ml

b) Tryptic soy broth (TSB) 3gm/100ml

c) Disl. Water 100ml [69].

5.2.4. Preparation of media

H_2O was introduced to a dehydrated substance comprising all the necessary components for the production of nutritional agar medium. Commercially available, there are powdered forms of nearly every substance.

Fig. 17: Preparation of media (DIU lab)

5.2.5. Sterilization of different equipment and media

The media, Petri dishes, and other glassware received sterilization using autoclave, which involved subjecting them to a 121°C temperature and a of 15 pounds pressure per square inch for 20 minutes. The blank discs, stored in covered Petri dishes, were sterilized using dry heat at a temperature of 160°C for 1 hour. Subsequently, they were exposed to UV light for 30 minutes in a laminar hood and The UV light was activated one hour before operating in the laminar hood as a precautionary measure against inadvertent contamination [25].

5.2.6. Preparation of extracts

100 mg plant extract was added in to 10 ml Methanol, and shack properly.

5.2.7. Preparation of Disc

Antibacterial screening included the utilization of 3 distinct kinds of discs: a) Sample discs; b) Standard discs and c) Blank discs

Following is a description of their preparations:

a) Sample discs: Aseptic filter paper discs measuring 5 mm in diameter were placed in an empty petri dish. The desired concentration of the sample solution was administered onto the discs

using a micropipette under sterile conditions. For this experiment, we utilized test solution discs with a concentration of 500 µg/disc.

b) Standard discs: The positive control was used to verify the effectiveness of the Standard antibacterial versus the test pathogens and to compare its reaction to the response of the test samples. The study used Ciprofloxacin standard discs as a reference.

c) Blank discs: The discs were used that the filter paper was not active themselves.

5.2.8. Determination of antibacterial activity

Test agent's antibacterial activity was measured after adequate incubation by recording the inhibitory zone diameter in millimeters using digital slide calipers [26].

In silico Study

6. In silico study

The usage of in silico methods for the detection of possible hits has gained popularity in the vicinity of computer-aided discovery of drug. This approach effectively reduces the search for potential lead compounds from large compound databases by employing high-throughput molecular docking. It also enables the understanding of the mechanistic interaction of potential hits, aiding in the rationalization and optimization of bioactivity [27].

6. 1. Literature studies and ligand-receptor selection criteria

Investigating drug-target interactions is crucial for comprehending biological mechanisms and advancing the development of novel therapeutic compounds. The rise in ligand-binding assays has occurred simultaneously with advancements in reagents, detection methods, and instrumentation technologies. This is due to the growing number of therapeutic targets and the growing recognition of the biochemical aspects of drug-target interactions, which play an essential part in deciding the clinical effectiveness of medicinal substances. Currently, ligand-

binding assays have the capability to ascertain all aspects of numerous drug–target interactions [28]. The docking approach is mathematical aspect used in drug design to forecast small-molecule binding mechanisms and affinities within specific receptor targets. It is commonly employed for virtual screening studies and optimization of lead compound to identify new molecules that are biologically active. A docking methodology relies on two fundamental components: a search algorithm and an energy scoring function. These components work together to generate and assess the positions of ligands. This paper provides a comprehensive examination of scoring systems and search algorithms. that are often employed in contemporary molecular docking techniques, with a specific emphasis on their application in protein-ligand interactions [29].

6.2. Genomic and protein structure of the cell wall of bacteria

Bacterial biological processes interact with the chromosome, forming distinct units like operons, replicators, or macrodomains. These interactions catalyze genome structure and organization, resulting in adaptive features. Genome organization allows gene repertoires to change rapidly, while chromosomal structure remains stable. Comparative genomics and experimental modifications reveal factors contributing to this stability. Understanding chromosomal organization can help explain diverse biological processes. Genomics plays a crucial role in molecular microbiology, with DNA sequencing enabling genetic manipulation and uncovering functional schemas. Prokaryotic genomes evolve new functions through distinctive chromosomes, duplication of genes, and highly compartmentalized cellular processes. Gene repertoires rise through horizontal gene transfer, and in gene sequence composition and density chromosomes are identical. The *Escherichia coli* and *Bacillus subtilis* genome maps are more comparable than genomes of yeast, resulting in complex and plastic bacterial chromosomes with conservative organizational features. Evidence suggests that cellular processes interacting with DNA shape genome structure, imposing constraints and selecting favorable genomic configurations. This necessitates fine-tuned organizations to manage affected regions [30]. Studying the peptidoglycan structure is crucial for accurately describing novel taxa of Gram-positive bacteria. Structural differences of the peptidoglycan in specific genera facilitate differentiation even at the species level. To analyze peptidoglycan, one must first isolate and purify this polymer, which is cross-linked in three dimensions. This process involves using

several analytical techniques to identify and measure its components, as well as having particular knowledge on how to interpret structural information from the collected data. While there are numerous review publications detailing the intricate process of assessing the peptidoglycan structure, the corresponding procedures are not generally employed, and many laboratories must seek collaboration or professional assistance for peptidoglycan analysis. Peptidoglycan is a heteropolymer with glycan strands cross-linked by peptides, with a glycan backbone of N-acetylmuramic acid and N-acetylglucosamine, with partial substitution of N-acetyl by N-glycolyl groups [31].

6.3. Phytocompounds derived from *Artocarpus heterophyllus* and pharmacological evidence

This study was the first to report the impact of enhancing the effectiveness of antibiotics by combining them with *A. heterophyllus* fixed almond oil. The oil exhibited antibacterial properties, particularly against *S. aureus*, and showed significant synergistic effects by reducing the minimum inhibitory concentrations (MICs) of various types of antibiotics against multidrug-resistant (MDR) strains of bacteria. The GC-FID analysis revealed that the primary acids present in the fixed oil composition of jackfruit, namely palmitic acid, myristic acid, oleic acid, and lauric acid, are commonly located inside other oils with significant biological qualities. Further investigations are warranted to confirm whether the primary components found inside the fixed oil's composition of chemicals possess independent antibacterial properties and demonstrate modulation potential. The chemical analysis revealed that the oil contains fatty acids commonly found in other fixed oils. These fatty acids have notable antibacterial properties, either on their own or when combined with antibiotics from different classes, like fluoroquinolones, aminoglycosides, and β-lactams. The primary acids in the oil are palmitic acid, myristic acid, oleic acid, and lauric acid. Further investigations are necessary to confirm whether the primary chemicals found in the oil, when isolated, possess antibacterial properties and exhibit the observed modulation capability in the conducted tests with the fixed oil [32].

7. Method and material

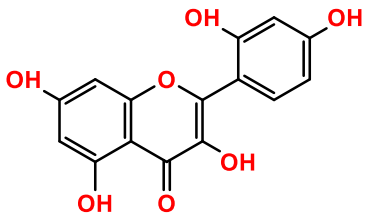
7.1. Ligand preparation and molecular optimization

The optimization method was developed to maximize molecular docking's effectiveness and the molecules arrangement in a framework that is three-dimensional. Initially, a three-dimensional structure of molecules is gathered from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Figure 3) [33]. This provided the observation of the three-dimensional structure. Before conducting molecular docking, the ligands received optimization utilizing the software named Gaussian09 using DFT/B3LYP-6311G technique. Nevertheless, optimization of aqueous operations was not performed, since ligand optimization does not need it. In order to facilitate the process of molecular docking of the ligands, these optimizations were carried out. The molecular docking was utilized to identify postures with the utmost binding affinity (measured in kcal/mol) among substances and the peptidoglycan glycosyltransferase (PDB ID 2OQO). Afterwards, the stances that were chosen as the best go through molecular dynamics simulation. There was no independent optimization of the ligands in either of the complex structures. Charmm-gui was used to create structural topology files, hence removing the necessity for an individual optimization process for the ligands. Ligands 02, 03, 04, and 08 have the highest antibacterial activity.

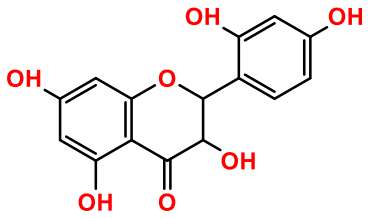
Table 3: Data of PASS prediction.

PubChem CID (Ligands)	**Antibacterial**	
	Pa	**Pi**
1) 5281670	0.387	0.033
2) 5458714	0.402	0.029
3) 5458461	0.477	0.019
4) 399491	0.501	0.016
5) 5481970	0.382	0.035
6) 64971	0.343	0.045
7)15298902	0.394	0.031
8) 370	0.418	0.026

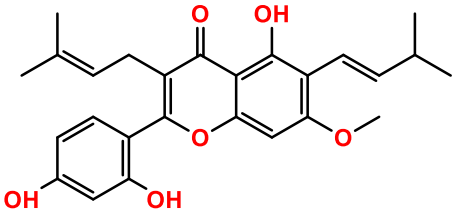
9) 10251	0.287	0.065
10) 15227962	0.242	0.087



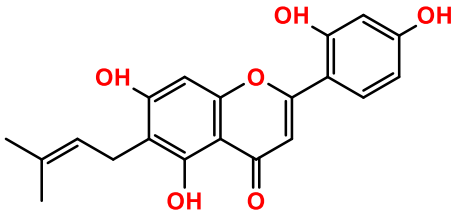
Morin



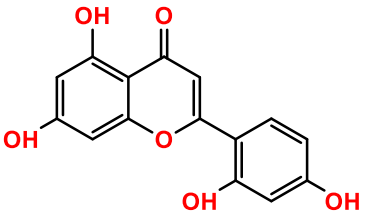
Dihydromorin



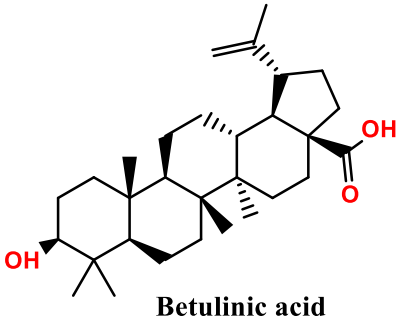
Artocarpin



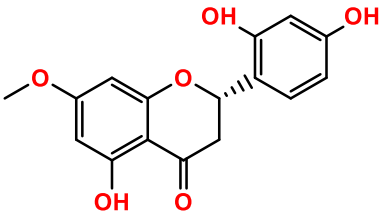
Artocarpesin



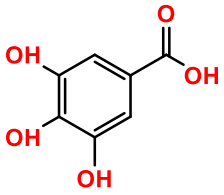
Norartocarpetin



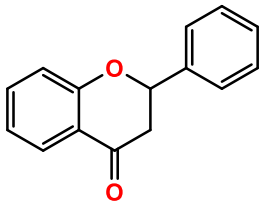
Betulinic acid



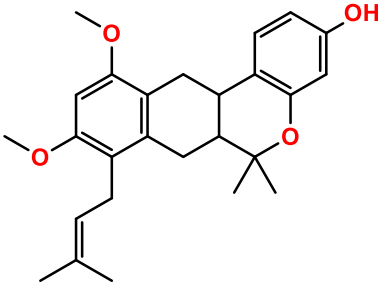
Artocarpanone



Gallic acid



Flavanone



Heterophylol

Figure 18: Chemical structures of studied ligand compounds.

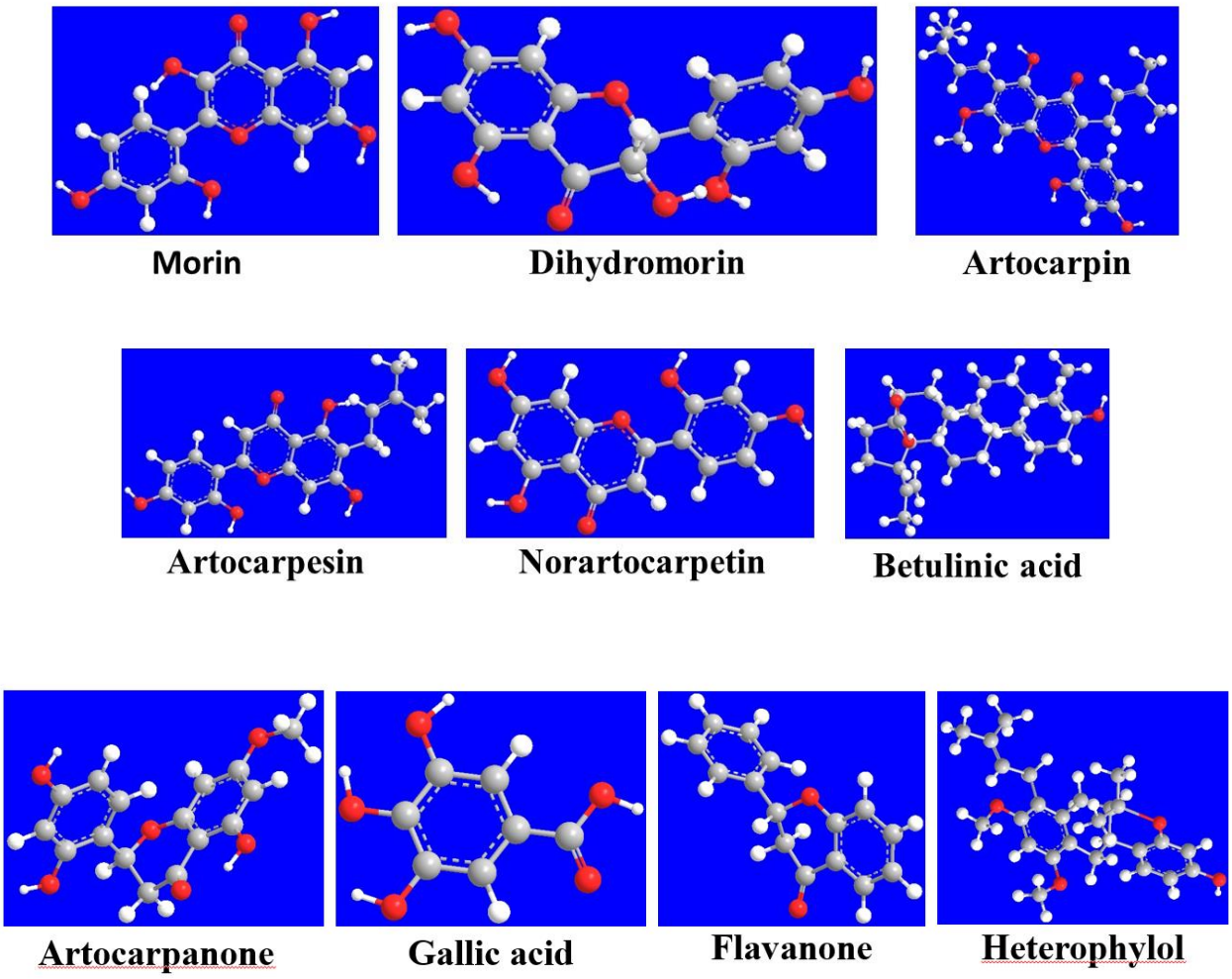
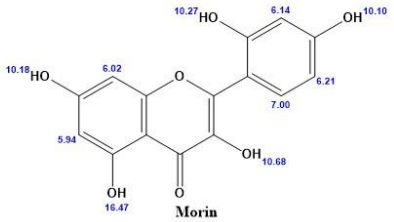


Figure 19: Chemical 3D structures of studied ligand compounds.

7.2. NMR study of ligand compounds

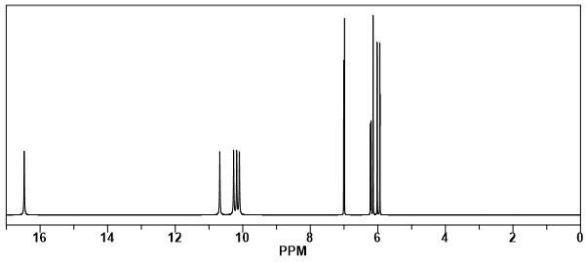
NMR spectroscopy is a widely recognized technique in the domain of pharmaceutical research. However, its effectiveness is generally believed to be limited to the initial phases of fragment-based drug design and hit discovery. In these stages, NMR (Fig. 21) provides specific benefits in analyzing the specific ways in which ligand molecules bind, that have relatively weak binding affinities and rapidly transition between bound and unbound states [34].

NMR ^{1}H Estimation

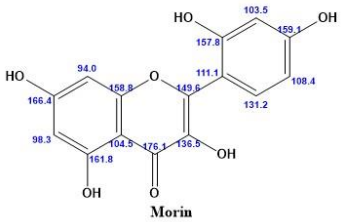


Morin

Estimation quality is indicated by color: good, medium, rough



NMR ^{13}C Estimation



Morin

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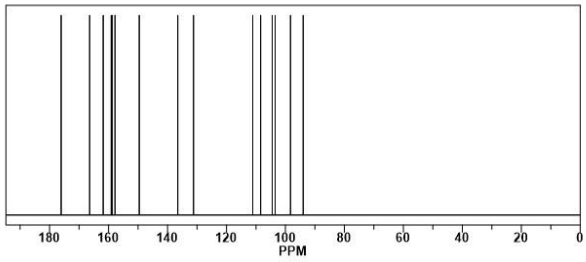
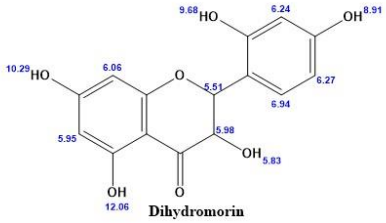


NMR ^{1}H Estimation



Dihydromorin

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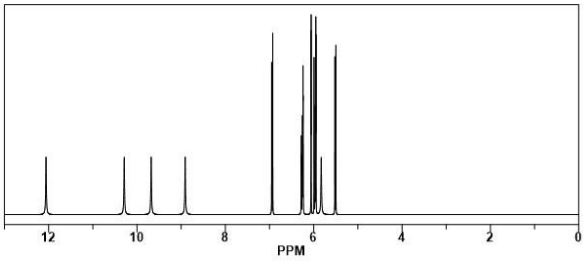
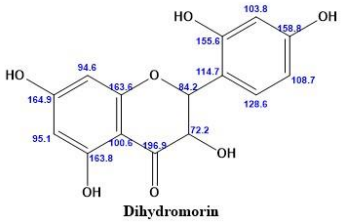


NMR ^{13}C Estimation



Dihydromorin

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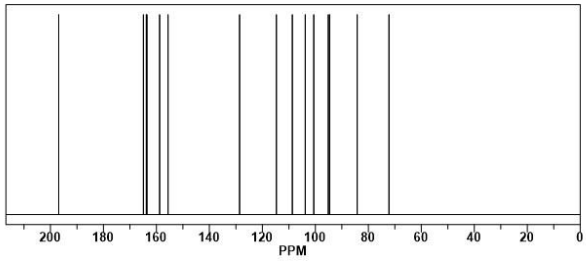

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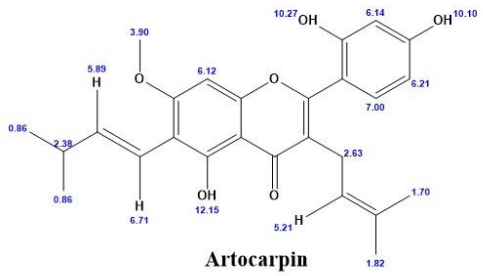

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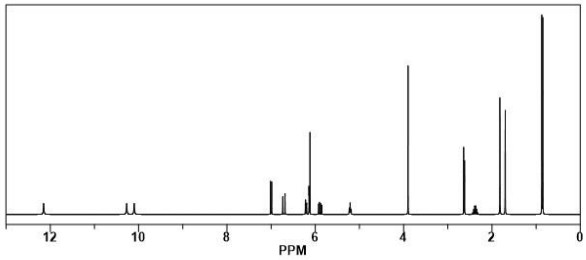
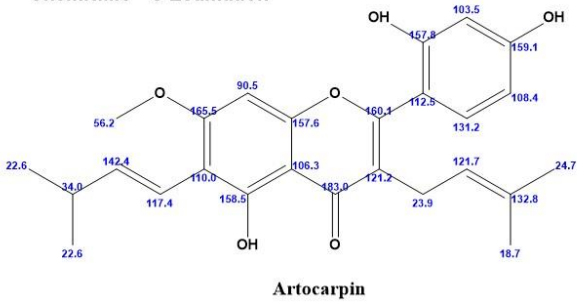


ChemNMR ^{13}C Estimation



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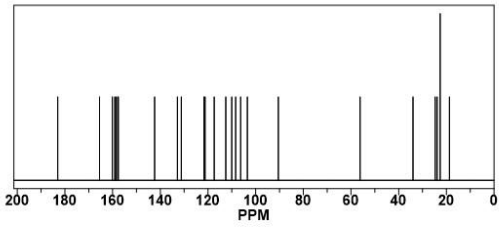
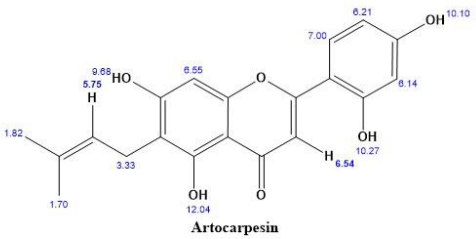


NMR ^{1}H Estimation



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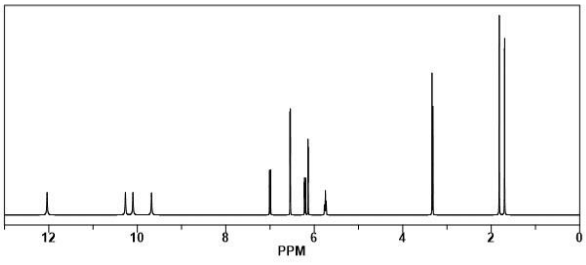
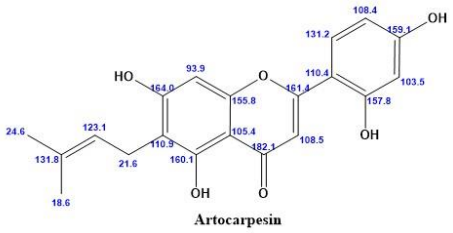


NMR ^{13}C Estimation



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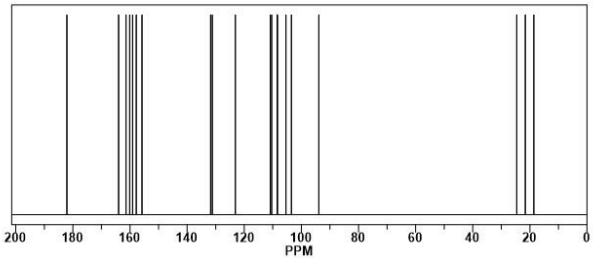
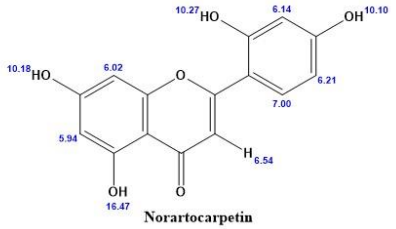


NMR ^{1}H Estimation



Norartocarpetin

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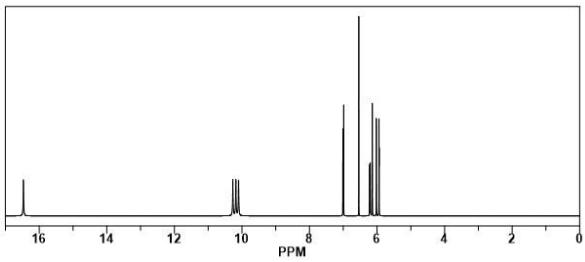
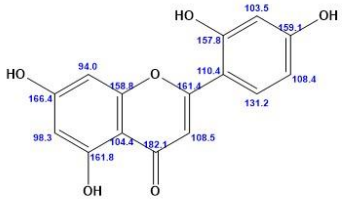


NMR ^{13}C Estimation



Norartocarpetin

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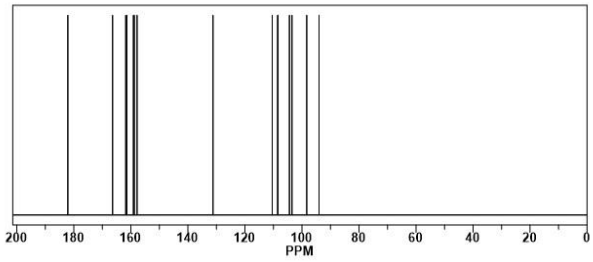
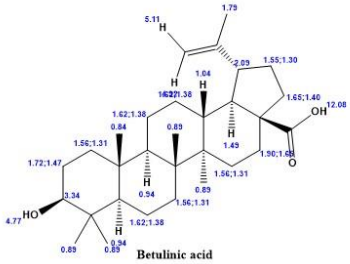
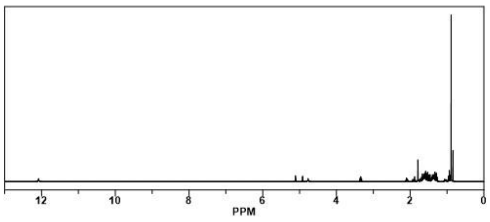


NMR ^{1}H Estimation

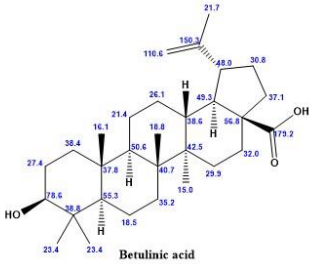


Betulinic acid

Estimation quality is indicated by color: good, medium, rough



NMR ^{13}C Estimation



Betulinic acid

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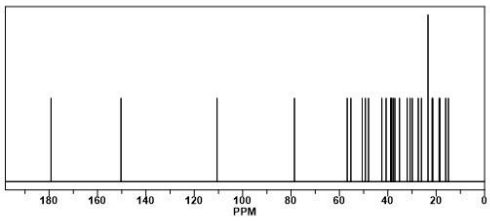

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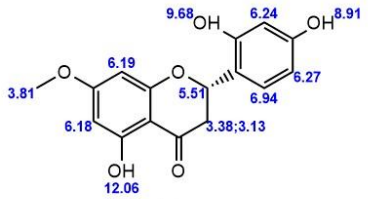

Artocarpanone

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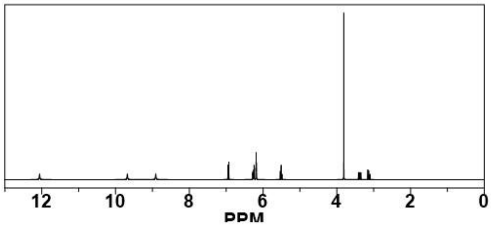
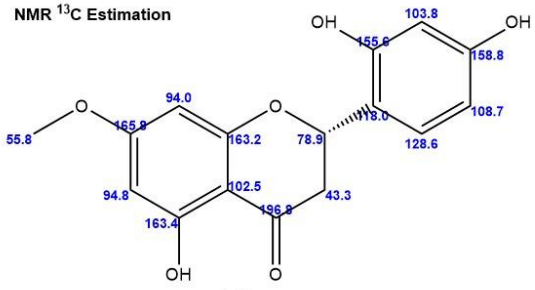


NMR ^{13}C Estimation



Artocarpanone

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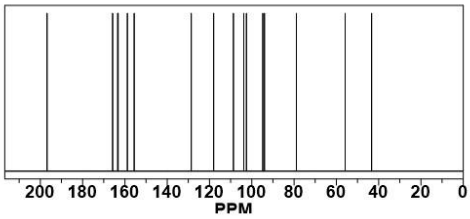
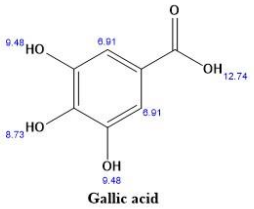
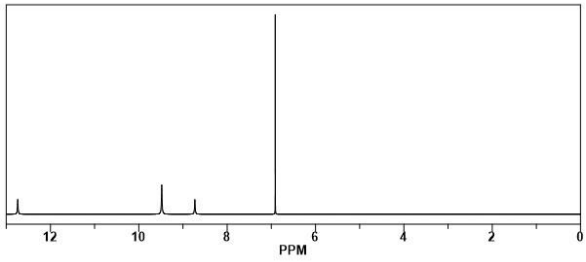


NMR ^{1}H Estimation

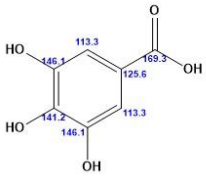


Gallic acid

Estimation quality is indicated by color: good, medium, rough



NMR ^{13}C Estimation



Gallic acid

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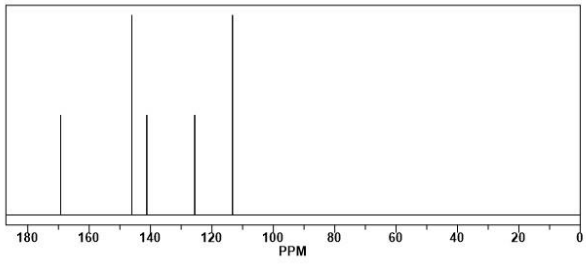


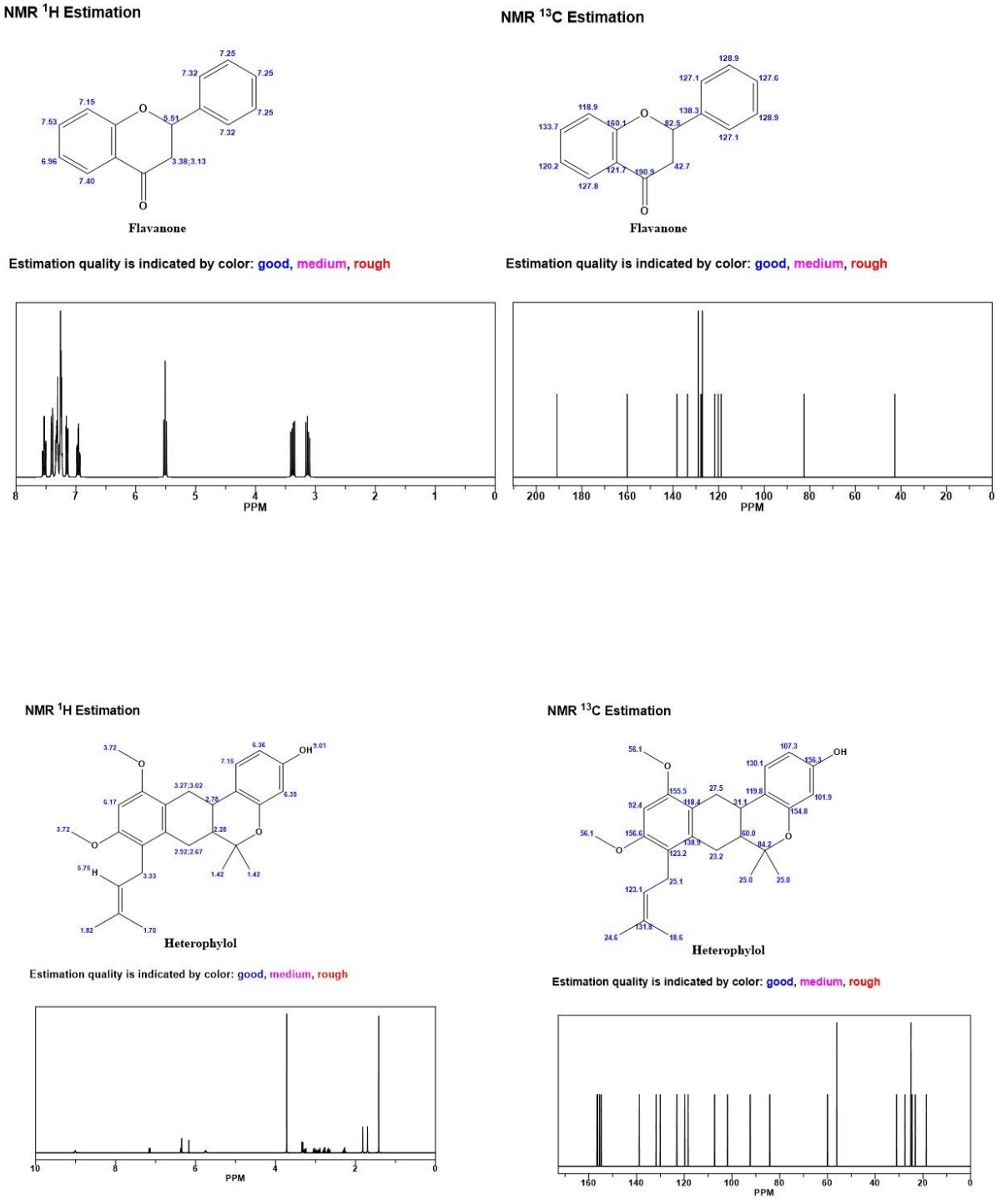
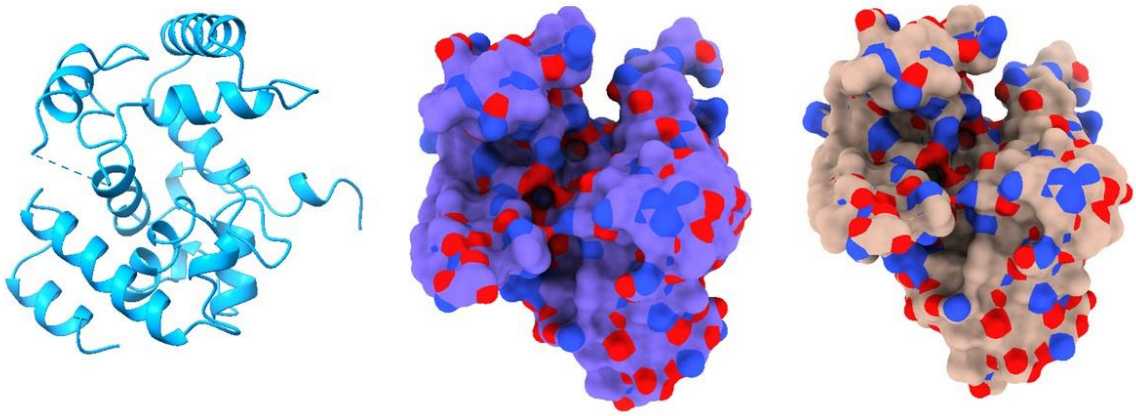


Fig. 20: NMR of ligand compounds

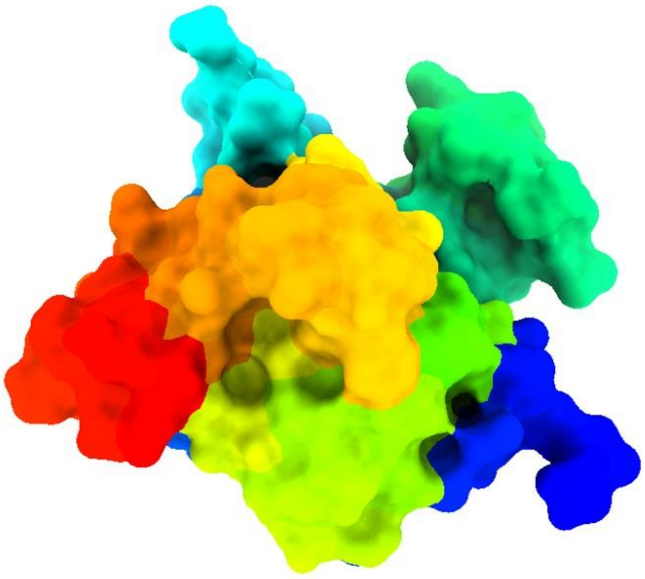
Physiological functions are primarily regulated by the mechanisms of intermolecular recognition that include interactions between proteins and either other proteins or small molecules known as ligands. A crucial method for investigating these exchanges is high-field solution nuclear magnetic resonance (NMR) using ligand-observed and protein-observed assays, which allow for selective detection of the protein receptor or the chemical molecules. NMR binding investigations involve comparing the NMR properties of molecules in their bound and free states. Ligand-observed approaches are highly versatile for analyzing protein-ligand interactions, as they are not constrained by the molecular scale of the protein. The utilization of these NMR techniques has significantly broadened in the past few years, including both chemical biology and drug development [35]. NMR has developed into a potent technique for analyzing protein-ligand interactions in a liquid state, closely resembling physiological settings. Currently, it is commonly utilized to evaluate the attraction and selectivity of contacts, pinpoint binding sites on proteins and ligands, and analyze the structural changes caused by binding [36]. NMR spectroscopy is a biophysical approach utilized during the process of searching for as well as creation of physiologically active chemicals seen as prospective medicines. The analysis pertains to contemporary NMR techniques utilized to analyze the low-molecular-mass drugs binding to targets that are biomacromolecular. This text discusses effective techniques for NMR screening and the tactics used to design rational leads. These pharmaceuticals were developed to design medications which have either been authorized for implementation in the field of medicine or are now in the advanced phases of clinical trials [37].

7.3. Protein preparation and Molecular docking study

The main goal of molecular docking methodology is to use computational methods to precisely predict the framework of the ligand-receptor complex. The docking procedure consists of two separate but interconnected processes. Initially it entails gathering data of several conformations that the ligand can assume when attached to the Protein's active site (Fig. 22). Furthermore, it involves the categorization of these remains according to a performance index. Molecular docking is achieved by executing a series of complex procedures that include the interaction between ligands and receptors. This process helps predict how they interact and assists in drug development and designing efforts [38].



Peptidoglycan glycosyltransferase (PDB ID 2OQO)



Peptidoglycan glycosyltransferase
(PDB ID 2OQO)

Fig. 21: Three-dimensional bacterial protein structure

7.4. Determination of ADMET, and pharmacokinetics, and drug-likeness

The pharmacokinetic (PK) properties of possible treatments, especially ADMET (absorption, distribution, metabolism, excretion, and toxicity), are clearly important elements to take into account during pharmaceutical development and research stage [39]. As an outcome, the SwissADME and pkCSM webservers were used to perform a crucial drug-likeness screening on the apigenin compounds that were being investigated [40]. Based on determined physicochemical and ADMET-related factors, this testing was conducted. The ligands' SMILES strings, which served as the molecular markers' input to both webpages. Unfavorable pharmacokinetic characteristics of possible medication candidates actually have a big role in the failure rate in clinical trials [41]. It assessed the compounds' absorption properties characteristics by considering their assimilation in the human digestive system, Caco-2 permeability, and solubility in water. The process of absorbing of drugs is contingent upon their water solubility; a greater degree of water solubility indicates enhanced absorption characteristics and increased bioavailability [42]. Compound 02 has a higher level of water solubility compared to compound 07, which has a slightly higher degree of water solubility. Caco-2 is a human cell line derived from colorectal adenocarcinoma which has been made immortal as well as mostly used like a standard representation for studying The barrier around the intestines [43]. Compound 11 exhibits the highest Caco-2 permeability with a value of 2.107, while Compound 05 has the lowest permeability with a value of -0.967. The rate at which a medicine is absorbed into the human intestines, known as the rate of human intestinal absorption (HIA), is crucial for forecasting its oral absorption efficiency [44]. After medication distribution, the liver metabolizes the chemical via many enzymatic pathways. The process of medication metabolism and biotransformation is carried out by the isoenzyme cytochrome P450. The significance of cytochrome P450 drug metabolism arises from concerns regarding medicine toxicity and pharmacological effects. Drug molecules can be expelled from the body by various pathways, including the liver, bile, and kidneys. The overall drug molecule's rate of clearance is a essential data for predicting excretion of drug. The term refers to the quantity of a substance eliminated per unit by the collective action of the kidney and liver [45]. Therefore, it's crucial to perform pre-clinical assessments of the drug-like qualities and pharmacokinetic properties of potential treatments.

7.5. Calculation of QSAR and pIC50

QSAR is an acronym for Quantitative Structure–Activity Relationship, a method that creates a correlation amongst the biological activity and the chemical structure of substances. QSAR, short for Quantitative Structure-Activity Relationship, is a computational approach in quantum chemistry that accurately forecasts the effectiveness of chemical compounds in pharmaceutical research and development. We utilized the ChemDes website (http://www.scbdd.com/chemopy_desc/index/) for doing QSAR and pIC50 analyses. This website is accessible to anyone without any cost. The web services offer various types of data, including Chiv5 molecular connectivity, bcutm1 indicates burden descriptors, GATSv4 which indicates autocorrelation descriptors, MRVSA9, MRVSA6, as well as PEOEVSA5 which are MOE type descriptors. The last two parameters, J and diameter, suggest topological descriptors of drug molecules for the reported ligands. In order to ascertain as well as compute the QSAR and pIC50, the specified values were initially gathered from the ChemDes database. Subsequently, a multiple linear regression (MLR) model was constructed in an Excel spreadsheet and the calculations were performed [45]. The Quantitative Structure–Activity Relationship (QSAR) is a computational simulation method employed in pharmaceutical exploration and development to forecast the pharmacological effects of chemical substances by analyzing their structures at the molecular level. This process is mostly employed in the advancement of novel pharmaceuticals, specifically within the domain of computer-assisted drug design. The process entails creating mathematical models that establish a connection between the physicochemical characteristics also molecular structural properties and biological effects [46]. The QSAR standard ranges are typically below 10. According to the hypothesis, any molecule with a size smaller than 10 is considered potential [47].

$$\text{pIC50 (Activity)} = -2.768483965 + 0.133928895 \times (\text{Chiv5}) + 1.59986423 \times (\text{bcutm1}) + (-0.02309681) \times (\text{MRVSA9}) + (-0.002946101) \times (\text{MRVSA6}) + (0.00671218) \times (\text{PEOEVSA5}) + (-0.15963415) \times (\text{GATSv4}) + (0.207949857) \times (\text{J}) + (0.082568569) \times (\text{Diametert})$$ [48].

CHAPTER- 06

Results and Discussion

8. Results and Discussion

8.1. Phytochemical test

The following table shows the quantity of dehydrated extract, physical features, and analysis of chemicals based on their qualitative properties of various *Artocarpus heterophyllus* extracts:

Table 4: Phytochemical study of *Artocarpus heterophyllus*.

Phytochemical constituents	**Methanolic extraction of leaves**
1. Alkaloids (Mayer's Test) (Dragendroff's Test)	 + +
2. Carbohydrates (Benedict test) (Fehling's Test)	 + +
3. Steroids	+
4. Gums	-
5. Flavonoid (Molisch test)	+
6. Saponins	+
7. Glycoside	+

We determined the various phytochemical group test in our laboratory. All phytochemical constituents provided positive result except gum.

8.2. Determination of antibacterial activity

Table 5: Antibacterial activity of gram (+) and gram (-) bacteria are determined.

Bacterial Strains	**Type of bacteria**		**Diameter of zone of inhibition**		
		Blank	**Ciprofloxacin**	**Sample**	**Sample** (500

			(50 µg/disc)	(250µg/disc)	µg/disc)
Bacillus megaterium	Gram (+)	----	24mm	6mm	10mm
Staphylococcus aureas	Gram (+)	----	22mm	9mm	12mm
Pseudomonas aeruginosa	Gram (-)	----	25mm	11mm	15mm
Escherichia coli	Gram (-)	----	28mm	9mm	14mm

We determined the diameter of zone of inhibition by two concentration sample (250µg/disc) and (500µg/disc) on gram-positive and gram-negative bacteria. The maximum diameter of zone of inhibition is 11mm and the minimum is 6mm for concentration of 250µg/disc. At the same way, the maximum diameter of zone of inhibition is 15mm and the minimum is 10mm for concentration of 500µg/disc.

8.3. Lipinski Rule and Pharmacokinetics

The SwissADME online server has provided calculations for the drug-likeness and pharmacokinetics features, which are displayed in Table 6. Lipinski's criterion requires a drug-like compound's molecular weight not exceeding 500 Daltons. Lipophilicity, a drug's attraction to lipid-rich habitats, is crucial in the pharmaceutical sector. The consensus logarithm of partition coefficient was computed. Lipinski's law mandates a partition coefficient (Po/w) of less than 5 for oral medication administration, and all the derivatives under consideration met this requirement. However, Ligand 06 did not conform to the Lipinski rule regarding the quantity of hydrogen bond donors and acceptors. Therefore, by ignoring these factors, we continued. The term 'bioavailability' denotes the degree wherein a chemical or treatment can target its intended biological recipient.

Table 6: Data of Lipinski rule, pharmacokinetics

PubChem CID	Molecular weight	Hydrogen bond acceptor	Hydrogen bond donor	Consensus Log $P_{o/w}$	Lipinski rule		Bioavailability
					Result	violation	
1) 5281670	302.24	7	5	1.20	Yes	0	0.55
2) 5458714	304.25	7	5	0.59	Yes	0	0.55
3) 5458461	436.50	6	3	4.78	Yes	0	0.55
4) 399491	354.35	6	4	3.22	Yes	0	0.55
5) 5481970	286.24	6	4	1.74	Yes	0	0.55
6) 64971	456.70	3	2	6.14	Yes	1	0.85
7) 15298902	302.28	6	3	1.84	Yes	0	0.55
8) 370	170.12	5	4	0.21	Yes	0	0.56
9) 10251	224.25	2	0	2.93	Yes	0	0.55
10) 15227962	408.53	4	1	5.18	Yes	0	0.55

Ligand 06 did not conform to the Lipinski rule regarding the quantity of hydrogen bond donors and acceptors. All ligands followed the Lipinski rule except ligand 06 and provided the exact bioavailability. All ligands are suitable for their molecular weight.

8.4. Molecular docking analysis against targeted receptor

Firstly, the compounds were evaluated for PK, ADMET, and drug-like features. It has been recorded that neither of the molecules have contravened the Lipinski rule, PK, or the ADMET computations. Consequently, these substances underwent molecular docking and subsequent analysis. The binding affinity is assessed in order to quantify the strength of inhibition or bonding between medicines and specific proteins throughout the creation of a complex structure. Ligand 01, 05, and 07 have the highest binding affinity (Table 7).

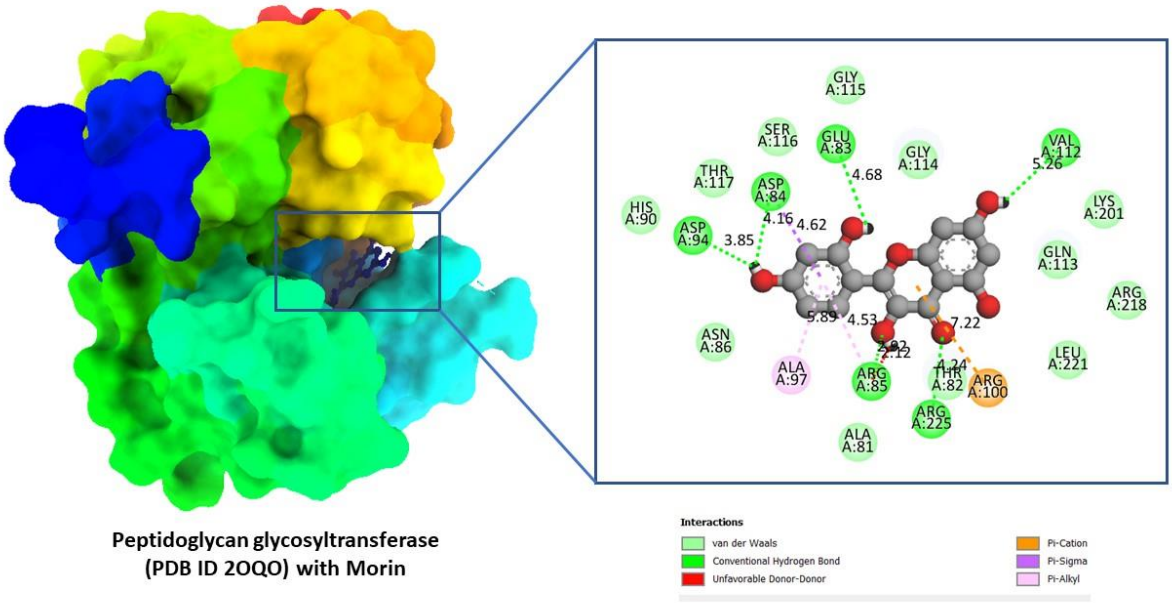
Table 7: Binding affinity against targeted protein

Chemical Name with PubChem CID	**Peptidoglycan glycosyltransferase (PDB ID 2OQO)**
	Binding Affinity(kcal/mol)
1. Morin (CID: 5281670)	-8.7
2. Dihydromorin (CID: 5458714)	-7.3
3. Artocarpin (CID: 5458461)	-7.7
4. Artocarpesin (CID: 399491)	-7.4
5. Norartocarpetin (CID: 5481970)	-8.1
6. Betulinic acid (CID: 64971)	-7.3
7. Artocarpanone (CID: 15298902)	-7.7
8. Gallic acid (CID: 370)	-6.6
9. Flavanone (CID: 10251)	-7.2
10. Heterophylol (CID: 15227962)	-7.2

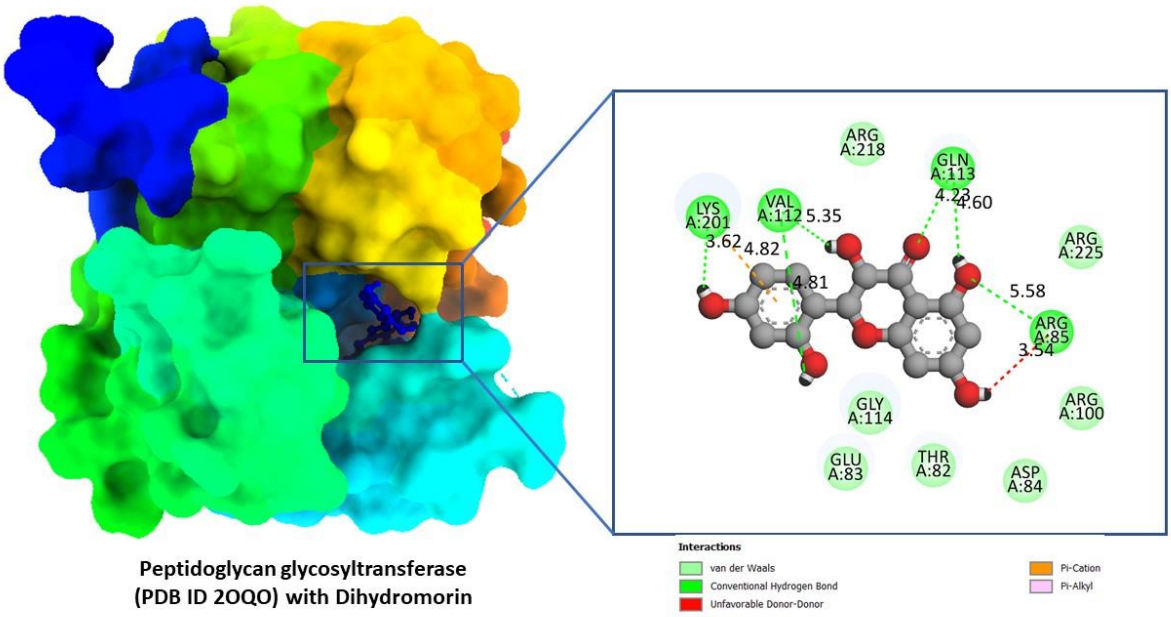
Ligand 01, 05, and 07 have the highest binding affinity to the targeted bacterial protein. For this reason, these ligands have been considered for molecular dynamic simulation.

8.5. Molecular docking pose and active site analysis

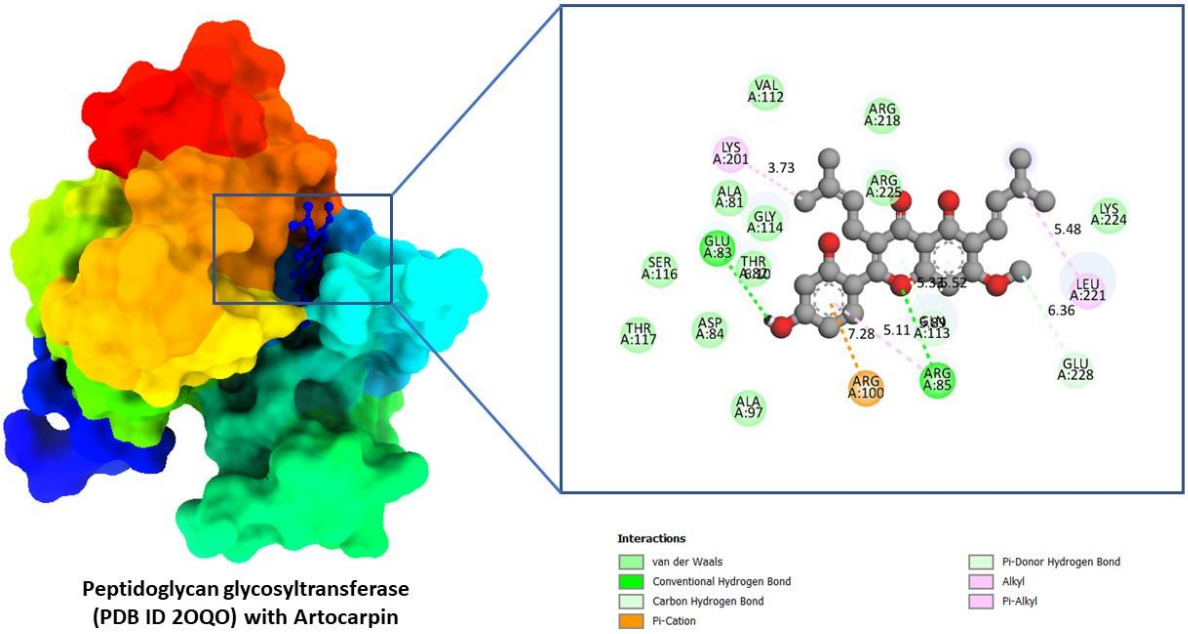
The process of active site analysis and molecular docking pose was conducted utilizing the Discovery Studio and Chimera X applications. Understanding and seeing the precise amino acid residue wherein ligand interacts to generate drug-protein complex is beneficial (Fig. 23). The study visualizes the top two complexes by utilizing the greatest binding energy.



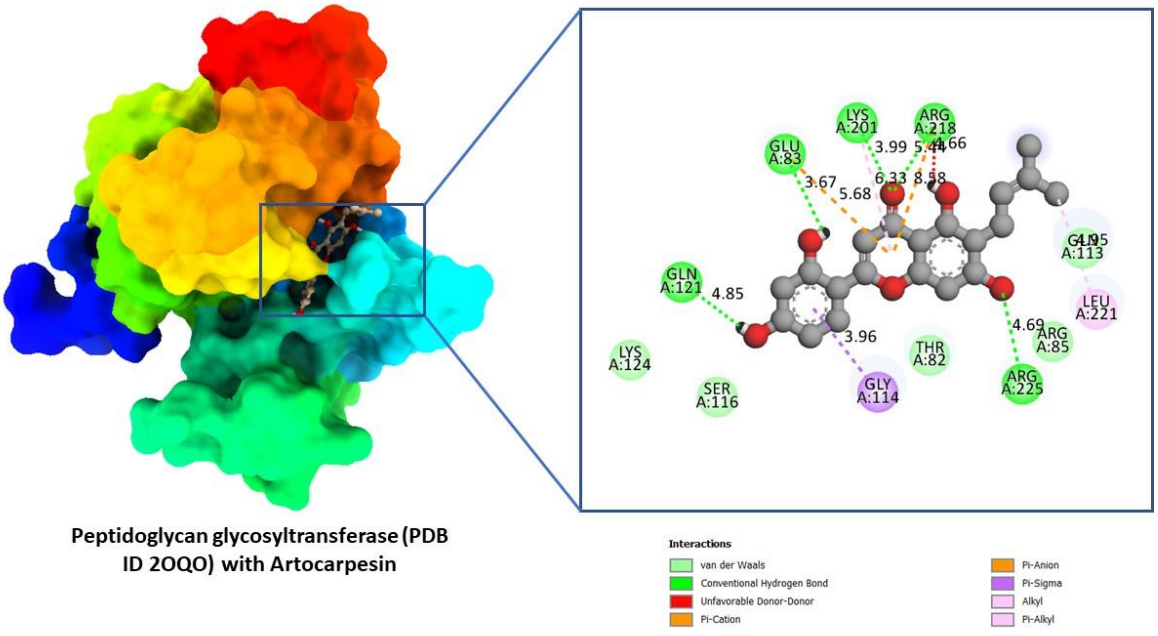
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Morin



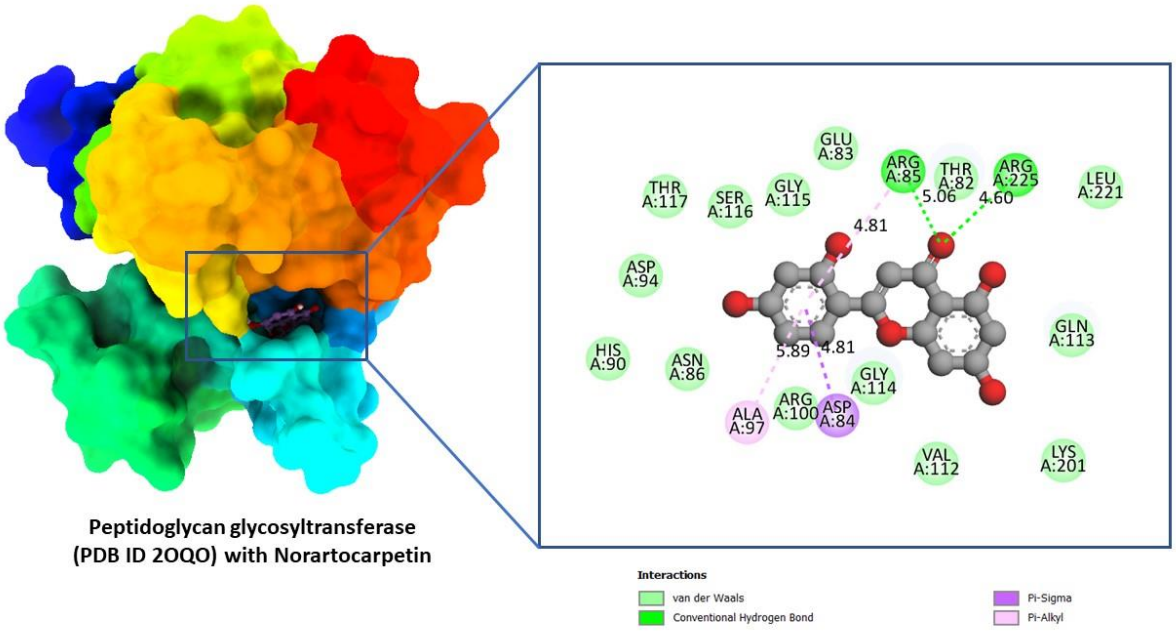
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Dihydromorin



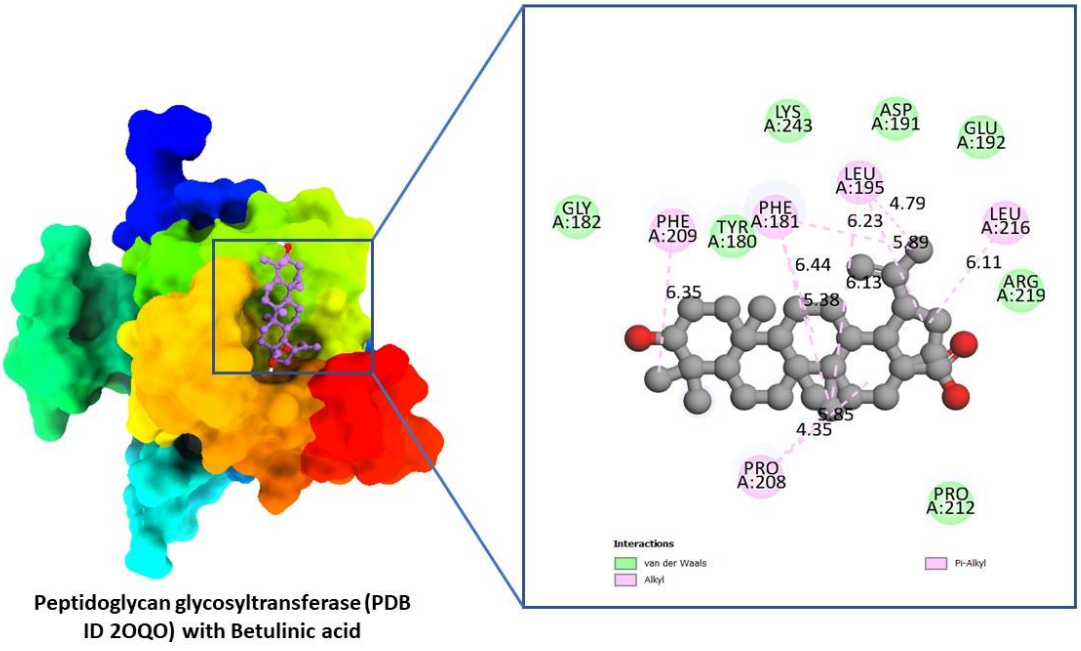
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Artocarpin



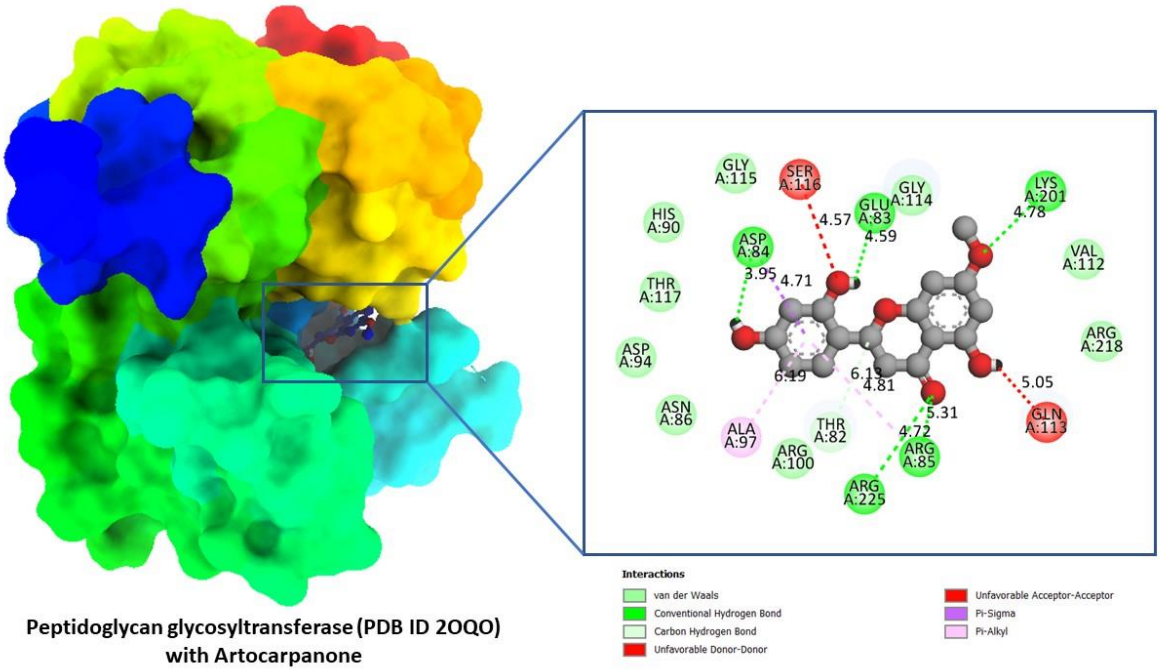
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Artocarpesin



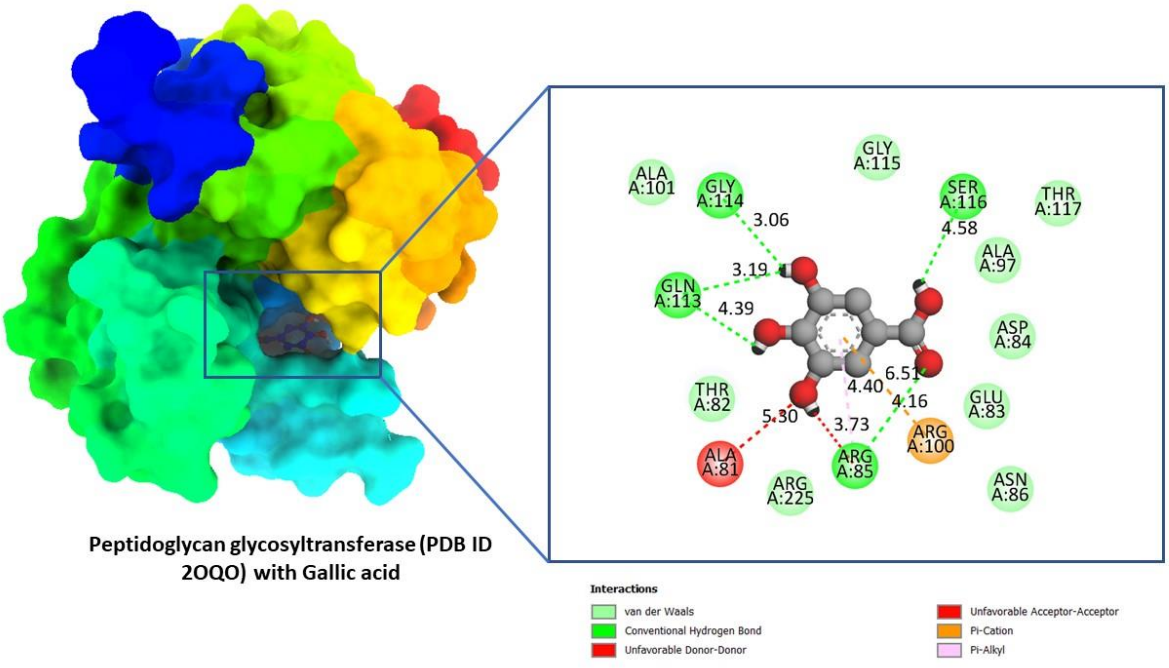
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Norartocarpetin



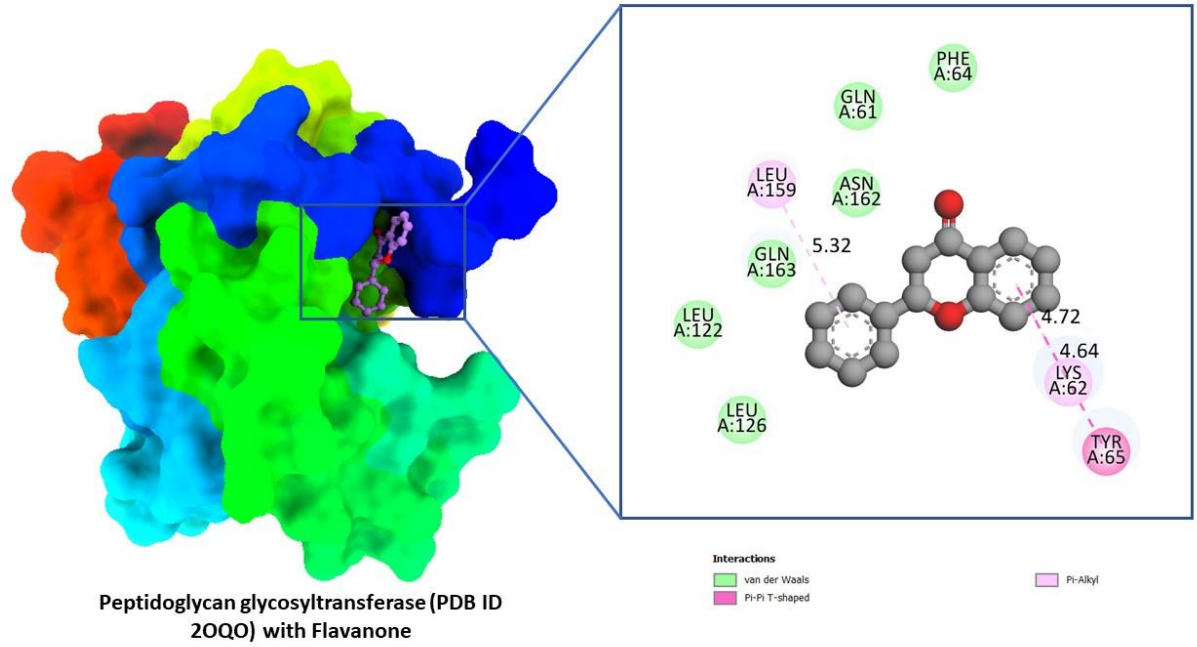
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Betulinic acid



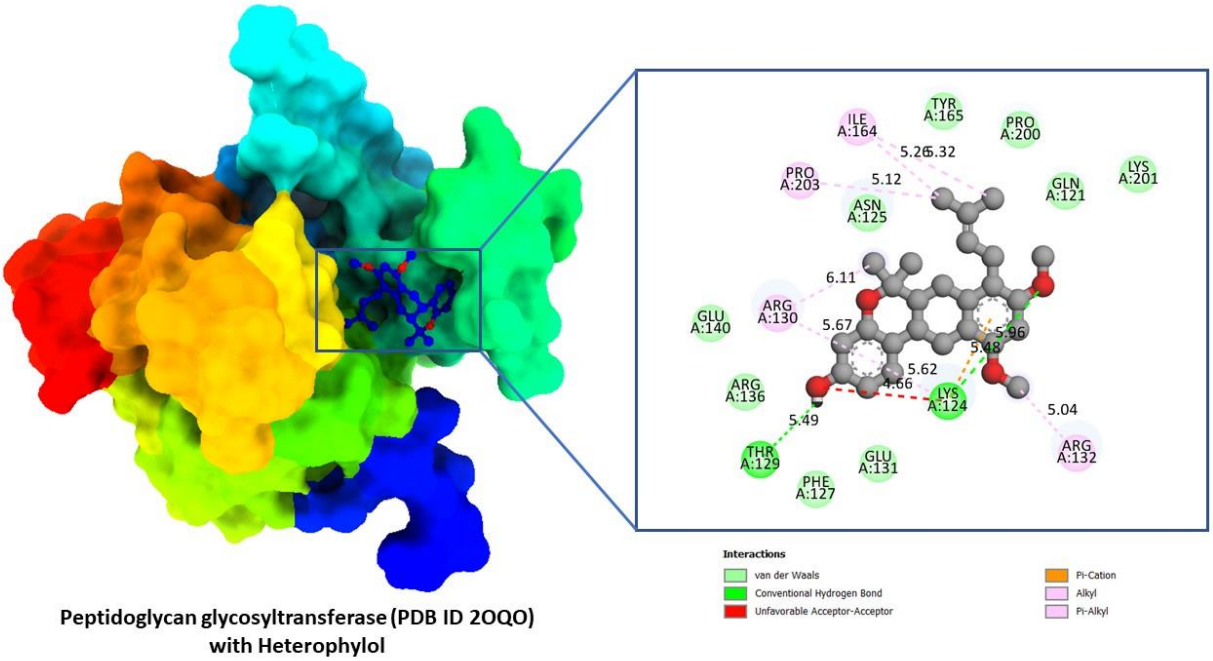
Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Artocarpanone



Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Gallic acid



Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Flavanone



Peptidoglycan glycosyltransferase (PDB ID 2OQO) with Heterophylol

Fig. 22: Molecular docking complex between bacterial protein and ligands

Ligand 01, 05, and 07 (Morin, Norartocarpetin, and Artocarpanone) have the highest binding affinity to the targeted bacterial protein. Molecular docking pose and active site analysis are showed by molecular docking. These ligands can be considered for the drug design and drug discovery.

8.6. Theoretical ADMET Data analysis of ligands

Computational approaches are used to anticipate the pharmacokinetic and toxicological properties of a chemical in theoretical ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) data analysis for ligands. By doing virtual screening and analyzing ligand-receptor interactions, this process facilitates drug development by promptly identifying possible problems, optimizing primary compounds, and reducing the need for extensive experimentation. Ligands 03 and 06 have hepatotoxicity only. For this reason, these ligands have to be avoided (Table 8).

Table 8: ADMET data analysis of ligands

PubChem CID	Absorption			Distribution		Metabolism		Excretion		Toxicity		
	Water solubility Log S	Caco-2 Permeability x 10^{-6}	Human Intestinal Absorption (%)	VDss (human)	BBB Permeability	CYP450 1A2 Inhibitor	CYP450 2D6 Substrate	Total Clearance (ml/min/kg)	Renal OCT2 substrate	AMES toxicity	Skin Sensitization	Hepatotoxicity
5281670	-2.978	-0.294	75.408	1.229	-1.18	Yes	No	0.486	No	No	No	No
5458714	-3.079	-0.216	65.43	1.501	-0.839	No	No	0.372	No	No	No	No
5458461	-3.703	0.282	92.022	-0.764	-1.243	Yes	No	0.429	No	No	No	Yes
399491	-3.249	-0.161	79.951	0.706	-1.272	Yes	No	0.322	No	No	No	No
5481970	-3.191	0.03	87.843	0.782	-1.066	Yes	No	0.578	No	No	No	No
64971	-3.122	-3.122	99.763	-1.18	-0.322	No	No	0.116	No	No	No	Yes
15298902	-3.239	0.458	84.005	0.403	-1.088	Yes	No	0.529	No	No	No	No
370	-2.56	-0.081	43.374	-1.855	-1.102	No	No	0.518	No	No	No	No
10251	-3.89	1.219	97.7	0.1	0.221	Yes	No	0.086	No	No	No	No
15227962	-2.892	0.923	79.603	0.011	0.017	No	No	-66.962	No	Yes	No	No

Ligands 03 and 06 have hepatotoxicity only. Other ligands have no any hepatotoxicity, for this reason they can be considered for the further investigation in laboratory. Compounds have no any skin sensitization toxicity. Ligand 10 can be avoided for its AMES toxicity.

8.7. Quantitative structure-activity relationship and pIC50

Quantitative Structure-Activity Relationship (QSAR) is a computer method used in medicinal chemistry to model and analyze chemical compounds. It establishes a relationship between the characteristics of a chemical structure and its biological effects, allowing for the prediction of a compound's strength. pIC50 is a quantitative measure of the inhibitory concentration of a chemical, which serves as an indicator of its efficacy. QSAR is a useful tool for improving drug design and forecasting pharmacological action by analyzing structural characteristics.

Table 9: Data of QSAR analysis and calculation

Ligand	Chiv5	Bcutm1	(MRVSA9)	(MRVSA6)	(PEOEVSA5)	GATSv4	J	Diameter	PIC50
1	1.41	4.114	10.969	40.555	0.0	0.951	1.76	10.0	4.67
2	1.623	3.966	5.783	41.459	0.0	0.951	1.76	10.0	4.58
3	2.447	4.138	17.045	63.341	37.649	0.877	1.835	14.0	5.25
4	1.819	4.116	10.969	57.768	11.649	0.882	1.66	12.200431	4.93
5	1.346	4.103	10.969	46.622	0.0	0.848	1.69	10.0	4.63
6	9.326	4.019	5.969	12.152	46.771	0.743	1.552	12.0	6.25
7	1.687	3.961	5.783	41.459	0.0	0.825	1.689	11.0	4.67
8	0.417	3.873	5.969	17.696	0.0	1.543	2.533	6.0	4.07
9	1.495	3.923	5.783	65.724	42.465	1.295	1.629	9.0	4.54
10	4.132	4.027	0.0	58.168	17.715	0.734	1.61	13.0	5.47

Our ongoing analysis reveals that both the QSAR and pIC50 values are positive (Table 9) and fall within the acceptable range. The maximum and minimum values of pIC50 are 6.25 and 4.07, respectively. The PIC50 result indicates that the substance has the potential stability and to be effective in treating the specific disease.

CHAPTER- 07

Conclusion

9. Conclusion

Artocarpus heterophyllus leaf extract exhibits strong antibacterial activity against gram-positive and gram-negative bacteria, with strong binding affinities between active compounds and major bacterial proteins, suggesting potential mechanisms. Using two concentration samples, the disk diffusion method was used to determine the zone of inhibition on gram-positive and gram-negative bacteria. The maximum diameters for the two concentration samples were 11 mm and the lowest diameters were 6 mm for 250 µg/disc, and 15 mm and 10 mm for 500 µg/disc. We evaluated the antibacterial activity of 10 leaves through the literature review, PASS prediction data analysis, molecular docking, and QSAR study for stability and stability calculation. The study reveals that ligands 01, 05, and 07 have low molecular weight, high binding affinity to Peptidoglycan glycosyltransferase, stability, and no hepatotoxicity, offering potential for innovative drug development.

CHAPTER- 08

Reference

Reference

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