CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Investigating the Antioxidant, Antimicrobial and Anticancer Potential of *Berberis lycium* and *Solanum surattense*

by

Remaisha Ali

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

2024

In deepest appreciation, this thesis is dedicated to my family, whose unwavering support provided the foundation, and to my esteemed teachers, whose guidance and expertise illuminated the path to completion.

CERTIFICATE OF APPROVAL

Investigating the Antioxidant, Antimicrobial and Anticancer Potential of *Berberis lycium* and *Solanum surattense*

by

Remaisha Ali

(MBS223017)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. M. Sheeraz Ahmed	PMAS-AAUR, Rawalpindi
(b)	Internal Examiner	Dr. Sohail Ahmad Jan	CUST, Islamabad
(c)	Supervisor	Dr. Muhammad Asad Anwar	CUST, Islamabad

Dr. Muhammad Asad Anwar
Thesis Supervisor
September, 2024

Dr. Syeda Marriam Bakhtiar
Head
Dept of Bioinformatics and Biosciences
September, 2024

Dr. Sahar Fazal
Dean
Faculty of Health and Life Sciences
September, 2024

Author's Declaration

I, **Remaisha Ali** hereby state that my MS thesis titled "**Investigating the Antioxidant, Antimicrobial and Anticancer Potential of *Berberis lycium* and *Solanum surattense***" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Remaisha Ali)
Registration No: MBS223017

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Investigating the Antioxidant, Antimicrobial and Anticancer Potential of *Berberis lycium* and *Solanum surattense***" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Remaisha Ali)
Registration No: MBS223017

Acknowledgement

I would like to express my sincere gratitude to Almighty Allah (SWT) for granting me the strength and guidance to complete this thesis. I am deeply indebted to my family for their unwavering love and support throughout my academic journey. My deepest appreciation goes to Dr. Muhammad Asad Anwar (Assistant Professor, Department of Bioinformatics and Biosciences, CUST) whose invaluable guidance, encouragement, and dedication of time significantly contributed to the successful completion of this work.

Thanks to all.

(Remaisha Ali)

Abstract

Throughout history, various plants have been utilized in traditional medicine to address a wide range of health concerns. They offer a vast array of bioactive compounds with potential health benefits. This study aimed to explore the potential health benefits of *Berberis lycium* and *Solanum surattense* by evaluating their phytochemical content, antioxidant, antibacterial, anticancer, and antifungal activities. FT-IR spectroscopy analysis revealed the presence of key functional groups, including alcohols, phenols, alkanes, and carbonyl compounds in both extract. Total phenolic and flavonoid contents were determined using spectrophotometric methods. The antioxidant capacity of the extracts was assessed using the DPPH radical scavenging assay, with IC_{50} values calculated to compare their effectiveness. The Alamar Blue assay evaluated the antibacterial activity against various bacteria, while the agar well diffusion method investigated antifungal activity against *Aspergillus niger*. The MTT assay measured cytotoxicity towards human prostate cancer cells (PC3).*Berberis lycium* exhibited significantly higher total phenolic content (193.83 ± 3.93 mg GAE/g) and total flavonoid content (37.47 ± 0.37 mg QE/g) compared to *Solanum surattense* (50.69 ± 3.53 mg GAE/g and 19.41 ± 0.33 mg QE/g, respectively), suggesting a richer profile of antioxidant compounds in *Berberis lycium*. Consistent with this, *Berberis lycium* extract displayed stronger DPPH radical scavenging activity (IC50 = 4.10 ± 0.06 μg/mL) compared to *Solanum surattense* (IC50 = 4.43 ± 0.11 μg/mL). Evaluation of antibacterial activity revealed moderate inhibition of *Staphylococcus aureus* by *Berberis lycium* extract (60.21% inhibition at 3000 μg/mL). While *Solanum surattense* did not exhibit significant inhibition against any of the tested bacteria (*Escherichia coli, Bacillus subtilis, Salmonella typhi*) at the tested concentration (3000 μg/mL), further exploration at different concentrations might be beneficial.Neither *Berberis lycium* nor *Solanum surattense* extracts exhibited significant antifungal activity against *Aspergillus niger* at the tested concentrations (250 μg/mL and 500 μg/mL).*Berberis lycium* extracts displayed low cytotoxic effects against human prostate cancer cells (PC3), with only 4.61% inhibition at 30 μg/mL. *Solanum surattense* extract also showed minimal inhibition (35.93% at 30

μg/mL).These findings suggest that *Berberis lycium* may be a promising source of natural antioxidants due to its higher content of phenolics and flavonoids.

Contents

List of Figures

List of Tables

Abbreviations

B. lycium	*Berberis lycium*
DMSO	Dimethylsulfoxide
DPPH Assay	2,2-diphenyl-1-picrylhydrazyl assay
FBS	Fetal bovine serum
FC reagent	Folin-Ciocalteu reagent
MABA	Microplate Alamar Blue Assay
MHB	Mueller-Hinton Broth
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
OD	optical density
PC3 Cells	prostate cancer cells
S. surattense	*Solanum surattense*
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

Chapter 1

Introduction

Plants act like natural chemical factories, producing a vast array of beneficial compounds essential for human health [1]. These are known as medicinal plants, and they're a treasure trove of potential ingredients for new drugs. The reason plants can be used to treat so many ailments is because they contain unique molecules called phytochemicals. Interestingly the World Health Organization reports that a staggering 80% of the world's population still relies on traditional medicine for their healthcare needs which often utilizes herbs for common illnesses and overall well-being [2]. There are several advantages to using plant-based medicines: they're generally safe, effective, have fewer side effects, and are often well-accepted culturally [3].

The hunt for natural cures goes way back - humans have been scouring nature for medicinal plants since the very beginning. Just like animals instinctively nibble on healing plants, our ancestors likely did the same [4]. Back then, with limited understanding of diseases or specific plant properties, treatment relied heavily on trial and error. Over time, this experience-based approach gradually evolved. As people began to grasp the reasons behind illnesses and the effects of particular plants, the use of medicinal plants shifted from pure observation to a more targeted, evidence-based approach. Prior to the 16th century, when iatrochemistry was developed, plants were source of cure and prophylaxis [5].

Approximately 300–400 medicinal plants were listed by Hippocrates (5th century B.C.) in his writings. Dioscorides, too, documented the medical applications of many different plant species in his De Materia Medica, a book on medicinal plants, which he authored in the first century B.C. [6]. Herbal medicine has a 5,000-year tradition in China [7, 8]. Numerous plant species with antiseptic and therapeutic characteristics, such frankincense and myrrh, are also mentioned in the Bible [9].

Approximately 250,000–500,000 different plant species can be found globally. Though less than 10% of these plants have been thoroughly studied by scientists, many of these plants are utilized by both human and animal species for a variety of reasons, including meals and medicines [10]. Herbal medicine is becoming more and more common in both developed and developing countries [11]. The effectiveness of herbal medicines as antibacterial agents and/or their qualities have been the subject of several investigations carried out worldwide [12–16].

The need for herbal remedies has grown recently since numerous plants and herbs have been shown by science to contain bioactive chemical or compounds, serving as safer substitutes for synthetic medications that have negative impacts on the environment and biological systems [18]. Worldwide, herbal medications have utilized to treat a extensive range of infectious illnesses in both humans and animals [18]. Due to their low cost, enhanced cultural acceptance, enhanced body compatibility, and minimal side effects, herbal medications are currently in high demand in poor countries [9, 19].Moreover, plant-based active ingredients may be present in blood thinners, laxatives, antibiotics, and anti-malarial medications. Lead molecules for drug design and development can also be obtained from medicinal plants [20–23]. Herbal medications are utilized in addition to antimicrobial therapy to treat age-related conditions such as immune system problems, memory loss, and osteoporosis [9]. Studies have shown they can act as analgesics (pain relievers), antibacterials (fight bacteria), deodorizers (eliminate odors), febrifuges (reduce fever), fungicides (kill fungus), antiseptics (fight germs), antidepressants (improve mood), astringents (tighten tissues), diuretics (increase urination), galactagogues (promote milk production), insecticides (kill insects), antipyretics (reduce

fever), antimicrobials (fight microbes), and sedatives (calm the nervous system) [24, 25].

With about 6000 wild plant species and 1572 genera, Pakistan has a wealth of floral diversity. The majority of these species are prevalent in the Hindukush, Himalaya, and Karakorum regions [26–28]. Numerous representative studies have found that Pakistani rural populations use about 600 distinct species of medicinal plants to treat common ailments [29]. The indigenous tribal groups in Northern Pakistan have extensive knowledge of the distribution and traditional uses of medicinal herbs. Records on the usage of medicinal plants in different parts of Pakistan have been gathered from numerous ethnobotanical studies conducted in the northwest of the country [30–36]. Particularly the elders (male and female) and traditional healers (male) have long been aware of the use of herbs to cure a range of common ailments [37].

1.1 Selected Plants

1.1.1 *Berberis lycium Royle*

One of the largest dicotyledonous genus in the Berberidaceae family is the Berberis. Members of this family often grow as bushes and are woody with prickly leaves that are evergreen. This family contains 650 species and 17 genera, according to recent data [38, 39]. Primarily found in America ,Asia, Europe and Africa, the genus Berberis is widely distributed worldwide. This genus is distributed between 1400 and 3500 meters above sea level throughout the majority of Pakistan's mountainous regions, namely in the northern regions of Swat and Azad Kashmir and the provinces of Balochistan regions the Khyber Pakhtunkhwa province, and Punjab. Both traditional and alternative medicine make use of its chemical constituents [38, 39].

Berberis lycium among the Berberis species, Royle has a specific place in many traditional medicines used all over the world. *Berberis lycium* is known as "Kashmal" or "Kasmal" in Urdu in Pakistan. Other common names for it are English barberry, Indian barberry, and Indian lycium [40]. The tribe people who live in the Kashmir valley primarily used herbal remedies to cure hemorrhagic dysentery. Every portion of *Berberis lycium* has unlimited medicinal value [41].

Berberis species are used for their bark, which is tonic and anti-periodic, and for their roots, which are antipyretic, diaphoretic, and anti-cyclical. In addition, plants in this genre have shown hepatoprotective, cardiovascular, anti-cancerous, and antibacterial properties. They have also been shown to be a safer treatment for gastrointestinal diseases in people [41]. *Berberis lycium* is used to treat diarrhea and jaundice with its stem powder, cure rheumatic and muscular discomfort with its root powder, and make tea from its leaves.The plant extract is used to cure intestinal problems, burning eyes, spleen diseases, cough, throat, diarrhea, chest and fractured bones [41, 42]. The fruits of *B. lycium* are used in anti-inflammatory, anti-tumor, hypo-glycemic, coagulant, and febrifuge drugs. The whole plant is used by the people to cure rheumatism, ulcers, broken bones, jaundice, and eye pain [43, 44]. Fruit extract, root bark, and stem bark have all been shown in animal models to have biological activities, including blood sugar regulation, cholesterol management, and anticancer properties. Additionally, reports suggest antioxidant, antitumor,antiurolithic and wound-healing capabilities [41, 45, 46].

This species is threatened because it is a woody plant found in hilly places without natural gas supplies, such as District Shangla. Despite its curative properties, the indigenous populations burn it to cook food. The local people have begun to rely on modern methods, nevertheless, as a result of the development of communication networks and educational resources, and it is possible that in the near future, modern knowledge may completely replace the traditional understanding of this plant [40]. As such, it is imperative to maintain the conventional knowledge base while integrating it with contemporary methodologies. Crucial phytoconstituents found in *B. lycium* include umbellatine, punjabine, sindamine, chinabine, gilgitine, jhelumine, baluchistanamine, berberine, plamitine, and berbamine. The primary

phytochemical among them is berberine, an isoquinoline alkaloid that is derived from the roots and bark.

Scientific research has identified the presence of alkaloids like *berberine* and palmatine in *B. lycium* extracts, which have demonstrated antioxidant and potentially beneficial effects against certain microorganisms and cancer cells [47]. Reviving the significance of this endangered plant species is, thus, the goal of the current study [48, 49]. The phytochemical composition and antioxidant potentials of the gathered ethnopharmacological data have been connected [45, 50]. This work will provide new insight into the chemical profile and biological activity of *B. lycium*, as well as aid in the recognition of the plant's medicinal significance.

1.1.2 *Solanum surattense*

The vast family Solanaceae, which comprises tiny trees, shrubs, and herbs, has 2300 species. For this family, the availability of natural items with medicinal properties is well established [51]. The Solanaceae family plant *Solanum surattense* is referred to by the following names: bhejibaugana, kantakari, kandangati, nelamulaka, and kateli [52]. Polynesian culture the Southeast Asian region, Malaysia, Ceylon, Australia, and India are home to this plant [53]. *Solanum surattense* is a woody-based, prickly perennial herb that grows to a height of 1.2 meters.

Solanum surattense leaves are oval-shaped with a slightly pointed tip and deeply indented edges. The base of the leaf tapers to a narrow point, and both the veins and margins are lined with sharp spines [54]. The stem itself is heavily branched and grows in a somewhat zig-zag pattern. Young branches are covered in a dense layer of fine hairs, while the mature stems have straight, smooth, and shiny prickles that can reach lengths of 1-3 centimeters.The plant produces clusters of pinkish-blue flowers that bloom on stalks arising from the angles between the stem and leaves. Each flower has five separate, pointed sepals with prickly tips that form the calyx. The corolla, or main flower body, is composed of five pointed lobes that are typically broad at the base and triangular towards the tip. The center of the flower holds a structure called the meniscus, which is likely a reference to a

specific floral part but the exact term might require further research. Finally, the plant bears spherical berries that start out green with white stripes and mature to a yellow color. Inside these berries lie numerous round seeds with a silky texture [54].

Solanum surattense has a long and rich history in Indian traditional medicine. For centuries, it's stayed to address extensive range of ailments with respiratory problems, fever, digestive issues, and even rheumatism and gonorrhea [55, 56]. Interestingly, the fruit of the plant has also been traditionally used to promote wound healing, with studies suggesting the effectiveness of its ethanol extract [57, 58]. Beyond these documented uses; there are anecdotal reports of *Solanum surattense* being used for various other purposes:

- As an aphrodisiac [60, 61]
- To expel intestinal worms [59]
- To aid sleep [59]
- As a laxative [59]
- To treat liver problems [59]
- For pain relief [64]
- To combat fungal infections [63]

One interesting traditional practice involves using the fumes from *Solanum surattense* seeds to relieve toothaches and gum swelling [64].

S. surattense has the following pharmacological properties: hypoglycaemic [68], larvicidal [69], antioxidant [66], antibacterial, antifungal, and antinociceptive [65, 67]. Numerous alkaloids have been found in *Solanum surattense* phytochemical research [70]. *S. surattense* seed vapors are an excellent treatment for gingival swelling pain as well as tooth pain, according to Pandey [64]. Pharmacologically, *S. surattense* possesses larvicidal, hypoglycemic, antioxidant, antibacterial, antifungal, antinociceptive, and antidepressant qualities [66–69]. *Solanum surattense*

has been the subject of numerous phytochemical studies, revealing a diverse range of potentially bioactive compounds. These studies have identified the presence of alkaloids, saponins, phenols, gums, sterols, flavonoids, and specific alkaloids like solamargine, solasurine, and solasonine [70, 73, 75–78]. Notably, research by Saiyed and Kanga (1936) [79] isolated glycoalkaloids (solanosine), steroidal compounds (carpesterol), and steroidal alkaloids (caffeic acid, coumarins, and triterpinoids) from the fruit of *S. surattense*. Subsequent studies confirmed the presence of steroidal alkaloids like solamorgine, solanocarpine, and solanocarpidine in the fruits [80]. Additionally, the roots of *S. surattense* are reported to contain flavonoids, alkaloids, triterpenoids, saponins, tannins, glycosides, and steroids [81, 82].

These diverse phytochemical constituents have been linked to various biological activities of *S. surattense*. Several studies have reported promising antibacterial potential against a range of pathogens. The ethanolic leaf extract, for instance, has demonstrated activity against bacteria like *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Staphylococcus aureus*, and *Streptococcus* species [83]. Similarly, ethanol and methanol extracts have shown effectiveness against *Pseudomonas aeruginosa* [84]. Studies investigating the fruit extract also indicate potential growth inhibition against various bacteria [85].

Beyond antibacterial properties, *S. surattense* extracts have also been explored for their antifungal activity. Research has focused on fungi like *Aspergillus niger, A. fumigatus, A. flavus,* and *Trichoderma viride* [85]. Mahmood et al. (2011) [87] specifically investigated the effect of *S. surattense* extract on the growth of *Aspergillus fumigates* and *A. niger*. Furthermore, the methanolic seed extract demonstrated strong activity against *Rhizopus oryzae* and *Aspergillus fumigates* [88].

1.2 Bioactive Properties of Plants: Antimicrobial, Anticancer, and Antioxidant Activities

For centuries, traditional medicine has offered a valuable source of affordable and effective treatments with fewer side effects compared to modern medications. This rich heritage stems from the natural world, where plants produce a diverse array of secondary metabolites. These plant chemicals, essential for plant health and survival, hold immense potential for human health as well. Phenolic compounds, a prominent group within these metabolites, are widely distributed throughout the plant kingdom and have been linked to range of beneficial effects, including anti-allergic, anti-cancer, anti-microbial, anti-inflammatory, anti-diabetic, and antioxidant properties. This highlights the exciting potential of plants as a source for developing novel drugs and therapeutic agents [89].

An estimated 9.6 million people tragically succumbed to cancer in 2018, solidifying its place as the second leading cause of death globally. Breast, lung, and colorectal cancers are among the most prevalent forms. There's a growing interest in traditional medicine and herbal-derived phytochemicals as potential tools in the fight against cancer. Recent clinical studies suggest that combining these herbal treatments with conventional therapies may improve patient outcomes, including survival rates, immune system function, and quality of life. The diverse array of phytochemicals found in herbs, such as phenolic compounds, terpenoids, lignans, tannins, and alkaloids, holds promise due to their potent antioxidant properties. These properties offer potential to inhibit cancer cell proliferation and stimulate the immune system, ultimately aiding in cancer prevention and treatment [90].

1.3 Antibiotic Resistance

The emergence of antibiotic resistance (ADR) is a growing public health crisis in the 21^{st} century.Antibiotic resistance is not a looming threat, but a "serious threat, not a prediction for the future". It's a problem impacting people "across

the globe, and it can affect anyone, anywhere" [90]. This widespread resistance undermines the very foundation of modern medicine, jeopardizing our ability to treat common infections in both hospitals and communities.Experts warn that the next pandemic might not be caused by a specific disease, but rather by the ineffectiveness of current medications in combating even minor injuries. The specter of a "post-antibiotic" world looms large, where routine procedures like surgery, cancer treatment, and organ transplants become high-risk endeavors due to the lack of effective antibiotics to combat infections [91]. Preserving the efficacy of antibiotics is paramount. We must act swiftly and collaboratively to prevent this potentially catastrophic future.

The emergence of ADR is a natural phenomenon. However, human actions significantly accelerate the development and spread of resistant strains. The misuse of antibiotics in both human medicine and animal agriculture (aquaculture, livestock farming) fosters the emergence and selection of resistant bacteria. Furthermore, inadequate measures to prevent and control infections contribute to the problem as well.International organizations like the World Health Organization, the Food and Agriculture Organization, and the World Organisation for Animal Health are working together to promote best practices for preventing the spread of antibiotic resistance. Their efforts focus on encouraging the responsible use of antibiotics in both humans and animals to combat this growing threat [92].

The rise of multidrug-resistant bacteria, coupled with a dwindling pipeline of new antibiotic classes (no new classes have been introduced in the past 30 years, with no promising replacements on the horizon), necessitates exploration of alternative solutions. This has spurred renewed interest in the field of botanical medicine, with researchers investigating the latent of medicinal plants as a source of new antimicrobial agents. Herbs and their essential oils have a long history of use for their antimicrobial properties, making them a promising avenue for further exploration [93].

1.4 Problem Statement

Given the increasing threats of antibiotic resistance, cancer, and fungal infections, coupled with limited access to effective treatments in Pakistan, there is an urgent need for novel and affordable therapeutic agents. This research aims to explore the potential of medicinal plants as a source of such compounds.

1.5 Scope of Study

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

1.6 Hypothesis

Extracts from the selected plants will exhibit antioxidant, antimicrobial and anti-cancer activity.

1.7 Aim

Investigating the antioxidant, antimicrobial and anticancer Potential of *Berberis lycium* and *Solanum surattense*.

1.8 Objectives

1. To determine total phenolic content and total flavonoid content

2. To investigate antioxidant, antimicrobial and anticancer activities of *Berberis lycium* and *Solanum surattense* extracts.

Chapter 2

Literature Review

2.1 Medicinal Plants

One of the elements thought to be essential to human life on Earth is the plant kingdom. They can provide all of the essential needs for both humans and animals, including gums and lubricants, food, energy, medicine, clothes, and shelter. Both from the standpoint of the environment as a whole and medicine, plants are enormous treasures. Because of their amazing and miraculous properties, plants can be used as food, decorative, and medicinal elements all at once. From every angle in life, they are appreciated. Every plant, from a prickly shrub to a towering evergreen tree, serves a purpose for humans in some way. These essential medicinal and aromatic plants have significant therapeutic value since early times [94].

Medicinal plants, a diverse group with therapeutic properties, have served as the foundation of healthcare for millennia. These plants offer a vast reservoir of compounds that hold immense potential for developing new drugs [95]. From seeds and roots to leaves, fruits, and even entire plants, various parts are utilized for medicinal purposes [96]. The ”active ingredients” within these plants, often referred to as ”active compounds,” are what give them their therapeutic value. These naturally

occurring molecules produce physiological effects on living organisms. Traditionally, humans have relied heavily on unprocessed plant materials to address their medical needs and treat various illnesses [97].

Medicinal plants represent the oldest form of medicine, with a rich history of use in traditional practices across numerous cultures for thousands of years. Over generations, this knowledge of medicinal plants and their benefits has been passed down through communities, fostering a shared understanding of their healing properties [98].

The significance of medicinal plants extends beyond traditional practices. They also serve as a crucial source of inspiration for modern pharmacology. Many contemporary medications used in modern treatment have their roots in traditional herbal medicine [99]. Pakistan boasts a rich flora with an estimated 6,000 plant species [100]. Notably, 600 of these plants are documented as having medicinal properties. Traditional medicine practices in Pakistan heavily rely on 135 of these medicinal plants. However, despite this wealth of medicinal flora, Pakistan imports a significant amount of high-value medicinal plants and related products. In 2020 alone, the country spent an estimated USD 216.392 million on these imports [101].In fact, estimates suggest that over 50,000 plant species, constituting more than 10% of all known plant life, are currently employed in the production of medicinal and cosmetic products [102, 103]. However, the distribution of these medicinal plants is uneven globally. A considerable portion of these therapeutic herbs are sourced from wild populations, leading to an increased demand that has risen by 8-15% annually in recent decades across Europe, North America, and Asia [104].

2.2 The History of Medicinal Plant Use

The use of plants for medicinal purposes stretches back to the very dawn of human civilization. While pinpointing the exact moment is difficult, evidence suggests our ancestors began domesticating medicinal plants around 60,000 years ago [105].

Some of the earliest documented uses of herbal remedies appear in ancient Egypt, China, and India, dating back nearly 5,000 years. Similar practices emerged in Greece and Central Asia at least 2,500 years ago [106].

Initially, plant use in medicine was largely instinctive, driven by trial and error much like the use of animal products for healing [107]. With limited knowledge of disease origins, specific medicinal plants, or proper treatment methods, practices relied heavily on empirical observations. Over time, however, this approach gradually gave way to a more rational understanding. As people discovered the underlying reasons why certain plants alleviated specific ailments, herbal medicine transitioned from a purely empirical approach to a knowledge-based practice [108].

The earliest known written record of medicinal plant use comes from a Sumerian clay tablet discovered in Nagpur, dating back approximately 5,000 years [108]. Some historical inscriptions suggest the Egyptians and Chinese may have been the earliest human civilizations to leverage plants for medicinal purposes, with evidence placing their practices around 27 centuries BC [109]. The ancient Greeks, including the famed physician Hippocrates and his student Aristotle, were also known to utilize medicinal plants in their healing practices. Greek scientist Theophrastus even established a dedicated School of Medicinal Plants [110]. Following these advancements, Pedanius Dioscorides, a physician who lived from approximately 75 to 45 BC, compiled a comprehensive encyclopedia titled ”De Materia Medica.” This landmark work documented the medicinal properties of over 600 plants, solidifying the foundation of scientific study in herbal medicine [110, 111].

2.3 Medicinal Plants: Universal Applications

Due to the remarkable growth of traditional medicine and the rising popularity of herbal remedies, the usage of medicinal plants is expanding globally. In addition to being used to cure certain illnesses and ailments, plants are utilized in medicine to preserve and improve physical, mental, and spiritual health. Traditional medicine

plays a vital role in healthcare, particularly in developing nations. In Africa, Asia, and Latin America, it fulfills a significant portion of basic healthcare needs, with up to 80% of Africans relying on traditional practices for primary care. Developed nations often refer to adapted traditional medicine as "alternative" or "complementary" medicine, highlighting its growing popularity worldwide.The global herbal medicine market, currently valued at around $80 billion annually, is steadily expanding. For instance, in Nigeria, Ghana, Mali, and Zambia, 60% of children with high fevers from malaria are treated initially with herbal remedies at home [112].

The Himalayan region, encompassing parts of Afghanistan, Bangladesh, Bhutan, China, Nepal, Myanmar, India, and Pakistan, is a recognized biodiversity hotspot for medicinal plants [113, 114]. Currently, the major international markets for medicinal plants are concentrated in the United States, China, France, Japan, the United Kingdom, and Italy [115]. While many Asian countries utilize medicinal herbs in their traditional practices, only a select few, including China, India, Indonesia, Nepal, Bangladesh, Iran, and Pakistan, have established large-scale cultivation capabilities [115].

2.4 Applications of Therapeutic Herbs In Pakistan

Pakistan is a treasure trove of medicinal plants, ranking as the seventh-largest producer in Asia [115, 116]. Boasting a rich floral diversity, the country harbors over 1572 genera and a staggering 6000 wild plant species, with many thriving in the majestic Hindu Kush, Himalayan, and Karakorum mountain ranges [117, 119]. This translates to around 600 documented medicinal plant species traditionally used by Pakistani people to treat various ailments [120]. The knowledge of these medicinal plants and their uses is particularly strong among indigenous tribal groups residing in northern Pakistan [121].

2.5 Plant Active Molecules With Antimicrobial Properties

Worldwide plant screening is currently being done as a source for alternative antibacterial medicines. Active substances such as quinones, phenols, alkaloids, flavonoids, terpenoids, essential oils, tannins, lignans, glucosinolates, and certain secondary metabolites are thought to give plants their antimicrobial qualities. The peptides that make up plant defense systems are another source of antimicrobial agents; they act and are structurally comparable to human antimicrobial peptides. The following is an overview of the extensive discussions on several plant-active compounds:

Quinones, naturally occurring compounds with a characteristic conjugated cyclic dione structure, are found in plants. They are not only responsible for the browning seen in cut fruits and vegetables but also play a role in the antimicrobial properties of some plants. For instance, **quinine** (sometimes referred to as lawsone), a type of quinone present in henna (*Lawsonia inermis*), contributes to its coloring properties and exhibits antibacterial activity against *Pseudomonas aeruginosa* [122]. Similarly, **hypericin**, an anthraquinone derived from St. John's wort (*Hypericum perforatum*), demonstrates broad-spectrum antibacterial activity, even against drug-resistant strains of Staphylococcus [123]. These findings highlight the potential of quinones as natural antimicrobial agents.

Plants offer a wealth of natural health compounds, including **alkaloids**, diverse chemicals found mainly in flowering plants (Angiosperms). The discovery of morphine from the opium poppy (*Papaver somniferum*) exemplifies their therapeutic potential. Berberine, present in barberry (*Berberis spp.*) and other plants, disrupts bacterial membranes by interacting with DNA and increasing permeability, making it effective against Streptococcus agalactiae [124]. Similarly, **hasubanalactam alkaloid** extracted from *Stephania glabra* tubers combats various bacteria like *Staphylococcus aureus* and fungi like *Trichophyton rubrum*, highlighting the promise of alkaloids as natural antimicrobials [125].

Flavonoids, natural plant chemicals abundant in fruits, vegetables, and beverages like tea, possess a diverse range of health benefits. These well-recognized properties include antiviral, antibacterial, anti-allergic, and anti-inflammatory effects [126]. Specific flavonoids like quercetin, rutin, and kaempferol have been shown to combat fungal infections [127]. Additionally, dihydrofuranoisoflavones found in legumes exhibit antifungal activity against specific strains [128]. This highlights the potential of dietary sources like fruits and vegetables to provide natural antimicrobial solutions.

Flavonoids, a class of plant phenolics, can be further categorized as flavones with a single carbonyl group. Research has shown promise for their antibacterial properties. Scientists isolated flavones from plants like Viscum album and Galium fissurense, demonstrating effectiveness against multidrug-resistant bacteria, raising hope for new options in combating antibiotic resistance [129].

Coumarins, natural plant compounds containing fused benzene and pyrone rings, offer promising antimicrobial potential. Studies have isolated coumarins from plants like *Angelica lucida* and *Ferulago campestris* that demonstrate activity against various bacteria, including oral pathogens [130, 141]. Notably, coumarins from *Ferulago campestris* showed effectiveness against both Gram-positive and Gram-negative bacteria [131].

Essential oils are a type of secondary metabolites derived from plants that typically have the formula $C_{10}H_{16}$ and contain chemicals with an isoprene structure, which is also referred to as terpenes. Hemiterpenes, sesquiterpenes, triterpenes, and tetraterpenes are a few of the known terpene varieties. Terpenoids are the chemicals that include oxygen as an extra ingredient.

Terpenoids, sometimes called isoprenoids, are essentially distinct class of terpene-like naturally occurring organic compounds. The basic carbon chains and functional groups of these compounds, which have multicyclic structures, are different from one another. All groups of living organisms contain them, which make up the biggest group of natural goods. Because of their fragrant properties, plant

terpenoids are employed. In addition to being studied for their potential pharmacological properties, such as antibacterial and antineoplastic properties, they are used in traditional herbal treatments. Terpenoids are responsible for the distinctive scent of eucalyptus, which includes notes of ginger, cloves, and cinnamon. Citral, camphor, menthol, salvinorin A from *Salvia divinorum*, and the cannabinoids in *cannabis* are a few examples of well-known terpenoids.

Terpenes, also called terpenoids, are natural compounds found in plants with a wide range of antimicrobial properties. Studies have shown effectiveness against viruses, bacteria, fungi, and even protozoa. Examples include terpenoids isolated from *Trichodesma amplexicaule* and *Acacia nilotica* bark, which demonstrated antibacterial activity against common pathogens like *E. coli* and *S. aureus* [132]. However, the effectiveness of terpenes can vary, as seen with Cymbopogon citratus essential oil, which showed limited activity against some bacterial and fungal strains [133]. This highlights the need for further research to explore the diverse antimicrobial potential of terpenes.

The term "**tannic acid**" is derived from the German word "*tanna*," which means "oak" or "fir tree." Tannic acid is obtained from the wood tannins found in oak galls. The preserving of animal hides into leather is the outcome of tannins' capacity to interact with proteins. Large polyphenolic molecule with carboxyl and hydroxyl groups is what tannin is chemically. Certain plant substances, such as red wine and unripe fruits, include tannins that have astringent (cleaning and constricting the skin pores) effects and make the mouth feel puckered when consumed.Plants that contain tannins have a protective function against animal predators.Plant extracts that include tannins have anti-infective properties and activate phagocytic cells. By attaching to the bacterial cell wall, tannins have the ability to prevent ruminal bacteria from growing and from producing proteases [134]. *Sorghum tannin* exhibits antibacterial properties against *S.aureus, Salmonella typhimurium, A.niger, A.flavus and Saccharomyces cerevisae* [135].

2.6 Plant Active Molecules with Antioxidant Properties

Medicinal plants serve as a natural defense against cellular damage, packed with antioxidant compounds that neutralize harmful reactive oxygen and nitrogen species (ROS/RNS). These damaging molecules, a byproduct of normal cellular processes, can lead to oxidative stress. The antioxidant power comes from diverse secondary metabolites within the plants, categorized into three main groups: flavonoids and related phenolics (known for their antioxidant strength), terpenoids (a diverse class with many beneficial antioxidants), and nitrogen-containing alkaloids and sulfur-containing compounds [136].

2.7 Plant Active Molecules with Anticancer Properties

A growing body of research suggests that natural products derived from plants may hold promise in the fight against cancer. This exploration centers on the prospective of herbal medicine as a source of **anticancer compounds**. Traditional practices utilized various plants, such as *Phaleria macrocarpa* (**Mahkota dewa**) and *Fagonia indica* (**Dhamasa**), for their purported anticancer properties. These plants are believed to contain active ingredients that can trigger apoptosis (programmed cell death) in cancer cells. Notably, **gallic acid**, isolated from the fruit of P. macrocarpa, has exhibited anti-cancer effects in laboratory experiments involving lung cancer, leukemia, and colon cancer cell lines. This natural antioxidant, belonging to the class of **polyhydroxy phenolic compounds**, is also found in fruits like grapes and strawberries, vegetables, and green tea. Furthermore, other plant-derived compounds like vinca alkaloids, **podophyllotoxin**, and **camptothecin** are already being utilized in conventional cancer treatment [137].

2.8 Pharmaceutical Company Contentment

Pharmaceutical corporations may invest in medicinal plants and natural items to develop new antimicrobial medications for five main reasons:

1. The high price of creating new chemical compounds with antibacterial properties in contrast to the inexpensive production of antibacterial agents using natural ingredients [138].

2. Due to a lack of economic incentives and earnings, large pharmacological corporations have gone the synthetic antibiotic industry [139].

3. The proliferation of bacterial infections that have developed a high level of resistance has not been stopped by synthetic antibiotics [140].

4. Numerous phytochemical compounds that have been identified from therapeutic plants and shown to be effective against bacterial infections [141].

5. Novel antibacterial medications can now be produced from plants with remarkable efficiency thanks to recent developments in biotechnology [142].

2.9 Need for Revival of Herbal Antimicrobials

Herbal remedies are experiencing a global resurgence, finding favor in both developed and developing nations [143]. This renewed interest has fueled scientific inquiry into the potential of plants as antimicrobial agents [144, 146]. Researchers are investigating a wide range of botanical candidates, with promising results from plants like yarrow, clove, and rosemary [147]. These studies highlight the potential of herbal medicine to provide natural solutions for combating infections.

It is frequently demonstrated that the essential oils of several medicinal plants have antibacterial properties when used in herbal medicinesThe most effective of all the oils have been found to be the essential oils of carvacrol, which is the active ingredient in the latter two plants, thyme, and cinnamon [148]. Since many plants

and herbs have been shown by science to contain bioactive compounds, there has been a rise in demand for herbal remedies as a safer alternative to synthetic medications that have negative impacts on the environment and biological systems. For centuries, herbal remedies have battled infections in humans and animals globally [149]. Developing nations especially embrace them due to affordability, cultural fit, perceived gentleness, and fewer side effects compared to conventional drugs [150, 151].

Beyond traditional antimicrobial therapy, herbal medications are playing an expanding role in addressing age-related concerns. These concerns include issues with the immune system, memory decline, and even osteoporosis. Interestingly, the world of pharmaceuticals also relies on plants – laxatives, blood thinners, antibiotics, and anti-malarial drugs can all hold active ingredients derived from botanical sources [151]. Furthermore, medicinal plants serve as valuable starting points for the discovery and development of new drugs [152–154, 165]. Plant volatile oils, in particular, have shown promise for a wide range of therapeutic benefits. Pain relief, antimicrobial activity, deodorizing properties, and even mood-boosting effects are just some of the potential advantages associated with these volatile oils [156, 157].

Turmeric's curcumin has been shown to prevent *H. pylori* from forming biofilms in cell cultures. On the other hand, after prolonged incubation, *H. pylori* may regain the capacity to build biofilm [158]. Herbal remedies have a plethora of applications, thus new research is required to create substitute antimicrobial medications for the treatment of infectious disorders. One strategy is to look for potential antibacterial qualities in native medicinal plants; these active molecules will be crucial in the future. An alternative strategy could involve identifying the plant compounds that enhance the effectiveness of current antibiotics by working in concert, blocking efflux pumps, or deactivating the enzymes in bacteria that break down medicines.

2.10 Medicinal Plants Studied in Present Study

Berberis lycium Royle and *Solanum surattense* were chosen for this investigation due to their rich history of medicinal use in traditional practices. The study intended to evaluate the potential antimicrobial, antioxidant and anticancer properties of these plants.

2.10.1 *Berberis lycium Royle*

Taxonomic classification

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Berberidaceae

Genus: Berberis

Species: *Berberis lycium*

FIGURE 2.1: Collection of *Berberis lycium* from field

The Berberidaceae family is a diverse group of flowering plants (angiosperms) with a global presence, particularly in the northern hemisphere. This family boasts 17 genera and a whopping 650 species. One such member is *Berberis lycium Royle*, a plant inherent to the Himalayan region, especially Nepal. However, its reach extends beyond the Himalayas, as it can be found in temperate and subtropical climates around the world, with the exception of Australia. Within the Himalayas, its distribution stretches from Kashmir in the west to Uttarakhand in the east, occupying the outer northern and western regions [159]. This adaptable shrub thrives in various environments, from small wooded areas to roadsides. Remarkably, it can flourish in diverse soil types, including clay, loam, sand, and even nutrient-poor soil [160].

2.10.1.1 Morphology

The plant is a compact shrub, typically reaching between 1.0 and 2.5 meters in height. Its robust, woody branches are covered in a thin layer of brittle bark [161]. The leaves are coriaceous and have a lanceolate or slightly obovate-oblong form. On the upper side, they are dull green, while on the lower side, they are pale and glaucous. There are a few noticeable spinous teeth on the plant's stem that are positioned alternately [159]. *Berberis lycium* stands out as a valuable plant with both medicinal and edible uses. This compact shrub typically reaches heights between 1.0 and 2.5 meters. Its flowers, arranged in attractive clusters called corymbose racemes, boast 11 to 16 pale yellow blooms each (typically bisexual) and are pollinated by insects. The flowering season adorns the plant from March to July, with the first blooms appearing in early March and full flowering happening by April. Fruit development follows closely behind, starting to ripen in mid-May and reaching its peak in June. These mature fruits, a beautiful bluish-purple color, are packed with nutrients and enjoyed by locals in various ways. Traditionally, they are consumed fresh or incorporated into dishes, beverages, jellies, and even preservatives [160].

2.10.1.2 Traditional Use

Beyond its ornamental value, *Berberis lycium* holds a significant place in traditional medicine worldwide [162]. Every part of the plant boasts potential medicinal uses. In Kashmir, tribal communities traditionally relied on the roots to treat severe dysentery [160]. The root powder finds use in relieving rheumatic and muscular discomfort, while leaves are used to make tea, and the stem powder tackles jaundice and diarrhea. Extracts from the entire plant address various ailments - spleen issues, coughs, lung and throat problems, digestive troubles, diarrhea, eye irritation, and even broken bones [163, 164].

The fruits of *Berberis lycium* shine as coagulants, anti-inflammatory agents, and fighters against cancer and high blood sugar. Locals traditionally use the whole plant to heal ulcers, rheumatism, jaundice, eye problems, and fractures [165, 176]. Scientific studies using animal models have shown promise for extracts from fruits, root bark, and stem bark. These extracts demonstrate potential benefits against high cholesterol, high blood sugar, kidney stones, cancer, parasitic worms, and wound healing [160, 167, 168]. Research also suggests various *Berberis lycium* parts may possess antiseptic, diuretic, carminative, diaphoretic, expectorant, and fever-reducing properties [169–172]. This highlights the possible of *Berberis lycium* as a source of natural remedies for a wide range of health concerns.

2.10.1.3 Biologically Active Compounds and Their Activities

Numerous physiologically active substances, including tannins, saponins, and alkaloids, are found in *B. lycium* [173]. Of them, the most significant biologically active ingredient is an isoquinoline alkaloid berberine that was extracted from bark & roots of *B. lycium* [162]. Clinical trials and animal investigations have demonstrated that beberine therapy improves cardiac contractility, prevents ischemia-induced ventricular tachyarrhythmia, and lowers blood pressure and peripheral vascular resistance [174]. Numerous biological uses for *B. lycium* were demonstrated, including wound healing, pesticidal, hepatoprotective, antihyperlipidemic, antioxidant, and antibacterial properties [165].

In addition, the herb has historically been used to treat ocular, skin, cough, liver, and stomach issues. Because of the plant's enormous nutritional and medicinal properties, it is being overexploited for consumption and commerce, which stances a severe threat to the plant's existence in natural habitat.

The species' habitat is being reduced by other human activities including deforestation, building and road construction, expanding agricultural land in mountainous areas, and so forth, which is causing a decline in population [175]. Therefore, conservation measures must be taken to protect this *B. lycium* species from more harm.

Berberine is the main isoquinoline alkaloid constituent of, and it is this compound that gives the plant its biological properties. Numerous biological uses for this chemical have been demonstrated, including hepatoprotective, anti-oxidant, anti-microbial, anti-inflammatory, anti-cancer, and antihyperlipidemic properties. *B. lycium* exhibits an extensive range of biological activity [163].

2.10.1.4 Phytochemistry

The phytochemicals found in *B. lycium* are diverse and serve a range of functions. Table 2.1 lists several phytochemicals that were separated from *B. lycium*.

TABLE 2.1: Phytochemicals of B. lycium

Plant Part	Reported Phytochemicals	References
Whole plant	Berberine, berbamine, palmatine, jhelumine, punjabine, sindamine, organic acids (maleic acid, ascorbic acid), alkaloids, flavonoids, phenolics, tannins, terpenoids, resin, and fat	[176, 177]
Leaf	Berberine, saponins, minerals (potassium, iron, sodium, phosphorus)	[178, 183, 184]
Stem	Berberine, minerals (copper, manganese)	[181, 185]
Shoot	Minerals (sodium, iron, potassium, phosphorus)	[178]
Fruit	Saponins, tannins, alkaloids(including berbamine, chinamine, balauchistanamine, sindamine), berberine, minerals (zinc and sulfur)	[186–188]

Table 2.1 continued from previous page

Plant Part	Reported Phytochemicals	References
Root	Alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, carbohydrates, fat, proteins, vitamin C, anthocyanins, specific alkaloids (berberine, palmatine, β-sitosterol), other identified compounds (Jatrorrhizine, butyl-3- hydroxypropyl phthalate, 4, 4- dimethylhexadeca-3-ol, 4-methyl, 7-hydroxy coumarin, and 3-(4' -(6-methyl butyl) phenyl) Propan-1-ol) , minerals (sulfur, zinc, manganese, copper, sodium, potassium, iron, phosphorus)	[177, 178, 178, 180–182]

2.10.1.5 Antimicrobial Activity

Using the microdilution method, Singh et al. [189] investigated the antibacterial activity of a 50% hydroalcoholic extract of air-dried *B. lycium* root and stem. *Micrococcus luteum, Enterobacter aerogenus, Escherichia coli, Bacillus cereus, Bacillus subtilis, Proteus mirabilis, Streptococcus pneumonia, Klebsiella pneumonia, Micrococcus luteum,* and *Pseudomonas aeruginosa* were some of the bacteria that the extract demonstrated antibacterial activity against.

Root and stem extracts inhibited *Aspergillus terreus* fungus strains. Strong *anti-A. flavus* and *anti-A. spinulosus* activity was demonstrated using root extract. When it came to bacterial strains, the hydroalcoholic extract outperformed it when it came to fungal strains [189, 190].

In terms of immunological performance, *B. lycium* fared better against infectious bursal disease, infectious bronchitis, and new castle disease. The amount of coccidial oocysts per gram of feces was significantly reduced by this plant [191]. This plant was often added to drinking water containers, along with other medicinal herbs, to help eliminate germs and lessen illness [165]. Berberine is one possible treatment for COVID-19 [192].

Hepatitis C Virus (HCV)-infected $HepG_2$ cells were exposed to 70% methanolic plant extract, this extract was benign, but at 40 μg/ml and higher levels, it demonstrated mild toxicity and severe toxicity, respectively [193].

2.10.1.6 Anticancer Activity

Khan et al. [194] investigated the anti-cancer properties of *Berberis lycium* root extracts and isolated compound berberine against human leukemia cells (HL-60). Their study compared the effectiveness of various extracts (water, ethanol, and n-butanol) with pure berberine. The n-butanol extract displayed the strongest cytotoxicity, followed by the ethanol and water extracts. Notably, berberine itself exhibited a lower IC50 (1.2 μg/ml) compared to most extracts.

While berberine demonstrated significant anti-cancer activity, the n-butanol extract proved even more effective. Further analysis revealed that both berberine and the butanol extract arrested the cell cycle at the S-phase, hindering cell division. All extracts and berberine induced apoptosis in HL-60 cells, with the n-butanol extract demonstrating the strongest pro-apoptotic effect.

Importantly, neither the extracts nor berberine caused DNA damage. Interestingly, both treatments activated specific signaling pathways (p38-MAPK and Chk2 phosphorylation), potentially linked to the observed cell cycle arrest. These findings echo the effort of Issat et al. [195], who reported on the growing evidence for berberine's potential in cancer treatment due to its antiproliferative and pro-apoptotic effects.

2.10.1.7 Antioxidant Activity

Berberis lycium, commonly known as barberry, is rich in free radical scavenging chemicals, suggesting its potential as a natural antioxidant [196]. Studies have confirmed this potential, with research by Ahmed and Shakeel [197] demonstrating significant antioxidant activity in *B. lycium* extracts. Different root extracts exhibited varying degrees of activity, with petroleum ether showing the strongest

effect (82% inhibition of DPPH free radical) followed by chloroform and methanol extracts.

Interestingly, the n-butanol extract displayed minimal antioxidant activity. This research highlights the impending of *B. lycium* as a source of natural antioxidants. Even beverages made from its berries, like wine, have been shown to possess significant antioxidant capacity [198]. These findings warrant further investigation into the specific bioactive components responsible for *B. lycium*'s antioxidant properties and their potential applications in promoting human health.

2.10.2 *Solanum surattense*

Taxonomic classification

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *Solanum surattense Burm. f.*

FIGURE 2.2: Collection of *Solanum surattense* from field.

The prickly *Solanum surattense* is a perennial herb, adding to the diverse Solanaceae family that includes potato, tomato, and eggplant. This plant goes by several names, including yellow-fruit nightshade, Surattense nightshade, and Indian nightshade. Its geographical reach is impressive, spanning across Polynesia, Australia, India, Southeast Asia, North Africa, and even parts of Pakistan [199].

A closer look reveals *Solanum surattense* typically thrives in arid regions, finding a home along roadsides and even wastelands [200].

2.10.2.1 Morphology

Solanum surattense is a vibrant green perennial herb with a woody base. This prickly character stands out with straight, yellow spines over 1.3cm long. Younger branches are densely hairy, and leaves (5-10 per plant) come in various shapes and sizes (2.5-5.7cm). They can be wavy or lobed, hairy initially, but smooth later. All leaves have sharp prickles for defense. Green berries with white stripes transform into yellow or white spheres with green veins as they ripen. The intricate flowers have a hairy calyx with sharp lobes. *Solanum surattense* showcases remarkable botanical complexity [201].

2.10.2.2 Traditional Used

In Indian traditional medicine, the plant *Solanum surattense* frequently used to treat variety of conditions, counting gonorrhea, rheumatism, respiratory illnesses, fever, asthma, and constipation [202].

The root of *Solanum surattense*, a tonic for nursing women, is a component in the ayurvedic preparation of dashmularishta [203]. The fruit of *S. surattense* has historically been used to treat wounds [204]. Compared to other solvent extracts, the ethanol extract of *S. surattense* demonstrated a more marked ability to cure wounds [205]. According to Ahmed et al. [206], the fruit extract of *S. surattense* greatly increased urine production in a dose-dependent manner and exhibited diuretic and serum electrolyte control qualities. Kidney stones and urinary tract

infections are treated by *S. surattense*, according to reports from Chauhan et al., [207].

In addition, the plant known as *S. surattense* is used to cure a variety of ailments, including the common cold, worms, sleeplessness, laxatives, liver enlargement, aphrodisiac activities, anti-nociceptives, molluscicidal activities, and antifungal activities [208]. The use of *S. surattense* seed vapors in cure of dental ache and pain associated with gingival swelling has been described by Pandey [209]. Traditional Indian Mukunda tribes in Rajasthan have been treating hernias with the root sections of *S. surattense* [210].

More antiulcer potentiality [211] has been reported for alcoholic extracts of *S. surattense* leaf than for other solvent extracts. Antibacterial, antifungal, antinociceptive, antioxidant, and antidepressant are among *S. surattense*'s pharmacological actions [212, 213], antidepressant activity [214], hypoglycaemic [215], and larvicidal [216].

2.10.2.3 Phytochemistry

Solanum surattense boasts a rich tapestry of phytochemical constituents. Extensive research has revealed the presence of various alkaloids, forming the cornerstone of its chemical makeup [217]. Beyond alkaloids, studies have identified a diverse range of other compounds, including saponins, phenols, gums, specific steroidal alkaloids like solamargine and solasonine, and the antioxidant vitamin C [218–221]. Additionally, torvoside K, torvoside L, khasianine, and sterols have been documented [222, 223]. Furthermore, *S. surattense* contains flavonoids and their glycosides, aculeatiside A, and solamargine [224, 225].

Saiyed and Kanga's (1936) research [226] sheds light on the bioactive components present in the fruit of *S. surattense*. These include a glycoalkaloid (solanosine), a steroidal substance (carpesterol), and steroidal alkaloids such as caffeic acid, coumarins, and triterpinoids. Further investigations confirmed the presence of steroid alkaloids like solamorgine, solanocarpine, and solanocarpidine in the

fruits [227].*Solanum surattense* also harbors a wealth of other potent phytochemicals, including triterpenoids, polyphenols (caffeic acid), coumarins (esculentin and aesculin), sapogenins (lupeol and diosgenin), and various steroids (carpesterol, campesterol, daucosterol, stigmasterol, stigma cycloortanol, and cholesterol) [228, 229]. The root of the plant is known to contain flavonoids, alkaloids, triterpenoids, saponins, tannins, glycosides, and steroids [230, 231]. Notably, Heble et al. (1984) [232] demonstrated the feasibility of extracting diosgenin and β-sitosterol from the callus tissue of *S. surattense*.

2.10.2.4 Biological Active Compound and Their Activity

Research on *Solanum surattense* is still rife, and new findings have shown off its fascinating potential. Solasodine, the primary alkaloid, may hold potential for the treatment of diabetes, according to studies like Chen et al.'s from 2020 [233].

Combining different plant bioactive components improves the plant's anti - inflammatory qualities, according to research by Verma et al. from 2021 [234]. This suggests possible synergies for improved therapeutic benefits. Furthermore, the plant's potential as a natural weapon against drug-resistant bacteria was emphasized by Kumari et al. in 2022, providing a glimmer of hope in the fight against antibiotic resistance [235].

2.10.2.5 Antimicrobial Activity

Numerous investigations have demonstrated the plant products' potent antibacterial properties. Potential antibacterial activity against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Staphylococcus aureus*, and Streptococcus species has been reported for the ethanolic leaf extract of *Solanum surattense* [236]. Strong antibacterial activity against *Pseudomonas aeruginosa* was demonstrated by the ethanol and methanol extracts of *S. surattense* [237].

Bacteria including *Micrococcus luteus*, *Staphylococcus aureus*, *E. coli*, *S. typhi*, *Pasteurella multifida*, and *Vibrio cholera* showed potential growth in the fruit extract of *Solanum surattense* [238]. The antifungal efficacy of *S. surattense* extract against Trichodermaviride, *A. fumigates*, *A. flavus*, and *Aspergillus niger* has been assessed [239]. The antifungal activity of *S. surattense* against the growth of *Aspergillus fumigates* and *A. niger* has been assessed by Mahmood et al., [240]. *Rhizopus oryzae* and Aspergillus fumigates were both significantly inhibited by the methanolic seeds extract of *S. surattense* [241].

2.10.2.6 Anticancer Activity

Plants hold promise as a source of cancer-fighting drugs. The Indian plant, *Solanum surattense*, shows particular potential due to its unique secondary metabolites like lupeol, apigenin, solamargine, and diosgenin. Studies have shown that solamargine, a steroidal alkaloid, has anti-tumor effects [242]. Fruit extracts from *S. surattense* also show promise, with a positive correlation observed between the amount of flavonoids (like apigenin, quercetin, fisatin, and luteolin) and the inhibition of cancer cell growth [243].

Diosgenin isolated from *Solanum surattense* has been shown to induce apoptosis in human colon cancer cells. Interestingly, research suggests that the presence of a specific sugar molecule (2-rhamnose moiety) in solamargine and solasonine is crucial for this effect. While these findings are promising, further research is needed to fully understand how these plant compounds induce cell death and to develop them into potential cancer therapies [244].

2.10.2.7 Antioxidant Activity

Growing concerns about the safety of synthetic antioxidants have fueled interest in natural alternatives, particularly plants rich in polyphenols like *Solanum surattense* [245]. Studies suggest this plant's methanolic and ethanolic extracts possess potential antioxidant properties, possibly by amplifying the activity of antioxidant

enzymes like superoxide dismutase and glutathione peroxidase in animal models [246, 247]. These findings, coupled with the established link between antioxidants and reduced oxidative damage, highlight *Solanum surattense* as a promising candidate for further research [245]. By identifying the specific bioactive components responsible for this potential antioxidant activity, we may unlock new avenues for developing natural antioxidant therapies to combat free radical damage and associated health problems.

2.11 Antibiotic Resistance

Modern antibiotherapy, as it relates to antibacterial medications, was first introduced in the 1930s with the discovery of sulfonamides by Fleming and his 1928 discovery of penicillin [248]. The early 1940s saw the rapid widespread adoption of penicillin. The 1950s marked the beginning of what is known as the ”golden era” of antibiotic progression and application, during which several latest antibiotic classes stayed introduced and continued to be used until the 1970s [249].

However, after that remarkable time, antibiotic resistance has been steadily increasing, leading to the current severe worldwide situation.The overdo and inadequate use of antibiotics, in addition to the pharmaceutical industry's lack of innovative drug research, have been the main causes of this problem [250]. The ubiquity of antibiotic-resistant germs worldwide has recently, in 2023, drawn much alarm. The unsettling pattern is exacerbated by the lack of novel antibiotic classes being created, leading to the creation of the so-called ”antibacterial crisis” [251]. There is a renewed focus on medicinal plants as a possible source of novel antibiotics due to the growing concern of antibiotic resistance. These plants include an abundance of different chemical constituents, such as phenolics, alkaloids, flavonoids, and terpenoids. Numerous of these substances appear to have strong antibacterial qualities, which makes them excellent candidates for the creation of new antibiotics, according to research [252].

Chapter 3

Material and Methods

3.1 Plant Collection

Plant samples of *Berberis lycium* and *Solanum surattense* were collected in [May, 2024] from the Kotli Sattian district of Rawalpindi, Pakistan.

3.2 Plant Identification

- **Field Identification**: Initial identification in the field was based on local experts familiar with the region's flora.
- **Museum Verification**: The collected plant samples were brought to the Pakistan Museum of Natural History (PMNH) in Islamabad for confirmation of their identity. Collaborating with qualified botanists or herbarium staff at the PMNH, the plant specimens were compared with herbarium collections and taxonomic keys to ensure accurate identification. This verification process confirmed the following:
  - The sample designated as *Solanum surattense* was identified as *Solanum surattense* with accession number 047509 at the PMNH.
  - The sample designated as *Berberis lycium* was identified as *Berberis lycium* with accession number 047510 at the PMNH.

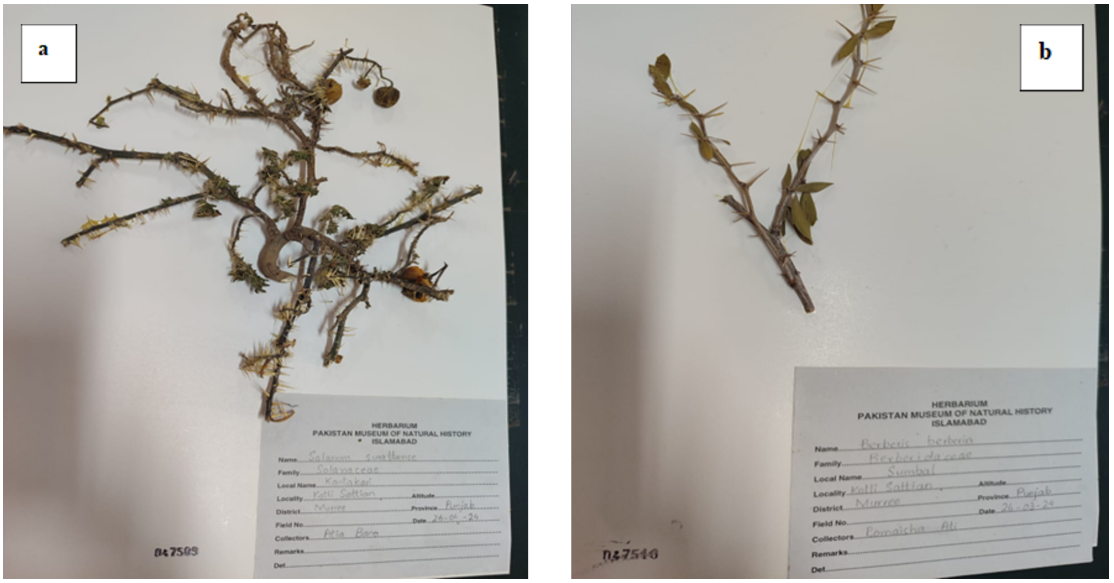

FIGURE 3.1: Herbarium specimens identified by the PMNH as: (a) *Solanum surattense* (PMNH 047509) and (b) *Berberis lycium* (PMNH 047510). These specimens were used for the extraction of bioactive compounds.

3.3 Sample Preparation

3.3.1 Drying

The collected plant materials were spread evenly on clean trays in a well-ventilated area with good air circulation and indirect sunlight.The drying process was continued until the plant material reached constant weight, indicating complete dryness.

3.3.2 Grinding

To facilitate further analysis, the dried plant materials were finely ground using an electric grinder.The grinder was thoroughly cleaned between samples to prevent cross-contamination.

3.4 Powder Storage

The powdered plant materials were stored in airtight containers made of inert materials such as amber glass or aluminum foil. These containers were labeled with the plant name (as confirmed by the Pakistan Museum of Natural History - *Solanum surattense* and *Berberis lycium*), collection date, and location to ensure traceability. The powders were stored in a cool, shady place (e.g., refrigerator at 4^{o}C or freezer at -20^{o}C) to minimize moisture uptake, light exposure, and degradation of bioactive compounds.

3.5 Extraction

Based on a review of relevant literature [253] and preliminary tests, we opted for methanol as the primary solvent for extraction. Methanol is a well-established solvent for extracting a broad spectrum of bioactive compounds from plant materials due to its polarity.

3.5.1 *Solanum surattense* Extraction

A specific amount (27.22 g) of the powdered *Solanum surattense* sample, as weighed precisely, was added to a conical flask and extracted with 100 mL of methanol

3.5.2 *Berberis lycium* Extraction

Similarly, a precise weight (49.24 g) of the powdered *Berberis lycium* sample was added to a clean, dry conical flask. 150 mL of methanol was then added to this flask.

3.5.3 Common Extraction Procedure

Following the addition of methanol to each flask containing the respective plant material (*Solanum surattense* and *Berberis lycium*), the flasks were sealed with stoppers. They were then placed on a shaking incubator set at 150 rpm for 72 hours at room temperature (25^{o}C) to ensure efficient extraction of bioactive compounds. Following 72-hour incubation, the extracts were passed through Whatman filter paper No. 1 to remove any solid plant material. Next, a rotary evaporator was employed to concentrate the filtrates. This process utilizes reduced pressure and a slightly elevated temperature (40^{o}C) to remove the methanol solvent. The concentrated extracts were then weighed to determine the yield, or the quantity of extract obtained from the starting plant material. Finally, they were stored at -20^{o}C for future analysis.

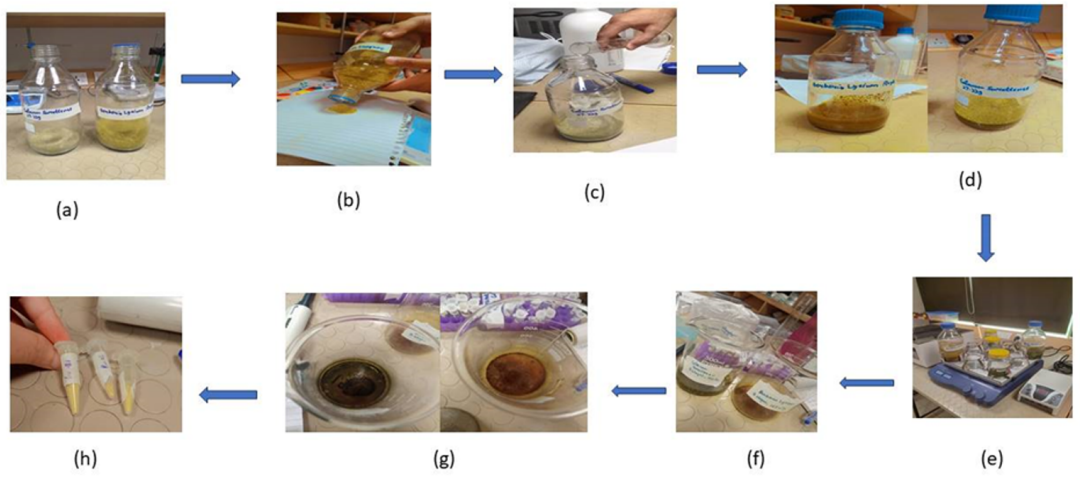

FIGURE 3.2: Plant material extraction process (a-h): (a) Dried, powdered plant sample is obtained. (b) The dried sample is weighed. (c) The dried sample is mixed with solvent in a flask. (d) The mixture is transferred to airtight bottles. (e) The mixture in the bottles is shaken on a shaker. (f) The mixture is strained using a muslin cloth, and the extract is passed through filter paper. (g) The filtrate (the extract after filtration) is transferred into a beaker for drying in a fume hood. (h) The dried extract is obtained.

3.6 Phytochemical Analysis

After the extraction process, the following phytochemical analyses were conducted on the concentrated extracts of both *Solanum surattense* and *Berberis lycium*:

3.6.1 Total Phenolic Content (TPC) Assay

To quantify the total phenolic content (TPC) of *Solanum surattense* and *Berberis lycium* extracts, a modified Folin-Ciocalteu method was employed. Diluted extracts were first reacted with the Folin-Ciocalteu reagent, followed by the addition of a sodium carbonate solution. This addition triggers the development of color within the mixture. The absorbance of these colored solutions was then measured at a wavelength of 725 nanometers using a spectrophotometer. To convert the measured absorbance values into a quantifiable amount of total phenolics, a standard curve prepared with gallic acid was used. This conversion provides the TPC results expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract weight [254].

3.6.2 Total Flavonoid Content (TFC) Assay

To quantify the total flavonoid content (TFC) in *Solanum surattense* and *Berberis lycium* extracts, a modified aluminum chloride colorimetric assay was implemented. Diluted extracts (1 mg/mL in DMSO) were combined with a pre-mixed solution of methanol, aluminum chloride, potassium acetate, and water. This mixture underwent incubation at room temperature for 30 minutes, allowing the formation of colored complexes between flavonoids and aluminum chloride. The resulting solution's absorbance was then measured at 415 nm using a spectrophotometer. To convert these absorbance values into a quantifiable measure of total flavonoids, a standard curve prepared with varying concentrations of quercetin solutions (15.6-250 μg/mL) was employed. This conversion provided the TFC results expressed as milligrams of quercetin equivalents (QE) per gram of dry extract weight [256].

3.6.3 FT-IR Analysis

The functional group composition of *Solanum surattense* and *Berberis berberis* extracts was investigated using Fourier-Transform Infrared (FT-IR) spectroscopy. FT-IR spectra of the extracts were acquired on a Nicolet iS10 FT-IR instrument within a wavelength range of 400-4000 cm^{-1}. This analysis aimed to detect the characteristic functional groups present in the bioactive compounds of each plant extract. The spectra will be compared to reference databases to assign specific peaks to various functional groups (e.g., alcohols, phenols, alkenes, carbonyls) present in the extracts. This information will provide valuable insights into the potential bioactive constituents responsible for the observed biological activities (e.g., antibacterial or antioxidant properties) of *Solanum surattense* and *Berberis lycium* [256]

3.7 Biological Activities

3.7.1 Antibacterial Activity Assay

3.7.1.1 Microbial Strains

The following bacterial strains were used to assess the antibacterial activity of the plant extracts:

- *Salmonella typhi* (ATCC 14028)
- *Escherichia coli* (ATCC 25922)
- *Pseudomonas aeruginosa* (ATCC 27853)
- *Staphylococcus aureus* (ATCC 25923)
- *Bacillus subtilis* (ATCC 6633)

3.7.1.2 Microplate Alamar Blue Assay (MABA)

The microplate Alamar Blue assay was employed to assess the antibacterial activity of the *Solanum surattense* and *Berberis lycium* extracts [257, 258].

3.7.1.3 Preparation of Bacterial Cultures

- Fresh cultures of each bacterial strain were prepared in appropriate broth media (e.g., Mueller-Hinton Broth - MHB) and incubated at 37^{o}C for a specified time (18-20 hours) to achieve an exponential growth phase.
- The bacterial cultures were then standardized to a specific optical density (OD) of 0.5-0.6 at a chosen wavelength of 600 nm (OD600) using a spectrophotometer. This ensures a consistent inoculum for the assay.

3.7.1.4 Preparation of Extract Solutions

- Stock solutions of the concentrated *Solanum surattense* and *Berberis lycium* extracts were prepared at a defined concentration (3000 μg/mL) in a suitable solvent (e.g.DMSO).

3.7.1.5 Microplate Assay Setup

- A sterile 96-well microplate was used for the assay.
- Each well received a specific volume of broth media containing a standardized inoculum of the test bacteria.
- Extract solution was then added to designated wells to achieve the desired final concentrations in the assay wells.
- Control wells included those with media only, media with bacteria only (growth control), and media with a known antibacterial agent (positive control).

3.7.1.6 Incubation and Alamar Blue Reduction

- The microplate was incubated at 37^{o}C for a specified time (24 hours) to allow bacterial growth in the presence or absence of the extracts.
- After incubation, Alamar Blue solution was added to each well.
- The microplate was further incubated for a defined period (24 hours) to allow the metabolically active bacteria to reduce the Alamar Blue.

3.7.1.7 Measurement and Analysis

- The fluorescence intensity of the solution in each well was measured at specific excitation and emission wavelengths using a fluorescence microplate reader.
- The bacterial growth inhibition percentge was calculated for each extract concentration compared to the growth control wells [257, 258].

3.7.2 Antioxidant Activity Determination

The antioxidant capacity of *Berberis lycium* and *Solanum surattense* extracts was evaluated using the DPPH radical scavenging assay.

3.7.2.1 Free Radical Scavenging Activity by DPPH Assay

The capability of the extracts and fractions to scavenge free radicals was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay, utilizing a well-documented protocol [259].

3.7.2.2 Preparation of DPPH Solution

To test free radical scavenging, a strong DPPH solution (60 μM) was made in methanol (2.4mg in 100mL). The solution was then diluted with methanol to absorb less light at 517nm (optimal for the assay).

3.7.2.3 Assay Procedure

The diluted DPPH solution (2 mL) was mixed with various concentrations (1-50 μg/mL) of each extract/fraction (200 μL). After vigorous mixing (vortexing) and a 30-minute incubation in darkness, the pending DPPH was measured at 517 nm (spectrophotometer) to assess free radical scavenging activity. This process was repeated three times (triplicate) for each sample for reliable results.

3.7.2.4 Evaluation of Antioxidant Activity

The antiradical potential of the crude extract and fractions was calculated using a specific formula. This formula translates the measured absorbance into a value representing the free radical scavenging capacity of the samples.

$$\%Scavenging = 1 - \frac{Absorbance of Extract}{Absorbance of Ascorbic Acid} \times 100$$

The IC50 value, determined using a DPPH free radical scavenging assay, indicated the concentration of extract or fraction required to scavenge half of the free radicals present. Ascorbic acid was employed as a reference standard for comparison.

3.7.3 Antifungal Activity Testing: Agar Well Diffusion Method

This study utilized the agar well diffusion method, a well-established and efficient technique, to investigate the antifungal activity of a plant extract against *Aspergillus niger*. This method offers a reliable preliminary screening tool for identifying potential antifungal agents.

3.7.3.1 Fungal Spore Preparation

- **Selecting the Right Age:** A 2-3 day old *Aspergillus niger* culture was chosen to ensure actively growing fungal cells. Younger or older cultures may have reduced viability, potentially affecting results.

- **Harvesting the Spores:** Spores were harvested from the culture using a sterile technique. The specific method may vary depending on the fungal species. Common approaches include scraping the culture surface with a sterile loop or suspending the culture in a sterile solution followed by filtration to isolate the spores.

- **Standardization for Consistency:** The harvested spores were suspended in a sterile broth (e.g., saline solution) to create a standardized inoculum. The suspension was then adjusted to a 0.5 McFarland standard. This turbidity-based method ensures consistent fungal spore concentration across replicates, crucial for data reproducibility.

3.7.3.2 Media and Plate Preparation

1. **Media Selection:** Two commonly used mycological media, Potato Dextrose Agar (PDA) or Sabouraud Dextrose Agar (SDA), were chosen for this study. Both media provide essential nutrients for fungal growth.

2. **Inoculation:** Creating a Uniform Lawn: The standardized spore suspension was spread evenly over the surface of solidified PDA/SDA plates using a sterile spreader. This ensures a uniform distribution of fungal cells across the plate, creating a consistent ”lawn” for potential inhibition zone visualization.

3. **Drying for Optimal Conditions:** The inoculated plates were allowed to dry for a short period. This prevents excessive media well flooding and ensures proper well formation, crucial for accurate sample application.

3.7.3.3 Well Creation and Sample Application

1. **Well Formation with Consistency:** Sterile cork borers of a defined diameter (e.g., 6 mm) were used to create wells in the solidified agar. Maintaining a consistent well size is essential for ensuring data reproducibility.

2. **Sample Application:** Introducing the Unknown: The plant extract solutions were prepared at two different concentrations (250 μg/mL and 500 μg/mL) in a suitable solvent (e.g., water, dimethyl sulfoxide). These solutions were then carefully added to separate wells using sterile micropipettes.

3. **Positive Control:** Confirmation and Comparison: Fluconazole discs, a standard antifungal medication, were placed on separate wells as a positive control. The presence of a clear inhibition zone around the Fluconazole disc confirms the viability of the fungal inoculum and the effectiveness of the assay itself.

3.7.3.4 Incubation and Measurement

1. **Incubation:** Time for Growth and Inhibition: The inoculated plates were incubated at a constant temperature (28^{o}C) for 4-5 days. This allows sufficient time for fungal growth and the development of potential inhibition zones around the wells containing the plant extract. The optimal incubation temperature and duration may vary depending on the specific fungal species under investigation.

2. **Zone Measurement:** Quantifying the Inhibition: After incubation, the diameters of any clear inhibition zones around the wells containing the plant extract solutions and the Fluconazole disc were measured using calipers. The presence and size of these zones indicate the antifungal activity of the plant extract. A larger zone diameter suggests a stronger antifungal effect [260].

3.7.4 Anticancer Activity: The MTT Assay

This protocol outlines a method for evaluating the cytotoxic activity (cell-killing ability) of compounds using the MTT assay.

3.7.4.1 Cell Culture and Seeding

Human prostate cancer cells (PC_3 line) were cultured in flasks containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) for essential nutrients, penicillin to prevent bacterial contamination, and streptomycin to inhibit bacterial growth. The flasks were maintained in a controlled environment incubator set at 37^oC with 5% CO_2 to ensure optimal cell growth.

For the experiment, exponentially growing cells were harvested, counted, and diluted in fresh medium to achieve a desired density of 100,000 cells per milliliter (1 $\times$ 10^5 cells/ml). This cell suspension was then dispensed at 100 microliters per well into 96-well plates for overnight incubation.

3.7.4.2 Treatment and Analysis

Following the overnight incubation, the culture medium was removed from the wells and replaced with fresh medium containing varying concentrations (1 to 30 micromolar) of the test compounds (200 microliters per well). These plates were then incubated for 48 hours to allow for compound interaction with the cells.

3.7.4.3 Cell Viability Assay (MTT)

To assess cell viability after treatment, an MTT assay was employed. MTT is a tetrazolium dye that is metabolized by metabolically active cells into formazan crystals. After the 48-hour incubation with test compounds, an MTT solution (200 microliters per well) was added to each well and incubated for an additional 4 hours. To stop the reaction and solubilize the formazan crystals formed by

viable cells, a solution of DMSO (100 microliters per well) was added. Finally, the extent of MTT reduction to formazan by viable cells was quantified by measuring the absorbance of light at 570 nanometers using a microplate reader. Higher absorbance values indicate a greater number of viable cells and, consequently, lower cytotoxicity caused by the test compound.

3.7.4.4 Evaluating Cell Death: The IC_{50} Value

The cytotoxicity of the tested compounds was determined by measuring the concentration required to inhibit the growth of PC_3 cells by 50%, known as the IC_{50} value. A lower IC_{50} value indicates greater cytotoxicity (cell-killing ability) of the compound.

3.7.4.5 Percent Inhibition Calculation

A specific formula was used to calculate the percentage of growth inhibition caused by the compounds at different concentrations. This value helps compare the effectiveness of various compounds in terms of their anti-cancer potential.

% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)) [261].

Chapter 4

Results

4.1 Total Phenolic and Flavonoid Contents & Extraction Yield of *Berberis lycium* & *Solanum surattense*

The total phenolic contents, total flavonoid contents, and extraction yield of *Berberis lycium* and *Solanum surattense* were determined (Table 4.1). *Berberis lycium* exhibited significantly higher ($p < 0.05$) total phenolic content (193.83 ± 3.93 mg GAE/g of dry extract) compared to *Solanum surattense* (50.69 ± 3.53 mg GAE/g of dry extract).

A similar trend was observed for total flavonoid content, with *Berberis lycium* showing a significantly higher content (37.47 ± 0.37 mg QE/g of dry extract) compared to *Solanum surattense* (19.41 ± 0.33 mg QE/g of dry extract). The extraction yield for both plants was comparable (around 3.5%).

These findings suggest that *Berberis lycium* is a richer source of antioxidant compounds compared to *Solanum surattense*.

The higher content of phenolics and flavonoids in *Berberis lycium* could be attributed to its specific biosynthetic pathways or environmental factors.

TABLE 4.1: Total phenolic contents, total flavonoid contents and extraction yield of *Berberis lycium* and Solanum surattense

Plants	**Total Phenolic Contents (mg GAE/g of Dry Extract)**	**Total Flavonoid Contents (mg QE/g of Dry Extract)**	**Extraction Yield (%)**
Berberis lycium	193.83±3.93a	37.47±0.37a	3.43
Solanum surattense	50.69±3.53b	19.41±0.33b	3.52

The data in the table are presented as means ± standard deviation (SD) based on three replicates (n = 3) per treatment. Statistical analysis was performed to compare means within each column. Values that do not share a letter superscript are significantly different from each other at a p-value threshold of less than 0.05.

4.2 FTIR Analysis of Phytochemical Functional Groups in *Berberis lycium* & *Solanum surattense* Extracts

Fourier-Transform Infrared (FTIR) spectroscopy was employed to characterize the functional groups present in the methanol extract of *Berberis lycium* and *Solanum surattense*. FTIR analysis revealed the presence of alcohols, alkanes, and carbonyl-containing compounds. Such chemical constituents are often associated with a broad spectrum of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. The spectrums were recorded in the range of 4000-500 cm^{-1}.

4.2.1 FTIR Analysis of *Berberis lycium* Methanol Extract

The FTIR spectrum of Berberis lycium methanol extract revealed a complex profile indicative of a diverse chemical composition. Prominent absorption bands were observed at approximately 3400 cm^{-1}, characteristic of O-H stretching vibrations,

suggesting the presence of alcohols or phenols. Aliphatic C-H stretching vibrations were evident at around 2900 cm^{-1}. The presence of a carbonyl functional group was indicated by a strong absorption band near 1650 cm^{-1}, potentially attributed to ketones, aldehydes, or carboxylic acids. Aromatic C-H bending vibrations were observed at approximately 1500 cm^{-1}. Additionally, C-O stretching vibrations, typically associated with alcohols, ethers, or esters, were observed in the fingerprint region below 1500 cm^{-1}. The complex nature of the fingerprint region also suggested the potential presence of other functional groups, such as amines, amides, or nitro compounds, requiring further analysis for definitive identification. A detailed analysis of the spectrum is presented in Table 4.2 and Figure 4.1.

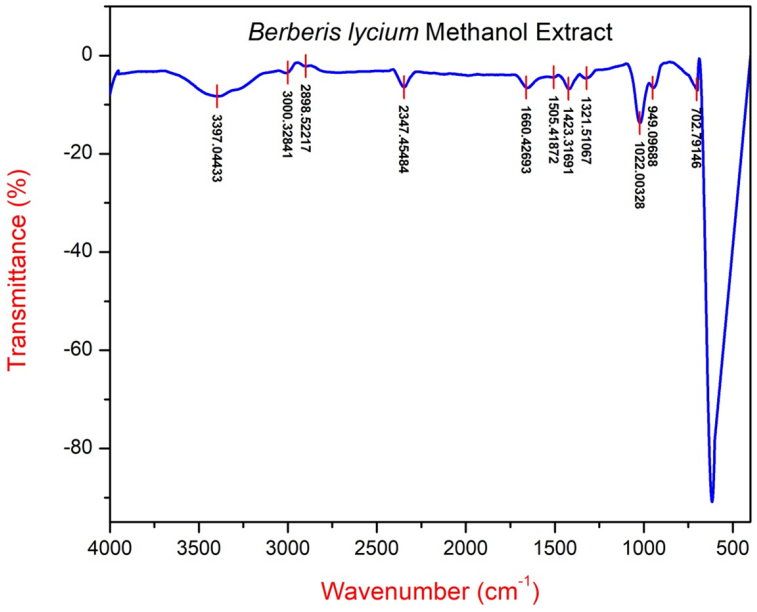

FIGURE 4.1: FTIR Spectrum of *Berberis lycium* Methanol Extract

TABLE 4.2: FTIR Spectral Data of Berberis lycium Methanol Extract

Peak Position (cm^{-1})	**Possible Functional Group**
3397.04433	O-H stretching (alcohols, phenols)
3000.32841	Aromatic C-H stretching
2898.52217	C-H stretching (aliphatic)
2347.45484	CO2 (atmospheric interference)
1660.42693	C=O stretching (carbonyl compounds)

Table 4.2 continued from previous page

Peak Position (cm^{-1})	Possible Functional Group
1505.41872	Aromatic C=C stretching
1423.31691	C-H bending (methylene)
1321.51067	C-O stretching (alcohols, esters)
1022.00328	C-O stretching (alcohols, ethers)
949.09688	C-H bending (aromatic)
702.79146	C-H bending (aromatic)

4.2.2 FTIR Analysis of *Solanum surattense* Methanol Extract

The FTIR spectrum of *Solanum surattense* methanol extract revealed the presence of key functional groups indicative of a complex chemical composition. Prominent peaks corresponding to O-H stretching, C-H stretching (both aliphatic and aromatic), and C=O stretching suggest the presence of alcohols, phenols, alkanes, and carbonyl-containing compounds such as ketones, aldehydes, or carboxylic acids. Additionally, the spectrum exhibited characteristic peaks associated with C-O stretching, potentially indicating the presence of esters or other oxygenated compounds. The fingerprint region below 1500 cm^{-1} displayed a complex pattern, suggesting the presence of various functional groups, including C-N, C-O, and C-C bonds, which would require further analysis for definitive assignments. A detailed analysis of the spectrum is presented in Table 4.3 and Figure 4.2.

TABLE 4.3: FTIR Spectral Data of Solanum surattense Methanol Extract

Peak Position (cm^{-1})	Possible Functional Group
3425.94417	O-H stretching (alcohols, phenols)
2990.47619	C-H stretching (aliphatic)
2903.77668	C-H stretching (aliphatic)
2337.60263	CO2 (atmospheric interference)
1984.23645	CO2 (atmospheric interference)

Table 4.3 continued from previous page

Peak Position (cm^{-1})	Possible Functional Group
1645.97701	C=O stretching (carbonyl compounds)
1413.4647	C-H bending (methylene)
1312.31527	C-O stretching (alcohols, esters)
1026.60099	C-O stretching (alcohols, ethers)
939.24466	C-H bending (aromatic)
702.79146	C-H bending (aromatic)

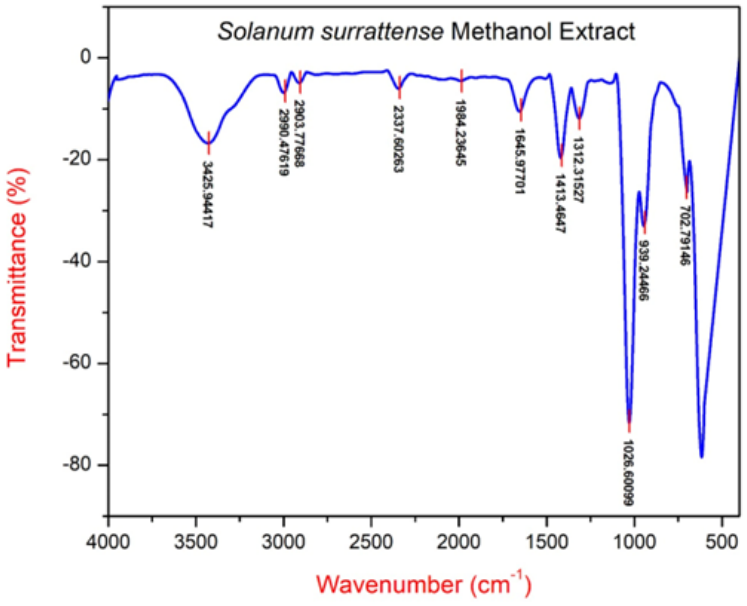

FIGURE 4.2: FTIR Spectrum of *Solanum surattense* Methanol Extract

4.3 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of *Berberis lycium* and *Solanum surattense* methanol extracts was evaluated at different concentrations (Figure 4.3). The results demonstrated a dose-dependent response, where the percentage of DPPH radical scavenging activity increased with increasing extract concentration (each value represents a mean ± SD, n = 3).

The figure depicted the concentration of the extracts on the x-axis labeled "Concentration of Extract" with units of μg/mL. The range tested appears to be from 1

μg/mL to likely 50 μg/mL based on the tick marks on the axis. As the concentration of both extracts increased, their percentage of DPPH radical scavenging activity also increased steadily, demonstrating the dose-dependent response. *Berberis lycium* extract exhibited a stronger DPPH radical scavenging activity compared to *Solanum surattense* extract at all tested concentrations. The percentage of DPPH radical scavenging activity amplified steadily with growing concentration for both extracts. *Berberis lycium* extract appears to reach a higher percentage of DPPH radical scavenging activity than *Solanum surattense* extract at the highest concentration tested.

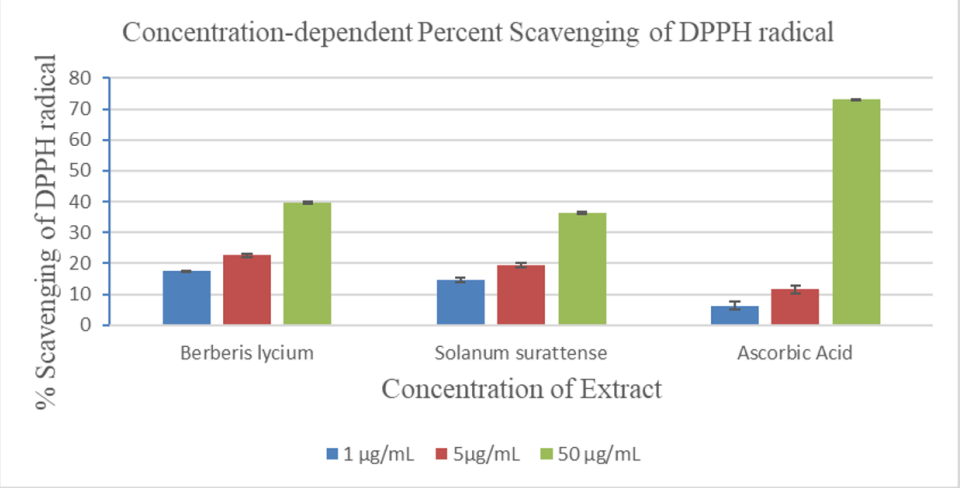

FIGURE 4.3: Scavenging activity of DPPH radicals by methanol extracts of *Berberis lycium* and *Solanum surattense* at different concentrations. Each value represents a mean ± SD (n = 3).

4.4 Antioxidant Effect (IC_{50}) on DPPH Radical of Methanol Extracts of *Berberis lycium* and *Solanum surattense*

The antioxidant capacity of *Berberis lycium* and *Solanum surattense* extracts were assessed using the DPPH radical scavenging assay. Ascorbic acid, a known antioxidant compound, was included for comparison (Table 4.4). Ascorbic acid displayed the strongest free radical scavenging activity, with the lowest IC50 value (3.28

± 0.02 μg/mL). Berberis lycium extract exhibited moderate antioxidant activity (IC50 = 4.10 ± 0.06 μg/mL), while Solanum surattense extract showed the weakest activity among the tested samples (IC50 = 4.43 ± 0.11 μg/mL).

These findings suggest that *Berberis lycium* extract possesses significant antioxidant potential. The lower IC_{50} value compared to *Solanum surattense* indicates that *Berberis lycium* may be a richer source of antioxidant compounds.

TABLE 4.4: Antioxidant effect (IC_{50}) on DPPH radical of methanol extracts of Berberis lycium and Solanum surattense

Plants	**IC_{50} values (μg/mL)**
Berberis lycium	4.10±0.06b
Solanum surattense	4.43±0.11a
Ascorbic Acid	3.28±0.02c

This table shows the mean ± SD (n = 3) for each treatment. Statistical analysis ($p < 0.05$) was performed to compare means within each column. Values that lack a common letter superscript differ significantly.

4.5 Anti-Bacterial Activity of *Berberis lycium* and *Solanum surattense*

The antibacterial potential of *Berberis lycium* and *Solanum surattense* extracts were assessed against various bacteria (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella typhi) using the Alamar Blue assay (Table 4.5). *Ofloxacin*, a widely used antibiotic, served as a positive control. The results indicated that Ofloxacin displayed the strongest inhibition against all tested bacteria. Neither *Solanum surattense* nor *Berberis lycium* extract exhibited significant inhibition against *E. coli, Bacillus subtilis, or Salmonella typhi* at the tested concentration (3000 μg/mL). However, *Berberis lycium* extract did demonstrate moderate inhibitory activity against Staphylococcus aureus (60.21%). Interestingly, berberine, a key alkaloid found in *Berberis lycium*, displayed strong inhibitory activity against *Staphylococcus aureus* (84.55%) at the

same concentration (3000 μg/mL). This finding suggests that berberine may be a key contributor to the antibacterial activity observed in *Berberis lycium* extracts, but these effects are weaker compared to the standard antibiotic Ofloxacin. The antibacterial properties of plant extracts can be influenced by various factors, such as the extraction method and solvents used.

TABLE 4.5: The anti-bacterial effects of *Berberis lycium* and *Solanum surattense* using Alamar Blue assay.

Bacteria	Percent (%) Inhibition			
	Solanum surattense (3000 μg/mL)	*Berberis lycium* (3000 μg/mL)	Berberine (3000 μg/mL)	Ofloxacin (50 μg/mL)
Escherichia coli	-	-	-	89.19
Bacillus subtilis	-	-	-	88.27
Staphylococcus aureus	-	60.21	84.55	85.94
Pseudomonas aeruginosa	-	-	-	92.21
Salmonella typhi	-	-	-	90.3

4.6 Antifungal Activity of *Solanum surattense* and *Berberis lycium*

The agar well diffusion method was employed to assess the antifungal activity of a plant extract against *Aspergillus niger*. Fluconazole discs were used as positive controls. Fungal spore suspensions were adjusted to a 0.5 McFarland standard and spread onto solidified media to create a uniform lawn of growth. After the introduction of wells and the addition of plant extract solutions at concentrations of 250 μg/mL and 500 μg/mL, the plates were incubated for 4-5 days at 28^{o}C.

Unfortunately, **no measurable inhibition** zones were observed around the wells containing the plant extract at either concentration. In contrast, the Fluconazole discs displayed clear zones of inhibition, indicating their effectiveness against *Aspergillus niger*. These findings suggest that, under the conditions of this experiment, the plant extract lacked antifungal activity against this particular fungal strain.

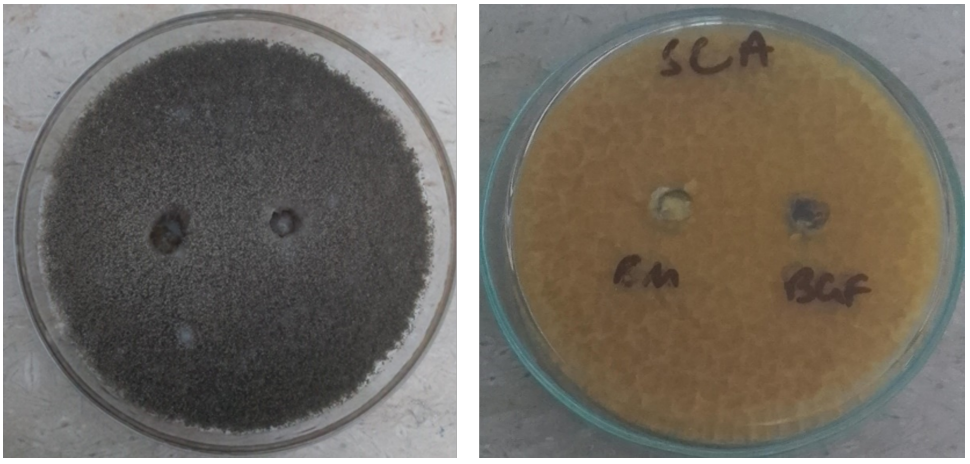

FIGURE 4.4: Antifungal activity of *Berberis lycium* and *Solanum surattense* against *Aspergillus niger*

4.7 Anticancer Activity of *Solanum surattense* and *Berberis lycium*

The results of the MTT assay to determine the anticancer activity of *Solanum surattense* and *Berberis lycium* extracts against PC_3 cells were promising, but ultimately showed low inhibition at the concentration tested (30 μg/mL). *Solanum surattense* extract exhibited a moderate inhibition of 35.93%, while *Berberis lycium* extract showed minimal inhibition of only 4.61% (Table 4.6).

For comparison, Doxorubicin, a well-known chemotherapeutic drug used as a positive control, resulted in a much higher inhibition rate of 80.8%. Even Berberine, an isolated compound found in plant like *Berberis lycium*, showed a moderately higher inhibition (22.15%) than the crude *Berberis lycium* extract itself. These

findings suggest that while the crude extracts may have some anti-cancer properties, further investigation is needed to isolate and purify the active components responsible for the observed inhibition.

TABLE 4.6: Percent inhibition of PC_3 cells treated with methanol extracts (30 μg/mL) of *Solanum surattense* and *Berberis lycium* in MTT assay

Plant	**% Inhibition**
Solanum surattense	35.93
Berberis lycium	4.61
Berberine	22.15
Doxorubicin	80

Chapter 5

Discussion

5.1 Antibacterial Activity

To assess the potential antimicrobial properties of *Berberis lycium* and *Solanum surattense*, we employed the Alamar Blue assay, a cell viability indicator. This assay is a non-destructive viability indicator that utilizes the reduction of a blue resazurin dye (Alamar Blue) to a pink resorufin product by metabolically active bacteria. While Ofloxacin, the standard antibiotic, effectively inhibited all tested bacteria, Our study investigated the antibacterial activity of *Solanum surattense* and *Berberis lycium* extracts against various bacterial strains, including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi*. While previous research reported promising results with these plants against these specific bacterial strains, often using ethanol or hydroalcoholic extracts [73, 74, 189], our findings with methanol extracts showed limited antibacterial effects at the tested concentration (3000 μg/mL). Antibacterial activity of *Berberis lycium* extracts revealed moderate inhibition against *Staphylococcus aureus* but no significant effect on *Escherichia coli, Bacillus subtilis,* or *Salmonella typhi* at the tested concentration (3000 μg/mL). Interestingly, berberine, a key alkaloid in *Berberis lycium*, displayed strong inhibition against the same bacteria at the same concentration. This finding highlights the potential of berberine as a key contributor to the antibacterial activity of *Berberis lyceum*.

and *Solanum surattense* did not reveal significant inhibitory effects at the tested concentration (3000 μg/mL).

Several factors can influence the potency of these extracts, including the extraction method and solvent used. Our study employed a methanol extract, whereas previous research utilized different methods, such as ethanol or hydroalcoholic extractions [73, 74, 189]. This variation can significantly affect the concentration and profile of bioactive compounds within the extracts, potentially explaining the observed difference in antibacterial activity. Additionally, variations in the plant source, such as harvest season, geographical location, and soil composition, can impact the plant's secondary metabolite profile and thus the extract's potency against different bacterial strains.This concept is further supported by a recent study demonstrating significant seasonal variations in the antibacterial potential of *Berberis lycium*, with winter and early spring harvests exhibiting the strongest activity [262].

5.2 Antioxidant Activity

Our study investigated the antioxidant properties of *Berberis lycium* and *Solanum surattense* extracts, employing the DPPH free radical scavenging assay with ascorbic acid as a positive control. *Berberis lycium* extract exhibited stronger free radical scavenging activity compared to *Solanum surattense* extract, suggesting a richer profile of antioxidant compounds in *Berberis lycium*.

Phenolic and flavonoid compounds are well-established contributors to plant antioxidant activity. Their ability to scavenge free radicals and chelate metal ions is a key mechanism for mitigating oxidative stress [77]. The analysis of total phenolic and flavonoid contents sheds light on the potential reasons behind the observed differences in antioxidant capacity between *Berberis lycium* and *Solanum surattense* extracts.The findings for *Berberis lycium* align with previous research attributing its antioxidant potential to compounds like berberine [196]. While *Solanum surattense* exhibited a lower content of phenolics and flavonoids, previous

research has documented the presence of a diverse range of potentially bioactive compounds within this plant [70, 73, 75–78]. Notably, studies have identified alkaloids, saponins, and steroidal compounds like solamargine, solasurine, and solasonine in S. surattense [70, 80]. These compounds may contribute to the observed, although less pronounced, antioxidant activity in *Solanum surattense* extracts.

FTIR analysis also revealed comparable functional group profiles for both *Solanum surattense* and *Berberis lycium* methanol extracts, with prominent peaks indicating the presence of alcohols, alkanes, and carbonyl-containing compounds. Such chemical constituents are often associated with a broad spectrum of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties.

5.3 Anticancer Activity

Our study investigated the anti-cancer activity of methanol extracts from *Solanum surattense* and *Berberis lycium* against human prostate cancer cells (PC_3) using the MTT assay. As expected, the assay measured the metabolic activity of viable cells through the conversion of MTT into formazan crystals. However, at the tested concentration of 30 μg/mL, both plant extracts exhibited low anti-cancer activity against PC_3 cells.

These findings are partially in line with previous studies on the anti-cancer properties of these plants. *Solanum surattense* extracts rich in flavonoids and solamargine have been shown to possess anti-tumor activity, but likely at higher concentrations or against different cancer cell lines [242, 243]. Similarly, berberine, an isolated alkaloid from *Berberis lycium*, has demonstrated potent cytotoxicity against human leukemia cells (HL-60), but the crude extract we tested may lack sufficient berberine or other active components for strong activity against PC_3 cells [194].

The observed low cytotoxicity of *Solanum surattense* and *Berberis lycium* extracts in our study warrants further investigation to reconcile the discrepancies with previous reports. Several factors might contribute to these differences. Variations in plant source (geographic origin, growth conditions) and extraction methods can

significantly impact the concentration and composition of bioactive compounds within the extracts [245]. These differences might explain the lack of observed activity in our extracts compared to those used in prior studies. Additionally, cancer cells exhibit diverse biological properties, making them susceptible to certain compounds more than others [246]. *Solanum surattense* and *Berberis lycium* extracts might be more effective against different cancer cell lines than PC_3 cells. Furthermore, the concentration of 30 μg/mL used in our study might not have been strong enough to induce a measurable cytotoxic response in PC_3 cells. Future studies with a wider range of concentrations and different cancer cell lines would be necessary to definitively assess the anti-cancer potential of these plant extracts.

5.4 Antifungal Activity

Our study employed the agar well diffusion method to evaluate the potential antifungal activity of *Solanum surattense* and *Berberis lycium* extracts against *Aspergillus niger*. This technique relies on the principle of diffusion, where the extract is introduced into a well created in the agar medium containing fungal spores. If the extract possesses antifungal properties, it will diffuse through the agar and inhibit the growth of the fungus around the well, forming a clear zone of inhibition. The diameter of this zone is often used as a semi-quantitative measure of antifungal activity [260].

However, our investigation yielded no significant antifungal zones of inhibition for either extract at the tested concentrations (250 μg/mL and 500 μg/mL). This finding appears to contradict previous research suggesting the potential of *Solanum surattense* extracts against Aspergillus species, including A. niger [239, 240]. One study identified *Berberis lycium* to have antifungal activity against *Aspergillus niger*, with an IC50 value of 399.64 μg/mL [264]. However, our findings with *Berberis lycium* extracts did not demonstrate such activity.

Several factors likely contribute to the discrepancy between our findings and previous research suggesting antifungal activity of *Solanum surattense* and *Berberis*

lycium extracts against *Aspergillus niger*. Firstly, the antifungal properties of plant extracts are highly influenced by the specific plant source. Variations in the plant's genetic makeup and environmental factors during growth, such as geographical location, soil composition, and sunlight exposure, can all impact the production of secondary metabolites with antifungal activity [262].

Chapter 6

Conclusion and Future Work

In conclusion, this study aimed to explore the potential health benefits of *Berberis lycium* and *Solanum surattense* by evaluating their phytochemical content, antioxidant, antibacterial, anticancer, and antifungal activities.Total phenolic and flavonoid contents were determined using spectrophotometric methods. FTIR analysis revealed comparable functional group profiles for both *Solanum surattense* and *Berberis lycium* methanol extracts, with prominent peaks indicating the presence of alcohols, alkanes, and carbonyl-containing compounds. Such chemical constituents are often associated with a broad spectrum of biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. The antioxidant capacity of the extracts was assessed using the DPPH radical scavenging assay, with IC_{50} values calculated to compare their effectiveness. The Alamar Blue assay evaluated the antibacterial activity against various bacteria, while the agar well diffusion method investigated antifungal activity against *Aspergillus nige*r. The MTT assay measured cytotoxicity towards human prostate cancer cells (PC_3). *Berberis lycium* exhibited a richer profile of antioxidant compounds, demonstrated by higher total phenolic and flavonoid content and stronger DPPH radical scavenging activity compared to *Solanum surattense*. This aligns with the potential of *Berberis lycium* as a natural source of antioxidants.

The antibacterial assessment revealed moderate inhibition of *Staphylococcus* aureus by *Berberis lycium* extract, but neither plant extract showed significant activity against other tested bacteria at the chosen concentration.Further investigation is needed to determine the minimum effective concentration and explore its efficacy against a broader range of bacteria, including those resistant to antibiotics. Understanding how *Berberis lycium* targets *Staphylococcus aureus* could also provide valuable insights

Evaluation of anticancer activity against human prostate cancer cells (PC_3) did not show significant cytotoxicity for either extract. Discrepancies with previous reports suggest the need for further investigation considering variations in plant source and extraction methods. Exploring the effectiveness of these extracts against different cancer cell lines and a broader range of concentrations may provide valuable insights.

Finally, the antifungal activity assessment against *Aspergillus niger* using the agar diffusion method did not yield any significant zones of inhibition. Future studies should explore the influence of extraction methods, plant source variations, and concentration range to understand the potential antifungal properties of these plants.

Overall, this study highlights the potential of *Berberis lycium* as a source of natural antioxidants. Further research is necessary to optimize extraction methods and explore the antibacterial and anticancer activities of these plants against a broader range of pathogens and cancer cell lines while considering potential variations in plant source and extraction procedures.

6.1 Future Perspectives and Recommendations

This study provided valuable insights into the potential health benefits of *Berberis lycium* and *Solanum surattense* extracts. However, further exploration is crucial to fully understand their therapeutic potential.

6.1.1 Future Directions

- **Optimization of extraction methods**: Investigating alternative extraction techniques, such as supercritical fluid extraction or enzyme-assisted extraction, may yield extracts with higher concentrations of bioactive compounds, potentially enhancing their observed activities.

- **Exploration of antibacterial activity**: Testing these extracts against a broader panel of bacterial strains and a wider range of concentrations could identify more susceptible pathogens and optimize their antibacterial efficacy. In vitro studies could be complemented with in vivo models to assess their effectiveness in combating bacterial infections.

- **Evaluation of anticancer activity**: Expanding research to include different cancer cell lines is recommended. Additionally, exploring various concentration ranges and employing combination therapy with established anticancer drugs could provide valuable insights into their potential for cancer treatment.

- **Investigation of antifungal activity**: Utilizing different fungal strains and exploring alternative antifungal assays could unveil the potential antifungal properties of these extracts. Further research should also consider the impact of extraction methods and plant source variations on their antifungal activity.

- **Mechanism of action studies**: Understanding the underlying mechanisms by which these extracts exert their biological activities (antioxidant, antibacterial, etc.) is crucial for future drug development efforts.

6.2 Recommendations

- **Standardization of plant materials**: Developing standardized protocols for plant collection, processing, and storage is essential for ensuring consistent quality and reproducibility of research findings.

- **Clinical trials**: If further in vitro and in vivo studies show promising results, well-designed clinical trials are necessary to evaluate the safety and efficacy of these extracts in human populations.
- **Synergistic effects**: Exploring potential synergistic effects between *Berberis lycium* and *Solanum surattense* extracts or with other therapeutic agents could lead to more potent therapeutic strategies.

By pursuing these future directions and recommendations, researchers can gain a deeper understanding of the therapeutic potential of *Berberis lycium* and *Solanum surattense* extracts. This knowledge could pave the way for the development of novel and effective natural products for promoting human health.

Bibliography

[1] A. Singh and M. Gupta, "Phytochemical investigation of different solvent extracts of *Berberis lycium* fruits," Rasayan J. Chem., vol. 11, no. 1, pp. 228-231, Jan. 2018.

[2] S. Sen and R. Chakraborty, "Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future," J. Tradit. Complement. Med., vol. 6, no. 4, pp. 1-11, 2016.

[3] S. Tagbota and S. Townson, "Antiparasitic properties of medicine and other natural occurring products," Adv. Parasitol., vol. 50, pp. 199-205, 2001.

[4] N. Stojanoski, Development of Health Culture in Veles and Its Region from the Past to the End of the 20th Century (in Macedonian), Society of Science and Art, Veles, 1999, pp. 13-34.

[5] K. Kelly, History of Medicine, Facts on File, New York, 2009, pp. 29-50.

[6] G. B. Mahady, "Are medicinal plants a potential alternative for conventional antibiotics in animal husbandry?" Thai J. Phytopharm., vol. 9, pp. 50-62, 2002.

[7] G. B. Mahady, "Application of the Napralert database in the study of traditional Chinese medicine," pp. 74-89, 1997.

[8] Z. X. Shen, "Recent research and developments in traditional Chinese medicine in China," World Health Organization, Geneva, 1996, pp. 7-13.

[9] V. P. Kamboj, "Herbal medicine," Curr. Sci., vol. 78, no. 1, pp. 35-38, 2000.

[10] M. M. Cowan, "Plant products as antimicrobial agents," Clin. Microbiol. Rev., vol. 12, no. 4, pp. 564-582, 1999.

[11] N. T. Ujam, A. N. Oli, M. N. Ikegbunam, M. U. Adikwu, and C. O. Esimone, "Antimicrobial resistance evaluation of organisms isolated from liquid herbal products manufactured and marketed in South Eastern Nigeria," British J. Pharmac. Res., vol. 3, no. 4, pp. 548-562, 2013.

[12] A. Z. Almagboul, A. K. Bashir, A. K. M. Salih, A. Farouk, and S. A. Khalid, "Antimicrobial activity of certain Sudanese plants used in folkloric medicine for their antibacterial activity (In-Vitro Tests)," J. Appl. Sci. Res., vol. 7, no. 7, pp. 235-256, 2011.

[13] N. Artizzu, L. Bonsignore, F. Cottiglia, and G. Loy, "Studies on the diuretic and antimicrobial activity of Cynodondactylon essential oil," Fitoterapia, vol. 67, no. 2, pp. 174-176, 1996.

[14] M. Ikram and I. Inamul, "Screening of medicinal plants for antimicrobial activities," Fitoterapia, vol. 55, no. 1, pp. 62-64, 1984.

[15] A. A. Izzo, G. Di Carlo, D. Biscardi, R. De Fusco, N. Mascolo, et al., "Biological screening of Italian medicinal plants for antibacterial activity," Phytother. Res., vol. 9, no. 4, pp. 281-286, 1995.

[16] E. E. Schapoval, S. M. Silveira, M. L. Miranda, C. B. Alice, and A. T. Henriques, "Evaluation of some pharmacological activities of Eugenia uniflora L.," J. Ethnopharmacol., vol. 44, no. 1, pp. 137-142, 1994.

[17] G. G. Nascimento, J. Locatelli, P. C. Freitas, and G. L. Silva, "Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria," Braz. J. Microbiol., vol. 31, no. 4, pp. 247-256, 2000.

[18] S. Verma and S. P. Singh, "Current and future status of herbal medicines," Vet. World, vol. 1, no. 11, pp. 347-350, 2008.

[19] S. K. Pal and Y. Shukla, "Herbal medicine: current status and the future," Asian Pac. J. Cancer Prev., vol. 4, no. 4, pp. 281-288, 2003.

[20] D. J. Newman, G. M. Cragg, and K. M. Snader, "Natural products as sources of new drugs over the period 1981-2002," J. Nat. Prod., vol. 66, no. 8, pp. 1022-1037, 2003.

[21] M. A. Bisi-Johnson, D. O. Kolawole, and A. O. Shittu, "Epidemiological analysis of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria," Pak. J. Biol. Sci., vol. 8, no. 7, pp. 1016-1020, 2005.

[22] M. N. Alekshun and S. B. Levy, "Molecular mechanisms of antibacterial multidrug resistance," Cell, vol. 128, no. 6, pp. 1037-1050, 2007.

[23] M. A. Bisi-Johnson, C. L. Obi, J. N. Eloff, B. B. Samuel, K. Baba, et al., "Can herbal remedies be the answer to multidrug resistance? Profile of drug resistance in Salmonella species in Eastern Cape, South Africa," J. Experi. Integrative Med., vol. 2, no. 4, pp. 147-153, 2012.

[24] M. M. Blanco, C. A. Costa, A. O. Freire, J. G. Santos Jr., and M. Costa, "Neurobehavioral effect of essential oil of Cymbopogon citratus in mice," Phytomedicine, vol. 16, no. 3, pp. 265-270, 2009.

[25] K. Revathi, C. A. Kumar, and K. Thamizhavahan, "Combined antimicrobial activity of lemon grass oil and Tulasi oil," Int. J. Preclinical Pharm. Res., vol. 3, no. 1, pp. 79-81, 2012.

[26] A. M. Abbasi, M. A. Khan, M. Ahmad, M. Zafar, J. Sarwat, and S. Sultana, "Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan," J. Ethnopharmacol., vol. 128, no. 2, pp. 322-335, 2010.

[27] K. Sheikh, T. Ahmad, and M. A. Khan, "Use, exploitation and prospects for conservation: people and plant biodiversity of Naltar Valley, Northwestern Karakorums, Pakistan," Biodivers. Conserv., vol. 11, no. 5, pp. 715-742, 2002.

[28] J. Tardio and M. Pardo-de-Santayana, "Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain)," Econ. Bot., vol. 62, no. 1, pp. 24-39, 2008.

[29] T. Wondimu, Z. Asfaw, and E. Kelbessa, "Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia," J. Ethnopharmacol., vol. 112, no. 1, pp. 152-161, 2007.

[30] A. M. Abbasi, M. A. Khan, M. Ahmad, and M. Zafar, Medicinal Plant Biodiversity of Lesser Himalayas-Pakistan, Springer, New York, 2012. ISBN 978-1-4614-1574-9.

[31] A. M. Abbasi, M. A. Khan, N. Khan, and M. H. Shah, "Ethnobotanical survey of medicinally important wild edible fruits species used by tribal communities of Lesser Himalayas-Pakistan," J. Ethnopharmacol., vol. 148, no. 2, pp. 528-536, 2013.

[32] R. Abe and K. Ohtani, "An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines," J. Ethnopharmacol., vol. 145, no. 1, pp. 554-565, 2013.

[33] D. Abebe and A. Ayehu, Medicinal Plants and Enigmatic Health Practices of Northern Ethiopia, B. S. P. E, Addis Ababa, Ethiopia, 1993, p. 511.

[34] M. Adnan, S. Begum, A. Latif, A. M. Tareen, and L. J. Lee, "Medicinal plants and their uses in selected temperate zones of Pakistani Hindukush-Himalaya," J. Med. Plants Res., vol. 6, no. 40, pp. 4113-4127, 2012.

[35] N. Akhtar, A. Rashid, W. Murad, and E. Bergmeier, "Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan," J. Ethnobiol. Ethnomed., vol. 9, no. 1, p. 25, 2013.

[36] S. I. Ali and M. Qaiser, "A phytogeographical analysis of the phanerogams of Pakistan and Kashmir," Proc. R. Soc. Edinb. Biol., vol. 89B, no. 1, pp. 89-101, 1986.

[37] G. Jan, M. A. Khan, F. G. Farhatullah Jan, M. Ahmad, M. Jan, and M. Zafar, "Ethnobotanical studies on some useful plants of Dir Kohistan valleys, KPK, Pakistan," Pak. J. Bot., vol. 43, no. 4, pp. 1849-1852, 2011.

[38] I. Khan, S. Najeebullah, M. Ali, and Z. K. Shinwari, "Phytopharmacological and ethnomedicinal uses of the Genus Berberis (Berberidaceae): A review," Trop. J. Pharm. Res., vol. 15, no. 8, pp. 2047-2057, 2016.

[39] T. Khan and I. Khan, "A review on Berberis species reported from Gilgit-Baltistan and Central Karakoram National Park, Pakistan," J. Med. Plants Stud., vol. 3, no. 1, pp. 16-20, 2014.

[40] H. Shah, A. A. Shad, S. Perveen, S. Khattak, and K. F. Khattak, "Physio-chemical Composition of Wild Medicinal Plant *Berberis lycium*," Appl. Sci., vol. 3, no. 4, pp. 370-375, 2003.

[41] P. Sood, R. Modgil, M. Sood, and B. Kapoor, "*Berberis lycium* a Medicinal Plant with Immense Value," Indian J. Pharm. Biol. Res., vol. 1, no. 1, pp. 27-37, 2013.

[42] H. Ali, S. Uddin, and S. Jalal, "Chemistry and Biological Activities of *Berberis lycium* Royle," J. Biol. Act. Prod. Nat., vol. 5, no. 2, pp. 295-312, 2015.

[43] S. Ahmed, M. Shuaib, K. Ali, S. Ali, and F. Hussain, "Evaluation of different parts of Berberis lyceum and their biological activities: A review," Pure Appl. Biol., vol. 6, no. 4, pp. 897-907, 2017.

[44] A. Shabbir, M. Shahzad, Y. Arfat, L. Ali, R. S. Aziz, G. Murtaza, and S. A. Waqar, "*Berberis lycium* Royle: A review of its traditional uses, phyto-chemistry and pharmacology," Afr. J. Pharm. Pharmacol., vol. 6, no. 12, pp. 2346-2353, 2012.

[45] S. Sabir, K. Tahir, N. Rashid, S. Naz, B. Masood, and M. A. Shah, "Phytochemical and antioxidant studies of *Berberis lycium*," Pak. J. Pharm. Sci., vol. 26, no. 6, pp. 1165-1172, 2013.

[46] B. Salehi, Z. Selamoglu, B. Sener, M. Kilic, A. K. Jugran, N. De Tommasi, C. Sinisgalli, L. Milella, J. Rajkovic, M. F. B. Morais-Braga, et al., "Berberis Plants-Drifting from Farm to Food Applications, Phytotherapy, and Phytopharmacology," Foods, vol. 8, no. 11, p. 522, 2019.

[47] V. Jamwal, S. Gupta, and M. Bhagat, "Analysis of phytochemical and biological potential of *Berberis lycium* roots," Arch. Pharm. Biol. Sci., vol. 4, no. 2, pp. 117-123, 2016.

[48] N. Jabeen, A. Saleem, S. Anwaar, and H. Hussain, "*Berberis lycium* Royle (Royle, 1837): A Threatened Medicinal Plant and Its Biological Activities," EC Agric., vol. 1, no. 2, pp. 100-108, 2015.

[49] M. Hamayun, A. S. Khan, Y. H. Kim, I. C. Na, and J. I. Lee, "Traditional knowledge and ex situ conservation of some threatened medicinal plants of Swat Kohistan, Pakistan," Int. J. Botany., vol. 2, no. 4, pp. 205-209, 2006.

[50] P. Sharma, A. Awasthi, and I. Gupta, "Phytochemical investigation and evaluation of anti-oxidant potential of *Berberis lycium* roots from Himachal Pradesh," J. Pharmacogn. Phytochem., vol. 8, no. 8, pp. 4740-4745, 2019.

[51] R. Hema, S. Kumaravel, and K. Alagusundaram, "GC-MS study on the bioactive components and anti-cancer activities of *Solanum surattense*," Cancer Biol., vol. 1, no. 1, pp. 13-17, 2011.

[52] C. S. Barik, N. Parida, D. Sahu, and A. R. Sahoo, "*Solanum surattense* evaluation of anthelmintic activity," J. Pharma. Adv. Res., vol. 1, no. 5, pp. 240-246, 2018.

[53] S. K. Tekuri, S. K. Pasupuleti, K. K. Konidala, S. R. Amuru, N. Bassaiahgari, and P. Pabbaraju, "Phytochemical and pharmacological activities of *Solanum surattense* Burm. F.- A review," Journal of Applied Pharmaceutical Science, vol. 9, no. 3, pp. 126-136, 2019.

[54] O. M. Singh and T. P. Singh, "Phytochemistry of Solanum xanthocarpum: an amazing traditional healer," J. Sci. Ind. Res., vol. 69, no. 9, pp. 732-740, 2010.

[55] A. K. Nadkarani, Indian Material Medica, Vol. I, 3rd ed., Popular Book Depot, Bombay, India, 1954, pp. 1153-1158.

[56] N. Sharma, A. K. Sharma, and R. Zafar, "Kantikari: a prickly medicinal weed-ecosensorium," J. Phytol. Res., vol. 9, no. 1, pp. 13-17, 2010.

[57] N. Kumar, D. Prakash, and P. Kumar, "Wound healing activity of Solanum xanthocarpum schrad and Wendl fruits," Indian J. Nat. Prod. Res., vol. 1, no. 4, pp. 470-475, 2010.

[58] H. Dewangan, M. Bais, V. Jaiswal, and V. K. Verma, "Potential wound healing activity of the ethanolic extract of Solanum xanthocarpum schrad and wendl leaves," Pak. J. Pharm. Sci., vol. 25, no. 1, pp. 189-194, 2012.

[59] D. Mathur and R. C. Agrawal, "Withania congulans: A review on the morphological and pharmacological properties of the shrub," World J. Sci. Technol., vol. 1, no. 10, pp. 30-37, 2011.

[60] R. K. Gupta, T. Hussain, G. Panigrahi, A. Das, G. N. Singh, K. Sweety, M. Faiyazuddin, and C. V. Rao, "Hepatoprotective effect of Solanum xanthocarpum fruit extract against CCl4 induced acute liver toxicity in experimental animals," Asian Pacific J. Trop. Med., pp. 964-968, 2011.

[61] K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants, 2nd ed., Bisen Singh Mahendra Pal Singh, Dehradun, India, 2005, pp. 1759-1762.

[62] P. Kumar, "Molluscicidal efficacy of medicinal plant *Solanum surattense* against Fasciola vector snail, Lymnaea acuminata," Int. J. Biol. Innov., vol. 3, no. 1, pp. 120-126, 2021.

[63] K. K. Bhutani, A. T. Paul, W. Fayad, and S. Linder, "Apoptosis inducing activity of steroidal constituents from Solanum xanthocarpum and Asparagus racemosus," Phytomedicine, vol. 17, no. 8-9, pp. 789-793, 2010.

[64] H. P. Pandey, "Seed fume of *Solanum surattense*: A traditional panacea for teeth and gums," Indian J. Tradit. Knowl., vol. 3, no. 2, pp. 206-207, 2004.

[65] M. T. Rahman, M. Ahmed, M. Alimuzzaman, and J. A. Shilpi, "Antinociceptive activity of the aerial parts of Solanum xanthocarpum," Fitoterapia, vol. 74, no. 1-2, pp. 119-121, 2003.

[66] S. Siddarthan, Y.-Z. Chen, H. Cheng, and S. Mei, "Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants," Food Chem., vol. 102, no. 4, pp. 938-953, 2007.

[67] A. W. Khan and A. U. Khan, "Antidepressant effect of *Solanum surattense* Burn F," The J. Anim. Plant Sci., vol. 29, no. 4, pp. 1188-1192, 2019.

[68] D. M. Kar, L. Maharana, S. Pattnaik, and G. K. Dash, "Studies on hypoglycemic activity of Solanum xanthocarpum fruit extract in rats," J. Ethnopharmacol., vol. 108, no. 2, pp. 251-256, 2006.

[69] L. M. S. Sharma and C. N. Srivastava, "Comparative efficacy of S. xanthocarpum extracts alone and in combination with a synthesis pyrethroid cypermethrin, against malaria vector, Anapheles stephensi," South Asian J. Trop. Med. Public Health, vol. 38, no. 4, pp. 256-260, 2007.

[70] S. Siddiqui and S. Faizi, "Studies in the chemical constituents of the fresh berries of Solanum xanthocarpum," J. Chem. Soc. Pakistan, vol. 5, no. 1, pp. 99-101, 1983.

[71] E. Sheeba, "Antibacterial activity of *Solanum surattense* Burn F," Kathmandu Univ. J. Sci., Eng. Technol., vol. 6, no. 1, pp. 1-4, 2010.

[72] A. Gawande, S. Wankhade, K. V. Shankhopal, and G. B. Shinde, "Isolation, purification and characterization of SolasodineA steroidal alkaloid from *Solanum surattense* berries," Indian Drugs, vol. 28, no. 4, pp. 149-150, 1991.

[73] M. C. Mali and N. Harsh, "Nutritional value estimation of the leaves and seeds of *Solanum surattense*," J. Med. Plants Stud., vol. 3, no. 1, pp. 27-29, 2015.

[74] Y. Iida, Y. Yanai, M. Ono, T. Ikeda, and T. Nohara, "Three unusual 22-6-0-23-hydroxy-(5a)-spirostanol glycosides from the fruits of Solanum torvum," Chem. Pharm. Bull., vol. 53, no. 9, pp. 1122-1125, 2005.

[75] G. Kusano, J. Beister, and Y. Sato, "Steroidal constituents of Solanum xanthocarpum," Phytochemistry, vol. 12, no. 2, pp. 397-401, 1973.

[76] P. Debey and P. C. Gupta, "A new flavonol glycoside from the flowers of Solanum xanthocarpum," Phytochemistry, vol. 17, no. 4, p. 613, 1936.

[77] M. Weissenberg, "Isolation of solasodine and other steroidal alkaloids and sapogennins by direct hydrolysis-extraction of Solanum plants or glycosides," Phytochemistry, vol. 58, no. 4, pp. 501-508, 2001.

[78] I. Z. Saiyed and D. D. Kanga, "Chemical examination of the fruits of Solanum xanthocarpum," Proc. Indian Acad. Sci. Sect. A, vol. 4, no. 3, pp. 255-260, 1936.

[79] K. Jayakumar and K. Murugan, "Solanum alkaloids and their pharmaceutical roles: a review," J. Pharma. Res., vol. 3, no. 6, p. 00075, 2016.

[80] S. Khanam and R. Sultana, "Isolation of β-sitosterol and stigmasterol as active immunomodulatory constituent from the fruits of Solanum xanthocarpum (Solanaceae)," Int. J. Pharm. Sci. Res., vol. 3, no. 4, pp. 1057-1040, 2012.

[81] O. M. Singh and T. P. Singh, "Phytochemistry of Solanum xanthocarpum an amazing traditional healer," J. Sci. Ind. Res., vol. 69, no. 9, pp. 732-740, 2010.

[82] S. Ghildiyal and V. K. Joshi, "Pharmacognostical studies on *Solanum surattense* burn F. root," Int. J. Pharm. Sci. Res., vol. 3, no. 4, pp. 240-245, 2014.

[83] E. Sheeba, "Antibacterial activity of *Solanum surattense* Burn F," Kathmandu Univ. J. Sci., Eng. Technol., vol. 6, no. 1, pp. 1-4, 2010.

[84] D. M. Kar, L. Maharana, S. Pattnaik, and G. K. Dash, "Studies on hypoglycemic activity of Solanum xanthocarpum fruit extract in rats," J. Ethnopharmacol., vol. 108, no. 2, pp. 251-256, 2006.

[85] K. Abbas, U. Niaz, T. Hussain, M. A. Saeed, Z. Javaid, A. Idrees, and S. Rasool, "Antimicrobial activity of fruit of Solanum nigrum and Solanum xanthocarpum," Acta Pol. Pharm., vol. 71, no. 3, pp. 415-421, 2014.

[86] S. K. Tekuri, S. K. Pasupuleti, K. K. Konidala, S. R. Amuru, and N. Bassaiahgari Pabbaraju, "Phytochemical and pharmacological activities of *Solanum surattense* Burm. F.: A review," J. Appl. Pharm. Sci., vol. 9, no. 03, pp. 126-136, 2019.

[87] A. Mahmood, A. Mahmood, and M. Mahmood, "In vitro biological activities of most common medicinal plants of family Solanaceae," World Appl. Sci. J., vol. 17, no. 8, pp. 1020-1032, 2012.

[88] E. David, E. K. Flumalai, C. Sivakumar, S. V. Therasa, and T. Thirumalai, "Evaluation of antifungal activity and phytochemical screening of *Solanum surattense* seeds," J. Pharm. Res., vol. 3, no. 4, pp. 684-687, 2010.

[89] Tran, N., Nguyen, M., Le, K. P. B., Nguyen, N., Tran, Q., & Le, L. (2020). Screening of antibacterial activity, antioxidant activity, and anticancer activity of Euphorbia hirta Linn. extracts. Applied Sciences, 10(23), 8408.

[90] Y. Xiang, Z. Guo, P. Zhu, J. Chen, and Y. Huang, "Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science," Cancer Med., vol. 8, no. 12, pp. 1958-1975, 2019.

[91] World Health Organization, "Antimicrobial resistance: global report on surveillance," World Health Organization, 2014.

[92] A. Fleming, "Penicillin," Nobel lecture, 1945.

[93] R. N. Chopra, S. L. Nayar, and I. C. Chopra, "Glossary of Indian Medicinal Plants," CSIR, New Delhi, 1956.

[94] M. Adnan, J. Hussain, M. T. Shah, Z. K. Shinwari, F. Ullah, A. Bahader, et al., "Proximate and nutrient composition of medicinal plants of humid and sub-humid region of North-West Pakistan," J. Med. Plant Res., vol. 4, no. 6, pp. 339-245, 2010.

[95] B. A. Rasool Hassan, "Medicinal plants (importance and uses)," Pharmaceut Anal Acta, vol. 3, no. e139, pp. 1-2, 2012, doi: 10.4172/2153-2435.1000e139.

[96] J. D. Phillipson, "Phytochemistry and medicinal plants," Phytochemistry, vol. 56, no. 3, pp. 237-243, 2001.

[97] D. B. Jack, "One hundred years of aspirin," Lancet, vol. 350, no. 9075, pp. 437-439, 1997, doi: 10.1016/s0140-6736(97)07087-6.

[98] H. Khan, "Medicinal plants in light of history: Recognized therapeutic modality," J. Evid. Based Integr. Med., vol. 19, pp. 216-219, 2014.

[99] B. Patwardhan, A. Vaidya, M. Chorghade, and S. Joshi, "Reverse pharmacology and systems approaches for drug discovery and development," Curr. Bioact. Compd., vol. 4, no. 3, pp. 201-212, 2008.

[100] M. Aslam, "Conservation, Cultivation and Trade of Herbal Medicine in Pakistan," presented at the International workshop on Health Challenges of 21st Century and Traditional Medicines in SAARC Region, 2018.

[101] M. Aslam, "Guidelines for cultivation, collection, conservation and propagation of medicinal herbs," Ministry of Food, Agriculture and Livestock, Islamabad, Pakistan, 2016.

[102] H. Huang, "Plant diversity and conservation in China: planning a strategic bioresource for a sustainable future," Bot. J. Linn. Soc., vol. 166, no. 3, pp. 282-300, 2011.

[103] M. Rafieian-Kopaei, "Medicinal plants and the human needs," J. HerbMed Pharmacol., vol. 1, no. 1, pp. 1-2, 2012.

[104] S. Verma and S. P. Singh, "Current and future status of herbal medicines," Vet. World, vol. 1, no. 11, pp. 347-350, 2008, doi: 10.5455/vetworld.2008.347-350.

[105] R. Solecki and Z. A. Shanidar, "A Neanderthal flower burial in Northern Iraq," Science, vol. 190, no. 4217, pp. 880-881, 1975, doi: 10.1126/science.190.4217.880.

[106] M. K. Ang-Lee, J. Moss, and C. S. Yuan, "Herbal medicines and perioperative care," JAMA, vol. 286, no. 2, pp. 208-216, 2001.

[107] J. Qiu, "Traditional medicine: a culture in the balance," Nature, vol. 448, no. 7150, pp. 126-128, 2007, doi: 10.1038/448126a.

[108] U. W. Schippmann, D. Leaman, and A. B. Cunningham, "A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects," Front. Ecol. Environ., vol. 17, no. 1, pp. 75-95, 2006.

[109] H. L. Madsen and G. Bertelsen, "Spices as antioxidants," Trends Food Sci. Technol., vol. 6, no. 8, pp. 271-277, 1995, doi: 10.1016/S0924-2244(00)89112-8.

[110] J. L. Rios and M. C. Recio, "Medicinal plants and antimicrobial activity," J. Ethnopharmacol., vol. 100, no. 1-2, pp. 80-84, 2005, doi: 10.1016/j.jep.2005.04.025.

[111] A. Zargari, Medicinal Plants , Tehran University Press, Tehran, Iran, 1992.

[112] O. So, O. Akinyemi, and K. A. Jimoh, "Medicinal plants and sustainable human health: a review," Hort. Int. J., vol. 2, no. 1, pp. 1-?, 2018.

[113] N. Rashid, R. C. Gbedomon, M. Ahmad, V. K. Salako, M. Zafar, and K. Malik, "Traditional knowledge on herbal drinks among indigenous communities in Azad Jammu and Kashmir, Pakistan," J. Ethnobiol. Ethnomed., vol. 14, no. 1, p. 16, 2018.

[114] Z. K. Shinwari and M. Qaisar, "Efforts on conservation and sustainable use of medicinal plants of Pakistan," Pak. J. Bot., vol. 43, no. 1, pp. 5-10, 2011.

[115] S. Hussain, F. Malik, N. Khalid, M. A. Qayyum, and H. Riaz, "Alternative and traditional medicines systems in Pakistan: History, Regulation, Trends, Usefulness, Challenges, Prospects and Limitations," in A Compendium of Essays on Alternative Therapy, InTech, London, UK, 2012, pp. 67.

[116] H. Kanwal and B. A. Sherazi, "Herbal medicine: Trend of practice, perspective, and limitations in Pakistan," Asian Pac. J. Health Sci., vol. 4, no. 1, pp. 6-8, 2017.

[117] A. M. Abbasi, M. A. Khan, M. Ahmad, M. Zafar, J. Sarwat, and S. Sultana, "Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan," Pak. J. Ethnopharm., vol. 128, no. 2, pp. 322-335, 2010.

[118] K. Sheikh, T. Ahmad, and M. A. Khan, "Use, exploitation and prospects for conservation: people and plant biodiversity of Naltar Valley, Northwestern Karakorums, Pakistan," Biodivers. Conserv., vol. 11, no. 4, pp. 715-742, 2002.

[119] J. Tardio and M. Pardo-de-Santayana, "Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain)," Econ. Bot., vol. 62, no. 1, pp. 24-39, 2008.

[120] T. Wondimu, Z. Asfaw, and E. Kelbessa, "Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopia," J. Ethnopharmacol., vol. 112, no. 1, pp. 152-161, 2007.

[121] A. M. Abbasi, M. A. Khan, M. Ahmad, and M. Zafar, Medicinal Plant Biodiversity of Lesser Himalayas-Pakistan, Springer USA, 2012, pp. 1-220. ISBN 978-1-4614-1574-9.

[122] O. Habbal, S. S. Hasson, A. H. El-Hag, Z. Al-Mahrooqui, N. Al-Hashmi, M. S. Al-Balushi, et al., "Antibacterial activity of Lawsonia inermis Linn (Henna) against Pseudomonas aeruginosa," Asian Pac. J. Trop. Biomed., vol. 1, no. 2, pp. 173-176, 2011.

[123] T. Dadgar, M. Asmar, S. Saifi, M. Mazandarani, H. Bayat, A. Moradi, et al., "Antibacterial activity of certain Iranian medicinal plants against methicillin resistant and methicillin sensitive *Staphylococcus aureus*," Asian J. Plant Sci., vol. 5, no. 6, pp. 861-866, 2006.

[124] L. Peng, S. Kang, Z. Yin, R. Jia, X. Song, L. Li, et al., "Antibacterial activity and mechanism of berberine against Streptococcus agalactiae," Int. J. Clin. Exp. Pathol., vol. 8, no. 8, pp. 5217-5223, 2015.

[125] D. K. Semwal and U. Rawat, "Antimicrobial hasubanalactam alkaloid from Stephania glabra," Planta Med., vol. 75, no. 5, pp. 378-380, 2009.

[126] M. Gabor, "Anti-inflammatory and anti-allergic properties of flavonoids," in Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships, V. Cody, V. E. Middleton, and J. B. Harbourne, Eds., Alan R. Liss: New York, NY, USA, 1986, pp. 471-480.

[127] M. Beschia, A. Leonte, and I. Oancea, "Phenolic compounds with biological activity," Bull. Univ. Galati Faso, vol. 6, pp. 23-27, 1984.

[128] S. Thara, J. Ingham, S. Nakahara, J. Mizutani, and J. B. Harborne, "Fungitoxic dihydrofuranoisoflavones and related compounds in white lupin, Lupinus albus," Phytochemistry, vol. 23, no. 8, pp. 1889-1900, 1984.

[129] B. Ozcelik, O. D. Deliorman, S. Ozgen, and F. Ergun, "Antimicrobial Activity of Flavonoids against Extended-Spectrum Beta Lactamase (ESBL)-Producing Klebsiella pneumoniae," Trop. J. Pharm. Res., vol. 7, no. 7, pp. 1151-1157, 2008.

[130] J. Widelski, M. Popova, K. Graikou, K. Glowniak, and I. Chinou, "Coumarins from Angelica lucida L.— Antibacterial Activities," Molecules, vol. 14, no. 7, pp. 2729-2734, 2009.

[131] A. Basile, S. Sorbo, V. Spadaro, M. Bruno, A. Maggio, N. Faraone, et al., "Antimicrobial and antioxidant activities of Coumarins from the roots of Ferulago campestris (Apiaceae)," Molecules, vol. 14, no. 3, pp. 939-952, 2009.

[132] A. Banso, "Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica," J. Med. Plants Res., vol. 3, no. 2, pp. 82-85, 2009.

[133] C. Y. Ragaso, "Antimicrobial and Cytotoxic terpenoid from Cymbopogon citratus Stapf," Philipp. Sci., vol. 45, no. 1, pp. 111-122, 2008.

[134] M. Serafini, A. Ghiselli, and A. Ferro-Luzzi, "Red wine, tea and antioxidants," Lancet, vol. 344, no. 8944, p. 626, 1994.

[135] A. A. Moneim, E. Suleman, F. M. Issa, and E. A. Elkhalifa, "Quantitative determination of tannin content in some sorghum cultivars and evaluation of its antimicrobial activity," Res. J. Microbiol., vol. 2, no. 6, pp. 284-288, 2007.

[136] Sayed A. El-Toumy, Samy M. Mohamed, Emad M. Hassan, Abdel-Tawab H. Mossa. Phenolic metabolites from Acacia nilotica flowers and evaluation of its free radical scavenging activity. J Am Sci 2011; 7(3):287-295.

[137] T. Khan, M. Ali, A. Khan, P. Nisar, S. A. Jan, S. Afridi, and Z. K. Shinwari, "Anticancer Plants: A Review of the Active Phytochemicals, Applications in Animal Models, and Regulatory Aspects," *Biomolecules*, vol. 10, no. 1, p. 47, 2020, doi: 10.3390/biom10010047.

[138] S. Gibbons, "Phytochemicals for bacterial resistance-strengths, weaknesses and opportunities," Planta Med., vol. 74, no. 8, pp. 594-602, 2008.

[139] B. Plackett, "Why big pharma has abandoned antibiotics," Nature, vol. 586, no. 7830, p. S50, 2020.

[140] E. Martens and A. L. Demain, "The antibiotic resistance crisis, with a focus on the United States," J. Antibiot., vol. 70, no. 6, pp. 520-526, 2017.

[141] M. M. Cowan, "Plant products as antimicrobial agents," Clin. Microbiol. Rev., vol. 12, no. 4, pp. 564-582, 1999.

[142] E. M. Abdallah, "Plants: An alternative source for antimicrobials," J. Appl. Pharm. Sci., vol. 1, no. 1, pp. 16-20, 2011.

[143] N. T. Ujam, A. N. Oli, M. N. Ikegbunam, M. U. Adikwu, and C. O. Esimone, "Antimicrobial resistance evaluation of organisms isolated from liquid herbal products manufactured and marketed in South Eastern Nigeria," British J. Pharmac. Res., vol. 3, no. 4, pp. 548-562, 2013.

[144] A. Z. Almagboul, A. K. Bashir, A. K. M. Salih, A. Farouk, and S. A. Khalid, "Antimicrobial activity of certain Sudanese plants used in folkloric medicine for their antibacterial activity (In-Vitro Tests)," Journal of Applied Sciences Research, vol. 7, no. 6, pp. 235-256, 2011.

[145] N. Artizzu, L. Bonsignore, F. Cottiglia, and G. Loy, "Studies on the diuretic and antimicrobial activity of Cynodondactylon essential oil," Fitoterapia, vol. 67, no. 3, pp. 174-176, 1996.

[146] E. E. Schapoval, S. M. Silveira, M. L. Miranda, C. B. Alice, and A. T. Henriques, "Evaluation of some pharmacological activities of Eugenia uniflora L.," J. Ethnopharmacol., vol. 44, no. 1, pp. 137-142, 1994.

[147] G. G. Nascimento, J. Locatelli, P. C. Freitas, and G. L. Silva, "Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria," Braz. J. Microbiol., vol. 31, no. 4, pp. 247-256, 2000.

[148] K. K. Aggarwal, A. Ahmed, T. R. K. Santha, N. Jain, S. K. Gupta, et al., "Antibacterial activity spectra of Pelargonium graveolens L. and Cymbopogon winterrianus jowitt oil constituents and acyl derivatives," J. Med. Aroma Plant Sci, vol. 22, no. 4, pp. 544-548, 2000.

[149] S. Verma and S. P. Singh, "Current and future status of herbal medicines," Vet. World, vol. 1, no. 11, pp. 347-350, 2008.

[150] S. K. Pal and Y. Shukla, "Herbal medicine: current status and the future," Asian Pac. J. Cancer Prev., vol. 4, no. 4, pp. 281-288, 2003.

[151] V. P. Kamboj, "Herbal medicine," Curr. Sci., vol. 78, no. 1, pp. 35-38, 2000.

[152] D. J. Newman, G. M. Cragg, and K. M. Snader, "Natural products as sources of new drugs over the period 1981-2002," J. Nat. Prod., vol. 66, no. 7, pp. 1022-1037, 2003.

[153] M. A. Bisi-Johnson, D. O. Kolawole, and A. O. Shittu, "Epidemiological analysis of clinical isolates of *Staphylococcus aureus* in Ile-Ife, Nigeria," Pak. J. Biol. Sci., vol. 8, no. 8, pp. 1016-1020, 2005.

[154] M. N. Alekshun and S. B. Levy, "Molecular mechanisms of antibacterial multidrug resistance," Cell, vol. 128, no. 6, pp. 1037-1050, 2007.

[155] M. A. Bisi-Johnson, C. L. Obi, J. N. Eloff, B. B. Samuel, K. Baba, et al., "Can herbal remedies be the answer to multidrug resistance? Profile of drug resistance in Salmonella species in Eastern Cape, South Africa," J. Experi Integrative Med, vol. 2, no. 4, pp. 147-153, 2012.

[156] M. M. Blanco, C. A. Costa, A. O. Freire, J. G. Santos Jr., and M. Costa, "Neurobehavioral effect of essential oil of Cymbopogon citratus in mice," Phytomedicine, vol. 16, no. 3, pp. 265-270, 2009.

[157] K. Revathi, C. A. Kumar, and K. Thamizhavahan, "Combined antimicrobial activity of lemon grass oil and Tulasi oil," Int. J. Preclinical Pharm. Res., vol. 3, no. 1, pp. 79-81, 2012.

[158] P. Pattiyathanee, R. K. Vilaichone, and N. Chaichanawongsaroj, "Effect of curcumin on Helicobacter pylori biofilm formation," African J. Biotechnol., vol. 8, no. 23, pp. 5106-5115, 2009.

[159] M. Gupta, S. Ajay, and C. H. Joshi, "*Berberis lycium* multipotential medicinal application, An overview," Int. J. Chem. Stud., vol. 3, no. 4, pp. 10-13, 2015.

[160] P. Sood, R. Modgil, and M. Sood, "*Berberis lycium* a medicinal plant with immense value," Ind. J. Pharmaceut. Biol. Res., vol. 1, no. 1, pp. 27-37, 2013.

[161] K. Mustafa, H. Mohamed, M. A. Shah, S. Yu, M. Akhlaq, H. Xiao, et al., "In-vitro anticancer potential of *Berberis lycium* Royle extracts against human Hepatocarcinoma (HepG2) cells," Biomed. Res. Int., vol. 2020, pp. 1-12, 2020.

[162] N. Nazir, A. Rahman, F. Uddin, A. A. Khan Khalil, M. Zahoor, et al., "Quantitative Ethnomedicinal status and phytochemical analysis of Berberis lyceum Royle," Agronomy, vol. 11, no. 1, p. 130, 2021.

[163] H. Ali, S. Uddin, and S. Jalal, "Chemistry and biological activities of *Berberis lycium* Royle," J. Biol. Active Products Nature, vol. 5, no. 5, pp. 295-312, 2015.

[164] M. A. Neag, A. Mocan, J. Echeverría, R. M. Pop, C. I. Bocşan, G. Crişan, et al., "Berberine, Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders," Front. Pharmacol., vol. 9, p. 557, 2018.

[165] S. Ahmed, M. Shuaib, K. Ali, S. Ali, and F. Hussain, "Evaluation of different parts of Berberis lyceum and their biological activities, a review," Pure Appl. Biol. (PAB), vol. 6, no. 3, pp. 897-907, 2017.

[166] S. Arham, S. Muhammad, A. Yasir, A. Liaqat, S. A. Rao, et al., "*Berberis lycium* Royle, A review of its traditional uses, phytochemistry and pharmacology," Afr. J. Pharm. Pharmacol, vol. 6, no. 31, pp. 2346-2353, 2012.

[167] S. Sabir, K. Tahir, N. Rashid, S. Naz, B. Masood, et al., "Phytochemical and antioxidant studies of *Berberis lycium*," Pak. J. Pharm. Sci., vol. 26, no. 6, pp. 1165-1172, 2013.

[168] B. Salehi, Z. Selamoglu, B. Sener, M. Kilic, A. K. Jugran, et al., "Berberis plants—drifting from farm to food applications, phytotherapy, and phytopharmacology," Foods, vol. 8, no. 10, p. 522, 2019.

[169] S. Ijaz, A. Perveen, S. Ashraf, A. Bibi, and Y. Dogan, "Indigenous wild plants and fungi traditionally used in folk medicine and functional food in district Neelum Azad Kashmir," Environ. Dev. Sustain., vol. 23, no. 6, pp. 8307-8330, 2021.

[170] C. Kaur and S. B. Maini, "Fruits and vegetables... Health foods for new millennium," Indian Horticulture, vol. 45, no. 4, pp. 29-32, 2001.

[171] Absolutely, here are the converted references in IEEE format for the additional references you provided:

[172] M. Hamayun, S. A. Khan, E. Y. Sohn, and I. J. Lee, "Folk medicinal knowledge and conservation status of some economically valued medicinal plants of District Swat, Pakistan," Lyonia, vol. 11, no. 2, pp. 101-113, 2006.

[173] F. Shamsa, A. Ahmadiani, and R. Khosrokhavar, "Antihistaminic and anticholinergic activity of barberry fruit (Berberis vulgaris) in the guinea-pig ileum," J. Ethnopharmacol., vol. 64, no. 2, pp. 161-166, 1999.

[174] M. C. Furrianca, M. Alvear, T. Zambrano, L. Barrientos, V. Fajardo, and L. A. Salazar, "Medicinal value of the Berberis genus as hypoglycemic agent," Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas, vol. 14, no. 5, pp. 423-441, 2015.

[175] N. Chand, F. R. Durrani, M. S. Qureshi, and Z. Durrani, "Role of *Berberis lycium* in reducing serum cholesterol in broilers," Asian Australas. J. Anim. Sci., vol. 20, no. 4, pp. 563-568, 2007.

[176] S. M. Fiaz Bukhari, G. Ali, S. R. Abbas, Z. Anjum, N. Ahmed, et al., "Ethnobotanical and biochemical study of *Berberis lycium* Royle collected from different areas of Azad Jammu and Kashmir," Evid. Based Complement. Alternat. Med., vol. 2021, no. 1, pp. 1-9, 2021.

[177] M. Gupta, S. Ajay, and C. H. Joshi, "*Berberis lycium* multipotential medicinal application, An overview," Int. J. Chem. Stud., vol. 3, no. 4, pp. 10-13, 2015.

[178] M. C. Furrianca, M. Alvear, T. Zambrano, L. Barrientos, V. Fajardo, and L. A. Salazar, "Medicinal value of the Berberis genus as hypoglycemic agent," Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas, vol. 14, no. 5, pp. 423-441, 2015.

[179] P. Sood, R. Modgil, and M. Sood, "Physico-chemical and nutritional evaluation of indigenous wild fruit Kasmal, *Berberis lycium* Royle," Int. J. Agric. Res. Hist., vol. 1, no. 3, pp. 362-366, 2010.

[180] M. Gulfraz, M. Arshad, N. Nayyer, N. Kanwal, and U. Nisar, "Investigation for bioactive compounds of *Berberis lycium* Royle and Justicia Adhatoda L," Ethnobotanical Leaflets, vol. 1, p. 5, 2004.

[181] S. Sabir, K. Tahir, N. Rashid, S. Naz, B. Masood, et al., "Phytochemical and antioxidant studies of *Berberis lycium*," Pak. J. Pharm. Sci., vol. 26, no. 6, pp. 1165-1172, 2013.

[182] S. K. Srivastava, V. Rai, M. Srivastava, A. K. S. Rawat, and S. Mehrotra, "Estimation of heavy metals in different Berberis species and its market samples," Environ. Monit. Assess., vol. 116, no. 1, pp. 315-320, 2006.

[183] P. Sharma, A. Awasthi, and I. Gupta, "Phytochemical investigation and evaluation of anti-oxidant potential of *Berberis lycium* roots from Himachal Pradesh," J. Pharmacognosy Phytochem., vol. 8, no. 6, pp. 4740-4745, 2019.

[184] M. Ahmed, A. Alamgeer, T. Sharif, C. H. M. Zabta, and A. Akbar, "Effect of *Berberis lycium* Royle on lipid profile in alloxan induced diabetic rabbits," Ethnobotanical Leaflets, vol. 6, p. 4, 2009.

[185] H. Wang, C. Zhu, Y. Ying, L. Luo, D. Huang, and Z. Luo, "Metformin and berberine, two versatile drugs in treatment of common metabolic diseases," Oncotarget, vol. 9, no. 11, pp. 10135-10146, 2018.

[186] H. Wang, C. Zhu, Y. Ying, L. Luo, D. Huang, and Z. Luo, "Metformin and berberine, two versatile drugs in treatment of common metabolic diseases," Oncotarget, vol. 9, no. 11, pp. 10135-10146, 2018.

[187] K. Mustafa, H. Mohamed, M. A. Shah, S. Yu, M. Akhlaq, H. Xiao, et al., "In-vitro anticancer potential of *Berberis lycium* Royle extracts against human Hepatocarcinoma (HepG2) cells," Biomed. Res. Int., vol. 2020, pp. 1-12, 2020.

[188] S. Ahmed, M. Shuaib, K. Ali, S. Ali, and F. Hussain, "Evaluation of different parts of Berberis lyceum and their biological activities, a review," Pure Appl. Biol. (PAB), vol. 6, no. 3, pp. 897-907, 2017.

[189] M. Ahmed, A. Alamgeer, T. Sharif, C. H. M. Zabta, and A. Akbar, "Effect of *Berberis lycium* Royle on lipid profile in alloxan induced diabetic rabbits," Ethnobotanical Leaflets, vol. 6, p. 4, 2009.

[190] M. Singh, S. Srivastava, and A. K. S. Rawat, "Antimicrobial activities of Indian Berberis species," Fitoterapia, vol. 78, no. 7-8, pp. 574-576, 2007.

[191] M. Singh, S. Srivastava, and A. K. S. Rawat, "Antimicrobial studies of stem of different Berberis species," Nat. Prod. Sci., vol. 15, no. 2, pp. 60-65, 2009.

[192] H. Nidaullah, F. R. Durrani, S. Ahmad, I. U. Jan, and S. Gul, "Aqueous extract from different medicinal plants as anticoccidial, growth promotive and immunostimulant in broilers," J. Agric. Biol. Sci., vol. 5, no. 1, pp. 53-59, 2010.

[193] A. K. Katare, B. Singh, P. Shukla, S. Gupta, B. Singh, et al., "Rapid determination and optimisation of berberine from Himalayan *Berberis lycium* by soxhlet apparatus using CCD-RSM and its quality control as a potential candidate for COVID-19," Nat. Prod. Res., vol. 36, no. 3, pp. 868-873, 2022.

[194] T. Yousaf, S. Rafique, F. Wahid, S. Rehman, A. Nazir, et al., "Phytochemical profiling and antiviral activity of Ajuga bracteosa, Ajuga parviflora, *Berberis lycium* and Citrus lemon against Hepatitis C Virus," Microb. Pathog., vol. 118, pp. 154-158, 2018.

[195] Khan, M., Giessrigl, B., Vonach, C., Madlener, S., Prinz, S., Herbaceck, I., ... Quereshi, R. A. (2010). Berberine and a *Berberis lycium* extract inactivate Cdc25A and induce α-tubulin acetylation that correlate with HL-60 cell cycle inhibition and apoptosis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 683(1-2), 123-130.

[196] Issat, T., Jakobisiak, M., & Golab, J. (2006). Berberine, a natural cholesterol reducing product, exerts antitumor cytostatic/cytotoxic effects independently from the mevalonate pathway. Oncology Reports, 16(6), 1273-1276.

[197] Z. Mashwani, M.A. Khan, S. Irum, M. Ahmad, Antioxidant potential of root bark of *Berberis lycium* Royle. from Galliyat, western Himalaya, Pakistan, Pakistan J. Botany 45 (231) (2013) 4.

[198] S. Ahmed, F. Shakeel, Voltammetric determination of antioxidant character in *Berberis lycium* Royle, Zanthoxylum armatum and Morus nigra Linn plants, Pak. J. Pharm. Sci. 25 (3) (2012).

[199] A. Rana, H.P. Singh, Bio-utilization of wild berries for preparation of high valued herbal wines, Indian J. Nat. Prod. Resour. 4 (2013) 165–169.

[200] Z. Yousaf, M. A. Khan, and Z. K. Shinwari, "A new variety of *Solanum surattense* Burm. From Pakistan," Pakistan J. Bot., vol. 41, no. 5, pp. 2097-2103, 2009.

[201] A. L. Vijay, N. Srinivasan, S. Abburi, K. Karthikeyan, and S. Mahalaxmi, "Evaluating the Analgesic Efficacy of *Solanum surattense* (Herbal Seed Extract) in Relieving Pulpal Pain - An In-vivo Study," Dentistry, vol. 5, p. 288, 2015.

[202] K. R. Kirtikar and B. D. Basu, Indian Medicinal Plants, International Book Distributors, vol. III, 2005, pp. 1759-1761.

[203] S. Andleeb, M. M. Ahmed, F. Saqib, M. Hussain, M. Hanif, and S. N. H. Shah, "Folkloric use of *Solanum surattense* Burm. Validates its pharmacological activity in constipation and diarrhea," 20th ISTANBUL International conference on Agricultural, Biological and Medical Sciences (IABMS-19), 2019.

[204] N. Sharma, A. K. Sharma, and R. Zafar, "Kantikari: a prickly medicinal weed-ecosensorium," J. Phytol. Res., vol. 9, no. 1, pp. 13-17, 2010.

[205] N. Kumar, D. Prakash, and P. Kumar, "Wound healing activity of Solanum xanthocarpum schrad and Wendl fruits," Indian J. Nat. Prod. Res., vol. 1, no. 4, pp. 470-475, 2010.

[206] H. Dewangan, M. Bais, V. Jaiswal, and V. K. Verma, "Potential wound healing activity of the ethanolic extract of Solanum xanthocarpum schrad and wendl leaves," Pak. J. Pharm. Sci., vol. 25, no. 1, pp. 189-194, 2012.

[207] M. M. Ahmed, S. Andleeb, F. Saqib, M. Hussain, M. N. Khatun, et al., "Diuretic and serum electrolyte regulation potential of aqueous methanolic extract of Solanum xanthocarpum fruit validates its folkloric use in dysuria," BMC Complement Altern Med., vol. 16, no. 1, p. 166, 2016.

[208] N. Chauhan, D. Kumar, and M. Kasana, "Medicinal plants of Muzaffarnagar district used in treatment of urinary tract and kidney stones," Ind. J. Tradit. Knowl., vol. 8, no. 2, pp. 191-195, 2009.

[209] K. K. Bhutani, A. T. Paul, W. Fayad, and S. Linder, "Apoptosis inducing activity of steroidal constituents from Solanum xanthocarpum and Asparagus racemosus," Phytomedicine, vol. 17, no. 8, pp. 789-793, 2010.

[210] H. P. Pandey, "Seed fume of *Solanum surattense*: A traditional panacea for teeth and gums," Indian Journal of Traditional Knowledge, vol. 3, no. 2, pp. 206-207, 2004.

[211] R. K. Pandey, S. S. Shukla, A. Jain, V. B. Gupta, and L. Deb, "Evaluation of comparative immunomodulatory potential of Solanum xanthocarpum root and fruit on experimental animal," Indian J. Pharma. Educ. Res., vol. 52, no. 4 Suppl 2, pp. S237-S245, 2018.

[212] Y. Bahuguna, V. Juyai, and K. Gusain, "Pharmacological evaluation of *Solanum surattense* leaves for antiulcer activity," J. Pharma. Res., vol. 2, pp. 253-259, 2008.

[213] M. T. Rahman, M. Ahmed, M. Alimuzzaman, and J. A. Shilpi, "Antinociceptive activity of the aerial parts of Solanum xanthocarpum," Fitoterapia, vol. 74, no. 1-2, pp. 119-121, 2003.

[214] S. Siddarthan, Y. Z. Chen, H. C. Liu, and S. Mei, "Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants," Food Chem., vol. 102, no. 4, pp. 938-953, 2007.

[215] A. W. Khan and A. U. Khan, "Antidepressant effect of *Solanum surattense* Burn F," The Journal of Animal and Plant Sciences, vol. 29, no. 4, pp. 1188-1192, 2019.

[216] D. M. Kar, L. Maharana, S. Pattnaik, and G. K. Dash, "Studies on hypoglycemic activity of Solanum xanthocarpum fruit extract in rats," J. Ethnopharmacol., vol. 108, no. 2, pp. 251-256, 2006.

[217] L. M. S. Sharma and C. N. Srivastava, "Comparative efficacy of S. xanthocarpum extracts alone and in combination with a synthesis pyrethroid cypermethrin, against malaria vector, Anapheles stephensi," South Asian Journal of Tropical Medicine and Public Health, vol. 38, pp. 256-260, 2007.

[218] S. Siddiqui and S. Faizi, "Studies in the chemical constituents of the fresh berries of Solanum xanthocarpum," Journal of Chemical Society of Pakistan, vol. 5, pp. 99-101, 1983.

[219] S. Shahiladevi, G. Jayanthi, and M. Jegadeesan, "Preliminary phytochemical studies on *Solanum surattense* burn F. seeds," Anc. Sci. Life, vol. 26, no. 1, pp. 59-64, 2006.

[220] E. Sheeba, "Antibacterial activity of *Solanum surattense* Burn F," Kathmandu University Journal of Science, Engineering and Technology, vol. 6, no. 1, pp. 1-4, 2010.

[221] A. Gawande, S. Wankhade, K. V. Shankhopal, and G. B. Shinde, "Isolation, purification and characterization of SolasodineA steroidal alkaloid from *Solanum surattense* berries," Indian Drugs, vol. 28, pp. 149-150, 1991.

[222] M. C. Mali and N. Harsh, "Nutritional value estimation of the leaves and seeds of *Solanum surattense*," J. Med. Plants Stud., vol. 3, pp. 27-29, 2015.

[223] Y. Iida, Y. Yanai, M. Ono, T. Ikeda, and T. Nohara, "Three unusual 22-6-0-23-hydroxy-(5a)-spirostanol glycosides from the fruits of Solanum torvum," Chem. Pharm. Bull., vol. 53, no. 9, pp. 1122-1125, 2005.

[224] G. Kusano, J. Beister, and Y. Sato, "Steroidal constituents of Solanum xanthocarpum," Phytochemistry, vol. 12, no. 2, pp. 397-401, 1973.

[225] P. Debey and P. C. Gupta, "A new flavonol glycoside from the flowers of Solanum xanthocarpum," Phytochemistry, vol. 17, no. 5, p. 613, 1936.

[226] M. Weissenberg, "Isolation of solasodine and other steroidal alkaloids and sapogennins by direct hydrolysisextraction of Solanum plants or glycosides," Phytochemistry, vol. 58, no. 4, pp. 501-508, 2001.

[227] I. Z. Saiyed and D. D. Kanga, "Chemical examination of the fruits of Solanum xanthocarpum," Proc. Indian Acad. Sci., vol. 4, no. 3, pp. 255-260, 1936.

[228] K. Jayakumar and K. Murugan, "Solanum alkaloids and their pharmaceutical roles: a review," Journal of Pharmacognosy and Research, vol. 3, no. 6, pp. 00075, 2016.

[229] S. Khanam and R. Sultana, "Isolation of β-sitosterol and stigmasterol as active immunomodulatory constituent from the fruits of Solanum xanthocarpum (Solanaceae)," International Journal of Pharmaceutical Sciences and Research, vol. 3, no. 4, pp. 1057-1040, 2012.

[230] O. M. Singh and T. P. Singh, "Phytochemistry of Solanum xanthocarpum an amazing traditional healer," Journal of Scientific & Industrial Research, vol. 69, no. 9, pp. 732-740, 2010.

[231] S. Ghildiyal and V. K. Joshi, "Pharmacognostical studies on *Solanum surattense* burn F. root," International Journal of Current Research, vol. 3, no. 4, pp. 240-245, 2014.

[232] T. Sahle and G. Okbatinsae, "Phytochemical investigation and antimicrobial activity of the fruit extract of Solanum incanum grown in Eritrca," Ornamental Med. Plants, vol. 1, no. 1, pp. 15-25, 2017.

[233] M. R. Heble, S. Narayanaswamy, and M. S. Chadha, "Hormonal control of steroid synthesis in Solanum xanthocarpum tissue cultures," Phytochemistry, vol. 10, no. 10, pp. 2393-2394, 1971.

[234] Q. Chen, J. Xu, Y. Li, and Z. Liu, "Solasodine improves glucose metabolism and insulin sensitivity in diabetic mice," Biomedicine & Pharmacotherapy, vol. 126, p. 110102, 2020.

[235] S. K. Verma, A. Singh, and V. R. Patel, "Exploration of Anti-inflammatory Potential of Different Bioactive Fractions from *Solanum surattense*," Current Research in Pharmaceutical Sciences, vol. 2, no. 2, pp. 100-105, 2021.

[236] S. Kumari, R. K. Singh, and A. K. Verma, "Antibacterial activity of *Solanum surattense* extracts against extended-spectrum β-lactamase producing Enterobacteriaceae," Journal of Applied Pharmaceutical Science, vol. 12, no. 3, pp. 238-242, 2022.

[237] E. Sheeba, "Antibacterial activity of *Solanum surattense* Burn F," Kathmandu University Journal of Science, Engineering and Technology, vol. 6, no. 1, pp. 1-4, 2010.

[238] D. M. Kar, L. Maharana, S. Pattnaik, and G. K. Dash, "Studies on hypoglycemic activity of Solanum xanthocarpum fruit extract in rats," J. Ethnopharmacol., vol. 108, no. 2, pp. 251-256, 2006.

[239] K. Abbas, U. Niaz, T. Hussain, M. A. Saeed, Z. Javaid, et al., "Antimicrobial activity of fruit of Solanum nigrum and Solanum xanthocarpum," Acta Pol. Pharma., vol. 71, no. 3, pp. 415-421, 2014.

[240] S. K. Tekuri, S. K. Pasupuleti, K. K. Konidala, S. R. Amuru, and N. B. Pabbajara, "Phytochemical and pharmacological activities of Solanum nigrum L," International Journal of Pharmaceutical Sciences and Research, vol. 6, no. 6, pp. 1845-1851, 2015.

[241] Mahmood, A., Mahmood, A., & Mahmood, M. (2012). In vitro biological activities of most common medicinal plants of family Solanaceae. World Applied Science Journal, 17(8), 1020-1032.

[242] David, E., Flumalai, E. K., Sivakumar, C., Therasa, S. V., & Thirumalai, T. (2010). Evaluation of antifungal activity and phytochemical screening of *Solanum surattense* seeds. Journal of Pharmacy Research, 3(4), 684-687.

[243] Burger, T., Mokoka, T., Fouché, G., Steenkamp, P., Steenkamp, V., & Cordier, W. (2018). Solamargine, a bioactive steroidal alkaloid isolated from Solanum aculeastrum induces non-selective cytotoxicity and P-glycoprotein inhibition. BMC Complementary and Alternative Medicine, 18(1), 137.

[244] Kumar, S., & Pandey, A. K. (2014). Medicinal attributes of Solanum xanthocarpum fruit consumed by several tribal communities as food: an in vitro antioxidant, anticancer and anti HIV perspective. BMC Complementary and Alternative Medicine, 14, 1-8.

[245] Tekuri, S. K., Pasupuleti, S. K., Konidala, K. K., Amuru, S. R., Bassaiahgari, P., & Pabbaraju, N. (2019). Phytochemical and pharmacological activities of *Solanum surattense* Burm. f.–A review. Journal of Applied Pharmaceutical Science, 9(3), 126-136.

[246] Ozsoy, N., Can, A., Yanardag, R., & Akev, N. (2008). Antioxidant activity of Smilax excelsa L. leaf extracts. Food Chemistry, 108(1), 571-583.

[247] Meena, A. K., Rao, M. M., Kandale, A., Sharma, K., Singh, U., & Yadav, A. (2010). Evaluation of physiochemical and standardization parameters of Solanum xanthocarpum Schrad and wendl. International Journal of Chemical and Analytical Science, 1(3), 47-49.

[248] Poongothai, K., Ponmurugan, P., Ahmed, K. S., Kumar, B. S., & Sheriff, S. A. (2014). Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown and in vitro raised) extracts on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine, 4(10), 778-785.

[249] Goodman, L. S., & Gilman, A. (1943). The pharmacological basis of therapeutics: A textbook of pharmacology, toxicology and therapeutics for physicians and medical students (9th ed.). Macmillan.

[250] Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy Therapeutics, 40(4), 277-283.

[251] Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules, 28(7), 1845.

[252] Schcolnik-Cabrera, A. Current Approaches to Overcome Antimicrobial Resistance. Curr. Med. Chem. 2023, 30, 3–4.

[253] C. Bitwell, S. S. Indra, L. Chimuka, and K. K. Maseka, "A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants," Sci. Afric., vol. 19, p. e01585, 2023.

[254] Sun, W.; Shahrajabian, M.H. Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules 2023, 28, 1845.

[255] Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26(5), 343-356. doi: https://doi.org/10.1016/j.ijantimicag.2005.09.002

[256] Tabassum, S., Anwar, M. A., Gulfaraz, M., Arshad, M., Uteuliyev, Y. S., Sadykov, B. N., ... & Ahmad, M. S. (2019). Bioactivity evaluation and HPLC UV-VIS based quantification of antioxidant secondary metabolites from extract and fractions of Bistorta amplexicaulis rhizome. Cellular and Molecular Biology, 65(1), 1-26. doi: http://dx.doi.org/10.14715/cmb/2019.65.1.4

[257] Pettit, R.K., Weber, C.A., Kean, M.J., Hoffmann, H., Pettit, G.R., Tan, R., Franks K.S., and Horton, M.L. (2005) Microplate alamar blue assay for Staphylococcus epidermidis Biofilm susceptibility testing. Antimicrob Agents Chemother 49(7), 2612–2617.

[258] Sarkar, S.D., Nahar, L. and Kumarasamy, Y. (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42(4), 321–324.

[259] M. Oyaizu, "Studies on products of browning reaction prepared from glucose amine," J. Jpn. Soc. Nutr. Diet., vol. 44, no. 1, pp. 307-315, 1986.

[260] Janaki S, Vijayasekaram V. (1998). Antifungal activities of aglaia roxburghiana (W&A), MIQ, Var, Beddome.i. Biomedicine, 18 (2): 86-9.

[261] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth., 65, 55-63.

[262] Bibi, Z., Bibi, Z., Khanum, K., Zhou, L., Zafar, M., & Abbasi, A. M. (2023). Impact of seasonal variations on polyphenolic content and antibacterial activity in *Berberis lycium* Royle. Journal of Holistic Integrative Pharmacy, 4(1), 29-40. doi: 10.1016/S2707-3688(23)00093-6

[263] Muruhan, S., Selvaraj, S., & Viswanathan, P. K. (2013). In vitro antioxidant activities of *Solanum surattense* leaf extract. Asian Pacific Journal of Tropical Biomedicine, 3(1), 28-34.

[264] M. Sangral, D. Gupta, P. Singh, R. Lochan, N. Bhat, Kiranbala, and M. Bhagat, "Title of the article," School of Biotechnology, Univ. of Jammu, Jammu, India, 2024.