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In Silico Evaluation of
Antibacterial Potential of
Phytochemicals from *Berberis*
lycium

by

Rameen Ahmad

A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

Faculty of Health and Life Sciences

Department of Bioinformatics and Biosciences

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Abstract

Antimicrobial resistance has emerged as one of the most significant global public health challenges, reducing the effectiveness of existing antibiotics and increasing the demand for novel antibacterial agents. Medicinal plants have gained considerable attention as valuable sources of bioactive compounds with diverse pharmacological properties. *Berberis lycium* Royle is a medicinal plant rich in phytochemicals and has traditionally been used to treat various infectious diseases.

The present investigation focused on the computational screening of *Berberis lycium* Royle metabolites for their potential antibacterial activity, assessment of their binding interactions with the target protein, and selection of the metabolite demonstrating the most favorable docking performance as a prospective inhibitor.

This study investigated the antibacterial potential of phytochemicals from *Berberis lycium* Royle against DNA Gyrase B using *in silico* approaches. The phytochemicals were evaluated for drug-likeness and pharmacokinetic properties using Lipinski's Rule of Five and ADMET analysis. Molecular docking and protein–ligand interaction analysis were performed to identify promising lead compounds, which were compared with the reference inhibitor novobiocin.

The docking results demonstrated that all evaluated phytochemicals interacted effectively with the active site of DNA Gyrase B and exhibited favorable binding affinities. Among the investigated compounds, kaempferol showed the strongest binding affinity, with a docking score of -9.2 kcal/mol, slightly surpassing that of the reference inhibitor novobiocin (-9.0 kcal/mol). Quercetin and berberine also exhibited strong interactions with the target protein, whereas palmatine, oxyberberine, and jatrorrhizine displayed comparatively favorable docking scores. These findings highlight the antibacterial potential of *Berberis lycium* Royle phytochemicals and demonstrate the effectiveness of computational approaches in identifying promising lead compounds for antibacterial drug discovery.

Keywords: *Berberis lycium* Royle, DNA Gyrase B, Molecular Docking, Phytochemicals, Antibacterial Activity, ADMET Analysis, Drug-Likeness, *In Silico* Study.

Contents

Author's Declaration	iii
Plagiarism Undertaking	iv
Acknowledgement	v
Abstract	vi
List of Figures	xii
List of Tables	xiii
Abbreviations	xiv
1 Introduction	1
1.1 Selected Plant	5
1.1.1 <i>Berberis lycium</i> Royle	5
1.1.2 Therapeutic Potential of Medicinal Plants: Antimicrobial, Anticancer and Antioxidant Properties	8
1.1.3 Antibiotic Resistance and the Need for Novel Antibacterial Agents	9
1.2 Problem Statement	10
1.3 Scope of Study	10
1.4 Hypothesis	10
1.5 Aim and Objectives	11
2 Literature Review	12
2.1 Therapeutic Significance of Medicinal Plants	12
2.2 Historical Development of Medicinal Plant Use	14
2.3 Plant-Derived Bioactive Compounds with Antimicrobial Activity	15
2.4 Renewed Interest in Plant-Based Antimicrobial Agents	16
2.5 Medicinal Plants Studied in Present Study	18
2.5.1 <i>Berberis lycium</i> Royle	18
2.5.2 Morphological Characteristics	19

2.5.3	Traditional Use	20
2.5.4	Phytochemical Profile and Pharmacological Activities	21
2.5.5	Phytochemistry of <i>Berberis lycium</i>	22
2.5.6	Antibiotic Resistance	22
2.5.7	Antioxidant Activity	24
2.6	Introduction to DNA Gyrase and Biological Significance	24
2.6.1	Structure of DNA Gyrase	25
2.6.2	Negative DNA Supercoiling Mechanism	26
2.6.3	Role in DNA Replication, Transcription and DNA Gyrase B as an Antibacterial Target	28
2.7	Research Gap	29
3	Methodology	31
3.1	Ligand Acquisition	32
3.2	Ligand Optimization	32
3.3	Protein Structure Retrieval	32
3.4	Protein Refinement	33
3.5	Physicochemical Analysis	33
3.6	ADMET and Drug Likeness Assessment	34
3.7	Molecular Docking	34
3.7.1	Components of Molecular Docking	34
3.7.1.1	Ligand	35
3.7.1.2	Receptor	35
3.7.1.3	Binding Site	35
3.8	Ligand Preparation	36
3.9	Protein Preparation	36
3.10	Docking Study Evaluation	37
3.11	Comparison with Reference Ligand	38
4	Results	39
4.1	Protein Selection and Preparation	39
4.1.1	Protein 3D Structure	39
4.1.2	FASTA Sequence	40
4.1.3	Physico-Chemical Properties	41
4.1.4	Functional Domain Analysis	42
4.1.5	Protein Preparation	43
4.2	Ligand Selection and Preparation	44
4.2.1	Physicochemical Properties of Ligands	44
4.2.2	Refinement of Ligand	45
4.2.3	Virtual Screening through Lipinski Rule of Five	46
4.2.4	ADMET Properties	46
4.2.4.1	Absorption	47
4.2.4.2	Distribution	48
4.2.4.3	Metabolism	49
4.2.4.4	Excretion	50

4.2.4.5	Toxicity	51
4.3	Molecular Docking	53
4.4	Interaction Analysis of Protein–Ligands	55
4.4.1	Interaction Pattern of Berberine–DNA Gyrase B	55
4.4.2	Interaction Pattern of Palmatine–DNA Gyrase B	56
4.4.3	Interaction Pattern of Jatrorrhizine–DNA Gyrase B	58
4.4.4	Interaction Pattern of Kaempferol–DNA Gyrase B	59
4.4.5	Interaction Pattern of Quercetin–DNA Gyrase B	60
4.4.6	Interaction Pattern of Oxyberberine–DNA Gyrase B	61
4.5	Lead Compound Identification	62
4.5.1	Drug-Likeness Evaluation	63
4.5.2	ADMET Evaluation	63
4.5.2.1	Absorption	63
4.5.2.2	Distribution Profile	63
4.5.2.3	Metabolism Profile	64
4.5.2.4	Excretion Profile	64
4.5.2.5	Toxicity Profile	64
4.5.3	Docking Results and Interaction Analysis	65
4.6	Drug Selection	65
4.6.1	Novobiocin	65
4.6.2	Structure Retrieval	67
4.6.3	Chemical Information	67
4.6.4	Molecular Properties of Novobiocin	68
4.6.4.1	Absorption	68
4.6.4.2	Distribution	69
4.6.4.3	Metabolism	70
4.6.4.4	Excretion	70
4.6.4.5	Toxicity	71
4.6.5	Drug-Likeness	72
4.6.6	Molecular-Docking Analysis of Novobiocin-DNA Gyrase B	72
4.6.7	Interaction Pattern of Novobiocin-DNA Gyrase B	73
4.7	Novobiocin–Kaempferol Comparison	74
4.7.1	Physicochemical Characteristics Comparative Pattern	74
4.7.2	ADMET Properties Comparison of Novobiocin Kaempferol	75
4.7.2.1	Absorption Comparison	75
4.7.2.2	Distribution Comparison	76
4.7.2.3	Metabolism Comparison	77
4.7.2.4	Excretion Comparison	78
4.7.2.5	Toxicity Comparison	78
4.7.3	Docking Score Comparison	79
4.7.4	Interaction analysis comparison	80

6	Conclusion and Future Recommendations	88
6.1	Conclusion	88
6.2	Future Recommendations	89
	Bibliography	91

List of Figures

2.1	Representative plants of <i>Berberis lycium</i> Royle located in different areas of Azad Jammu and Kashmir (AJK) used in the current study [80].	18
2.2	Schematic representation of the DNA gyrase A ₂ B ₂ complex [105]. .	27
2.3	ATP-dependent catalytic cycle of DNA Gyrase B showing its role in bacterial DNA supercoiling [107].	27
3.1	Research methodology flowchart.	31
4.1	Three-dimensional structure of DNA Gyrase B (PDB ID: 4DUH) retrieved from the Protein Data Bank.	40
4.2	Retrieved amino acid sequence of the human BRCA1 protein (UniProt accession: P38398).	40
4.3	Functional domain analysis of DNA gyrase B predicted using the InterPro database. The analysis identifies the protein family, conserved ATPase domains, HATPase superfamily, and domain architecture across the 220-amino-acid sequence.	41
4.4	Prepared three-dimensional structure of DNA gyrase subunit B . .	43
4.5	Berberine–DNA Gyrase B Interaction–profile.	56
4.6	Palmatine–DNA Gyrase B Interaction–profile.	57
4.7	Jatrorrhizine–DNA Gyrase B interaction–profile.	58
4.8	Kaempferol–DNA Gyrase B interaction–profile.	59
4.9	Quercetin–DNA Gyrase B interaction–profile.	60
4.10	Oxyberberine–DNA Gyrase B interaction–profile.	61
4.11	(3D) structure of novobiocin.	67
4.12	Interaction profile of novobiocin–DNA gyrase B.	74

List of Tables

2.1	Scientific classification of <i>Berberis lycium</i> Royle [79].	18
2.2	Major phytochemicals reported in <i>Berberis lycium</i>	22
4.1	Physicochemical properties of the target protein.	42
4.2	Physicochemical properties and chemical structures of the selected phytochemicals from <i>Berberis lycium</i> Royle (PubChem).	45
4.3	Lipinski Rule of Five properties of ligands.	47
4.4	Absorption properties of ligands.	48
4.5	Distribution properties of ligands.	49
4.6	Metabolism properties of ligands.	50
4.7	Excretion properties of ligands.	51
4.8	Toxicity Properties of Selected Ligands (PkCsm).	52
4.9	Molecular docking results of selected ligands against DNA gyrase B (Part I).	53
4.10	Molecular docking results of selected ligands against DNA gyrase B (Part II).	54
4.11	Physicochemical characteristics of novobiocin.	68
4.12	Absorption properties of novobiocin predicted by pkCSM.	69
4.13	Distribution properties of novobiocin predicted by pkCSM.	69
4.14	Metabolism properties of novobiocin predicted by pkCSM.	70
4.15	Excretion properties of novobiocin predicted by pkCSM.	71
4.16	Toxicity properties of novobiocin predicted by pkCSM.	71
4.17	Drug-likeness evaluation of novobiocin based on Lipinski's Rule of Five.	72
4.18	Docking parameters of novobiocin against DNA gyrase B.	73
4.19	Comparative drug-likeness properties of kaempferol and novobiocin.	75
4.20	Comparative absorption properties of novobiocin and kaempferol.	76
4.21	Comparative distribution properties of novobiocin and kaempferol.	77
4.22	Comparative metabolism properties of novobiocin and kaempferol.	77
4.23	Comparative excretion properties of novobiocin and kaempferol.	78
4.24	Comparative toxicity profiles of novobiocin and kaempferol.	79
4.25	Docking Parameters Comparative Pattern.	80

Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
ATP	Adenosine Triphosphate
BBB	Blood–Brain Barrier
CYP	Cytochrome P450
DHFR	Dihydrofolate Reductase
DNA	Deoxyribonucleic Acid
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HERG	Human Ether-à-go-go-Related Gene
HIA	Human Intestinal Absorption
Lipinski’s RO5	Lipinski’s Rule of Five
LogP	Octanol–Water Partition Coefficient
MD	Molecular Dynamics
MM/GBSA	Molecular Mechanics/Generalized Born Surface Area
MM/PBSA	Molecular Mechanics/Poisson–Boltzmann Surface Area
MW	Molecular Weight
PDB	Protein Data Bank
P-gp	P-glycoprotein
QSAR	Quantitative Structure–Activity Relationship
RMSD	Root Mean Square Deviation
SMILES	Simplified Molecular Input Line Entry System

Chapter 1

Introduction

Plants are remarkable natural reservoirs of biologically active compounds that have played a fundamental role in maintaining human health for centuries. They synthesize a wide variety of secondary metabolites with significant therapeutic potential, making them an invaluable source for the discovery and development of pharmaceutical agents [1]. These medicinal plants contain diverse phytochemicals that exhibit numerous biological activities and serve as the basis for many traditional and modern medicines. The World Health Organization (WHO) reports that over 80% of the world's population still receives primary healthcare from traditional medicine, with medicinal herbs continuing to play a significant role in both illness prevention and treatment [2].

Plant-derived medicines are widely recognized for their therapeutic effectiveness, affordability, cultural acceptance, and comparatively lower incidence of adverse effects than many synthetic drugs [3]. Moreover, the growing prevalence of antimicrobial resistance has renewed scientific interest in medicinal plants as alternative sources of bioactive compounds capable of combating infectious diseases. Consequently, medicinal plants have become an important focus of pharmacological and phytochemical research aimed at identifying novel therapeutic agents.

The use of medicinal plants dates back to the earliest stages of human civilization, when people relied on naturally occurring vegetation to relieve pain, treat

infections, and promote healing. Similar to the instinctive behavior observed in animals that consume specific plants for self-medication, early humans gradually identified beneficial plant species through continuous observation and practical experience [4]. Initially, the selection of medicinal plants was largely based on trial and error because there was little understanding of disease mechanisms or the chemical constituents responsible for therapeutic effects. As scientific knowledge advanced, traditional herbal practices gradually evolved into a more systematic and evidence-based discipline supported by botanical, pharmacological, and chemical investigations. Before the emergence of iatrochemistry during the sixteenth century, medicinal plants represented the principal source of both treatment and disease prevention across many civilizations [5]. Even today, they continue to provide valuable lead compounds for modern drug discovery, highlighting their enduring importance in the development of safer and more effective therapeutic agents.

The use of medicinal plants has been documented since ancient civilizations, reflecting their long-standing importance in the prevention and treatment of human diseases. One of the earliest recorded contributions was made by Hippocrates during the fifth century B.C., who described approximately 300–400 medicinal plants in his medical writings. Likewise, Dioscorides compiled extensive information on the therapeutic applications of numerous plant species in his renowned work *De Materia Medica*, written during the first century B.C., which remained an authoritative reference on medicinal plants for many centuries [6].

Traditional herbal medicine has also been deeply rooted in Chinese civilization for over 5,000 years, where medicinal plants have been extensively utilized for maintaining health and managing a wide range of diseases [7, 8]. Similarly, several aromatic and medicinal plants, including frankincense and myrrh, are mentioned in the Bible because of their antiseptic, healing, and therapeutic properties, highlighting the universal reliance on natural remedies throughout human history [9]. These historical records demonstrate that medicinal plants have consistently served as an integral component of healthcare systems across different cultures and civilizations.

It is estimated that nearly 250,000–500,000 plant species exist worldwide, representing an enormous reservoir of biologically active compounds with diverse pharmacological properties. However, fewer than 10% of these species have been comprehensively investigated for their phytochemical composition and medicinal potential, leaving a substantial proportion of natural biodiversity unexplored [10]. Despite this limitation, countless plant species continue to be used by both humans and animals as sources of food, traditional remedies, and healthcare products.

In recent decades, the popularity of herbal medicine has increased considerably in both developed and developing countries due to growing awareness of the therapeutic potential of natural products, their affordability, and concerns regarding the adverse effects associated with synthetic drugs [11]. Moreover, the increasing prevalence of antimicrobial resistance has encouraged researchers to investigate medicinal plants as alternative sources of novel antibacterial compounds.

Consequently, numerous studies conducted worldwide have demonstrated the antibacterial efficacy and therapeutic potential of herbal medicines, emphasizing their importance in the search for safer and more effective antimicrobial agents [12–16].

The growing interest in herbal medicine is largely attributed to scientific evidence demonstrating that numerous medicinal plants contain biologically active compounds with significant therapeutic potential. These naturally occurring phytochemicals are increasingly regarded as safer alternatives to synthetic drugs, which are often associated with adverse effects on both human health and the environment [17].

Across the world, herbal medicines have been widely employed in the management of a broad spectrum of infectious diseases affecting both humans and animals [18]. Their increasing popularity, particularly in developing countries, is primarily due to their affordability, greater cultural acceptance, compatibility with the human body, and comparatively fewer side effects than conventional pharmaceuticals [9, 19]. In addition, medicinal plants continue to serve as valuable sources of bioactive molecules that contribute to the development of modern therapeutic agents.

Many clinically important drugs, including anticoagulants, laxatives, antibiotics, and antimalarial agents, either originate from or are derived from plant-based compounds. Furthermore, medicinal plants provide structurally diverse lead molecules that play a crucial role in drug discovery and the development of novel pharmaceuticals [20–23]. Besides their antimicrobial applications, herbal medicines have also been investigated for the management of age-related disorders, including impaired immune function, osteoporosis, and memory decline [9]. Numerous studies have reported that medicinal plants possess a wide range of pharmacological properties, functioning as analgesics, antibacterial agents, deodorizers, febrifuges, fungicides, antiseptics, antidepressants, astringents, diuretics, galactagogues, insecticides, antipyretics, antimicrobials, and sedatives, thereby highlighting their broad therapeutic significance in both traditional and modern healthcare systems [24, 25].

Pakistan possesses a rich and diverse flora, comprising approximately 6,000 wild plant species distributed among nearly 1,572 genera. A significant proportion of this botanical diversity is concentrated in the mountainous regions of the Hindukush, Himalaya, and Karakoram, which are recognized for their favorable ecological conditions and abundance of medicinal plant species [26–28]. Owing to this remarkable biodiversity, medicinal plants have long served as an important component of traditional healthcare practices throughout the country. Various ethnobotanical investigations have reported that nearly 600 medicinal plant species are routinely utilized by rural communities in Pakistan for the treatment and management of a wide range of common diseases and health conditions [29]. This extensive use reflects the cultural importance of herbal medicine and the dependence of local populations on naturally available therapeutic resources.

The indigenous communities residing in the northern mountainous regions of Pakistan possess valuable traditional knowledge regarding the identification, distribution, and medicinal applications of native plant species. Their understanding of herbal remedies has been transmitted across generations and continues to play a significant role in primary healthcare. Numerous ethnobotanical surveys conducted in northwestern Pakistan have systematically documented the therapeutic uses of medicinal plants practiced by these communities [30–36]. In particular,

elderly men and women, along with traditional male healers, have preserved extensive knowledge of herbal medicine and continue to employ locally available plants for the treatment of various common ailments and health-related disorders [37].

1.1 Selected Plant

1.1.1 *Berberis lycium* Royle

The genus *Berberis* is one of the largest dicotyledonous genera belonging to the family Berberidaceae. Species within this family are generally characterized as woody, evergreen shrubs bearing thorny branches and spiny leaves. According to recent taxonomic reports, the Berberidaceae family comprises approximately 17 genera and nearly 650 species, many of which possess considerable medicinal importance [38, 39]. The genus *Berberis* has a broad geographical distribution and is naturally found across Asia, Europe, Africa, and the Americas. In Pakistan, *Berberis* species predominantly occur in mountainous regions at elevations ranging from approximately 1,400 to 3,500 m above sea level. Their distribution extends throughout the northern mountainous areas, including Swat, Azad Jammu and Kashmir, Khyber Pakhtunkhwa, Balochistan, and parts of Punjab, where they have long been utilized in traditional and complementary healthcare systems because of their diverse bioactive constituents [38, 39].

Among the members of this genus, *Berberis lycium* Royle occupies a prominent position owing to its extensive medicinal applications in traditional healthcare practices. In Pakistan, it is commonly known as “Kashmal” or “Kasmal” in Urdu, while its English names include Indian barberry, English barberry, and Indian lycium [40]. Indigenous communities, particularly those inhabiting the Kashmir Valley, have traditionally employed this species for treating various ailments, including hemorrhagic dysentery and other gastrointestinal disorders. Every part of the plant, including the roots, stem, bark, leaves, and fruits, is considered therapeutically valuable and has been incorporated into traditional remedies for

generations [41]. The long history of its medicinal use reflects the confidence placed in this species as an important source of natural healing agents.

Traditionally, different parts of *B. lycium* have been used for the management of a wide range of diseases. The bark is regarded as a tonic and anti-periodic agent, whereas the roots are valued for their antipyretic, diaphoretic, and therapeutic properties in the treatment of cyclic fevers. Experimental and ethnopharmacological studies have further demonstrated that *Berberis* species possess hepatoprotective, cardioprotective, anticancer, and antibacterial activities, in addition to providing relief from several gastrointestinal disorders [41].

Stem powder has been traditionally administered for diarrhea and jaundice, root powder has been used to alleviate rheumatic and muscular pain, and herbal infusions prepared from the leaves have been consumed for general health benefits. Furthermore, extracts obtained from different plant parts have been employed in the treatment of intestinal disorders, eye irritation, splenic diseases, cough, sore throat, chest infections, diarrhea, and even fractured bones, highlighting the remarkable therapeutic versatility of this medicinal species [41, 42].

The fruits of *B. lycium* have also received considerable attention because of their broad pharmacological potential. They have been traditionally utilized for the preparation of remedies exhibiting anti-inflammatory, antitumor, hypoglycemic, coagulant, and febrifuge activities. Likewise, the whole plant has been used in indigenous medicine to manage rheumatism, ulcers, jaundice, bone fractures, and ocular disorders [43, 44].

Scientific investigations conducted using animal models have demonstrated that extracts prepared from the fruits, root bark, and stem bark possess multiple biological activities, including regulation of blood glucose levels, improvement of lipid metabolism, and inhibition of cancer cell growth. Additional studies have also reported significant antioxidant, antitumor, antiurolithic, and wound-healing properties, further supporting the traditional medicinal claims associated with this species [41, 45, 46].

Despite its remarkable medicinal importance, *B. lycium* is increasingly threatened in several mountainous regions of Pakistan. In remote hilly areas such as District Shangla, where access to natural gas and alternative fuel sources is limited, the woody stems of the plant are frequently harvested and used as firewood for cooking. Such unsustainable exploitation, coupled with habitat degradation, has contributed to a gradual decline in natural populations. Moreover, the expansion of modern healthcare facilities, educational opportunities, and communication networks has led to a gradual reduction in the transmission of traditional ethnobotanical knowledge among younger generations [40]. Consequently, conserving both the plant and the indigenous knowledge associated with its medicinal applications has become increasingly important to ensure its sustainable utilization in the future.

Phytochemical investigations have revealed that *B. lycium* contains a rich diversity of bioactive constituents, including umbellatine, punjabine, sindamine, chinabine, gilgitine, jhelumine, baluchistanamine, berberine, palmatine, and berbamine. Among these compounds, berberine, an isoquinoline alkaloid predominantly isolated from the roots and stem bark, is recognized as the principal bioactive constituent responsible for many of the plant's pharmacological activities. In addition to berberine, alkaloids such as palmatine have demonstrated significant antioxidant, antimicrobial, and potential anticancer properties in experimental studies [47].

The rich phytochemical composition of *B. lycium* provides a scientific basis for its extensive therapeutic use and supports its continued investigation as a valuable source of novel bioactive compounds. Therefore, renewed scientific interest in this medicinal species is essential not only for its conservation but also for exploring its pharmacological potential through modern approaches such as phytochemical profiling, molecular docking, and antibacterial drug discovery [48, 49].

Furthermore, the documented relationship between its ethnopharmacological uses, phytochemical constituents, and antioxidant capacity strengthens the medicinal significance of *B. lycium* and provides a strong foundation for future investigations into its biological activities and therapeutic applications [45, 50].

1.1.2 Therapeutic Potential of Medicinal Plants: Antimicrobial, Anticancer and Antioxidant Properties

Traditional medicine has served as an important component of healthcare for centuries by providing accessible, economical, and effective therapeutic options with comparatively fewer adverse effects than many synthetic drugs. Much of its therapeutic value originates from medicinal plants, which synthesize a wide range of secondary metabolites as part of their natural defense and survival mechanisms. Although these compounds primarily protect plants against environmental stress and pathogenic attacks, they also exhibit remarkable pharmacological activities that are beneficial to human health. Among these bioactive constituents, phenolic compounds are one of the most abundant and widely distributed groups found in the plant kingdom.

Extensive scientific investigations have demonstrated that these phytochemicals possess diverse biological properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antiallergic, and anticancer activities. Owing to their broad spectrum of therapeutic effects, medicinal plants continue to attract considerable attention as valuable sources of lead compounds for the discovery and development of novel pharmaceuticals and therapeutic agents [51].

Cancer is acknowledged as the second greatest cause of death globally and continues to be one of the most significant public health issues. An estimated 9.6 million deaths worldwide were linked to cancer in 2018, with colorectal, lung, and breast cancers being the most often diagnosed cancers. Traditional medicine and phytochemicals derived from plants have received more attention recently as supplementary methods for treating and preventing cancer.

Clinical findings indicate that the integration of herbal products with conventional therapeutic strategies may improve treatment efficacy, enhance immune function, increase survival rates, and improve the overall quality of life of cancer patients. The therapeutic potential of medicinal plants is largely attributed to their rich phytochemical composition, which includes phenolic compounds, alkaloids, terpenoids,

lignans, and tannins. These naturally occurring constituents exhibit strong antioxidant properties capable of reducing oxidative stress, suppressing the proliferation of cancer cells, and modulating immune responses. Consequently, plant-derived phytochemicals are increasingly being explored as promising candidates for the development of safer and more effective anticancer therapies [52].

1.1.3 Antibiotic Resistance and the Need for Novel Antibacterial Agents

Antibiotic resistance (ADR) has become one of the most significant public health challenges of the twenty-first century. Rather than being a future concern, it is now recognized as a serious global problem affecting people of all ages and regions [53]. The widespread occurrence of antibiotic-resistant bacteria has weakened the effectiveness of many commonly prescribed antibiotics, making the treatment of infectious diseases increasingly difficult in both hospitals and community settings.

As resistance continues to increase, routine medical procedures such as surgery, cancer therapy, and organ transplantation are becoming more challenging because of the higher risk of untreatable bacterial infections. The possibility of entering a post-antibiotic era, in which even minor infections may become life-threatening, further emphasizes the importance of preserving the effectiveness of existing antibiotics [54].

Although antibiotic resistance is a naturally occurring evolutionary process, its development and spread have been greatly accelerated by human activities. The excessive and inappropriate use of antibiotics in human healthcare, livestock production, and aquaculture promotes the selection of resistant bacterial strains. Likewise, inadequate infection prevention and control practices contribute to the continued spread of antibiotic resistance.

To address this global issue, international organizations such as the World Health Organization, the Food and Agriculture Organization, and the World Organisation for Animal Health have implemented strategies that encourage the responsible use

of antibiotics in both human and veterinary medicine and promote measures to reduce the spread of resistant microorganisms [55].

The increasing prevalence of multidrug-resistant bacteria, together with the limited discovery of new antibiotic classes during the past three decades, has created an urgent need to identify alternative therapeutic options.

As a result, medicinal plants have gained renewed scientific attention as potential sources of novel antimicrobial compounds. Herbs and their essential oils have been used traditionally for their antimicrobial properties and continue to be investigated for their potential in the development of new antibacterial agents [56].

1.2 Problem Statement

The growing challenge of antibiotic resistance has highlighted the need for new antibacterial agents. Therefore, this study aims to evaluate the antibacterial potential of phytochemicals from *Berberis lycium* Royle against DNA Gyrase B using an *in silico* approach.

1.3 Scope of Study

The scope of this study is to evaluate the antibacterial potential of *Berberis lycium* extracts, with the aim of identifying bioactive compounds that could lead to the development of new antibiotic drugs, which are urgently needed in the market due to rising antibiotic resistance, especially in regions like Pakistan with limited access to conventional treatments.

1.4 Hypothesis

Extracts from the selected plant *Berberis lycium* will exhibit antibacterial activity.

1.5 Aim and Objectives

This research aims to explore potential antibacterial metabolites from *Berberis lycium* using *in silico* approaches.

- i. To screen *Berberis lycium* metabolites for antibacterial potential using an *in silico* approach.
- ii. To analyze the *in silico* interaction between selected *Berberis lycium* metabolites and the target protein.
- iii. To identify the best-docked *Berberis lycium* metabolite as a potential inhibitor of the target protein through molecular docking analysis.

Chapter 2

Literature Review

2.1 Therapeutic Significance of Medicinal Plants

The plant kingdom is regarded as one of the most valuable natural resources for sustaining life on Earth. Plants fulfill a wide range of human and animal requirements by providing food, medicines, fuel, fibers, shelter, gums, and other economically important products. Their importance extends beyond environmental conservation, as they also represent an indispensable source of therapeutic agents.

Owing to their diverse biological and medicinal properties, plants serve multiple purposes, including nutritional, ornamental, and pharmaceutical applications. Whether they are small shrubs or large evergreen trees, every plant contributes in some way to human welfare. Since ancient times, medicinal and aromatic plants have been highly valued because of their remarkable healing potential and their role in the prevention and treatment of numerous diseases [57].

Medicinal plants comprise a diverse group of species recognized for their therapeutic properties and have remained an integral part of healthcare systems for thousands of years. They contain a wide variety of naturally occurring compounds that continue to provide valuable opportunities for the discovery of new pharmaceutical agents [58]. Different plant parts, including roots, seeds, leaves, fruits,

stems, and sometimes the entire plant, are utilized for medicinal purposes depending on the species and the desired therapeutic effect [59]. The medicinal value of these plants is primarily attributed to their bioactive constituents, commonly referred to as active compounds, which produce specific physiological responses in living organisms. Throughout history, unprocessed plant materials have been extensively used by humans to prevent and treat a variety of diseases and health disorders [60].

Medicinal plants represent one of the oldest forms of healthcare and have been extensively employed in traditional healing systems across different civilizations for centuries. Knowledge regarding their identification, preparation, and therapeutic uses has been preserved and transferred from one generation to another, allowing communities to maintain their long-standing reliance on herbal remedies. This continuous transmission of traditional knowledge has contributed significantly to the sustained use of medicinal plants in primary healthcare practices [61].

The importance of medicinal plants is not limited to traditional medicine, as they have also contributed substantially to the advancement of modern pharmacology. Numerous drugs currently used in clinical practice have originated from medicinal plants or were developed based on compounds initially identified in traditional herbal remedies [62]. Pakistan possesses a rich botanical diversity consisting of approximately 6,000 plant species, of which nearly 600 have been reported to possess medicinal importance [63].

Among these, around 135 species are routinely utilized in traditional healthcare practices throughout the country. Despite this considerable botanical wealth, Pakistan continues to depend on the import of high-value medicinal plants and herbal products. In 2020, the expenditure on these imports was estimated to be approximately USD 216.392 million [64].

Globally, more than 50,000 plant species, representing over 10% of the known flora, are currently utilized in the manufacture of medicinal and cosmetic products [18, 65]. However, these valuable medicinal resources are not uniformly distributed across the world, and a substantial proportion is collected directly from

wild populations. Consequently, the increasing global demand for medicinal plants has resulted in an annual growth of approximately 8–15% in Europe, North America, and Asia over recent decades [66].

2.2 Historical Development of Medicinal Plant Use

The medicinal use of plants dates back to the earliest stages of human civilization and has remained an integral part of healthcare throughout history. Although the exact beginning of herbal medicine is uncertain, available evidence indicates that humans began utilizing and domesticating medicinal plants nearly 60,000 years ago [67]. Some of the oldest documented records of herbal medicine originate from the ancient civilizations of Egypt, China, and India, where medicinal plants were used approximately 5,000 years ago. Similar traditions later became established in Greece and Central Asia around 2,500 years ago, demonstrating the widespread reliance on plant-based remedies across different cultures [68].

In the early stages of civilization, the medicinal use of plants was primarily based on observation and experience, following a process of trial and error similar to the therapeutic use of animal-derived products [69]. At that time, limited knowledge regarding disease mechanisms and the medicinal properties of plants meant that treatment practices relied largely on empirical evidence. As scientific understanding gradually advanced, people began recognizing the relationship between particular plants and their therapeutic effects. Consequently, herbal medicine evolved from an experience-based practice into a more systematic and knowledge-driven approach [70].

One of the earliest written records describing the medicinal use of plants is a Sumerian clay tablet discovered in Nagpur, which dates back nearly 5,000 years [71]. Historical evidence also indicates that the ancient Egyptian and Chinese civilizations extensively utilized medicinal plants as early as the twenty-seventh century BC [72]. In ancient Greece, renowned scholars such as Hippocrates and

his student Aristotle incorporated medicinal plants into their medical practices, while Theophrastus established a specialized School of Medicinal Plants that contributed significantly to botanical knowledge [26]. Later, the physician Pedanius Dioscorides, who lived between approximately 75 and 45 BC, compiled the influential encyclopedia *De Materia Medica*, in which the medicinal properties of more than 600 plant species were systematically documented. This work became one of the earliest scientific references on herbal medicine and laid the foundation for future studies on medicinal plants [26, 73].

2.3 Plant-Derived Bioactive Compounds with Antimicrobial Activity

Berberis lycium Royle is a medicinal plant well known for its rich phytochemical composition, particularly its abundance of bioactive alkaloids and phenolic compounds that contribute to its antimicrobial properties. Among these phytochemicals, berberine is considered the principal and most pharmacologically important isoquinoline alkaloid. Berberine has demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria through multiple mechanisms, including inhibition of bacterial DNA replication, disruption of cell division, suppression of biofilm formation, and interference with efflux pump activity [74].

Another major alkaloid, palmatine, also exhibits significant antibacterial, antifungal, and anti-inflammatory activities by increasing bacterial membrane permeability and enhancing the effectiveness of conventional antibiotics. Jatrorrhizine, a protoberberine alkaloid structurally related to berberine, has been reported to possess antimicrobial and antioxidant properties by inhibiting bacterial growth and reducing oxidative stress. Oxyberberine, an oxidized derivative of berberine, has attracted increasing attention due to its potent antibacterial and anti-inflammatory activities against several clinically important pathogenic microorganisms [74, 75].

In addition to alkaloids, *Berberis lycium* contains several flavonoids that contribute to its antimicrobial potential. Quercetin and kaempferol are two important flavonols identified in the plant and are recognized for their broad-spectrum antibacterial activity. These compounds inhibit bacterial nucleic acid synthesis, interfere with essential metabolic enzymes, disrupt microbial cell membranes, and prevent biofilm formation, thereby reducing bacterial survival and pathogenicity. Furthermore, the plant contains a variety of terpenoids, phenolic compounds, and other flavonoids, which collectively enhance its antimicrobial efficacy [76].

Phenolic compounds exert their antibacterial effects by damaging bacterial cell walls, denaturing cellular proteins, and scavenging reactive oxygen species, while terpenoids disrupt membrane integrity and inhibit microbial metabolism. The synergistic action of these diverse phytochemicals is believed to be responsible for the remarkable antimicrobial, antioxidant, anti-inflammatory, and therapeutic potential of *Berberis lycium* Royle, making it an important medicinal plant for the discovery of novel antibacterial agents and an excellent candidate for *in silico* drug discovery studies.

2.4 Renewed Interest in Plant-Based Antimicrobial Agents

Herbal medicines have gained renewed global attention and are increasingly being accepted in both developed and developing countries as valuable therapeutic alternatives [11]. This growing interest has encouraged extensive scientific research into the antimicrobial potential of medicinal plants [12, 16]. Numerous botanical species, including yarrow, clove, and rosemary, have been investigated for their antimicrobial activities, and many have demonstrated encouraging results against a variety of microbial pathogens [17]. These findings emphasize the potential of medicinal plants as natural sources of antimicrobial compounds for the management of infectious diseases.

Several medicinal plants have been reported to exhibit antibacterial activity, particularly through their essential oils, which are widely used in herbal medicine. Among these, the essential oils obtained from thyme and cinnamon, especially those containing carvacrol as a major active constituent, have shown remarkable antimicrobial effectiveness [77]. The increasing demand for herbal medicines is largely attributed to the presence of biologically active compounds that offer a safer alternative to synthetic drugs, which may adversely affect both the environment and biological systems. For centuries, herbal remedies have been used worldwide for the treatment of infectious diseases in both humans and animals [18]. Their affordability, cultural acceptance, compatibility with the human body, and relatively fewer side effects have made them particularly popular in developing countries [9, 19].

In addition to their role in antimicrobial therapy, herbal medicines are increasingly being explored for managing age-related health conditions, including impaired immune function, memory loss, and osteoporosis. Many modern pharmaceutical products, such as laxatives, anticoagulants, antibiotics, and antimalarial drugs, also contain active compounds derived from medicinal plants. Furthermore, medicinal plants continue to serve as valuable sources of lead molecules for drug discovery and development [20, 43]. Plant-derived volatile oils have attracted considerable attention because of their diverse therapeutic properties, including analgesic, antimicrobial, deodorizing, and mood-enhancing effects [24, 25].

Curcumin, the principal bioactive constituent of turmeric, has been reported to inhibit biofilm formation by *Helicobacter pylori* under *in vitro* conditions. However, prolonged incubation may enable the bacterium to regain its ability to produce biofilms [78]. Owing to the broad therapeutic potential of medicinal plants, continued research is required to identify alternative antimicrobial agents for the treatment of infectious diseases. Particular emphasis should be placed on investigating native medicinal plants for their antibacterial compounds, as well as identifying phytochemicals capable of enhancing the activity of existing antibiotics through synergistic interactions, inhibition of bacterial efflux pumps, or suppression of bacterial enzymes responsible for antibiotic degradation.

2.5 Medicinal Plants Studied in Present Study

Berberis lycium Royle was selected for this study because of its traditional medicinal importance. The research aims to evaluate the antibacterial potential of its phytochemicals using an *in silico* approach. Taxonomic classification is shown in Table 2.1.

TABLE 2.1: Scientific classification of *Berberis lycium* Royle [79].

Taxonomic Category	Scientific Classification
Kingdom	Plantae
Phylum (Division)	Tracheophyta
Class	Magnoliopsida
Order	Ranunculales
Family	Berberidaceae
Genus	<i>Berberis</i>
Species	<i>Berberis lycium</i>

2.5.1 *Berberis lycium* Royle



FIGURE 2.1: Representative plants of *Berberis lycium* Royle located in different areas of Azad Jammu and Kashmir (AJK) used in the current study [80].

The Berberidaceae family comprises a diverse group of flowering plants (angiosperms) that are widely distributed across the world, particularly throughout the Northern Hemisphere shown in Fig 2.1. According to current taxonomic records, this family includes approximately 17 genera and nearly 650 species. Among its important members is *Berberis lycium* Royle, a medicinal shrub native to the Himalayan region, especially Nepal. Its natural distribution, however, is not limited to the Himalayas, as the species is also found in temperate and subtropical regions across the world, with the exception of Australia.

Within the Himalayan range, *B. lycium* extends from Kashmir in the western region to Uttarakhand in the east, occupying the outer northern and western Himalayan zones [79]. The species demonstrates remarkable ecological adaptability and is commonly found in a variety of habitats, including forest margins, open woodlands, and roadsides. It is capable of growing successfully in different soil types, such as clay, loam, sandy, and even nutrient-deficient soils, reflecting its ability to thrive under diverse environmental conditions [41].

2.5.2 Morphological Characteristics

The plant is a little shrub that usually grows to a height of 1.0 to 2.5 meters. A thin layer of brittle bark covers its sturdy, woody branches [43]. The lanceolate or slightly obovate-oblong leaves are coriaceous. They are light and glaucous on the lower side and dull green on the top. On the stem of the plant, there are a few prominent, alternating spinous teeth [79]. *Berberis lycium* is a particularly useful plant having both culinary and medical applications. This little shrub usually grows to a height of 1.0 to 2.5 meters.

Insects pollinate its flowers, which are grouped in lovely clusters known as corymbose racemes and have 11 to 16 pale yellow blooms each (usually bisexual). The plant is adorned throughout the flowering season, which lasts from March to July. The first blooms appear in early March, and full flowering occurs by April. Fruit development comes next, peaking in June after beginning to ripen in mid-May. Locals enjoy these mature, nutrient-rich, gorgeous bluish-purple fruits in a variety

of ways. They are typically eaten raw or added to foods, drinks, jellies, and even preservatives [41].

2.5.3 Traditional Use

Berberis lycium Royle has long been recognized not only as an ornamental shrub but also as an important medicinal plant in traditional healthcare systems across different regions [80]. Almost every part of the plant has been utilized for therapeutic purposes. In the Kashmir region, indigenous communities have traditionally used its roots for the treatment of severe dysentery [41]. The powdered roots are commonly employed to relieve muscular and rheumatic pain, whereas the leaves are prepared as herbal tea and the stem powder is traditionally administered for the management of jaundice and diarrhea.

Furthermore, extracts prepared from various parts of the plant have been used to treat spleen disorders, cough, respiratory and throat infections, digestive disturbances, diarrhea, eye irritation, and fractured bones, demonstrating its broad range of traditional medicinal applications [80, 81].

The fruits of *Berberis lycium* have also been valued for their diverse therapeutic properties, including coagulant, anti-inflammatory, antihyperglycemic, and anti-cancer activities. Traditionally, local communities have used the entire plant to manage ulcers, rheumatism, jaundice, eye disorders, and bone fractures [43, 82]. Experimental studies conducted using animal models have further demonstrated that extracts obtained from the fruits, root bark, and stem bark possess beneficial biological activities, including antihyperglycemic, antihyperlipidemic, anti-urolithic, anticancer, anthelmintic, and wound-healing effects [41, 45, 46].

In addition, different parts of the plant have been reported to exhibit antiseptic, diuretic, carminative, diaphoretic, expectorant, and antipyretic properties, further supporting its significance as a valuable medicinal species with diverse therapeutic applications [83, 84].

2.5.4 Phytochemical Profile and Pharmacological Activities

Berberis lycium synthesizes a diverse array of bioactive secondary metabolites, including tannins, saponins, and various alkaloids [85]. Among these components, the isoquinoline alkaloid berberine, which is primarily isolated from the roots and bark of the plant, represents its most significant pharmacological constituent [80]. Evidence from both animal models and clinical trials indicates that berberine therapy enhances myocardial contractility, mitigates ischemia-induced ventricular tachyarrhythmias, and reduces both systemic blood pressure and peripheral vascular resistance [86]. Beyond these cardiovascular effects, *B. lycium* has demonstrated a broad spectrum of biomedical applications, including antioxidant, hepatoprotective, antihyperlipidemic, wound-healing, antimicrobial, and pesticidal activities [43].

Historically, this herb has also been utilized in traditional medicine to manage ophthalmic, cutaneous, respiratory, hepatic, and gastrointestinal disorders. However, due to its exceptional nutritional and therapeutic value, *B. lycium* is currently experiencing severe commercial overexploitation, which poses a critical threat to its survival in the wild. This vulnerability is further compounded by anthropogenic habitat destruction, such as deforestation, infrastructure development, and the encroachment of agricultural land into mountainous ecosystems, all of which are accelerating population declines [87]. Consequently, the immediate implementation of targeted conservation strategies is essential to preserve *B. lycium* from further ecological degradation.

As the primary isoquinoline alkaloid found within the species, berberine serves as the principal driver of the plant's medical efficacy. Rigorous evaluation of this isolated compound has confirmed its potent anti-inflammatory, antineoplastic, antioxidant, and broad-spectrum antibacterial characteristics, which ultimately account for the extensive pharmacological profile exhibited by *B. lycium* Table 2.2 [88].

2.5.5 Phytochemistry of *Berberis lycium*

TABLE 2.2: Major phytochemicals reported in *Berberis lycium*.

Plant Part	Major	Phytochemi- cals	Biological Properties	Ref
Root bark	Berberine, Jatrorrhizine,	Palmatine, Berbamine	Antibacterial, antioxi- dant, anti-inflammatory, hepatoprotective, anti- cancer	[44, 89]
Roots	Berberine, tine, β -Sitosterol, Tannins, Saponins	Palma- Jatrorrhizine, Flavonoids,	Antibacterial, antifun- gal, antioxidant, wound healing	[44, 85, 90]
Stem bark	Berberine, Berbamine	Palmatine,	Antimicrobial, antioxi- dant	[89, 91]
Leaves	Quercetin, Flavonoids, compounds	Kaempferol, Phenolic	Antioxidant, antibacte- rial, anti-inflammatory	[89, 92]
Fruits	Berberine, cyanins, Phenolic Vitamin C, Flavonoids	Antho- acids,	Antioxidant, antimicro- bial, cardioprotective	[91, 93]
Whole plant	Berberine, Jatrorrhizine, berine, Quercetin, Flavonoids, compounds	Palmatine, Oxyber- Kaempferol, Terpenoids, Phenolic	Broad-spectrum antibac- terial, antioxidant, anti- cancer, antidiabetic, hep- atoprotective	[44, 89, 91, 94]

2.5.6 Antibiotic Resistance

Modern antibacterial chemotherapy originated in the late 1920s and 1930s, anchored by Fleming's 1928 discovery of penicillin and the subsequent introduction

of sulfonamides [95]. By the early 1940s, penicillin witnessed widespread clinical implementation. This momentum culminated in the 1950s, initiating a “golden era” of antibiotic discovery and deployment that saw the introduction of numerous novel antimicrobial classes, a period of robust innovation that sustained clinical medicine until the 1970s [96]. In the subsequent decades, however, the steady escalation of antimicrobial resistance has precipitated a severe global health crisis.

This emergency is primarily driven by the systemic misuse and overprescription of existing antibiotics, compounded by stagnation in innovative drug discovery within the pharmaceutical sector [97]. By 2023, the global ubiquity of multi-drug resistant pathogens had triggered widespread international alarm. This alarming trajectory is further aggravated by a depletion in the pipeline of novel antibiotic classes, resulting in what is now widely termed the “antibacterial crisis” [98].

Berberis lycium is highly regarded as a foundational medicinal plant due to its complex matrix of naturally occurring phytochemicals that exhibit potent biological efficacy. The species’ robust antioxidant capacity is primarily mediated by key bioactive constituents, including alkaloids like berberine, palmatine, and jatrorrhizine, alongside quercetin, kaempferol, phenolic acids, flavonoids, tannins, and anthocyanins.

These compounds neutralize oxidative stress by directly scavenging reactive oxygen species (ROS), terminating destructive lipid peroxidation chain reactions, and shielding vital cellular macromolecules from oxidative degradation. Beyond the discrete therapeutic mechanisms of individual constituents, complex synergistic interactions among these varied phytochemicals significantly amplify the collective antioxidant efficacy of *B. lycium*. This molecular synergy validates its historical integration into traditional medicine and underscores its therapeutic viability in treating diseases mediated by oxidative stress.

The escalating threat of antibiotic resistance has revitalized scientific interest in medicinal plants as prospective reservoirs for novel antimicrobial agents. Botanicals synthesize an abundance of structurally diverse secondary metabolites, including phenolics, alkaloids, flavonoids, and terpenoids. Accumulating empirical

evidence indicates that many of these natural compounds possess potent antimicrobial properties, positioning them as highly promising candidates for the development of next-generation antibiotics [99].

2.5.7 Antioxidant Activity

Berberis lycium, commonly referred to as barberry, is recognized for its high content of phytochemicals capable of scavenging free radicals, highlighting its potential as a valuable natural antioxidant source [100]. Several studies have validated this antioxidant potential, including the work of Ahmed and Shakeel [101], who reported remarkable free radical scavenging activity in various extracts of *B. lycium*. The exceptional antioxidant capacity of *Berberis lycium* Royle is driven by a complex matrix of polar phenolics, flavonoids, anthocyanins, and isoquinoline alkaloids—chiefly berberine, palmatine, and jatrorrhizine—concentrated heavily within its root bark, leaves, and fruits. These bioactive constituents act synergistically to mitigate oxidative stress by directly scavenging reactive oxygen and nitrogen species, halting destructive lipid peroxidation chain reactions, and chelating transition metal ions. Furthermore, *in vivo* evaluations demonstrate that *B. lycium* extracts actively upregulate the expression of critical endogenous antioxidant enzymes, including superoxide dismutase, catalase, and reduced glutathione, while simultaneously reducing malondialdehyde levels. This multi-pathway mechanism shields vital cellular membranes, proteins, and DNA from oxidative degradation, providing a rigorous molecular validation for the plant's traditional use in managing hepatic, cardiovascular, and inflammatory disorders [102].

2.6 Introduction to DNA Gyrase and Biological Significance

DNA gyrase is a bacterial type II topoisomerase that plays an indispensable role in regulating DNA topology and maintaining chromosome integrity. It is the only

known bacterial enzyme capable of introducing ATP-dependent negative supercoils into DNA, a process essential for relieving torsional stress generated during DNA replication and transcription [103]. By controlling DNA supercoiling, the enzyme facilitates strand separation, supports efficient gene expression, and preserves chromosome stability. Inhibition of DNA gyrase results in the accumulation of positive supercoils, leading to impaired DNA replication, defective transcription, disrupted chromosome segregation, and ultimately bacterial cell death [104, 105].

Structurally, DNA gyrase functions as an A_2B_2 heterotetramer consisting of two GyrA and two GyrB subunits. GyrA mediates DNA cleavage and religation, whereas GyrB contains the ATP-binding and ATPase domains that provide the energy required for catalytic activity through ATP hydrolysis. The coordinated action of these subunits enables transient cleavage of DNA, passage of a second DNA segment through the break, and subsequent religation, thereby maintaining appropriate DNA topology without compromising chromosome integrity [105].

Since its discovery in the 1970s, DNA gyrase has been recognized as an essential enzyme for bacterial survival and an attractive target for antibacterial drug development. Several antibacterial agents, including fluoroquinolones and coumarin derivatives, exert their effects by inhibiting different functional regions of DNA gyrase. More recently, the ATPase domain of the GyrB subunit has gained considerable attention because inhibition of ATP hydrolysis effectively blocks DNA supercoiling and suppresses bacterial growth. The absence of an equivalent ATP-dependent gyrase in human cells further enhances the selectivity of GyrB-directed inhibitors, making DNA Gyrase B one of the most promising targets for molecular docking and antibacterial drug discovery [106].

2.6.1 Structure of DNA Gyrase

DNA gyrase is a heterotetrameric enzyme composed of two GyrA and two GyrB subunits, each contributing distinct functions to the catalytic cycle. The GyrA subunit contains the catalytic tyrosine residue responsible for reversible DNA

cleavage and religation, as well as a C-terminal DNA-wrapping domain that enables efficient introduction of negative supercoils. In contrast, the GyrB subunit contains the highly conserved Bergerat ATP-binding fold and ATPase domain, which hydrolyzes ATP to provide the energy required for DNA strand passage and conformational changes during catalysis [103]. The enzyme operates through three coordinated functional gates: the N-gate, DNA-gate, and C-gate. ATP binding to the GyrB ATPase domains induces closure of the N-gate, allowing capture of the transported DNA segment (T-segment). The DNA-gate then generates a transient double-stranded break in the gate DNA segment (G-segment), permitting passage of the T-segment before rapid religation of the DNA. Finally, the transported DNA exits through the C-gate, completing one catalytic cycle and introducing negative supercoils into the chromosome [104].

The ATP-binding pocket of GyrB is highly conserved among numerous pathogenic bacteria and has been extensively characterized through X-ray crystallography and cryo-electron microscopy. These structural studies have identified a well-defined ligand-binding cavity that accommodates ATP as well as ATP-competitive inhibitors such as novobiocin and coumermycin. Owing to its structural conservation, essential biological function, and minimal similarity to human enzymes, the ATPase domain of GyrB represents an ideal target for structure-based drug design, molecular docking, and the identification of novel antibacterial compounds from natural products, including phytochemicals isolated from *Berberis lycium* Royle [105]. Schematic diagram of DNA Gyrase A₂B₂ complex is shown in Figure 2.2.

2.6.2 Negative DNA Supercoiling Mechanism

Under normal conditions, ATP binds to the GyrB subunit, initiating conformational changes that enable DNA cleavage, strand passage, ATP hydrolysis, DNA religation, and the introduction of negative supercoils required for DNA replication, transcription, repair, and chromosome segregation. When ATP binding or hydrolysis is inhibited by antibacterial agents or bioactive phytochemicals, the

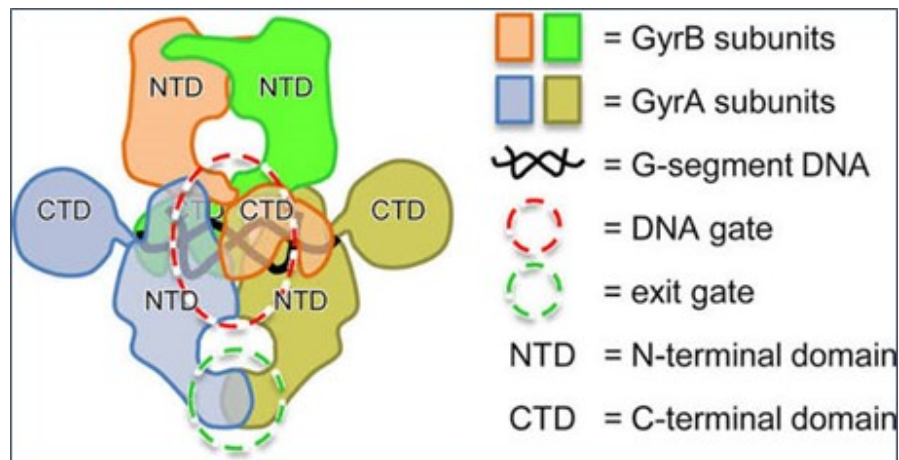


FIGURE 2.2: Schematic representation of the DNA gyrase A_2B_2 complex [105].

catalytic cycle is interrupted, preventing DNA supercoiling and blocking essential bacterial cellular processes. Consequently, bacterial growth is inhibited, ultimately leading to cell death [106].

This mechanism highlights DNA Gyrase B as an attractive molecular target for the development of novel antibacterial compounds, including phytochemicals derived from *Berberis lycium* Royle Fig2.3.

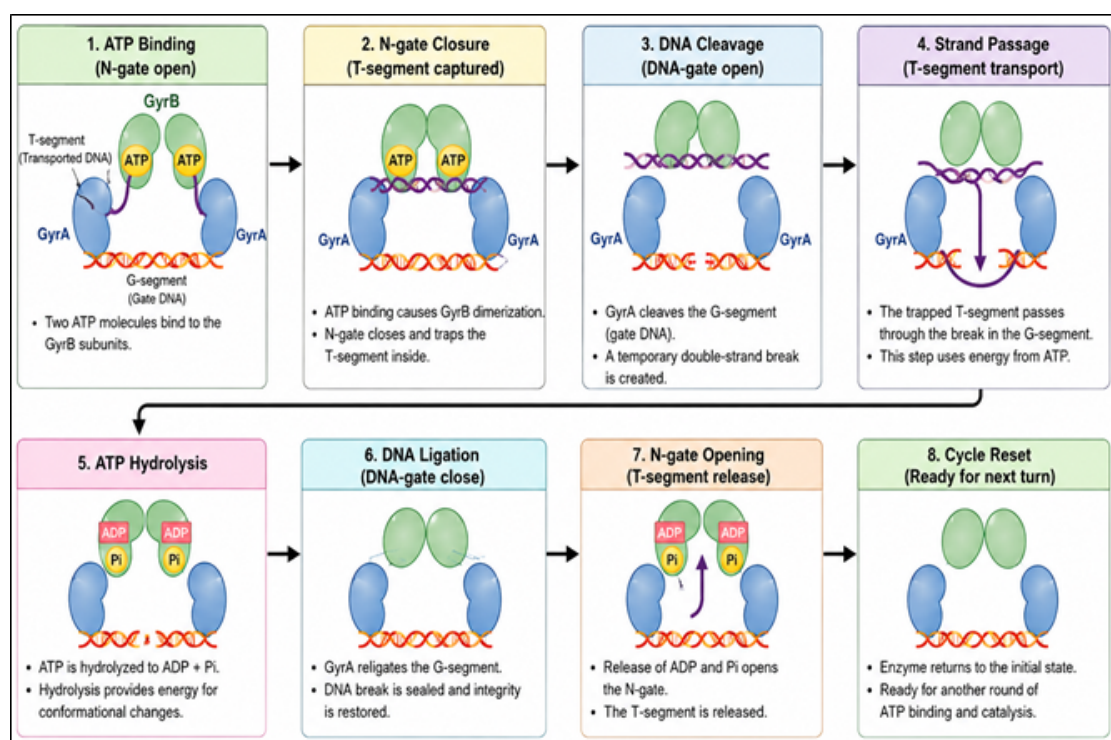


FIGURE 2.3: ATP-dependent catalytic cycle of DNA Gyrase B showing its role in bacterial DNA supercoiling [107].

2.6.3 Role in DNA Replication, Transcription and DNA Gyrase B as an Antibacterial Target

DNA gyrase is indispensable for bacterial chromosome duplication because it continuously regulates DNA topology during genome replication. As the replication machinery unwinds the double-stranded DNA, positive supercoils accumulate ahead of the replication fork, creating mechanical tension that can obstruct DNA polymerase movement. DNA gyrase relieves this topological stress by introducing negative supercoils in an ATP-dependent manner, thereby ensuring smooth progression of DNA synthesis and successful chromosome duplication. Besides replication, the enzyme also contributes significantly to transcription by maintaining an appropriate level of DNA supercoiling during RNA synthesis.

The movement of RNA polymerase alters DNA topology, and gyrase counteracts these changes to facilitate uninterrupted gene expression. In addition, the enzyme participates in chromosome segregation, homologous recombination, and DNA repair, making it essential for preserving bacterial genome stability. Since these biological processes are crucial for bacterial survival, inhibition of DNA gyrase disrupts several cellular functions simultaneously, ultimately preventing bacterial proliferation and leading to cell death [107].

The GyrB subunit has become a preferred molecular target for antibacterial drug discovery because it contains a highly conserved ATP-binding domain that supplies the energy required for DNA supercoiling. Unlike the catalytic GyrA subunit, GyrB possesses a well-defined ATPase pocket that is particularly suitable for molecular docking and structure-based drug design. Numerous crystallographic studies have characterized this binding site, enabling accurate prediction of ligand–protein interactions and facilitating the discovery of ATP-competitive inhibitors [103, 108].

In this context, phytochemicals isolated from *Berberis lycium* Royle, including berberine, palmatine, jatrorrhizine, oxyberberine, quercetin, and kaempferol, have attracted considerable interest because of their structural diversity and biological

activity. These natural compounds are capable of establishing hydrogen bonds, hydrophobic interactions, and aromatic stacking with amino acid residues within the ATP-binding pocket of GyrB. Such interactions may interfere with ATP binding or ATP hydrolysis, thereby interrupting the catalytic cycle of DNA gyrase. As a consequence, DNA supercoiling is impaired, resulting in inhibition of DNA replication and transcription, suppression of bacterial growth, and eventual bacterial cell death. These characteristics highlight the therapeutic potential of *B. lycium* phytochemicals as promising candidates for the development of novel antibacterial agents targeting DNA Gyrase B [109].

2.7 Research Gap

Despite the availability of numerous antibiotics, the rapid emergence of multidrug-resistant bacterial pathogens has significantly reduced the effectiveness of conventional antimicrobial therapies, creating an urgent need for the discovery of novel antibacterial agents. Although several studies have reported the antimicrobial and pharmacological activities of *Berberis lycium* Royle, most investigations have focused on crude extracts, isolated compounds, or general biological activities such as antioxidant, anti-inflammatory, and anticancer effects. Comparatively fewer studies have explored the antibacterial potential of its phytochemicals using computational approaches, particularly molecular docking against essential bacterial target proteins.

Similarly, while DNA Gyrase B has been extensively recognized as an important antibacterial target because of its indispensable role in DNA replication and transcription, limited information is available regarding the interaction of *B. lycium*-derived phytochemicals with the ATP-binding pocket of this protein. Therefore, additional *in silico* investigations are required to evaluate the binding affinity, molecular interactions, and inhibitory potential of these naturally occurring compounds. Such studies may facilitate the identification of promising lead molecules

for the development of safer and more effective antibacterial agents against drug-resistant bacterial pathogens [104, 106].

The literature reviewed in this chapter demonstrates that *Berberis lycium* Royle is a valuable medicinal plant containing diverse phytochemicals, including alkaloids, flavonoids, phenolic compounds, and terpenoids, which exhibit considerable pharmacological and antimicrobial potential. Previous studies have confirmed the antibacterial activity of several major constituents, particularly berberine, palmatine, jatrorrhizine, quercetin, kaempferol, and oxyberberine. The review also highlights the biological importance of DNA Gyrase B as an essential bacterial enzyme involved in DNA supercoiling, replication, and transcription, making it an attractive target for antibacterial drug discovery. Owing to its highly conserved ATP-binding domain and suitability for structure-based drug design, DNA Gyrase B provides an ideal protein target for molecular docking studies. Based on the findings presented in this chapter, the present study employs an *in silico* approach to investigate the binding interactions and antibacterial potential of selected phytochemicals from *Berberis lycium* Royle against DNA Gyrase B.

Chapter 3

Methodology

The methodology flowchart is represented in Fig 3.1

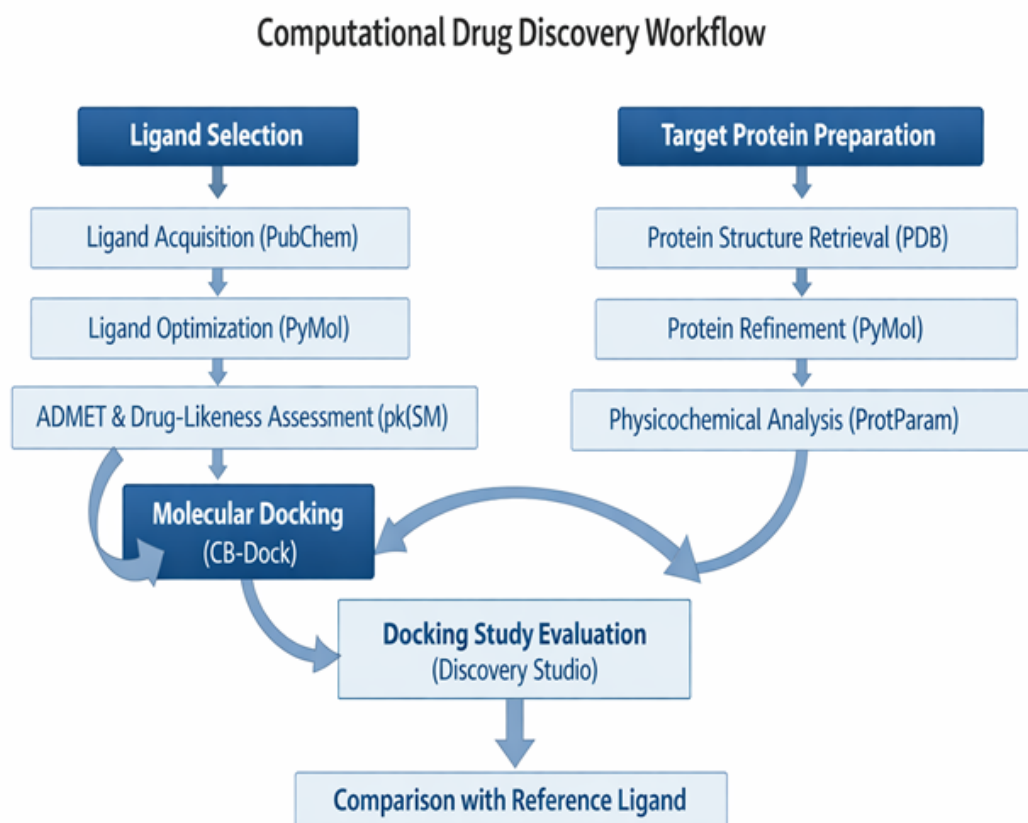


FIGURE 3.1: Research methodology flowchart.

3.1 Ligand Acquisition

Ligand acquisition marks the starting point of computational drug discovery, where candidate molecules are collected from the PubChem database. This resource provides extensive chemical information, including 3D molecular structures, formulas, and physicochemical attributes. The retrieved files, typically in formats such as SDF or MOL, serve as the foundation for further computational refinement.

This step ensures that the chosen ligands are authentic, well documented, and suitable for downstream analysis, thereby establishing a reliable basis for docking experiments.

3.2 Ligand Optimization

Following acquisition, ligands undergo optimization using PyMol, a molecular visualization and editing platform. This process involves energy minimization to eliminate steric clashes and adjust bond angles, resulting in a stable and biologically relevant conformation.

The optimized structure reflects the lowest energy state of the molecule, which is essential for accurate docking simulations. PyMol enhances the precision of ligand protein interactions and ensures computational stability. This step is critical for producing realistic models that can effectively predict binding behavior in drug discovery studies.

3.3 Protein Structure Retrieval

The process of protein structure retrieval begins with accessing the Protein Data Bank (PDB), which serves as a global repository of experimentally validated biomolecular structures. The downloaded PDB files contain atomic coordinates that define the three dimensional arrangement of the protein, which is essential

for computational docking studies. This step ensures that the receptor model is authentic, scientifically validated and suitable for interaction analysis. By using PDB, researchers guarantee that the protein structures employed in simulations are accurate and reliable for drug discovery applications.

3.4 Protein Refinement

Once retrieved, the raw protein structures undergo refinement using PyMol, a molecular visualization and editing platform. This process involves removing water molecules, heteroatoms, and unnecessary chains that may interfere with docking simulations. Hydrogen atoms are added to stabilize the protein and ensure proper bonding interactions [110].

The refined structure is then saved in PDB format, making it compatible with docking tools. PyMol's visualization features allow researchers to closely examine the protein's binding regions and confirm structural accuracy. This refinement step is critical as it produces a clean, biologically relevant protein model, thereby improving the precision and reliability of subsequent docking experiments.

3.5 Physicochemical Analysis

Physicochemical analysis is carried out using ProtParam, a web based computational tool that calculates essential biochemical parameters of the target protein. These include molecular weight, theoretical isoelectric point (pI), instability index, and aliphatic index, which collectively provide insights into the protein's stability and solubility under physiological conditions. By evaluating these properties, researchers can determine whether the protein is structurally stable and suitable for docking simulations.

ProtParam thus validates the protein's compatibility for computational studies and highlights its biochemical characteristics that may influence ligand binding.

This step ensures that the receptor model is not only structurally accurate but also functionally relevant for drug discovery applications.

3.6 ADMET and Drug Likeness Assessment

ADMET profiling represents a crucial stage in computational drug discovery, as it predicts how a compound behaves inside the human body. Using pkCSM, an online predictive platform, researchers evaluate pharmacokinetic parameters and drug likeness through molecular descriptors. Key properties such as intestinal absorption, blood brain barrier penetration, hepatotoxicity, and skin sensitization are assessed to ensure safety and efficacy.

This step also verifies compliance with Lipinski’s Rule of Five, confirming the compound’s potential as an orally active drug [111]. By filtering out molecules with poor bioavailability or high toxicity, pkCSM ensures that only the most promising candidates progress to docking simulations.

3.7 Molecular Docking

Molecular docking is performed using CB Dock 2, an automated server designed to predict ligand protein interactions. The tool identifies potential binding pockets and executes docking simulations. Results include binding affinity scores, interaction residues, and 3D visualizations of the ligand protein complex [112]. This analysis reveals how effectively a compound can interact with proteins, providing insights into its therapeutic relevance. By highlighting strong binding interactions and favorable docking scores, CB Dock 2 helps prioritize phytochemicals with the highest potential for therapeutic drug development.

3.7.1 Components of Molecular Docking

Molecular docking studies involve three major components:

3.7.1.1 Ligand

A ligand is the molecule that is evaluated for its biological activity during the docking process. Ligands can include:

- i. Natural products
- ii. Phytochemicals
- iii. Synthetic compounds
- iv. Drug molecules
- v. Peptides

In the present study, phytochemicals isolated from *Berberis lycium* Royle were selected as ligands to investigate their interactions with the DNA Gyrase B protein through molecular docking.

3.7.1.2 Receptor

The receptor is the target protein with which the ligand interacts during molecular docking. It usually contains one or more active regions that allow small molecules to bind and form stable interactions [112]. In this study, DNA Gyrase B was chosen as the receptor protein for docking analysis.

3.7.1.3 Binding Site

The binding site is the specific region of the receptor where the ligand binds. It is composed of amino acid residues that are important for molecular recognition and protein function [112]. Correct identification of the binding site is essential because it helps produce accurate and reliable docking results.

3.8 Ligand Preparation

Ligand preparation is a fundamental step in molecular docking because the reliability of docking results depends greatly on the quality of the ligand structures used. Before docking analysis, each selected phytochemical must be carefully processed to ensure that its chemical structure accurately represents its biologically active form. Proper ligand preparation minimizes structural errors and improves the prediction of ligand–protein interactions.

The process generally begins with retrieving the chemical structures of the selected phytochemicals from publicly available chemical databases such as PubChem. These structures are typically available in two-dimensional (2D) format and are subsequently converted into three-dimensional (3D) models suitable for docking studies.

The generated 3D structures undergo energy minimization to obtain the most stable molecular conformation. Additional preparation steps include the addition of hydrogen atoms, optimization of bond lengths and bond angles, correction of bond orders, and assignment of appropriate atomic charges.

Well-prepared ligands improve the accuracy of docking simulations by ensuring that each molecule adopts a realistic conformation before interacting with the target protein. In the present study, phytochemicals isolated from *Berberis lycium* Royle, including berberine, palmatine, berbamine, jatrorrhizine, and oxyacanthine, were considered potential ligands for evaluating their binding affinity against DNA Gyrase B.

3.9 Protein Preparation

Protein preparation is another critical stage of molecular docking because the quality of the receptor structure directly influences the accuracy of docking predictions. A properly prepared protein provides a reliable representation of the biological target and allows more accurate evaluation of ligand–protein interactions.

The three-dimensional crystal structure of the target protein is generally obtained from the Protein Data Bank (PDB), which serves as a comprehensive repository of experimentally determined protein structures. Before molecular docking, the protein undergoes several preprocessing steps to eliminate structural inconsistencies that may interfere with docking analysis.

These procedures typically involve the removal of crystallographic water molecules, elimination of co-crystallized ligands or other non-essential molecules, addition of missing hydrogen atoms, correction of incomplete amino acid residues where necessary, assignment of appropriate atomic charges, and energy minimization to stabilize the protein structure.

Since the objective of this study is to identify potential inhibitors of DNA Gyrase B, particular attention is focused on the ATP-binding pocket of the enzyme. This highly conserved active site plays an essential role in ATP hydrolysis and bacterial DNA supercoiling, making it one of the most suitable regions for evaluating the binding interactions of *Berberis lycium* phytochemicals through molecular docking.

3.10 Docking Study Evaluation

Docking evaluation is performed using Discovery Studio Visualizer, which provides a detailed examination of ligand–protein interactions. The software highlights hydrogen bonding, hydrophobic contacts, and binding energies, allowing researchers to assess the strength and specificity of molecular interactions.

It also enables comparison of multiple docked complexes and identification of critical amino acid residues involved in binding. This analysis confirms whether the ligand demonstrates effective binding affinity and therapeutic potential. By offering high quality visualizations and interaction data, Discovery Studio supports the validation of docking outcomes and provides a strong basis for selecting compounds for further experimental testing.

3.11 Comparison with Reference Ligand

The final stage of computational drug discovery involves comparing the docked phytochemicals with a reference ligand, typically a known inhibitor or clinically approved drug. This comparative analysis examines binding affinities, docking scores, and interaction profiles side by side to evaluate performance. By assessing hydrogen bonding, hydrophobic contacts, and overall docking energy, researchers can determine whether the new compounds demonstrate equal or superior activity compared to established drugs. This benchmarking process provides a scientific basis for identifying the most promising candidates. It also guides the selection of molecules for subsequent wet lab validation and potential drug development, ensuring that computational predictions align with therapeutic relevance.

Chapter 4

Results

4.1 Protein Selection and Preparation

This section presents the results obtained from the selection and preparation of the target protein for subsequent molecular docking analyses.

4.1.1 Protein 3D Structure

Proteins adopt specific three-dimensional (3D) conformations that govern their biological functions and interactions with other molecules. Precise knowledge of protein structure is therefore fundamental for understanding molecular mechanisms and performing structure-based drug discovery.

Advances in computational and experimental approaches have substantially improved the accuracy of protein structure determination, enabling reliable identification of ligand-binding sites and facilitating molecular docking studies [110].

Recent studies have also demonstrated that three-dimensional protein structures can be inferred from sequence variation and mutational data, highlighting the growing potential of genomics-based methods for structural prediction. In the present study, the three-dimensional structure of DNA gyrase subunit B was obtained from the Protein Data Bank (PDB) using the PDB ID 4DUH.

This experimentally resolved structure was employed as the target receptor for molecular docking following appropriate preparation, and its three-dimensional representation is shown in Figure 4.1.



FIGURE 4.1: Three-dimensional structure of DNA Gyrase B (PDB ID: 4DUH) retrieved from the Protein Data Bank.

4.1.2 FASTA Sequence

The FASTA sequence of DNA gyrase subunit B (PDB ID: 4DUH) was retrieved from the RCSB Protein Data Bank (RCSB PDB) and used for subsequent physicochemical characterization and functional domain analysis.

The retrieved amino acid sequence is presented below in Figure 4.2.

```
>4DUH_1|Chains A, B|DNA gyrase subunit B|Escherichia coli (83333)
MSNSYDSSSIKVLKGLDAVRKRPGMYIGDIDDGTGLHMMVFEVVDNAIDEALAGHCKEIIVTIHADN
SVSVQDDGRGIPTGIHPEEGVSAAEVIMTVLHAGGKFDDNSYKVSGLHGVGVSVVNALSQKLELVI
QREGKIHRQIYEHGVPQAPLAVTGTEKTGTMVRFWPSLETFTNVTEFEYEILAKRLRELSFLNSGV
SIRLRDKRDGKEDHFHYEG
```

FIGURE 4.2: Retrieved amino acid sequence of the human BRCA1 protein (UniProt accession: P38398).

FASTA sequence retrieved from RCSB Protein Data Bank (RCSB PDB).

4.1.3 Physico-Chemical Properties

The ExPASy ProtParam tool was used to predict the physicochemical characteristics of the chosen target protein, DNA gyrase subunit B. The outcomes are collected in Table 4.1. The protein has a molecular weight of 24,157.17 Da and is made up of 220 amino acid residues.

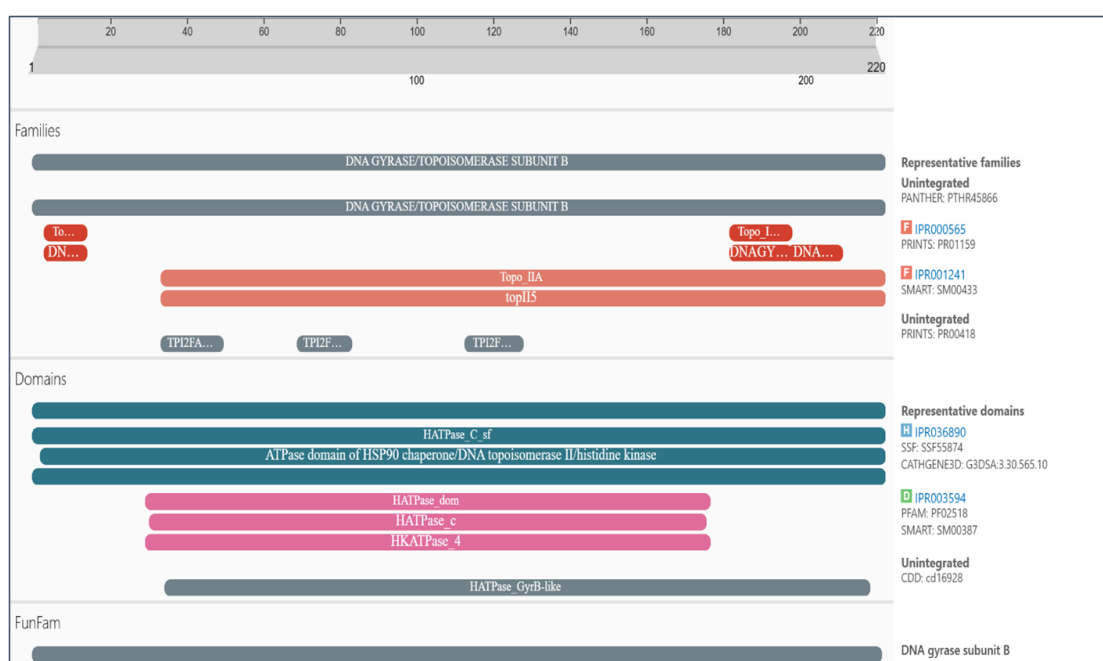


FIGURE 4.3: Functional domain analysis of DNA gyrase B predicted using the InterPro database. The analysis identifies the protein family, conserved ATPase domains, HATPase superfamily, and domain architecture across the 220-amino-acid sequence.

The protein has an overall acidic character, as shown by the predicted isoelectric point (pI) of 5.53. The protein was classified as stable with an instability value of 27.78. The protein's hydrophilic character was revealed by the negative GRAVY score (-0.310), whereas an aliphatic index of 87.64 showed a comparatively high degree of thermostability.

There are 23 positively charged residues (Arg + Lys) and 33 negatively charged residues (Asp + Glu) in the protein. Its chemical formula, which has a total of

3,378 atoms, was expected to be $C_{1061}H_{1678}N_{300}O_{333}S_6$. At a concentration of 1 g/L, the absorbance value was 0.598 and the computed extinction coefficient was $14,440 \text{ M}^{-1} \text{ cm}^{-1}$. Additionally, the protein was projected to have an estimated half-life of 30 hours in mammalian reticulocytes (*in vitro*), more than 20 hours in yeast (*in vivo*), and more than 10 hours in *Escherichia coli* (*in vivo*), showing sufficient protein stability under various biological settings.

TABLE 4.1: Physicochemical properties of the target protein.

Property	Value
Number of Amino Acids	220
Molecular Weight (Da)	24,157.17
Theoretical pI	5.53
Instability Index	27.78 (Stable)
Aliphatic Index	87.64
GRAVY	-0.310
Total Negatively Charged Residues (Asp + Glu)	33
Total Positively Charged Residues (Arg + Lys)	23
Molecular Formula	$C_{1061}H_{1678}N_{300}O_{333}S_6$
Total Number of Atoms	3378
Extinction Coefficient ($\text{M}^{-1} \text{ cm}^{-1}$)	14,440
Abs 0.1% (1 g/L)	0.598
Estimated Half-life (Mammalian Reticulocytes, <i>in vitro</i>)	30 h
Estimated Half-life (Yeast, <i>in vivo</i>)	>20 h
Estimated Half-life (<i>E. coli</i> , <i>in vivo</i>)	>10 h

4.1.4 Functional Domain Analysis

The functional domain analysis of DNA gyrase subunit B was performed using the InterPro database, and the predicted domain architecture is illustrated in

Figure 4.3. The analysis classified the protein as a member of the DNA gyrase/topoisomerase subunit B family, confirming its functional identity. Conserved domain analysis identified the HATPase_C superfamily extending across the protein sequence, along with the ATPase domain of HSP90 chaperone/DNA topoisomerase II/histidine kinase, indicating the presence of a highly conserved ATP-binding region.

Additional domain annotations included the HATPase_dom, HATPase_c, and HK ATPase_4 domains, while the Conserved Domain Database (CDD) recognized the protein as HATPase_GyrB-like. These conserved functional regions are characteristic of DNA gyrase subunit B and support its role as an ATP-dependent enzyme involved in bacterial DNA topology, making it a suitable molecular target for antibacterial drug discovery.

4.1.5 Protein Preparation

Before molecular docking research, PyMOL was used to construct the obtained three-dimensional structure of DNA gyrase subunit B. In order to prevent possible interference during ligand binding, water molecules, co-crystallized ligands, and other unnecessary heteroatoms were removed during protein production.



FIGURE 4.4: Prepared three-dimensional structure of DNA gyrase subunit B

In order to ensure a clean receptor model appropriate for interaction investigations, the generated protein structure was then adjusted for docking while maintaining the target protein chain.

For later molecular docking study, the modified protein structure was saved in PDB format. Figure 4.4 depicts the prepared structure.

4.2 Ligand Selection and Preparation

This section describes the selection and preparation of phytochemical ligands identified from *Berberis lycium* for subsequent computational and molecular docking studies.

4.2.1 Physicochemical Properties of Ligands

The physicochemical characteristics of the selected *Berberis lycium* phytochemicals are summarized in Table 4.2.

A total of six bioactive compounds, including berberine, palmatine, jatrorrhizine, kaempferol, quercetin, and oxyberberine, were chosen for subsequent *in silico* investigations.

The molecular weights of these compounds ranged from 286.24 to 352.4 g/mol, with kaempferol possessing the lowest molecular weight (286.24 g/mol) and palmatine the highest (352.4 g/mol).

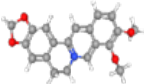
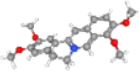
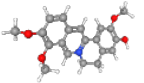
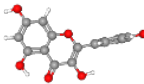
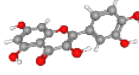
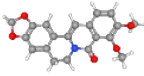
Variations in their molecular formulas reflected the structural diversity of the selected phytochemicals, particularly in the composition of carbon, hydrogen, oxygen, and nitrogen atoms.

The chemical structures of all compounds were obtained from the PubChem database using their respective Compound Identification (CID) numbers and were prepared for subsequent drug-likeness assessment, molecular docking, and protein–ligand interaction studies to evaluate their antibacterial potential.

4.2.2 Refinement of Ligand

The selected phytochemical ligands were refined using BIOVIA Discovery Studio

TABLE 4.2: Physicochemical properties and chemical structures of the selected phytochemicals from *Berberis lycium* Royle (PubChem).

Ligand	PubChem CID	MF	Mol. Weight (g/mol)	Structure
Berberine	2353	$C_{20}H_{18}NO_4^+$	336.4	
Palmatine	19009	$C_{21}H_{22}NO_4^+$	352.4	
Jatrorrhizine	72323	$C_{20}H_{20}NO_4^+$	338.4	
Kaempferol	5280863	$C_{15}H_{10}O_6$	286.24	
Quercetin	5280343	$C_{15}H_{10}O_7$	302.23	
Oxyberberine	11066	$C_{20}H_{17}NO_5$	351.4	

to perform energy minimization and obtain stable three-dimensional conformations suitable for molecular docking. The energy-minimized ligand structures were subsequently used for docking analysis.

4.2.3 Virtual Screening through Lipinski Rule of Five

The drug-likeness of the selected phytochemicals was assessed using the SwissADME web server according to Lipinski's RO5, which is widely applied to predict the oral bioavailability of potential drug candidates.

Based on Lipinski's criteria, an ideal orally active compound should possess a molecular weight of ≤ 500 g/mol, a LogP value of ≤ 5 , no more than five hydrogen bond donors (HBD), no more than ten hydrogen bond acceptors (HBA), and preferably ten or fewer rotatable bonds (RB) [111].

The findings of the drug-likeness evaluation are summarized in Table 4.3. All six selected phytochemicals, namely berberine, palmatine, jatrorrhizine, kaempferol, quercetin, and oxyberberine, satisfied all the parameters of Lipinski's Rule of 5 without exhibiting any violations.

The molecular weights, hydrogen bond donor and acceptor counts, rotatable bonds, and LogP values of each compound remained within the recommended limits, indicating favorable physicochemical characteristics for oral drug candidates.

The absence of any Lipinski violations suggests that these phytochemicals possess promising drug-like properties and acceptable pharmacokinetic profiles. These findings support their suitability for further computational investigations, including molecular docking and protein–ligand interaction analysis, to evaluate their potential antibacterial activity.

4.2.4 ADMET Properties

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the selected phytochemicals were predicted using the pkCSM web

TABLE 4.3: Lipinski Rule of Five properties of ligands.

Ligand	MW (g/mol)	HBA	HBD	RB	LogP	Lipinski	Violation
Berberine	336.36	4	0	2	2.82	Yes	0
Palmatine	352.40	4	0	4	2.93	Yes	0
Jatrorrhizine	338.38	4	1	3	2.59	Yes	0
Kaempferol	286.24	6	4	1	1.20	Yes	0
Quercetin	302.24	7	5	1	0.79	Yes	0
Oxyberberine	351.35	5	0	2	2.50	Yes	0

server to evaluate their pharmacokinetic behavior and safety profiles. ADMET analysis provides valuable information regarding the absorption, distribution, metabolic stability, excretion, and potential toxicity of compounds, which are important parameters in assessing their suitability as drug candidates.

4.2.4.1 Absorption

The absorption characteristics of the selected phytochemicals were predicted using the pkCSM web server, and the results are summarized in Table 4.4. The predicted water solubility values ranged from -4.218 to -2.925 log mol/L. Among the selected compounds, quercetin exhibited the highest aqueous solubility (-2.925 log mol/L), whereas oxyberberine showed the lowest water solubility (-4.218 log mol/L). The predicted Caco-2 permeability values varied across the phytochemicals, with berberine displaying the highest permeability (1.734 log Papp $\times 10^{-6}$ cm/s), followed by jatrorrhizine (1.234) and oxyberberine (1.077), indicating their greater potential for intestinal membrane permeation. Oxyberberine demonstrated the highest predicted intestinal absorption (100.00%), followed by berberine (97.15%) and palmatine (97.08%), while kaempferol exhibited the lowest intestinal absorption (74.29%). The predicted skin permeability (log Kp) values ranged from -2.554 to -2.741 , suggesting relatively low skin permeability for all evaluated compounds. Analysis of P-glycoprotein (P-gp) interactions revealed that all phytochemicals except oxyberberine were predicted to be P-gp substrates. In addition, palmatine and oxyberberine were identified as inhibitors of both P-gp I and P-gp II, whereas berberine and jatrorrhizine inhibited only

P-gp II. Kaempferol and quercetin were not predicted to inhibit either P-gp I or P-gp II. Overall, the absorption analysis indicated that oxyberberine possesses the most favorable intestinal absorption profile, whereas berberine exhibited the highest Caco-2 permeability, highlighting their promising absorption characteristics for further *in silico* evaluation.

TABLE 4.4: Absorption properties of ligands.

Ligand	Aqueous	Caco-2	Intestinal	Skin	P-gp	P-gp
	Solubility	Permeability	Absorption (%)	Permeability	Substrate	Inhibitor (I/II)
Berberine	-3.973	1.734	97.147	-2.576	Yes	No / Yes
Palmitine	-4.194	0.863	97.084	-2.554	Yes	Yes / Yes
Jatrorrhizine	-3.871	1.234	94.465	-2.741	Yes	No / Yes
Kaempferol	-3.040	0.032	74.290	-2.735	Yes	No / No
Quercetin	-2.925	-0.229	77.207	-2.735	Yes	No / No
Oxyberberine	-4.218	1.077	100.000	-2.623	No	Yes / Yes

4.2.4.2 Distribution

The distribution characteristics of the selected phytochemicals were predicted using the pkCSM web server, and the corresponding results are presented in Table 4.5. The predicted volume of distribution at steady state (VDss) ranged from -0.025 to $1.559 \log \text{ L/kg}$. Among the evaluated compounds, quercetin exhibited the highest VDss value ($1.559 \log \text{ L/kg}$), followed by kaempferol ($1.274 \log \text{ L/kg}$), indicating a greater tendency for distribution into body tissues. In contrast, oxyberberine showed the lowest VDss value ($-0.025 \log \text{ L/kg}$), suggesting comparatively limited tissue distribution.

The fraction unbound (Fu) values varied between 0.168 and 0.262, with berberine exhibiting the highest Fu value (0.262), indicating a relatively larger proportion of the compound remains unbound in plasma, whereas oxyberberine displayed the lowest value (0.168). Blood–brain barrier (BBB) permeability predictions

revealed that berberine had the highest permeability value (0.198), while quercetin exhibited the lowest BBB permeability (-1.098). Likewise, berberine showed the highest predicted central nervous system (CNS) permeability (-1.543), whereas quercetin demonstrated the lowest CNS permeability (-3.065). Overall, quercetin displayed the greatest potential for tissue distribution, while berberine exhibited the highest predicted ability to penetrate both the blood–brain barrier and the central nervous system, highlighting differences in the distribution profiles of the selected phytochemicals.

TABLE 4.5: Distribution properties of ligands.

Ligand	VDss (log L/kg)	Fraction Unbound (Fu)	BBB Permeability	CNS Permeability
Berberine	0.580	0.262	0.198	-1.543
Palmatine	0.641	0.245	-0.112	-1.535
Jatrorrhizine	0.539	0.182	-0.150	-2.142
Kaempferol	1.274	0.178	-0.939	-2.228
Quercetin	1.559	0.206	-1.098	-3.065
Oxyberberine	-0.025	0.168	-0.070	-2.116

4.2.4.3 Metabolism

The metabolic properties of the selected phytochemicals were predicted using the pkCSM web server, and the results are summarized in Table 4.6. None of the evaluated compounds were predicted to act as substrates of CYP2D6. Berberine, palmatine, jatrorrhizine, and oxyberberine were identified as CYP3A4 substrates, whereas kaempferol and quercetin were predicted to be non-substrates of this enzyme.

Regarding cytochrome P450 enzyme inhibition, all six phytochemicals were predicted to inhibit CYP1A2, indicating a potential interaction with this metabolic enzyme. Oxyberberine was the only compound predicted to inhibit both CYP2C19 and CYP2C9, suggesting a broader inhibitory profile compared with the other selected ligands. Berberine, palmatine, and jatrorrhizine were identified as CYP2D6

inhibitors, while CYP3A4 inhibitory activity was predicted only for berberine and oxyberberine.

Overall, the metabolism analysis demonstrated distinct interaction patterns with cytochrome P450 enzymes among the selected phytochemicals, with oxyberberine exhibiting the broadest inhibitory profile and berberine showing inhibitory activity against multiple CYP enzymes, indicating their potential influence on drug metabolism and possible drug–drug interactions.

TABLE 4.6: Metabolism properties of ligands.

Ligand	CYP2D6	CYP3A4	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
	Substrate	Substrate	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
Berberine	No	Yes	Yes	No	No	Yes	Yes
Palmatine	No	Yes	Yes	No	No	Yes	No
Jatrorrhizine	No	Yes	Yes	No	No	Yes	No
Kaempferol	No	No	Yes	No	No	No	No
Quercetin	No	No	Yes	No	No	No	No
Oxyberberine	No	Yes	Yes	Yes	Yes	No	Yes

4.2.4.4 Excretion

The excretion properties of the selected phytochemicals were predicted using the pkCSM web server, and the results are presented in Table 4.7. The predicted total clearance values ranged from 0.121 to 1.270 log mL/min/kg. Among the evaluated compounds, berberine exhibited the highest total clearance value (1.270 log mL/min/kg), followed by palmatine (1.246 log mL/min/kg) and jatrorrhizine (1.222 log mL/min/kg), suggesting a relatively faster elimination from the body.

In contrast, oxyberberine showed the lowest total clearance (0.121 log mL/min/kg), indicating a comparatively slow excretion rate. Kaempferol and quercetin displayed intermediate clearance values of 0.477 and 0.407 log mL/min/kg, respectively.

Assessment of renal organic cation transporter 2 (OCT2) substrate activity revealed that oxyberberine was the only compound predicted to be an OCT2 substrate, whereas all other phytochemicals were identified as non-substrates.

Overall, the excretion analysis demonstrated that berberine possesses the highest predicted clearance among the selected phytochemicals, while oxyberberine exhibits the lowest clearance and may undergo renal elimination through OCT2-mediated transport.

TABLE 4.7: Excretion properties of ligands.

Sr. No.	Ligand	Total Clearance	Renal OCT2
		(log mL/min/kg)	Substrate
1	Berberine	1.270	No
2	Palmatine	1.246	No
3	Jatrorrhizine	1.222	No
4	Kaempferol	0.477	No
5	Quercetin	0.407	No
6	Oxyberberine	0.121	Yes

4.2.4.5 Toxicity

The toxicity profiles of the selected phytochemicals were predicted using the pkCSM web server, and the results are presented in Table 4.8. The AMES toxicity assessment predicted that berberine and palmatine possess mutagenic potential, whereas jatrorrhizine, kaempferol, quercetin, and oxyberberine were identified as non-mutagenic compounds.

The predicted maximum tolerated dose ranged from -0.234 to 0.531 log mg/kg/day, with kaempferol exhibiting the highest tolerated dose (0.531 log mg/kg/day), while

TABLE 4.8: Toxicity Properties of Selected Ligands (PkCsm).

Property	Berberine	Palmatine	Jatrorrhizine	Kaempferol	Quercetin	Oxyberberine
AMES	Yes	Yes	No	No	No	No
Maximum Tolerated Dose (log mg/kg/day)	0.144	0.231	0.175	0.531	0.499	-0.234
hERG I	No	No	No	No	No	No
hERG II	No	Yes	Yes	No	No	No
LD ₅₀ (mol/kg)	2.571	2.551	2.445	2.449	2.471	2.394
LOAEL (log mg/kg/day)	1.890	1.536	1.356	2.505	2.612	2.226
Hepatotoxicity	Yes	Yes	Yes	No	No	Yes
Skin Sensitization	No	No	No	No	No	No
<i>T. pyriformis</i> Toxicity	0.354	0.388	0.385	0.312	0.288	0.414
Minnow Toxicity	-0.277	-0.313	0.177	2.885	3.721	0.165

oxyberberine showed the lowest value (-0.234 log mg/kg/day). None of the evaluated phytochemicals were predicted to inhibit the hERG I channel, whereas palmatine and jatrorrhizine were identified as hERG II inhibitors. The predicted acute oral toxicity (LD₅₀) values ranged from 2.394 to 2.571 mol/kg, with berberine exhibiting the highest LD₅₀ value and oxyberberine the lowest.

The predicted lowest observed adverse effect level (LOAEL) values ranged from 1.356 to 2.612 log mg/kg/day, with quercetin showing the highest LOAEL value and jatrorrhizine the lowest. Hepatotoxicity predictions indicated that berberine, palmatine, jatrorrhizine, and oxyberberine may exhibit hepatotoxic effects, whereas kaempferol and quercetin were predicted to be non-hepatotoxic. In addition, none of the selected compounds were predicted to cause skin sensitization.

The *Tetrahymena pyriformis* toxicity values ranged from 0.288 to 0.414 log μ g/L, with oxyberberine exhibiting the highest predicted toxicity and quercetin the lowest. Minnow toxicity values varied between -0.313 and 3.721 log mM, where quercetin showed the highest value and palmatine the lowest.

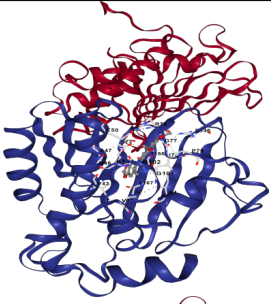
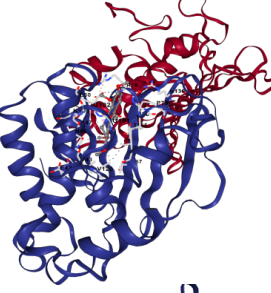
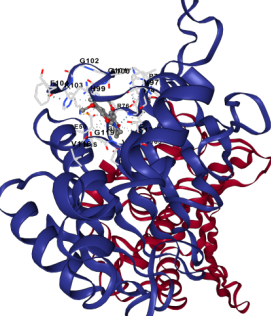
Overall, kaempferol and quercetin demonstrated comparatively favorable toxicity profiles among the selected phytochemicals, supporting their suitability for further

in silico and experimental investigations.

4.3 Molecular Docking

By measuring the binding energy of the resultant complex, molecular docking is a computational method for predicting the binding affinity and interaction pattern between a ligand and its target protein [112]. The binding interactions of the chosen phytochemicals from *Berberis lycium* with DNA gyrase subunit B were assessed in the current study using molecular docking.

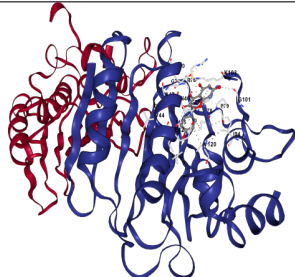
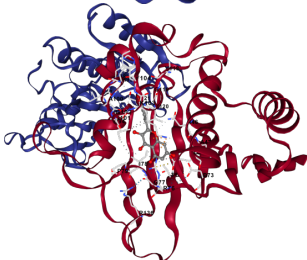
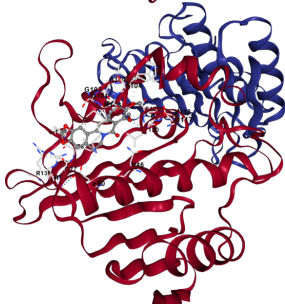
TABLE 4.9: Molecular docking results of selected ligands against DNA gyrase B (Part I).

Ligand	Vina Score	Cavity Volume (Å ³)	Docking Results
Berberine	-8.8	810	
Palmatine	-8.3	810	
Jatrorrhizine	-8.0	810	

The CB-Dock2 service, which automatically locates the receptor's possible binding cavities and carries out docking analysis, was used.

During the docking procedure, the ligands were treated as flexible, whereas the receptor protein was considered rigid. The most promising compounds with potential antibacterial activity were subsequently identified by analyzing the docking scores and protein–ligand interactions obtained.

TABLE 4.10: Molecular docking results of selected ligands against DNA gyrase B (Part II).

Ligand	Vina Score	Cavity Volume (Å ³)	Docking Results
Kaempferol	-9.2	810	
Quercetin	-8.9	1018	
Oxyberberine	-8.7	1018	

The molecular docking outcomes of the selected *Berberis lycium* phytochemicals against DNA Gyrase B are summarized in Tables 4.9 and 4.10. All six ligands exhibited favorable binding affinities toward the target protein, with docking scores

ranging from -8.0 to -9.2 kcal/mol, indicating the formation of stable protein–ligand complexes.

Among the evaluated compounds, kaempferol exhibited the strongest binding affinity, with the lowest docking score (-9.2 kcal/mol), followed by quercetin (-8.9 kcal/mol), berberine (-8.8 kcal/mol), oxyberberine (-8.7 kcal/mol), palmatine (-8.3 kcal/mol), and jatrorrhizine (-8.0 kcal/mol). Four ligands, namely berberine, palmatine, jatrorrhizine, and kaempferol, were docked within a binding cavity of $810 \text{ \AA}^3$, whereas quercetin and oxyberberine occupied a larger binding cavity with a volume of $1018 \text{ \AA}^3$. The negative docking scores indicate favorable interactions between the phytochemicals and the active site of DNA Gyrase B, with lower binding energy values corresponding to stronger binding affinity and greater complex stability. Based on these findings, kaempferol demonstrated the highest binding affinity toward DNA Gyrase B and emerged as the most promising phytochemical for further investigation as a potential antibacterial agent.

4.4 Interaction Analysis of Protein–Ligands

Using BIOVIA Discovery Studio Visualizer, the binding relationships between the target protein and the chosen *Berberis lycium* phytochemicals were investigated. Important contacts between the ligands and amino acid residues within the active site, including hydrogen bonds, hydrophobic interactions, and van der Waals forces, were identified using the 2D interaction maps. These patterns of interaction confirmed the docked compounds' potential antibacterial activity and explained their binding behavior.

4.4.1 Interaction Pattern of Berberine–DNA Gyrase B

The 2D interaction diagram presented in Figure 4.5 depicts the binding mode of berberine within the active site of DNA Gyrase B. Berberine achieved a docking

score of -8.8 kcal/mol, reflecting a favorable interaction with the target protein. The ligand established carbon–hydrogen bonds with Gly77 and Thr165, while Asp73, Lys103, and Glu50 contributed to electrostatic interactions, including π –anion, π –cation, and attractive charge interactions. Furthermore, Ile78 was involved in a π –sigma interaction, Asn46 formed an amide– π stacked interaction, and hydrophobic contacts through alkyl and π –alkyl interactions were observed with Pro79, Val43, Val120, and Val167. Collectively, these interactions indicate that berberine binds effectively within the catalytic pocket of DNA Gyrase B, highlighting its potential to inhibit the target protein and contribute to antibacterial activity.

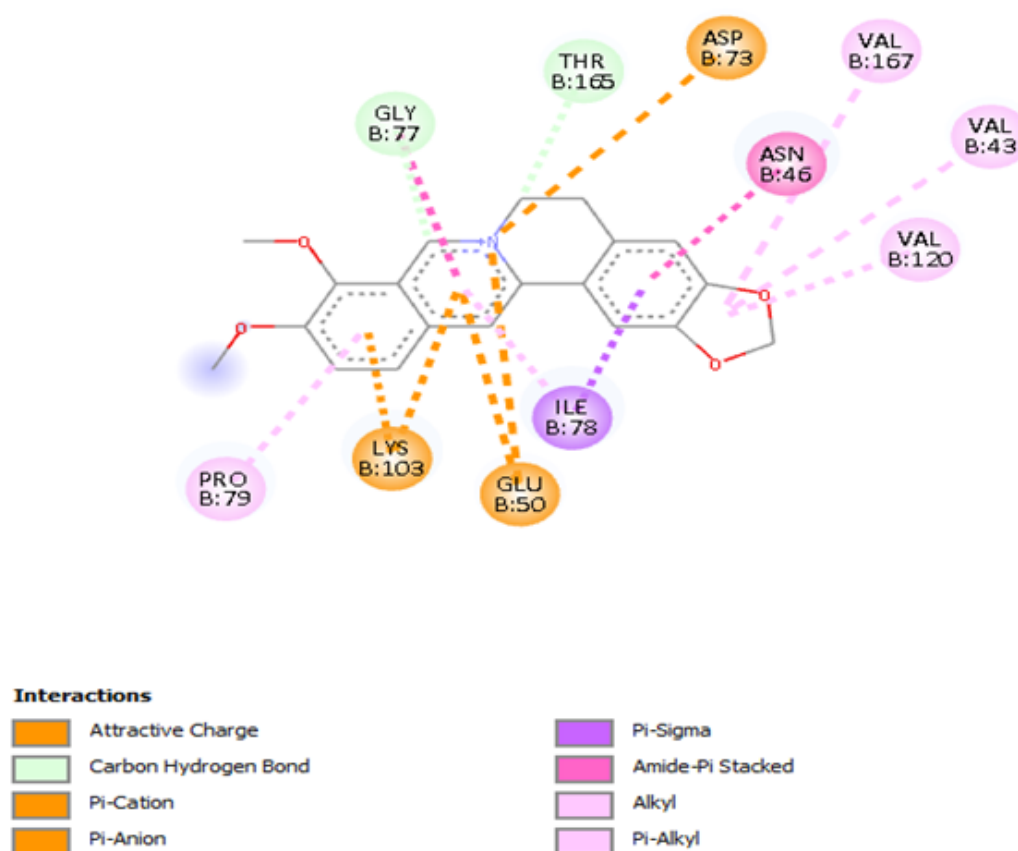


FIGURE 4.5: Berberine–DNA Gyrase B Interaction–profile.

4.4.2 Interaction Pattern of Palmatine–DNA Gyrase B

The 2D interaction diagram presented in Figure 4.6 illustrates the binding mode of palmatine within the active site of DNA Gyrase B. Palmatine achieved a docking

score of -8.3 kcal/mol, indicating a favorable binding affinity toward the target protein. The ligand formed a conventional hydrogen bond with Arg76, while carbon–hydrogen bond interactions were observed with Asp73 and Val43. Electrostatic interactions, including attractive charge, π -cation, and π -anion interactions, were established with Glu50 and Lys103, contributing to the stability of the protein–ligand complex. In addition, Ile78 participated in a π -sigma interaction, whereas Pro79, Ala47, and Val120 were involved in alkyl and π -alkyl hydrophobic interactions. These binding interactions suggest that palmatine is stably accommodated within the active site of DNA Gyrase B, supporting its potential as an antibacterial inhibitor.

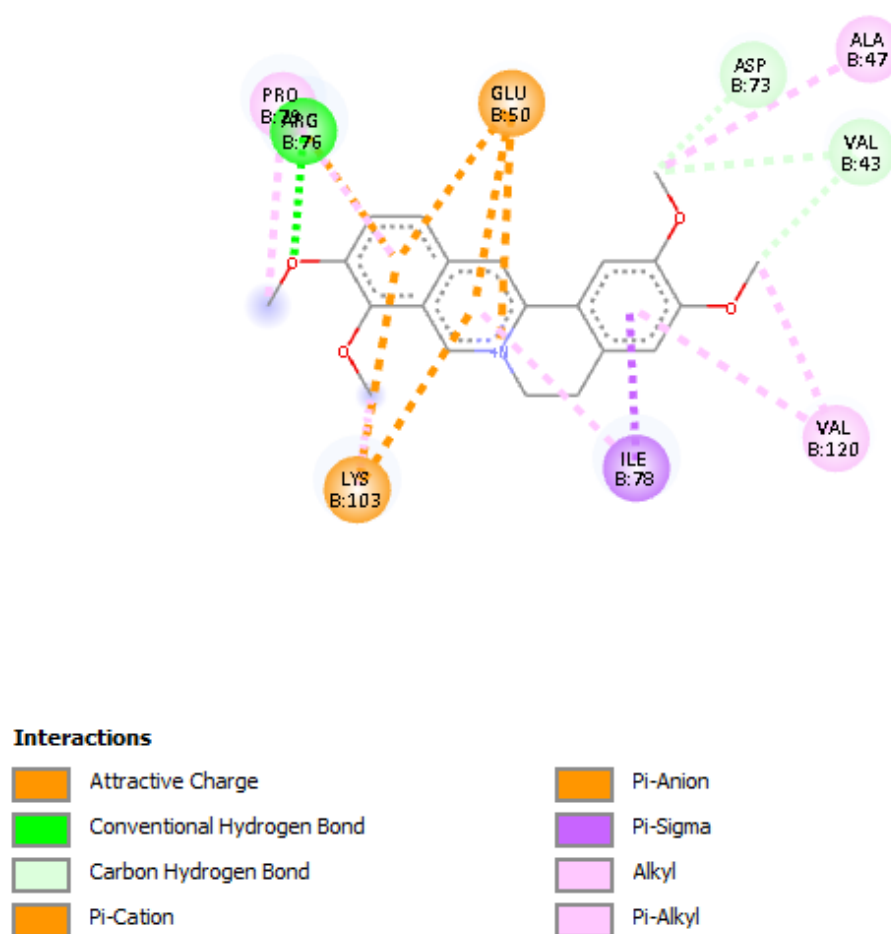


FIGURE 4.6: Palmatine–DNA Gyrase B Interaction–profile.

4.4.3 Interaction Pattern of Jatrorrhizine–DNA Gyrase B

The binding orientation of jatrorrhizine within the active site of DNA Gyrase B is depicted in the 2D interaction diagram in Figure 4.7. Jatrorrhizine achieved a docking score of -8.0 kcal/mol, indicating a favorable interaction with the target protein. Asp73 and Gly77 formed conventional hydrogen bonds with the ligand, whereas Ile94 formed a carbon–hydrogen bond. The complex was further stabilized by a π –lone pair interaction with Asn46 and a π –cation interaction with Lys103. In addition, Arg76 established an amide– π stacked interaction, whereas His99, Pro79, Ile78, and Ile94 participated in alkyl and π –alkyl hydrophobic interactions. The combination of these intermolecular contacts indicates that jatrorrhizine fits well within the active site of DNA Gyrase B, supporting its potential to inhibit the target enzyme and contribute to antibacterial activity.

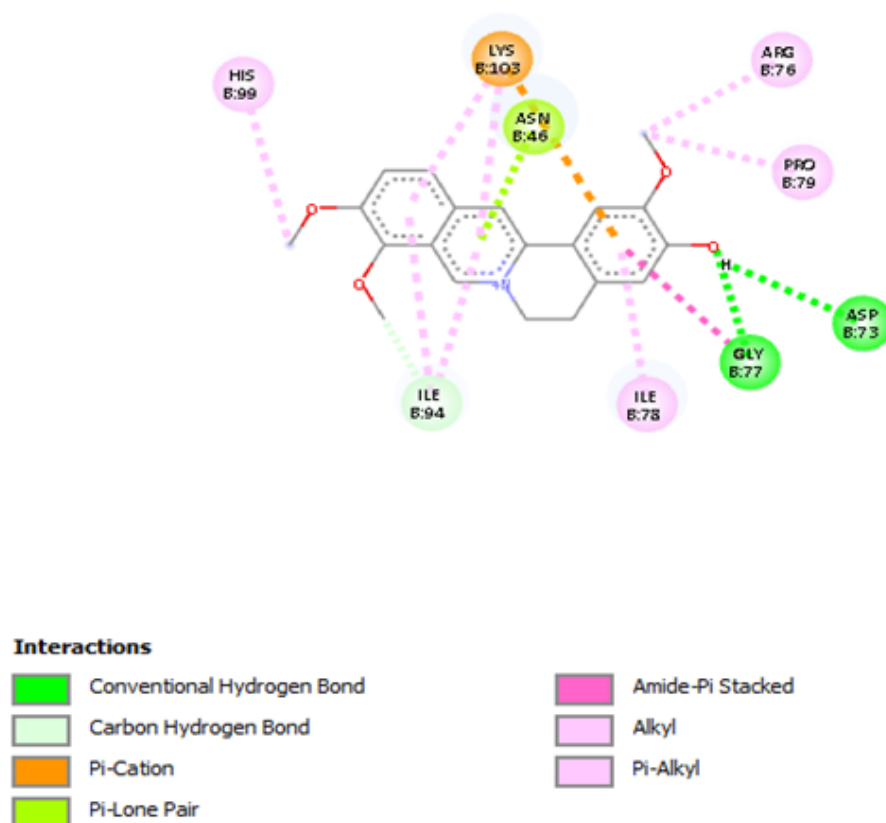


FIGURE 4.7: Jatrorrhizine–DNA Gyrase B interaction–profile.

4.4.4 Interaction Pattern of Kaempferol–DNA Gyrase B

The binding orientation of kaempferol within the active site of DNA Gyrase B is illustrated in the 2D interaction diagram shown in Figure 4.8. Kaempferol achieved a docking score of -9.2 kcal/mol, demonstrating the highest binding affinity among the selected phytochemicals. The ligand formed conventional hydrogen bonds with Asp73, Thr165, Gly77, and Arg76, contributing significantly to the stability of the protein–ligand complex. Electrostatic interactions, including attractive charge, π -cation, and π -anion interactions, were observed with Glu50 and Lys103. Furthermore, Ile78 participated in a π -sigma interaction, while Asn46 formed an amide- π stacked interaction. Hydrophobic stabilization was provided through π -alkyl interactions with Pro79 and Val120. The presence of these favorable intermolecular interactions indicates that kaempferol is firmly accommodated within the binding pocket of DNA Gyrase B, suggesting its potential to inhibit the target enzyme and contribute to antibacterial activity.

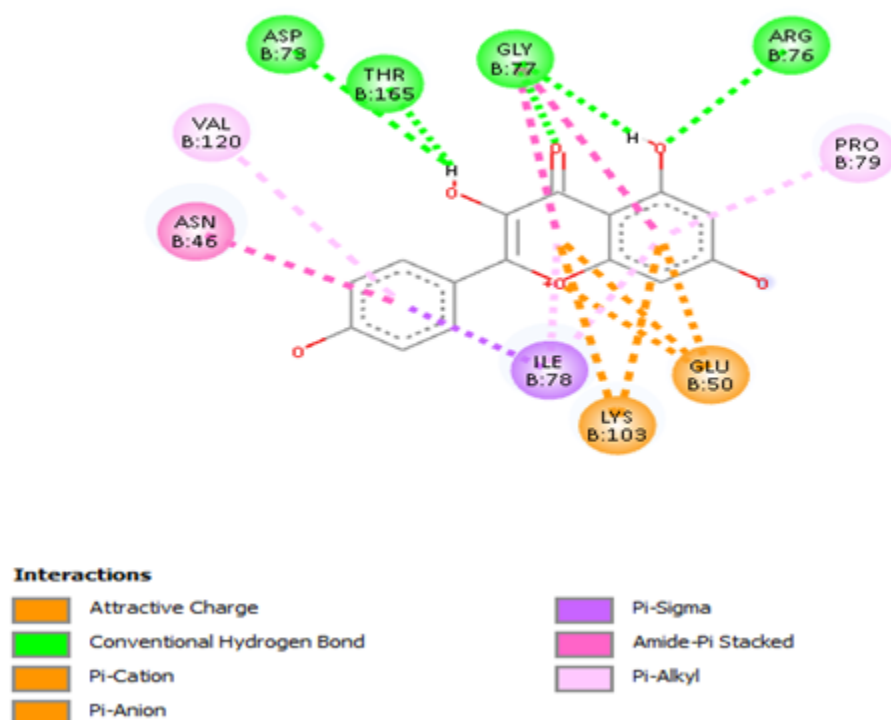


FIGURE 4.8: Kaempferol–DNA Gyrase B interaction–profile.

4.4.5 Interaction Pattern of Quercetin–DNA Gyrase B

The 2D interaction diagram shown in Figure 4.9 depicts the binding interactions of quercetin within the active site of DNA Gyrase B. Quercetin achieved a docking score of -8.9 kcal/mol, demonstrating a favorable binding affinity toward the target protein. The ligand formed conventional hydrogen bonds with Ala100, Gly101, Asp73, and Lys103, while carbon–hydrogen bond interactions were observed with Gly119, Pro79, Asn46, and Ala47. Electrostatic stabilization was provided through π -cation and π -anion interactions involving Lys103 and Glu50. In addition, Ala47 participated in a π -donor hydrogen bond, whereas Ile78 and Ile94 contributed π -alkyl hydrophobic interactions. An amide- π stacked interaction was also identified with Val120. The overall interaction profile suggests that quercetin binds effectively within the active site of DNA Gyrase B, indicating its potential to inhibit the target enzyme and supporting its antibacterial activity.

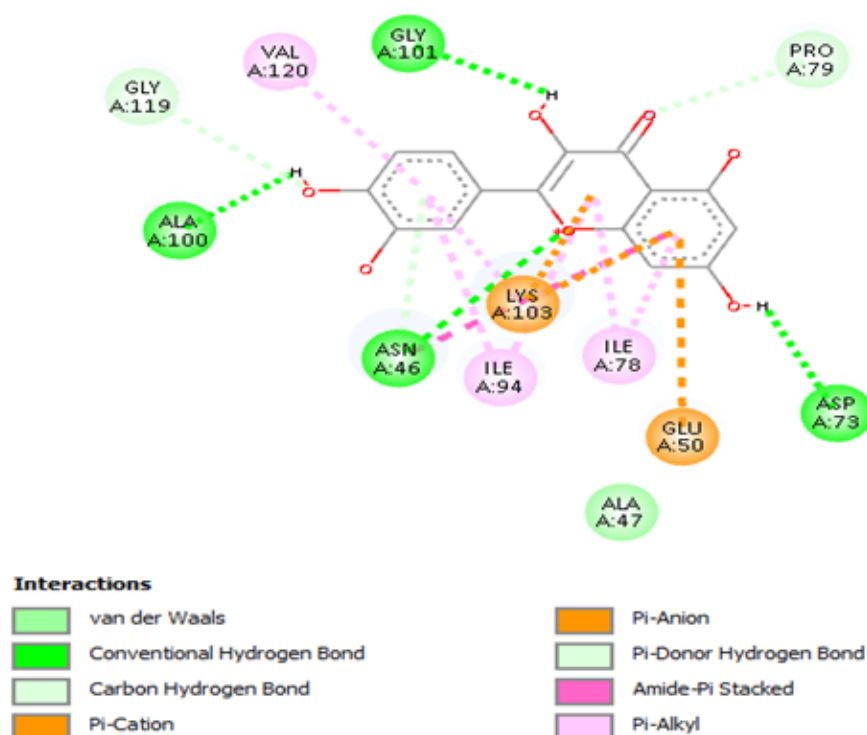


FIGURE 4.9: Quercetin–DNA Gyrase B interaction–profile.

4.4.6 Interaction Pattern of Oxyberberine–DNA Gyrase B

The binding interactions of oxyberberine with the active site of DNA Gyrase B are illustrated in the 2D interaction diagram shown in Figure 4.10. Oxyberberine achieved a docking score of -8.7 kcal/mol, indicating a favorable binding affinity toward the target protein. The ligand formed carbon–hydrogen bond interactions with Asn46 and Gly119. The ligand formed carbon–hydrogen bond interactions with Asn46 and Gly119.

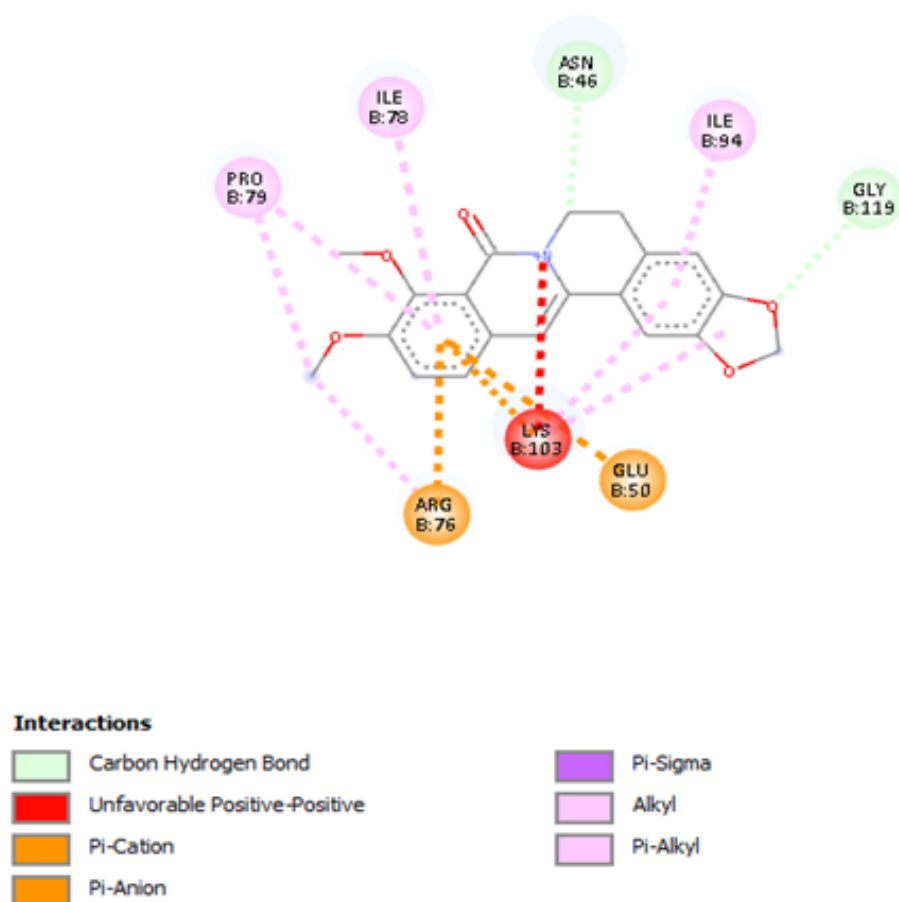


FIGURE 4.10: Oxyberberine–DNA Gyrase B interaction–profile.

Electrostatic stabilization was provided through π -cation and π -anion interactions involving Arg76 and Glu50.

In addition, Pro79, Ile94, and Lys103 participated in alkyl and π -alkyl hydrophobic interactions that contributed to stabilizing the ligand within the binding pocket, while Ile78 was involved in a π -sigma interaction.

A repulsive positive–positive interaction with Lys103 was also observed. Despite this unfavorable contact, the overall interaction network indicates that oxyberberine binds effectively within the active site of DNA Gyrase B, supporting its potential inhibitory effect against the target enzyme and its antibacterial activity.

Based on the combined findings of the molecular docking, protein–ligand interaction, and ADMET analyses, kaempferol was identified as the lead compound among the selected *Berberis lycium* phytochemicals.

It exhibited the strongest binding affinity (-9.2 kcal/mol), established several favorable interactions with the active-site residues of DNA Gyrase B, and demonstrated desirable drug-likeness and safety characteristics. These results highlight kaempferol as a promising candidate for further investigation as a potential antibacterial agent.

4.5 Lead Compound Identification

Among the phytochemicals investigated from *Berberis lycium*, kaempferol was identified as the most promising lead compound based on its overall *in silico* performance. The selection was supported by its superior molecular docking results, favorable protein–ligand interactions, compliance with drug-likeness criteria, and acceptable ADMET profile.

The antioxidant, anti-inflammatory, antibacterial, and anticancer properties of kaempferol, a naturally occurring flavonoid found in many medicinal plants, fruits, and vegetables, have been extensively documented. With a docking score of -9.2 kcal/mol, it showed the strongest binding affinity for DNA gyrase subunit B in the current investigation, suggesting that it may inhibit the target enzyme.

Furthermore, its relatively low molecular weight (286.24 g/mol) and complete adherence to Lipinski's Rule of Five (Ro5) highlight its suitability as a potential orally active drug candidate.

4.5.1 Drug-Likeness Evaluation

The drug-likeness assessment showed that kaempferol fulfilled all the requirements of Lipinski's Ro5 without any violations. The compound possesses a molecular weight of 286.24 g/mol, six hydrogen bond acceptors, four hydrogen bond donors, one rotatable bond, and a LogP value of 1.20. These physicochemical properties fall within the acceptable limits for orally administered drugs, suggesting that kaempferol has favorable characteristics for further pharmaceutical development.

4.5.2 ADMET Evaluation

The pharmacokinetic behavior of kaempferol was investigated using the pkCSM web server to evaluate its absorption, distribution, metabolism, excretion, and toxicity characteristics.

4.5.2.1 Absorption

The absorption analysis predicted that kaempferol has an intestinal absorption rate of 74.29%, indicating satisfactory oral uptake. The compound exhibited a water solubility of $-3.040 \log \text{ mol/L}$ and a Caco-2 permeability value of $0.032 \log \text{ Papp} \times 10^{-6} \text{ cm/s}$, reflecting acceptable membrane permeability. Although kaempferol was predicted to be a substrate of P-glycoprotein, it showed no inhibitory activity against either P-gp I or P-gp II, suggesting a relatively low probability of transporter-mediated pharmacokinetic interactions.

4.5.2.2 Distribution Profile

The predicted steady-state volume of distribution (VD_{ss}) for kaempferol was $1.274 \log \text{ L/kg}$, indicating efficient distribution into body tissues after systemic absorption. The fraction unbound (Fu) value of 0.178 suggests moderate plasma protein binding. Predictions also indicated limited penetration across the blood-brain

barrier (-0.939) and low central nervous system permeability (-2.228), implying that kaempferol is unlikely to accumulate significantly within the brain.

4.5.2.3 Metabolism Profile

The metabolic profile demonstrated that kaempferol is neither a CYP2D6 nor a CYP3A4 substrate. Among the cytochrome P450 enzymes evaluated, inhibition was predicted only for CYP1A2, whereas no inhibitory activity was observed against CYP2C19, CYP2C9, CYP2D6, or CYP3A4. This restricted interaction with major drug-metabolizing enzymes suggests a comparatively lower risk of metabolism-related drug–drug interactions.

4.5.2.4 Excretion Profile

A modest elimination rate was indicated by the estimated total clearance of kaempferol, which was $0.477 \log \text{ mL/min/kg}$. Furthermore, the compound was not predicted to be a substrate of the renal organic cation transporter 2 (OCT2), indicating that OCT2-mediated transport is unlikely to be necessary for its renal clearance.

4.5.2.5 Toxicity Profile

The toxicity assessment indicated a favorable safety profile for kaempferol. It was predicted to be non-mutagenic in the AMES test and showed no evidence of hepatotoxicity. Moreover, the compound was not predicted to inhibit either hERG I or hERG II potassium channels and exhibited no potential to induce skin sensitization. Kaempferol also displayed the highest maximum tolerated dose ($0.531 \log \text{ mg/kg/day}$) among the selected phytochemicals, supporting its comparatively favorable safety characteristics.

4.5.3 Docking Results and Interaction Analysis

Docking analysis revealed that kaempferol exhibited the strongest interaction with DNA gyrase subunit B, producing the lowest docking score (-9.2 kcal/mol) among all evaluated phytochemicals. The interaction analysis demonstrated that the ligand established several conventional hydrogen bonds with Asp73, Gly77, Thr165, and Arg76, which contributed significantly to complex stabilization. Additional electrostatic interactions involving Glu50 and Lys103, together with π -sigma, amide- π stacked, and π -alkyl hydrophobic interactions, further strengthened the binding within the active site. These intermolecular contacts indicate that kaempferol fits favorably into the ATP-binding pocket of DNA gyrase subunit B and may effectively interfere with its biological function.

4.6 Drug Selection

The reference antibacterial drug was selected through KEGG, also known as the Kyoto Encyclopedia of Genes and Genomes database, by identifying antibiotics that specifically target bacterial DNA gyrase. Among the reported DNA gyrase inhibitors, novobiocin was selected as the reference ligand because it is a well-established inhibitor of DNA gyrase subunit B (GyrB). Novobiocin binds to the ATP-binding domain of GyrB and inhibits ATP hydrolysis, thereby preventing DNA supercoiling and bacterial DNA replication. Owing to its well-characterized mechanism of action against DNA gyrase B, novobiocin was employed as the reference compound for comparison with the selected phytochemicals in subsequent molecular docking and protein-ligand interaction analyses.

4.6.1 Novobiocin

Novobiocin is a naturally occurring aminocoumarin antibiotic produced by *Streptomyces* species and was historically used for treating a variety of infections caused

primarily by Gram-positive bacteria, particularly *Staphylococcus aureus*. It was also investigated for its activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and was occasionally prescribed for uncomplicated urinary tract infections. Its antibacterial activity is mediated through the inhibition of bacterial DNA gyrase subunit B (GyrB), making it one of the most extensively studied inhibitors of this essential bacterial enzyme [113].

In order to prevent ATP hydrolysis, which is necessary for the energy-dependent supercoiling of bacterial DNA, novobiocin binds to the ATP-binding pocket of the GyrB subunit. DNA replication, transcription, recombination, and chromosome segregation are all disrupted when this process is inhibited because DNA gyrase cannot introduce negative supercoils into DNA. Inhibition of DNA gyrase ultimately limits bacterial growth and may result in bacterial cell death because these biological mechanisms are necessary for bacterial survival and proliferation.

Because of its specific interaction with the ATP-binding domain of DNA gyrase B, novobiocin has become a widely accepted reference inhibitor in studies aimed at discovering new GyrB-targeted antibacterial agents [113].

Despite its demonstrated antibacterial efficacy, the clinical use of novobiocin gradually declined because of the rapid emergence of bacterial resistance, which commonly develops through mutations in the *gyrB* gene that reduce the binding affinity of the drug. In addition, adverse effects such as gastrointestinal disturbances, hypersensitivity reactions, and hematological toxicity limited its therapeutic application. Consequently, novobiocin was withdrawn from routine clinical use and replaced by antibiotics with improved efficacy and safety profiles.

Although it is no longer prescribed in clinical practice, novobiocin continues to play an important role in antibacterial research. Its well-characterized mechanism of action and experimentally validated interaction with DNA gyrase subunit B have made it a valuable reference ligand for molecular docking, structure-based drug design, and investigations into antibiotic resistance mechanisms. Therefore, in the present study, novobiocin was selected as the standard reference compound

to compare the docking performance and protein–ligand interaction profiles of the selected *Berberis lycium* phytochemicals against DNA gyrase subunit B [114].

4.6.2 Structure Retrieval

The three-dimensional structure of novobiocin was downloaded from the PubChem database for use in the molecular docking study Figure 4.11.

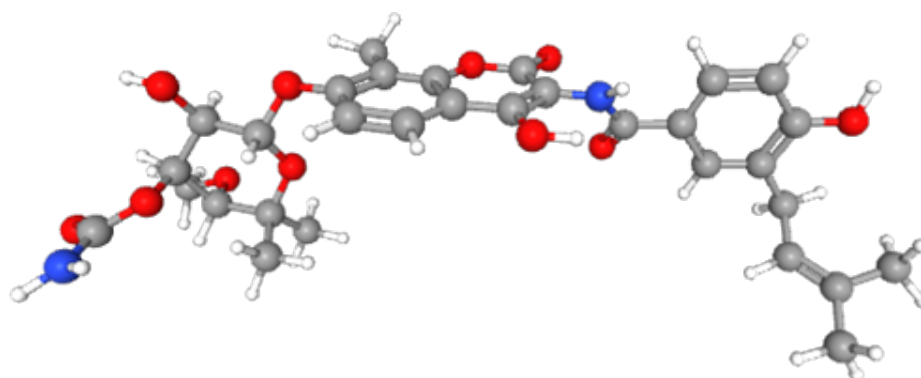


FIGURE 4.11: (3D) structure of novobiocin.

4.6.3 Chemical Information

The chemical properties of the reference ligand, novobiocin, are presented in Table 4.11. Novobiocin possesses the molecular formula $C_{31}H_{36}N_2O_{11}$ and a molecular weight of 612.632 g/mol, reflecting its relatively large and complex molecular structure.

The three-dimensional structure of the compound was obtained from the PubChem database using its Compound Identification (CID: 54675769) and was subsequently prepared for molecular docking studies. These physicochemical characteristics are important for understanding the interaction of novobiocin with DNA gyrase subunit B and provide a suitable reference for comparing the docking behavior and binding interactions of the selected *Berberis lycium* phytochemicals.

TABLE 4.11: Physicochemical characteristics of novobiocin.

Property	Value
Drug	Novobiocin
PubChem CID	54675769
Molecular Formula	C ₃₁ H ₃₆ N ₂ O ₁₁
Molecular Weight (g/mol)	612.632

4.6.4 Molecular Properties of Novobiocin

The molecular properties of novobiocin were predicted using the pkCSM web server, which provides a computational evaluation of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles.

4.6.4.1 Absorption

The absorption characteristics of novobiocin were predicted using the pkCSM web server, and the corresponding results are summarized in Table 4.12. Novobiocin exhibited a water solubility value of $-3.297 \log \text{ mol/L}$, indicating moderate aqueous solubility. The predicted Caco-2 permeability was $0.183 \log P_{\text{app}} \times 10^{-6} \text{ cm/s}$, suggesting moderate permeability across intestinal epithelial cells. The compound demonstrated a predicted intestinal absorption of 71.504%, reflecting acceptable oral absorption.

In addition, the skin permeability value ($-2.735 \log K_p$) indicated limited transdermal penetration. Novobiocin was also identified as a substrate of P-glycoprotein and an inhibitor of P-glycoprotein II, while no inhibitory activity was predicted against P-glycoprotein I.

TABLE 4.12: Absorption properties of novobiocin predicted by pkCSM.

Model	Outcome Value
Aqueous solubility (log mol/L)	-3.297
Caco-2 cell permeability (log Papp × 10 ⁻⁶ cm/s)	0.183
Human intestinal absorption (%)	71.504
Dermal permeability (log Kp)	-2.735
P-glycoprotein substrate status	Yes
P-glycoprotein I inhibition	No
P-glycoprotein II inhibition	Yes

4.6.4.2 Distribution

The distribution characteristics of novobiocin predicted by the pkCSM web server are presented in Table 4.13. The compound exhibited a predicted steady-state volume of distribution (VD_{ss}) of 0.329 log L/kg, indicating moderate distribution throughout body tissues. The fraction unbound (Fu) value was 0.002, suggesting extensive plasma protein binding and that only a very small proportion of the drug remains unbound in plasma. Furthermore, the predicted blood–brain barrier (BBB) permeability (−1.905) and central nervous system (CNS) permeability (−3.459) indicate that novobiocin is unlikely to readily cross the blood–brain barrier or accumulate within the central nervous system.

TABLE 4.13: Distribution properties of novobiocin predicted by pkCSM.

Model	Outcome Value
Steady-state volume of distribution (VD _{ss}) (log L/kg)	0.329
Fraction unbound (Fu)	0.002
Blood–brain barrier (BBB) permeability	-1.905
Central nervous system (CNS) permeability	-3.459

4.6.4.3 Metabolism

The predicted metabolic characteristics of novobiocin are summarized in Table 4.14. Novobiocin was identified as a substrate of CYP3A4 but was not predicted to be metabolized by CYP2D6. Evaluation of cytochrome P450 enzyme inhibition showed that the compound inhibits CYP3A4, whereas no inhibitory activity was predicted for CYP1A2, CYP2C19, CYP2C9, or CYP2D6.

These findings suggest that CYP3A4 is the principal enzyme involved in the metabolism of novobiocin and may influence its pharmacokinetic behavior.

TABLE 4.14: Metabolism properties of novobiocin predicted by pkCSM.

Model	Outcome Value
CYP2D6 substrate	No
CYP3A4 substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes

4.6.4.4 Excretion

The excretion properties of novobiocin predicted using the pkCSM web server are presented in Table 4.15. The compound exhibited a total clearance value of -0.452 log mL/min/kg, indicating a relatively slow rate of elimination from the body.

Furthermore, novobiocin was not predicted to be a substrate of the renal organic cation transporter 2 (OCT2), suggesting that OCT2-mediated renal transport is unlikely to play a significant role in its excretion.

TABLE 4.15: Excretion properties of novobiocin predicted by pkCSM.

Model	Outcome Value
Total clearance (log mL/min/kg)	-0.452
Renal OCT2 substrate	No

4.6.4.5 Toxicity

The predicted toxicity profile of novobiocin is presented in Table 4.16. The compound was classified as non-mutagenic according to the AMES toxicity prediction and exhibited a maximum tolerated dose of 0.475 log mg/kg/day. No inhibitory activity was predicted against the hERG I potassium channel; however, inhibition of hERG II was observed. The predicted acute oral toxicity (LD_{50}) was 2.714 mol/kg, while the lowest observed adverse effect level (LOAEL) was estimated to be 2.456 log mg/kg/day.

Novobiocin was also predicted to possess hepatotoxic potential but showed no evidence of causing skin sensitization. In addition, the predicted *Tetrahymena pyriformis* toxicity and minnow toxicity values were 0.285 log μ g/L and 3.902 log mM, respectively. Collectively, these findings indicate that although novobiocin demonstrates acceptable pharmacokinetic characteristics, its predicted hepatotoxicity and hERG II inhibition may contribute to the safety concerns associated with its clinical use.

TABLE 4.16: Toxicity properties of novobiocin predicted by pkCSM.

Model	Outcome Value
AMES toxicity	No
Maximum tolerated dose (log mg/kg/day)	0.475
hERG I inhibition	No
hERG II inhibition	Yes
LD_{50} (mol/kg)	2.714
LOAEL (log mg/kg/day)	2.456
Hepatotoxicity	Yes
Skin sensitization	No
<i>T. pyriformis</i> toxicity (log μ g/L)	0.285
Minnow toxicity (log mM)	3.902

4.6.5 Drug-Likeness

The drug-likeness profile of novobiocin was assessed using the SwissADME web server according to Lipinski’s Rule of Five (Ro5), and the corresponding results are summarized in Table 4.17. The compound possesses a molecular weight of 612.62 g/mol, 11 hydrogen bond acceptors, 5 hydrogen bond donors, 10 rotatable bonds, and a consensus LogP value of 2.01. Evaluation against Lipinski’s criteria revealed two violations, namely a molecular weight greater than 500 g/mol and the presence of more than 10 hydrogen bond acceptors. Despite these deviations, novobiocin remains an established antibacterial agent owing to its specific mechanism of action against DNA gyrase subunit B. However, the observed violations suggest that the compound may exhibit less favorable oral drug-likeness characteristics when compared with molecules that fully satisfy Lipinski’s parameters.

TABLE 4.17: Drug-likeness evaluation of novobiocin based on Lipinski’s Rule of Five.

Physicochemical Descriptor	Calculated Value
Molecular weight (g/mol)	612.62
Hydrogen bond acceptors	11
Hydrogen bond donors	5
Rotatable bonds	10
Consensus LogP	2.01
Lipinski evaluation	Failed (2 violations)

4.6.6 Molecular-Docking Analysis of Novobiocin-DNA Gyrase B

The molecular docking results of novobiocin with DNA gyrase subunit B are presented in Table 4.18. The reference ligand demonstrated a docking score of -9.0 kcal/mol, indicating a favorable binding affinity for the target protein. Novobiocin

was docked within a binding cavity having a volume of 319 Å³, suggesting effective accommodation within the active site of DNA gyrase B.

The negative binding energy reflects the formation of a stable protein–ligand complex, supporting the strong interaction of novobiocin with the ATP-binding pocket of the enzyme. These docking results are consistent with the known inhibitory activity of novobiocin against DNA gyrase subunit B and further validate its selection as the reference ligand for the present study.

TABLE 4.18: Docking parameters of novobiocin against DNA gyrase B.

Ligand	Vina Score	Cavity Volume (Å ³)	Center (x,y,z)	Docking Size (x,y,z)
Novobiocin	-9.0	319	16,16,20	29,29,29

4.6.7 Interaction Pattern of Novobiocin-DNA Gyrase B

The two-dimensional interaction diagram presented in Figure 4.12 demonstrates the binding interactions between novobiocin and DNA gyrase subunit B. The ligand formed several conventional hydrogen bonds with key amino acid residues, including Lys162, Glu58, Arg20, and Thr175, which play an important role in stabilizing the protein–ligand complex. Additional carbon–hydrogen bond interactions were observed with residues such as His147, Ser202, Thr30, Pro23, Phe176, Thr177, His215, Lys21, Gln135, Gln72, Asp17, Asp74, Ile59, and Asp73, further contributing to binding stability. Electrostatic interactions, comprising attractive charge and pi-anion contacts, were identified with Glu58, Glu174, and Arg204, enhancing the overall affinity of novobiocin for the active site. Moreover, hydrophobic interactions involving Ile60, Arg20, and His215 through alkyl and pi-alkyl contacts provided additional stabilization of the complex. Collectively, these interactions indicate that novobiocin binds firmly within the active site of DNA gyrase subunit B, supporting its well-established inhibitory activity against the enzyme.

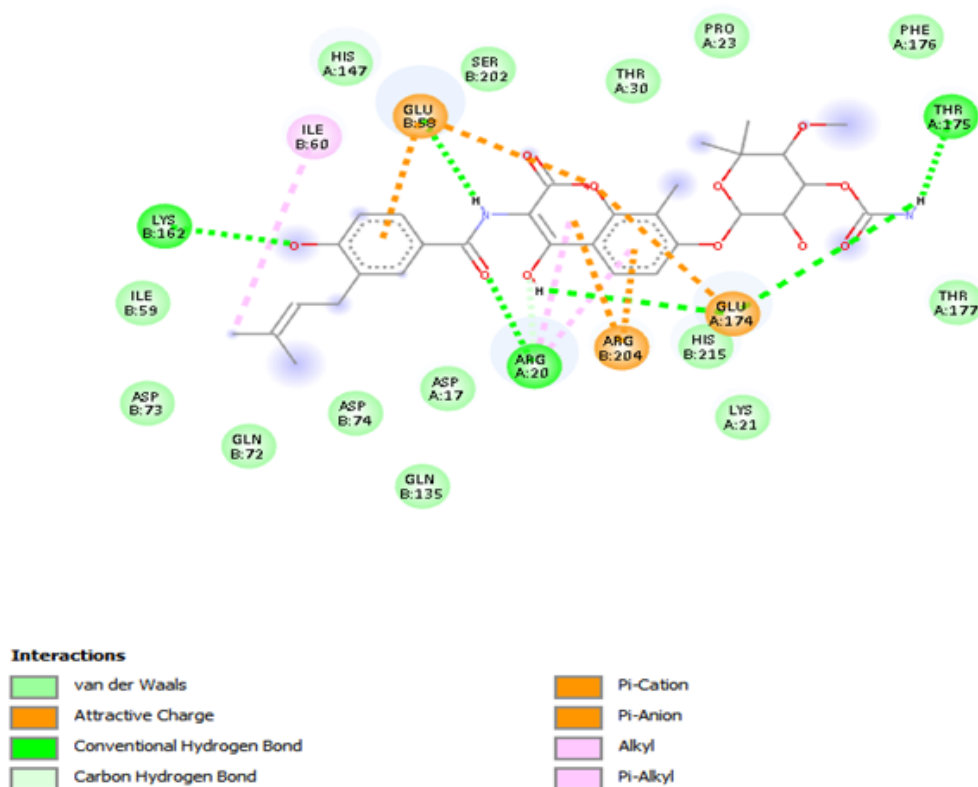


FIGURE 4.12: Interaction profile of novobiocin–DNA gyrase B.

4.7 Novobiocin–Kaempferol Comparison

4.7.1 Physicochemical Characteristics Comparative Pattern

The comparative drug-likeness profiles of kaempferol and novobiocin are presented in Table 4.19. Evaluation based on Lipinski's Rule of 5 demonstrated that kaempferol possesses more desirable physicochemical characteristics than the reference drug.

Kaempferol exhibited a molecular weight of 286.24 g/mol, which is well within the recommended limit for orally active compounds, whereas novobiocin had a considerably higher molecular weight of 612.62 g/mol. In addition, kaempferol

contained six hydrogen bond acceptors, four hydrogen bond donors, and only one rotatable bond, satisfying all of Lipinski's recommended criteria.

Conversely, novobiocin exceeded the permissible limits for molecular weight and hydrogen bond acceptors, resulting in two violations of the rule. Both compounds displayed acceptable lipophilicity, with consensus LogP values of 1.20 for kaempferol and 2.01 for novobiocin.

Overall, the complete compliance of kaempferol with Lipinski's Rule of 5 suggests that it has more favorable drug-like characteristics and a greater potential for oral bioavailability than the reference drug.

TABLE 4.19: Comparative drug-likeness properties of kaempferol and novobiocin.

Drug-Likeness Parameter	Kaempferol	Novobiocin
Molecular Weight (g/mol)	286.24	612.62
Hydrogen Bond Acceptors	6	11
Hydrogen Bond Donors	4	5
Rotatable Bonds	1	10
Consensus LogP	1.20	2.01
Lipinski Compliance	Yes (0 violations)	No (2 violations)

4.7.2 ADMET Properties Comparison of Novobiocin Kaempferol

4.7.2.1 Absorption Comparison

The comparative absorption characteristics of novobiocin and kaempferol are presented in Table 4.20. Both compounds were predicted to act as P-glycoprotein substrates and exhibited the same skin permeability value ($-2.735 \log K_p$). Kaempferol demonstrated a slightly greater intestinal absorption (74.29%) than novobiocin (71.50%), indicating a marginally improved oral absorption profile.

Although novobiocin showed higher Caco-2 permeability, both ligands displayed comparable water solubility. Furthermore, novobiocin was predicted to inhibit P-gp II, whereas no such inhibitory activity was observed for kaempferol, suggesting a reduced likelihood of transporter-associated interactions for the latter.

TABLE 4.20: Comparative absorption properties of novobiocin and kaempferol.

Absorption Parameter	Novobiocin	Kaempferol
Water Solubility (log mol/L)	-3.297	-3.040
Caco-2 Permeability	0.183	0.032
Intestinal Absorption (%)	71.504	74.290
Skin Permeability (log Kp)	-2.735	-2.735
P-gp Substrate	Yes	Yes
P-gp-I Inhibitor	No	No
P-gp-II Inhibitor	Yes	No

4.7.2.2 Distribution Comparison

The predicted distribution properties of novobiocin and kaempferol are summarized in Table 4.21. Kaempferol exhibited a considerably higher steady-state volume of distribution (1.274 log L/kg) than novobiocin (0.329 log L/kg), indicating a stronger tendency to distribute into body tissues. Additionally, kaempferol showed a higher proportion unbound in plasma, indicating that there was more free molecule available for pharmacological effect. Although both substances are anticipated to have restricted entry into the central nervous system, predictions of blood–brain barrier and central nervous system permeability also showed comparatively higher permeability for kaempferol.

TABLE 4.21: Comparative distribution properties of novobiocin and kaempferol.

Distribution Parameter	Novobiocin	Kaempferol
VDss (log L/kg)	0.329	1.274
Fraction Unbound (Fu)	0.002	0.178
BBB Permeability	-1.905	-0.939
CNS Permeability	-3.459	-2.228

4.7.2.3 Metabolism Comparison

The metabolic behavior of novobiocin and kaempferol is compared in Table 4.22. Novobiocin was predicted to function as both a substrate and an inhibitor of CYP3A4, indicating that its metabolism is primarily associated with this enzyme. In contrast, kaempferol was not predicted to interact with CYP3A4 as either a substrate or an inhibitor but demonstrated inhibitory activity against CYP1A2. Neither compound was identified as a substrate or inhibitor of CYP2D6, CYP2C19, or CYP2C9.

Overall, kaempferol exhibited fewer predicted interactions with cytochrome P450 enzymes, suggesting a lower potential for metabolism-related drug interactions.

TABLE 4.22: Comparative metabolism properties of novobiocin and kaempferol.

Metabolism Parameter	Novobiocin	Kaempferol
CYP-2D6 Substrate	Negative	Negative
CYP-3A4 Substrate	Positive	Negative
CYP-1A2 Inhibitor	Negative	Positive
CYP-2C19 Inhibitor	Negative	Negative
CYP-2C9 Inhibitor	Negative	Negative
CYP-2D6 Inhibitor	Negative	Negative
CYP-3A4 Inhibitor	Positive	Negative

4.7.2.4 Excretion Comparison

Table 4.23 displays the excretion characteristics of kaempferol and novobiocin. Compared to novobiocin (-0.452 log mL/min/kg), kaempferol had a higher estimated total clearance value (0.477 log mL/min/kg), suggesting a more quicker removal from the body. The renal organic cation transporter 2 (OCT2) was not anticipated to be a substrate of either molecule, indicating that this transporter is unlikely to play a major role in the renal excretion of either ligand.

TABLE 4.23: Comparative excretion properties of novobiocin and kaempferol.

Excretion Parameter			Novobiocin	Kaempferol
Total Clearance	(log mL/min/kg)		-0.452	0.477
Renal OCT2 Substrate			No	No

4.7.2.5 Toxicity Comparison

The predicted toxicity profiles of novobiocin and kaempferol are summarized in Table 4.24. Both compounds were classified as non-mutagenic in the AMES toxicity test and were not predicted to cause skin sensitization. Kaempferol exhibited a slightly higher maximum tolerated dose than novobiocin and was predicted to be free from hepatotoxic effects. In contrast, novobiocin demonstrated hepatotoxic potential and was identified as an inhibitor of the hERG II channel, whereas kaempferol showed no predicted inhibition of either hERG I or hERG II. Although novobiocin displayed a marginally higher LD50 value, kaempferol exhibited a slightly higher LOAEL, reflecting a favorable chronic toxicity profile. Collectively, these predictions indicate that kaempferol possesses a safer overall toxicity profile and improved pharmacokinetic characteristics compared with the reference drug, supporting its potential as a promising lead compound for further antibacterial studies.

TABLE 4.24: Comparative toxicity profiles of novobiocin and kaempferol.

Toxicity Parameter	Novobiocin	Kaempferol
AMES Mutagenicity	Negative	Negative
Maximum Tolerated Dose	0.475	0.531
hERG-I Inhibitor	Negative	Negative
hERG-II Inhibitor	Positive	Negative
LD50 (mol/kg)	2.714	2.449
LOAEL (log mg/kg/day)	2.456	2.505
Hepatotoxicity	Positive	Negative
Skin Sensitization	Negative	Negative
Tetrahymena pyriformis Toxicity	0.285	0.321
Minnow Toxicity	3.902	2.885

4.7.3 Docking Score Comparison

The comparative molecular docking results of novobiocin and kaempferol against DNA gyrase subunit B are presented in Table 4.25. Both ligands demonstrated strong binding affinities toward the target protein, as indicated by their negative docking scores. Novobiocin, used as the reference antibacterial drug, produced a docking score of -9.0 kcal/mol within a binding cavity of 319 Å³.

The docking pose was generated using a grid center of (16, 16, 20) and a docking box size of (29, 29, 29), reflecting a stable interaction with the ATP-binding region of the enzyme. Kaempferol exhibited a slightly lower docking score of -9.2 kcal/mol, indicating a stronger predicted binding affinity than the reference ligand. The compound was accommodated within a binding cavity of 810 Å³, with docking coordinates of (34, 8, 3) and a docking box size of (21, 21, 21).

These findings suggest that kaempferol interacts favorably with DNA gyrase subunit B and further support its selection as the lead phytochemical for subsequent analyses.

TABLE 4.25: Docking Parameters Comparative Pattern.

Ligand	Vina	Cavity	Center	Docking Size
	Score	Volume ($\text{\AA}^3$)	(x,y,z)	(x,y,z)
Novobiocin	-9.0	319	(16,16,20)	(29,29,29)
Kaempferol	-9.2	810	(34,8,3)	(21,21,21)

4.7.4 Interaction analysis comparison

The comparative interaction analysis demonstrated that both novobiocin and kaempferol formed stable complexes with DNA gyrase subunit B through multiple intermolecular interactions. Novobiocin established several conventional and carbon hydrogen bonds together with electrostatic and hydrophobic interactions involving residues such as Lys162, Glu58, Arg20, Thr175, His147, Phe176, Ile60, and His215. These interactions contributed to the stability of the protein–ligand complex and are consistent with the known inhibitory mechanism of novobiocin against DNA gyrase B.

Kaempferol also formed a stable binding complex with DNA gyrase subunit B through multiple conventional hydrogen bonds involving residues such as Asp73, Gly77, Thr165, and Arg76. Additional electrostatic interactions with Glu50 and Lys103, together with π -sigma, amide- π stacked, and π -alkyl hydrophobic interactions, further stabilized the ligand within the active site. The combination of strong hydrogen bonding and hydrophobic contacts, together with its favorable docking score, indicates that kaempferol exhibits a robust binding pattern with DNA gyrase subunit B, supporting its identification as the lead compound in the present study.

In summary, the combined findings from the drug-likeness evaluation, ADMET prediction, molecular docking, and protein–ligand interaction analyses identified kaempferol as the most promising lead compound among the investigated *Berberis*

lycium phytochemicals. The compound exhibited excellent binding affinity toward DNA gyrase subunit B, desirable pharmacokinetic characteristics, full compliance with Lipinski's Rule of Five, and a favorable predicted safety profile. Collectively, these results highlight the potential of kaempferol as a natural antibacterial candidate and provide a strong rationale for its further investigation through experimental validation and preclinical studies.

Chapter 5

Discussion

This study utilized computational techniques to assess the antibacterial potential of selected phytochemicals derived from *Berberis lycium* Royle by targeting DNA Gyrase B, a key bacterial enzyme responsible for DNA replication and maintaining DNA supercoiling. In order to examine the antibacterial activity of certain phytochemicals extracted from *Berberis lycium* Royle against DNA Gyrase B, a crucial bacterial enzyme involved in DNA replication and supercoiling, the current work used an in silico method [115].

The binding affinities of six phytochemicals, namely berberine, palmatine, jatrorrhizine, oxyberberine, quercetin, and kaempferol, were assessed using molecular docking. All of the chosen chemicals successfully connected with DNA Gyrase B's active site, according to the docking data, suggesting that they may block the enzyme and obstruct bacterial development. These results provide computational evidence for *Berberis lycium*'s potential utility in the development of antibacterial drugs and validate its traditional medical use as an antimicrobial plant.

Among the evaluated phytochemicals, kaempferol exhibited the strongest binding affinity with a docking score of -9.2 kcal/mol, followed by quercetin (-8.9 kcal/mol), berberine (-8.8 kcal/mol), oxyberberine (-8.7 kcal/mol), palmatine (-8.3 kcal/mol), and jatrorrhizine (-8.0 kcal/mol). The reference inhibitor, novobiocin, showed a docking score of -9.0 kcal/mol.

The slightly lower binding energy observed for kaempferol suggests that this compound possesses a binding affinity comparable to, and marginally stronger than, the standard inhibitor under the docking conditions employed in this study. Since lower docking scores generally indicate more stable ligand–protein complexes, these findings suggest that kaempferol may serve as a promising inhibitor of DNA Gyrase B.

In addition to docking scores, the interaction analysis demonstrated that the selected phytochemicals established several important molecular interactions within the ATP-binding pocket of DNA Gyrase B. Hydrogen bonds, hydrophobic interactions, and electrostatic contacts were observed between the ligands and amino acid residues located in the active site [107]. Such interactions play a crucial role in stabilizing ligand–protein complexes and contribute significantly to binding affinity.

The presence of multiple hydrogen bonds formed by kaempferol and other phytochemicals may explain their relatively strong docking performance. Similar observations have been reported in previous molecular docking studies, where stable hydrogen-bonding networks and hydrophobic interactions were associated with increased inhibitory activity against bacterial enzymes.

The favorable docking results obtained for quercetin and kaempferol are consistent with previous reports describing the antibacterial properties of flavonoids. Flavonoids have been shown to inhibit several bacterial enzymes through interactions with catalytic residues and ATP-binding domains, thereby interfering with essential cellular processes. Likewise, berberine, one of the major isoquinoline alkaloids present in *Berberis lycium*, has been extensively reported to possess broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria [76].

The strong docking affinity observed for berberine in the present study further supports these earlier findings and highlights its importance as one of the principal bioactive constituents of *Berberis lycium*. Previous investigations have also

demonstrated that berberine can inhibit bacterial nucleic acid synthesis and interfere with essential metabolic pathways, making it an attractive lead compound for antibacterial drug development.

Although palmatine, jatrorrhizine, and oxyberberine exhibited slightly lower docking scores than kaempferol and quercetin, their binding energies remained sufficiently favorable to indicate meaningful interactions with DNA Gyrase B. These compounds formed stable ligand–protein complexes within the active site, suggesting that they may also contribute to the antibacterial activity traditionally associated with *Berberis lycium*.

The variation in docking scores among the phytochemicals may be attributed to differences in their molecular structures, functional groups, flexibility, and ability to establish stabilizing interactions with amino acid residues present in the ATP-binding pocket. Therefore, the antibacterial potential of *Berberis lycium* is likely the result of the combined activity of multiple phytochemicals rather than a single constituent [109].

The use of DNA Gyrase B as the molecular target further strengthens the significance of the present study. DNA Gyrase B is an ATP-dependent enzyme that is indispensable for bacterial DNA replication, transcription, chromosome segregation, and repair. Because this enzyme is absent in human cells, it has become one of the most effective and promising targets for antibacterial drug discovery. Inhibiting the ATPase domain of DNA Gyrase B disrupts bacterial DNA supercoiling, ultimately preventing bacterial proliferation.

Consequently, the favorable interactions observed between the selected *Berberis lycium* phytochemicals and the ATP-binding pocket of DNA Gyrase B indicate their potential as natural inhibitors of this essential bacterial enzyme and support further investigation through experimental studies [104, 105].

The molecular docking approach employed in this study provides valuable preliminary insights into the possible mechanism through which *Berberis lycium* phytochemicals may exert antibacterial activity. Molecular docking has become an

essential component of computer-aided drug discovery because it enables the prediction of ligand–protein interactions before conducting laboratory experiments.

By estimating the binding affinity of phytochemicals toward a specific biological target, molecular docking assists in identifying compounds that are more likely to exhibit biological activity. Consequently, the present findings not only support the traditional medicinal use of *Berberis lycium* but also provide a scientific basis for selecting promising phytochemicals for future experimental investigations.

Among the phytochemicals evaluated, kaempferol demonstrated the highest binding affinity toward DNA Gyrase B. Its superior docking score may be attributed to its molecular structure, which allows the formation of multiple stabilizing interactions within the ATP-binding pocket of the enzyme [105].

While kaempferol's aromatic rings promote hydrophobic interactions with nearby amino acid residues, its hydroxyl groups can participate in the formation of hydrogen bonds. Together, these interactions support the stability of the ligand–protein complex and could account for the relatively higher binding affinity found during docking research.

Similar observations have been reported for flavonoids, where hydroxylated aromatic structures enhance protein-binding capability and improve inhibitory potential against bacterial enzymes.

The favorable docking score obtained for quercetin further supports the antibacterial potential of flavonoid compounds present in *Berberis lycium*. Quercetin has previously been reported to exhibit antimicrobial activity through multiple mechanisms, including inhibition of bacterial enzymes, disruption of membrane integrity, and interference with nucleic acid synthesis. The strong interaction of quercetin with DNA Gyrase B observed in the present study suggests that inhibition of this enzyme may represent one of the mechanisms responsible for its antibacterial effects.

Berberine, a major isoquinoline alkaloid found in *Berberis lycium*, also demonstrated strong binding affinity toward DNA Gyrase B. Berberine has long been

recognized as one of the principal bioactive constituents of the genus *Berberis* and has been extensively investigated for its antimicrobial, anti-inflammatory, antioxidant, and antidiabetic properties [74]. Previous experimental studies have shown that berberine inhibits the growth of several pathogenic bacteria by interfering with essential cellular processes. The docking results obtained in the present study provide additional computational evidence supporting its antibacterial potential and indicate that DNA Gyrase B may serve as one of its possible molecular targets.

Although palmatine, oxyberberine, and jatrorrhizine produced comparatively higher docking energies than kaempferol, they nevertheless exhibited favorable interactions with the active site of DNA Gyrase B. These findings indicate that all selected phytochemicals possess some degree of affinity for the target enzyme. The differences observed in docking scores are likely related to variations in molecular size, structural flexibility, functional groups, and the ability of each compound to establish stabilizing interactions with active-site residues. Such variations are expected in molecular docking studies and provide useful information for prioritizing compounds for further biological evaluation.

Another important aspect of the present investigation is the comparison of the selected phytochemicals with novobiocin, which was used as the reference inhibitor. Novobiocin is a well-established inhibitor of DNA Gyrase B and specifically targets the ATPase domain of the enzyme. The observation that kaempferol exhibited a docking score comparable to, and slightly better than, novobiocin suggests that naturally occurring phytochemicals may possess inhibitory potential similar to known antibacterial agents [116].

Although docking scores alone cannot confirm biological activity, they provide valuable preliminary evidence for identifying compounds that deserve further investigation through experimental studies. The docking scores obtained in this study are comparable with those reported in previous *in silico* investigations evaluating plant-derived compounds against DNA Gyrase B [117].

The observation that kaempferol exhibited a binding affinity comparable to or slightly stronger than the reference inhibitor novobiocin highlights the potential of naturally occurring phytochemicals as alternative antibacterial candidates. Although computational predictions alone cannot confirm biological activity, the consistency of these findings with published literature strengthens the reliability of the present study and provides a scientific basis for future experimental validation.

Overall, the molecular docking analysis demonstrates that *Berberis lycium* contains several phytochemicals capable of interacting effectively with DNA Gyrase B. The combination of favorable binding affinities and stable molecular interactions indicates that these naturally occurring compounds may interfere with the normal function of this essential bacterial enzyme. These findings strengthen the scientific basis for the traditional medicinal use of *Berberis lycium* and highlight its potential as a source of lead compounds for the development of novel antibacterial agents.

Chapter 6

Conclusion and Future Recommendations

6.1 Conclusion

The current study used molecular docking and in silico ADMET analysis to examine the antibacterial potential of several phytochemicals from *Berberis lycium* Royle against DNA Gyrase B. A total of six phytochemicals, namely berberine, palmatine, jatrorrhizine, oxyberberine, quercetin, and kaempferol, were evaluated for their binding affinity toward DNA Gyrase B. Molecular docking analysis demonstrated that all selected phytochemicals interacted with the ATP-binding pocket of the target protein through hydrogen bonding, hydrophobic interactions, and electrostatic contacts, indicating their potential to inhibit the normal function of DNA Gyrase B.

Among the investigated compounds, kaempferol exhibited the strongest binding affinity, with a docking score of -9.2 kcal/mol, slightly better than the reference inhibitor novobiocin (-9.0 kcal/mol) under the docking conditions employed in this study. Quercetin and berberine also demonstrated strong binding affinities, while oxyberberine, palmatine, and jatrorrhizine showed favorable interactions with the

active site of the protein. The ADMET and drug-likeness analyses further indicated that most of the selected phytochemicals exhibited acceptable pharmacokinetic characteristics and favorable drug-like properties. In particular, kaempferol showed compliance with Lipinski's Rule of Five and a favorable predicted safety profile.

Overall, the findings of this study provide computational evidence supporting the antibacterial potential of *Berberis lycium* Royle phytochemicals against DNA Gyrase B and identify kaempferol as a promising candidate for further investigation. However, molecular docking and ADMET prediction provide preliminary evidence only, and the predicted interactions and pharmacokinetic properties require confirmation through laboratory-based investigations before definitive conclusions regarding antibacterial efficacy can be made.

6.2 Future Recommendations

Based on the findings of the present in silico study, further computational and experimental investigations are recommended. The most promising phytochemicals, particularly kaempferol, quercetin, and berberine, should be subjected to molecular dynamics (MD) simulations to evaluate the stability, flexibility, and conformational behavior of the protein–ligand complexes. Binding free energy calculations, such as MM/PBSA or MM/GBSA analyses, may also be performed to provide a more accurate estimation of ligand–protein binding strength.

Future in silico studies should expand the phytochemical library by including additional compounds reported from different parts of *Berberis lycium* Royle. The selected phytochemicals should also be evaluated against additional bacterial targets, including Topoisomerase IV, Penicillin-Binding Proteins (PBPs), Mur enzymes, β -lactamases, and Dihydrofolate Reductase (DHFR), to provide broader insight into their possible mechanisms of action.

Finally, the computationally promising phytochemicals identified in this study should be subjected to in vitro antibacterial assays and further experimental investigations to validate their predicted activity, mechanism of action, pharmacokinetic properties, and safety. These investigations may contribute to the development of novel plant-derived antibacterial agents and support further research on *Berberis lycium* Royle.

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