WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL RESEARCH

Research Article

2025
Volume: 02
Issue: 07
Page: 82-135

INVESTIGATION OF ANTI-MICROBIAL ACTIVITY AND BIOACTIVE CONSTITUENTS IN ETHANOLIC EXTRACT OF *BERBERIS LYCIUM* ROYLE FRUITS

Lalit Kumar* and Dr. Nitin Jumnani

India.

Received: 06 May 2025 **Revised: 26 May 2025** **Accepted: 15 June 2025**

Corresponding Author: Lalit Kumar

Address: India.

Email Id: nitinjumnani6443@gmail.com,

INTRODUCTION

In the modern world, a significant portion of the global population continues to face challenges in accessing necessities that are critical for survival and quality of life. These include essential resources such as nutritious food, clean and safe drinking water, adequate education, reliable healthcare services, and a clean and sustainable environment. The lack of these fundamental needs is a pressing issue that affects millions, particularly in underdeveloped and developing regions. Governments at all levels are striving to address these challenges, which are further intensified by two interconnected factors: a rapidly growing global population and the ongoing deterioration of the environment. As populations expand, the demand for limited resources increases, placing immense pressure on the environment and compounding the difficulties faced by vulnerable communities. Amid these challenges, there has been a growing focus over the past two decades on exploring plant materials as sources of new antibacterial agents to address various health concerns. Medicinal plants, in particular, have emerged as a vital area of study due to their inherent healing properties and the wide range of phytochemical constituents they contain. These natural compounds have been shown to possess therapeutic properties that can be harnessed to treat and manage numerous human diseases. The World Health Organization (WHO) has recognized the importance of medicinal plants, emphasizing that they serve as one of the best sources for developing a diverse array of drugs and pharmaceutical compounds. Their accessibility and potential for sustainability make them invaluable in efforts to enhance global healthcare. To fully leverage the potential of medicinal

plants, it is imperative to conduct comprehensive research to understand their properties, safety, and efficacy. Rigorous investigation into their pharmacological and chemical composition can unlock their utility in combating infectious diseases and other health conditions. This knowledge can pave the way for the development of new, effective, and affordable treatments that are rooted in nature. Such advancements not only address immediate health challenges but also offer a sustainable and eco-friendly alternative to synthetic drugs, aligning with broader efforts to mitigate environmental degradation.[1]

In recent years, the prevalence of infections has surged significantly, posing a serious challenge to global health systems. Alongside this rise in infections, the phenomenon of antibiotic resistance has emerged as an increasingly critical therapeutic problem. The overuse and misuse of antibiotics in both healthcare and agricultural settings have accelerated the development of resistant strains of bacteria, rendering many conventional treatments ineffective. This alarming trend has not only complicated the management of common infections but also increased the risk of severe disease outbreaks and prolonged hospital stays, leading to higher healthcare costs and mortality rates. Addressing antibiotic resistance requires a multi-pronged approach that includes the development of new antimicrobial agents, stricter regulation of antibiotic usage, and widespread public health initiatives to promote awareness and preventive measures.[2] Natural products derived from higher plants have emerged as a promising source of new antimicrobial agents, offering a significant potential to combat infectious diseases with novel mechanisms of action. These plant-based compounds have demonstrated remarkable efficacy in treating a variety of infections, making them an attractive alternative to synthetic antimicrobials. One of their key advantages lies in their ability to mitigate many of the adverse side effects commonly associated with synthetic drugs, which often pose risks during long-term use or for individuals with sensitive health conditions. This makes plant-derived antimicrobials not only effective but also safer and more patient-friendly options for managing infectious diseases. To fully realize their potential, it is essential to conduct systematic and comprehensive screening of these plants. This involves exploring their traditional applications in folk medicine to identify those with therapeutic promise and isolating their active principles for in-depth characterization. Such investigations are crucial to understanding the specific bioactive compounds responsible for their medicinal properties. Phytochemicals such as alkaloids, flavonoids, tannins, and phenolic compounds are commonly found in medicinal plants and are renowned for their diverse biological activities. These compounds exhibit a range of therapeutic effects, including antimicrobial, anti-inflammatory, and antioxidant

properties, making them invaluable in the search for new treatments. The systematic study of these plant-derived compounds not only enhances our knowledge of their medicinal potential but also plays a critical role in addressing global health challenges, such as antibiotic resistance. By uncovering and characterizing these bioactive compounds, researchers can pave the way for the development of innovative drugs with unique mechanisms of action, offering effective solutions against resistant pathogens. This exploration of plant-based antimicrobials thus represents a vital step forward in pharmaceutical innovation and the advancement of sustainable approaches to managing infectious diseases. As the demand for safer and more effective treatments continues to grow, the role of medicinal plants in drug discovery becomes increasingly important, offering hope for a future with improved therapeutic options and healthier populations.[3]

Medicinal plants play a vital role in addressing multiple aspects of human and economic development, serving not only as a cornerstone for accessible medicine in healthcare systems but also as a source of livelihood for farmers and a significant contributor to national economies. To maximize these benefits, it is essential for countries to establish comprehensive systems for collecting, characterizing, and evaluating genetic resources from diverse biological sources, including plants, animals, insects, and microorganisms. These resources hold immense potential for pharmaceutical development; however, their utilization is often hindered by challenges related to the exchange of materials and technology. Such challenges arise due to the high commercial value associated with biological products and complex issues surrounding intellectual property rights. Despite these barriers, the critical and universal need for accessible medicines, which directly impacts the quality of life, highlights the importance of global collaboration. Cooperative efforts among nations and communities are indispensable to overcoming these constraints, ensuring equitable access to medicinal resources, and fully leveraging the value of biological diversity for the benefit of humanity.[4]

Medicinal plants, which grow naturally across the globe, have long been used for their healing properties, and they remain a crucial part of both traditional and modern medicine. These plants are easily accessible, culturally significant, and serve as the foundation for many healthcare systems, providing affordable and effective treatment options. Furthermore, they offer an essential livelihood for indigenous and rural populations who rely on them for income and sustenance. Over the past few decades, medicinal plant species found in natural environments have attracted growing scientific and commercial attention, with pharmaceutical industries

increasingly exploring these plants for new drug development. In the United States, for example, it is estimated that at least 118 out of 150 prescription drugs are derived from natural sources. A notable success story is the remarkable advancement in leukemia treatment: in 1960, a child diagnosed with leukemia had only a 10% chance of remission, but by 1997, that likelihood had risen to 95%, thanks to two drugs developed from a plant native to Madagascar. Despite these successes, the rising demand for medicinal plants has placed significant pressure on wild populations, leading to the overharvesting of many species. This overexploitation has caused the depletion and scarcity of certain medicinal plants in their natural habitats, underlining the critical need for sustainable harvesting practices and conservation measures to ensure the continued availability of these valuable resources.[5]

Medicinal plants have been utilized for centuries due to their natural therapeutic properties, with various bioactive compounds that can treat a wide range of diseases. Recently, as pathogens have developed resistance to conventional antibiotics, interest in these plants has surged as potential alternatives to synthetic medications. This shift has rekindled research into the biological activities of medicinal plants, particularly in terms of their ability to combat infections, inflammation, and chronic illnesses. Many plants have demonstrated promising effects in areas such as antimicrobial, antioxidant, anti-inflammatory, and even anticancer properties, positioning them as valuable candidates for the development of new, more sustainable treatments. In particular, plants used in traditional medicinal systems such as Ayurveda, Traditional Chinese Medicine (TCM), and Indigenous healing practices have garnered significant attention. Researchers are isolating and analyzing the active compounds from these plants, studying their mechanisms of action, and rigorously assessing their safety and efficacy. This renewed focus on medicinal plants not only provides opportunities for discovering new drugs but also helps preserve the knowledge embedded in traditional healing practices, offering more sustainable, plant-based healthcare solutions in response to the growing demand for alternatives to synthetic pharmaceuticals.[6] Medicinal plants are regarded as crucial natural resources for developing safe and effective drugs, playing an important role in promoting human health through their use as herbal medicines. In Pakistan, a wide variety of native plants have been traditionally utilized in herbal medicine to treat a range of infectious diseases and aid in the healing of various injuries. These plants are often celebrated for their extensive biological and pharmacological properties, which include the ability to alleviate inflammation, as well as exhibit potent antibacterial and antifungal activities. The diversity of medicinal flora in Pakistan highlights its potential as a valuable source of

natural remedies, offering effective and sustainable alternatives to conventional treatments. The therapeutic applications of these plants not only support the local healthcare practices but also present opportunities for further scientific exploration and development of plant-based medicines with a broad spectrum of health benefits.[7] In traditional medicine, various parts of medicinal plants, including the root, bark, leaves, flowers, and fruit, are extensively used to create natural remedies such as syrups and infusions. These plant extracts are carefully harvested and processed to capture their therapeutic properties, which have been recognized for centuries. The root, bark, leaves, flowers, and fruits of different plants are believed to contain bioactive compounds that can address a wide range of health issues. In the preparation of syrups and infusions, these extracts are often combined with other ingredients to enhance their effectiveness and provide a natural approach to healing. This practice reflects the longstanding tradition of utilizing the diverse parts of plants in creating potent, plant-based formulations that have been passed down through generations to promote health and well-being. The use of these natural preparations underscores the importance of plant-based treatments in maintaining holistic health.[8]

Medicinal and aromatic plants represent a significant and diverse group of plants that hold considerable economic value as they provide essential raw materials for a variety of industries, including medicine, food, pharmaceuticals, perfumes, flavors, and cosmetics. These plants are highly regarded for their wide-ranging applications, offering natural resources that contribute to the production of medicinal products, flavoring agents, and personal care items. Aromatic plants, in particular, are known for their distinctive and pleasing fragrances, which are primarily contained in their essential oils. These oils carry the characteristic scents of the plants and are utilized in the creation of perfumes, flavorings, and therapeutic remedies. In addition to their aromatic properties, many of these plants also serve as spices, enhancing the flavor and aroma of food. The unique combination of medicinal, aromatic, and culinary uses of these plants makes them invaluable not only in traditional medicine but also in modern industries, where they contribute to a wide array of products and applications.[9] Spices are defined as dried plant substances, known for their fragrant, aromatic, or pungent qualities, which can be used in their whole, broken, or ground forms. These plant-based ingredients play a vital role in enhancing the flavor and piquancy of foods and beverages. Whether adding depth to savory dishes or a distinctive sharpness to sweets, spices contribute significantly to the overall sensory experience of a meal. Their unique ability to transform the taste, aroma, and texture of food makes them an indispensable element in culinary traditions worldwide. Spices are valued not

only for their ability to improve the flavour of dishes but also for their potential health benefits and cultural significance.[10]

Medicinal plants are a vital resource for both healthcare and the economy, serving as a significant source of income for small industries and entrepreneurs. They contribute to foreign exchange earnings through the export of plant-based products, helping boost the economy. These plants are particularly valued for their rich content of secondary metabolites, which are organic compounds that play a key role in the development of therapeutic drugs. Secondary metabolites found in medicinal plants include alkaloids, glycosides, coumarins, flavonoids, and steroids, each possessing unique pharmacological properties. Alkaloids, such as morphine and quinine, are known for their pain-relieving and antimalarial effects, respectively. Glycosides, like digoxin from the foxglove plant, have vital cardiovascular effects, while coumarins are recognized for their anticoagulant properties. Flavonoids, with their antioxidant and anti-inflammatory effects, and steroids, which exhibit immune-modulating and anti-inflammatory activities, further enhance the therapeutic value of these plants. Herbal medicines are derived from various plant parts, including leaves, roots, flowers, stems, seeds, and fruits, each used for its medicinal properties. The process of preparing herbal drugs involves extracting or processing these plant components to harness their therapeutic potential. The growing use of medicinal plants offers an alternative to synthetic pharmaceuticals while also promoting biodiversity conservation and sustainable agricultural practices. With increasing recognition of their health and economic benefits, the global herbal medicine industry continues to expand, highlighting the importance of these plants in both healthcare and commerce.[11]

Approximately 30-40% of the medicines available in the market are derived from plants or other natural sources, highlighting the significant role of nature in modern pharmaceutical development. Many contemporary drugs have been inspired by or directly extracted from plant species, fungi, and microorganisms due to their potent medicinal properties. For example, Aspirin**,** a widely used pain reliever, was derived from salicylic acid found in the bark of the willow tree. Morphine**,** a powerful pain management drug, is extracted from the opium poppy, while Quinine, an essential treatment for malaria, originates from the bark of the cinchona tree. Furthermore, Taxol, a key chemotherapy drug, is derived from the Pacific yew tree. These examples demonstrate how nature's rich repository of bioactive compounds—such as alkaloids, flavonoids, terpenoids, and glycosides—serve as the foundation for numerous therapeutic applications. The continued exploration of plants and other natural resources

remains crucial for discovering new drugs, reinforcing the importance of biodiversity conservation to sustain the potential for future pharmaceutical innovations.[12] Medicinal plants have long been valued for their therapeutic potential, particularly within traditional medicine systems such as Ayurveda and Unani. India, known for its rich biodiversity, has historically relied on these plants for their healing properties, with many species demonstrating significant antibacterial, antifungal, and antiviral effects. With the rise of antibiotic resistance, medicinal plants are being increasingly explored as alternative or complementary treatments. Plants like Neem (*Azadirachta indica*)**,** with its powerful antimicrobial properties, Tulsi (*Ocimum sanctum*)**,** renowned for its immune-boosting and antimicrobial qualities, and Garlic (*Allium sativum*)**,** which is effective against a wide range of infections, are just a few examples of nature's remedies. Other plants such as Turmeric (*Curcuma longa*)**,** containing the active compound curcumin, are well known for their antibacterial and antifungal effects, while Ginger (*Zingiber officinale*) is utilized for its antimicrobial properties in treating infections. Cinnamon (*Cinnamomum verum*)**,** with its antibacterial and antifungal compounds, and *Echinacea* (*Echinacea purpurea*)**,** known for its immune-boosting and infection-fighting properties, also play crucial roles in managing infections. These plants offer a promising avenue for addressing the growing issue of antibiotic resistance, as they contain bioactive compounds that can help prevent and treat infections with fewer side effects compared to conventional antibiotics. The growing interest in medicinal plants provides a valuable alternative, not only in fighting infections but also in promoting overall health in a more sustainable and natural manner.

Over the past two decades, there has been a notable resurgence in interest regarding traditional medicine, particularly the use of plant-based remedies. While advancements in science and technology have led to the widespread use of synthetic drugs, the growing curiosity to uncover the scientific foundations behind traditional treatments has gained considerable momentum. This interest is not only focused on extracting useful compounds from plants but also on delving deeper into the underlying mechanisms that contribute to their therapeutic effects. A key area of focus is understanding the structure-function relationship of bioactive compounds present in plant extracts. By studying how the molecular structures of these compounds interact with biological systems, researchers aim to optimize their use, making them more effective while reducing potential side effects and toxicity commonly associated with synthetic pharmaceuticals. The scientific exploration of plant-based remedies holds the promise of developing safer, more sustainable alternatives to conventional drugs. By bridging traditional knowledge with modern scientific methodologies, we can unlock novel therapeutic agents that

harness the natural healing properties of plants, offering a more holistic approach to addressing contemporary health challenges.[13,14]

Numerous studies have explored the antimicrobial properties of various medicinal plants found in the plant kingdom, including *Berberis lycium***,** *Berberis vulgaris***,** *Curcuma longa*, and *Datura metel***.** These plants have been traditionally utilized for their healing properties, and modern scientific research has worked to validate their effectiveness against a wide range of microbial pathogens**.** *B. lycium***,** commonly known as Himalayan barberry, contains bioactive compounds like berberine, which is known for its antimicrobial, anti-inflammatory, and anti- parasitic effects.[15] Research has demonstrated that extracts from this plant exhibit significant antibacterial and antifungal activity, particularly against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida species*. Similarly, *B. vulgaris*, or European barberry, also contains berberine and other compounds that contribute to its antimicrobial properties. It has shown efficacy against various bacteria, fungi, and viruses, including *Helicobacter pylori*, *Salmonella*, and *Streptococcus pneumoniae*, and has demonstrated potential as an antiviral agent as well. *C. longa*, better known as turmeric, contains the active compound curcumin, which is widely celebrated for its potent antimicrobial, anti-inflammatory, and antioxidant properties. Numerous studies have found that curcumin has remarkable antimicrobial activity against a variety of pathogens, including bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as fungi like *Candida albicans*. In addition, curcumin has demonstrated antiviral properties against certain viruses, highlighting its broad- spectrum therapeutic potential. *D. metel*, or devil's trumpet, is traditionally used for its analgesic, anti-inflammatory, and antispasmodic properties. Some research has indicated its antimicrobial activity, showing promise against a range of bacterial and fungal pathogens. However, it is important to note that *D. metel* contains tropane alkaloids like atropine and scopolamine, which can be toxic in high doses. Therefore, while this plant may hold antimicrobial potential, its therapeutic use requires careful consideration of its toxicity and safety. However, these plants are of significant interest in the search for natural antimicrobial agents, especially in the context of rising antibiotic resistance. While their efficacy in combating microbial infections has been demonstrated, further research is necessary to fully understand their safety profiles and therapeutic applications.[16] Many scientists and researchers across various countries have conducted several experiments on the antimicrobial activity of plants.[16-18] Many plants demonstrate antibacterial and antifungal properties due to the compounds generated in their secondary metabolism, making them valuable for use. Key active ingredients in these medicinal plants, such as phenolic compounds, are essential components found in both essential oils and tannins.[19,20]

The genus *Berberis* holds a prominent position in traditional medicine systems across the globe, particularly for its therapeutic properties, and has been extensively documented in

Ayurvedic literature. In Ayurveda, the concentrated extract derived from the stems or roots of *Berberis* species, known as *Rasaut*, has long been revered for its healing abilities. The term "*Rasaut*" refers to the concentrated juice, which is believed to possess significant medicinal value. Among its notable uses, *Rasaut* has been particularly effective in treating eye diseases and indolent ulcers. Its application for eye conditions is due to its powerful anti-inflammatory and antimicrobial properties, helping to alleviate infections and reduce inflammation. Similarly, *Rasaut* is celebrated for its success in healing chronic, slow-healing ulcers, offering relief where conventional treatments may fail. In addition to its traditional uses, *Berberis* species contain the alkaloid berberine, a bioactive compound that has earned recognition in the British Pharmacopoeia for its medicinal benefits. Berberine is particularly effective in treating conditions like oriental sore, a type of skin infection, due to its antibacterial, anti-inflammatory, and immune-modulating properties. The therapeutic applications of both *Rasaut* and berberine highlight their enduring significance, not only in ancient healing practices but also in modern pharmacology, where they are acknowledged for their broad-spectrum medicinal effects.[21]

Berberis lycium, commonly known as Barberry, belongs to the *Berberidaceae* family and is an evergreen shrub found in various parts of the world, including Baluchistan. In this region, the leaves of the Barberry plant have been traditionally used for the treatment of jaundice, reflecting its long-standing use in folk medicine. Barberry is known for its rich content of alkaloids, particularly berberine, which has demonstrated significant antimicrobial properties, including antibacterial, antifungal, and anti-inflammatory effects. These therapeutic properties have attracted attention to Barberry as a potential natural remedy for a range of diseases. Despite its historical use and promising biological activities, there is still limited scientific research on the full medicinal value of Barberry. Its pharmaceutical importance remains underexplored, and further investigations are necessary to better understand its pharmacological mechanisms, therapeutic efficacy, and safety profile. Comprehensive studies are needed to evaluate its potential as a reliable treatment option in modern medicine, with the aim of developing standardized herbal formulations that could harness the plant's medicinal benefits for broader clinical applications.

Figure 1: Fruits of Berberis lyceum.

Common Names of *Berberis lycium*

B. lycium, an evergreen shrub belonging to the Berberidaceae family, is commonly known by various names across different languages and regions. In English, it is called Indian barberry, while in Hindi, it is referred to as kashmal or kasmal, and in Urdu, it is known as Ishkeen and Sumbal. In Pakistan, the plant is called Zyarh larghai, with its fruit often referred to as kashmal and its roots known as Daruhaldi. The plant was historically described by the Persian scholar Al-Biruni, who named it 'Ambaribis', and he also mentioned its Persian name, 'Zirkash'. The genus *Berberis* includes around 500 species, of which 77 are native to India. *B. lycium* has long been valued for its medicinal uses, with its fruit and roots playing a prominent role in traditional remedies throughout Asia.[22]

Morphology of *Berberis lycium*

B. lycium, commonly known as the Himalayan barberry, is an evergreen shrub that typically grows upright to a height of about 3 meters. This plant features a strong trunk, covered with a thin, brittle bark that branches out in various directions. It is both edible and medicinal, with a value rating of 2-3 for its properties. The leaves of *B. lycium* are narrow, thin, and typically measure between 2.5 and 7.5 cm in length and 0.7 to 1.8 cm in width. They are lanceolate or narrowly obovate-oblong in shape, with a coriaceous (leathery) texture, and are covered with spines along their entire length. The upper surface of the leaves is dull green, while the

underside is pale and glossy, with the secondary nerves being faintly visible. The plant retains its leaves throughout all seasons, making it evergreen. The twigs of *B. lycium* are pale yellowish in color, and its bark is grayish, rough, and deeply furrowed. The petiole, or leaf stalk, is either absent or can grow up to 2.5 mm in length. The plant's flowers are arranged in simple racemes, which range from 13 to 38 mm in length, and are bright yellow in color. The flowers are hermaphroditic, meaning each flower contains both male and female reproductive organs, and they are pollinated by insects, predominantly through self-pollination. The fruits of *B. lycium* are small, berry-like, and have an ovoid, long, or obovoid-subglobose shape. Each fruit is about 7 mm in length, 4 mm in diameter, and weighs approximately 227 mg. When ripe, the berries turn a bright red or purplish color. Every part of this plant, including the twigs, bark, leaves, and berries, is known for its medicinal value, making *B. lycium* an important plant in traditional healing practices.[22]

FLOWERING AND FRUITING SEASON

The blooming season of flowers and flowers changing into fruits of *Berberis lycium* is from the end of the month of February to the starting month of August. The first appearance of flowers is at the end of February and lasts up to April or May. The fruit starts coming from the end of April. The ripening of fruit starts from the second week of May and continues to do so throughout June, and they can be relished by July. The berries of *Berberis lycium* can be retained on the shrub for a longer period of time after ripening. When the rainy season starts, the berries fall off, thus resulting in the stoppage of the fruiting season.[23]

CULTIVATION AND PROPAGATION

The plant does not have a complex nutritional requirement but grows well in thin, dry, aerated, and shallow soil. It is a hardy plant. It suffers severe damage in the winter. It can be hybridized freely with the other members of the genus. They can be pruned severely, but they easily resprout from the base. The plant is propagated by means of seeds. The over raped fruit seeds will take longer time to germinate. The seedlings can be raised in a nursery and then transplanted when small plants are grown and are easy to handle. Damping off of the seeds can be prevented with proper ventilation. Fully ripened seeds are best to be sown in a cold frame; they germinate in late winter or early spring. Seeds from over-ripe fruit will take longer to germinate, while stored seeds may require cold stratification and should be sown in a cold frame as early in the year as possible. To prevent damping off, seedlings require proper ventilation. Cuttings of half-ripe wood are kept in July/August in a frame. Cuttings of mature

wood of the current season's growth are kept in October/November in a frame. *Berberis lycium* is found throughout the temperate and subtropical regions of the world (apart from Australia).

OCCURRENCE

Berberis lycium is widely distributed in various parts of the world and is also native to Nepal. It occurs in subtropical and temperate regions from Kashmir to Uttaranchal in the outer northern-western Himalayas. It shows its presence in the temperate and subtropical parts of Asia, Europe, and America. *Berberis lycium* is dispersed in the moderate and semitropical Asian, European, and American divisions. In Pakistan, it is extensively distributed in Balochistan, NWFP, Punjab, and northerly regions like Gilgit, Baltistan, Ghizer, Astore, Diamer, Swat, and Azad Kashmir at elevations of 900 to 2900 m. According to the International Union for Conservation of Nature (IUCN), the *Berberis lycium* species are vulnerable and are also stated as one of the endangered medicinal plant species in Pakistan. Due to a large climatic diversity in Pakistan, many medicinal plants are scattered abundantly over a large area, particularly in the Kurram and Kaghan valleys, in Gilgit, Chitral, Waziristan, Quetta, Azad Kashmir, and Himalayan and sub-Himalayan tracts.[24, 25]

LITERATURE REVIEW

Anti-mutagenic activity

Khan et al. (2010) examined the anti-neoplastic activity of *Berberis lycium* root extracts using p53-deficient HL-60 cells in combination with berberine and palmatine. The n-butanol extract exhibited the maximum toxicity against HL-60 cells, with an IC50 value of 2.3 µg extract/ml after 48 hours of treatment. This was followed by the ethanol extract (23.5 µg/ml) and the water extract (110 µg/ml). Berberine showed an IC50 of 1.2 µg/ml, while palmatine did not show any inhibitory effect on cell growth. Exposure of HL-60 cells to 5.5 µg/ml butanol extract and 0.6 µg/ml berberine for 48 hours resulted in a reduction of G1-phase cells and accumulation in the S-phase, indicating cell cycle disruption. The butanol extract induced the highest level of apoptosis, followed by the ethyl acetate and water extracts.[26]

Anti-arrhythmic activity

Lau et al. (2001) evaluated the effectiveness of berberine and its derivatives, 8-oxoberberine and tetrahydroberberine, through the blockade of K^+ channels (including delayed rectifier and K(ATP) channels) and the stimulation of the Na^+-Ca^{2+} exchanger. These actions have been shown to prolong the duration of the ventricular action potential, which is significant for maintaining proper cardiac function. Additionally, berberine demonstrates vasodilator activity,

which is attributed to multiple cellular mechanisms. The collective effects of berberine on the cardiovascular system suggest its potential clinical usefulness in the treatment of arrhythmias, as it can help regulate heart rhythm and improve cardiac performance.[27]

Anti-depressant activity

Kulkarni et al. (2008) demonstrated the neuropsychiatric study of berberine for effects on the central nervous system (CNS), demonstrating that berberine possesses antidepressant activity. The antidepressant effect was found to be related to the modulation of the L-arginine-NO-cGMP signaling pathway. This activity was confirmed through behavioral assessments, including the Forced-Swim Test (FST) and the Tail Suspension Test (TST). In these tests, a reduction in the immobility period was recorded over a 6-minute duration. Berberine (5-20 mg/kg, i.p.) led to a significant decrease in immobility time in both tests. Furthermore, co-administration of berberine (5 mg/kg, i.p.) with other typical antidepressants such as mianserin (32 mg/kg, i.p.) or trazodone (2 mg/kg, i.p.) enhanced the anti-immobility effect of sub-effective doses of these drugs in the FST without altering their effects. Additionally, berberine administration (5 mg/kg, i.p.) increased the levels of norepinephrine, serotonin, and dopamine in the mouse brain, further supporting its antidepressant potential.[28]

Anti-diabetic Activity

Ahmad et al. (2009) examined the antidiabetic properties of *Berberis lycium* using an alloxan-induced diabetic rabbit model. The simple powder of *Berberis lycium* reduced blood glucose levels in both diabetic and normal rabbits. Various extracts were prepared, including water, methanolic, aqueous methanolic, n-hexane, and chloroform, and their antidiabetic effects were tested. Among these, the water extract (500 mg/kg) demonstrated the most significant hypoglycemic activity, lasting for about six hours when administered orally. Methanolic, aqueous methanolic, and n-hexane extracts also reduced blood glucose levels, but the effects persisted for four hours. However, the chloroform extract showed no significant changes.[29]

In another study, Gulfraz et al. (2007) explored the antidiabetic effects of *Berberis lycium* in alloxan-induced diabetic rats. Oral administration of *Berberis lycium* root extracts (50 and 100 mg/kg) led to a decrease in blood glucose levels after three to five hours, with a more pronounced effect observed at higher doses. The oral glucose tolerance test revealed that these plant extracts decreased serum glucose levels in a dose-dependent manner, indicating an insulin-like effect likely due to enhanced peripheral glucose utilization.[30]

Anti-diarrheal activity

Sack and Froehlich (1982) investigated berberine, a bioactive compound found in *Berberis lycium*, which has demonstrated a range of therapeutic effects, particularly in gastrointestinal health. Studies have shown that berberine can reduce smooth muscle contraction, decrease intestinal motility, and delay intestinal transit time in humans, suggesting its potential use in managing conditions like diarrhea and irritable bowel syndrome (IBS). Berberine also has significant antimicrobial properties, directly inhibiting enterotoxins produced by *Escherichia coli* and *Vibrio cholerae*, which are known to cause severe gastrointestinal infections and dehydration. This effect is crucial in managing infectious diarrhea caused by these pathogens.[31]

Anti-hyperlipidemic activity

Ahmad et al. (2009) demonstrated the anti-hyperlipidemic effect of Berberis lycium Royle was studied in male albino rabbits using its roots. The results indicated that oral administration of 250 and 500 mg/kg of crude powder significantly reduced the levels of low-density lipoproteins (LDLs), total cholesterol, and triglycerides, while increasing high-density lipoproteins (HDLs). Furthermore, these doses also helped stabilize the weight of diabetic rabbits. The observed increase in HDL and decrease in LDL levels, following treatment with the plant root, suggests that this effect could help prevent heart problems in diabetic patients. Frequent administration of plant root bark powder showed promising results in managing hyperlipidemia associated with high blood glucose levels.[29]

Additionally, berberine, an active compound in *Berberis lycium*, has been extensively studied for its role in lipid metabolism. Lee et al. (2007) found that berberine could reduce lipid concentrations by enhancing the transcriptional activity of the LDL receptor (LDLR) promoter *via* the JNK pathway and stabilizing hepatic LDL-C receptors through an ERK-dependent pathway.[32]

Antimicrobial activity

Singh et al. (2007) described that *B. lycium* is highly effective against many microorganisms, particularly bacteria and fungi. Medicinal plants, such as *Berberis lycium*, have the potential to eliminate bacterial and fungal pathogens. It has been used to target various bacteria, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The hydroalcoholic extract of *B.*

lycium has been reported to exhibit a stronger and broader spectrum of activity against bacterial strains compared to fungal strains.[33]

Furthermore, singh et al. (2009) attained the effectiveness of plant extract for antimicrobial activity against *Enterobacter aerogenus, Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa, Micrococcus luteum, Escherichia coli, Bacillus cereus, Proteus mirabilis, Staphylococcus aureus, Streptococcus pneumonia,* and *Salmonella typhimurium*. The root extract showed effectiveness with MIC values of 2.50, 1.25, 0.62, 0.62, 1.25, 0.31, 2.50, 1.25, 0.62, 0.62, and 2.5 µg/ml, respectively, against each microorganism. In addition, the stem extract exhibit MICs were 1.25, 0.31,0.31, 0.31, 0.31, 0.62, 2.50, 0.31, 0.62, 1.25 and 0.62 µg/ml, respectively. The hydroalcoholic extract of *B. lycium* has been proven to be effective against bacterial strains as compared to fungal strains.[33]

Anti-protozoal Activity

The crude extracts of berberine, as shown by Kaneda, Tanaka, and Saw (1990), have proven to be more effective than its salts.

In a clinical trial by Choudhary, Sabir, and Bhide (1972), berberine administration improved gastrointestinal symptoms and resulted in a marked reduction in Giardia-positive stool. It was also effective at half the dose of the commonly used Giardiasis medication, metronidazole.[32]

Antioxidant activity

Singh et al. (2009) suggested that Reactive oxygen species (ROS), such as hydroxyl radicals (-OH), hydrogen peroxide, and superoxide anions, are known to contribute to diseases such as rheumatoid arthritis, inflammation, cancer, aging, and atherosclerosis.[33]

Gupta et al. (2009) described that the root extract of *B. lycium* has been demonstrated to possess significant antioxidant properties and a strong reduction potential. The root extract reduces potassium ferricyanide (Fe3+) to potassium ferrocyanide (Fe2+), which subsequently reacts with ferric chloride to form a ferric-ferrous complex that absorbs at a maximum wavelength of 700 nm. This method is inexpensive, simple, and effective for evaluating antioxidant activity.[34]

Hepatoprotective activity

Khan et al. (2008) tested the hepatoprotective effects of *Berberis lycium* in combination with *Galium aparine* and *Pistacia integerrima* on carbon tetrachloride-induced hepatotoxicity in

rats. The results showed that this combination exhibited potent anti-hepatotoxic effects, suggesting a high therapeutic efficacy as opposed to a mere protective effect. Additionally, Ahmad et al. (2008) assessed the hepatoprotective potential of *Berberis lycium* using methanolic extracts to treat paracetamol-induced hepatotoxicity in rabbits. The study demonstrated significant reductions in elevated levels of serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and alkaline phosphatase enzymes in hepatotoxic rabbits.[35]

Pesticidal Activity

Tewary et al. (2005) examined the pesticidal activity of *B.lycium* root, which was evaluated using petroleum ether and aqueous methanol extracts prepared through a Soxhlet apparatus. The extracts were tested at two higher doses (5000 and 10000 ppm) against pests. The petroleum ether extract exhibited a 25% mortality rate against *Helicoverpa armigera* and a 92% mortality rate against Aphis craccivora at the 5000-ppm dose.[36]

Wound Healing Activity

Asif et al. (2007) reported that the wound healing potential of *B. lycium* root extract was investigated in Swiss Wistar rats. Both methanolic and aqueous extracts of the roots were tested in various wound healing models, including excision, incision, and deceased wound space forms. The study demonstrated that both extracts promoted epithelialization and enhanced the breaking strength of the wounds. However, the methanolic extract proved to be more effective than the aqueous extract in promoting wound healing.[37]

AIMS & OBJECTIVES

AIM:

To evaluate the antimicrobial activity and phytochemical composition of the ethanolic extract of *Berberis lycium* fruits.

OBJECTIVES:

- To assess the antimicrobial potential of the ethanolic extract of *Berberis lycium* fruits against selected microorganisms.
- To identify and analyze the bioactive phytochemicals present in the ethanolic extract.

MATERIALS AND METHODS

MATERIALS

Plant Samples (*Berberis lycium*)

- Fruits

Chemical/Reagents

S. No.	Chemicals
1	Ethanol
2	Methanol
3	Propanol
4	CCl4
5	DMF
6	n-hexane
7	Distilled water
8	Standard Vitamin C
9	EDTA
10	BSA
11	Acetone
12	Phosphate buffer
13	Alkaline Na2CO3
14	Copper sulfate reagent
15	Alkaline copper sulphate reagent
16	Folin's reagent
17	TCA
18	NaOH
19	NBT
20	Trition X
21	Hydroxylamine HCl
22	2,6 Dichlorophenol indophenol
23	Standard Vitamin C

Microbiological media

S. No.	Media
1	Nutrient agar (NA)
2	Nutrient broth (NB)
3	Mueller Hinton Agar (MHA)
4	Potato Dextrose agar (PDA)

EXPERIMENTAL TEST ORGANISMS

The bacterial and fungal strains used in this study are detailed in Table 1 below: -

S. No.	Bacterial strains
1	*Escherichia coli*
2	*Staphylococcus aureus*
3	*Salmonella typhii*
4	*Proteus mirabilis*

5	*Klebsiella pneumonia*
6	*Enterococcus*
7	*Acinetobacter baumannii*
8	*Pseudomonas* spp.
Fungal strains	
1	*Aspergillus niger*
2	*Aspergillus fumigatus*
3	*Aspergillus cuboida*
4	*Candida albicans*

4.3 EQUIPMENT

- Glasswares
- Incubator
- Water bath
- Laminar air flow
- GC-MS
- Soxhlet assembly

METHODOLOGY

Collection of Plant material (Sampling site)

This work was carried out in Department of chemistry, Uttaranchal University, Dehradun. Fruits, flowers and roots of *Berberis lycium* were collected from allied hills of Mussoorie and Nainital during the month of March to July 2016.The botanical identity of plant was determined from the Botany Laboratory of Forest Research Institute (FRI) Dehradun. The plant was preserved in the advanced chemistry laboratory of Uttaranchal University. The flowers, fruits and roots of *Berberis lycium* was thoroughly washed with water and dried under shade for about 10-15 days. The dried plant samples were crushed in mortar pesil. The crushed samples were stored in an air sealed polyethylene bag at room temperature before extraction.

Sample Size

For this study, a sample size of approximately 25 grams was selected for each plant part under investigation. This quantity was deemed sufficient to conduct various analytical procedures while ensuring consistency and reliability in the results. The plant samples were carefully collected, weighed, and processed according to standardized methodologies to maintain uniformity across all experimental conditions. By selecting 25 grams as the sample size, the study aimed to obtain accurate and reproducible data while minimizing potential variations that could arise from discrepancies in sample weight. This approach also facilitated efficient

extraction, analysis, and evaluation of the plant's phytochemical, pharmacological, or biological properties, depending on the study's objectives.

Sample variables

The present study focused on evaluating the bioactive potential of fruit extracts by assessing their antimicrobial and antioxidant activities, as well as identifying their chemical composition using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The selected fruit extracts served as the primary sample variables and were systematically tested to determine their efficacy against microbial strains, measuring their ability to inhibit bacterial or fungal growth. Additionally, their antioxidant potential was analyzed to assess their free radical scavenging properties, which are crucial indicators of their potential health benefits and preservative qualities. Furthermore, the chemical profiling of these extracts was conducted through GC-MS analysis to identify the presence of bioactive compounds responsible for the observed biological activities. This comprehensive approach aimed to explore the therapeutic and functional properties of fruit-derived phytochemicals, contributing to the growing body of research on natural products with potential applications in medicine, food preservation, and pharmaceutical industries.

Storage

After the extraction process was completed using the Soxhlet assembly, the obtained extracts were carefully stored in a refrigerator at a temperature of approximately 4°C to maintain their stability and prevent any potential degradation. Proper storage conditions are essential to preserve the bioactive compounds present in the extract, ensuring their efficacy for subsequent analysis. When required for antimicrobial activity testing and compound detection, the stored extracts should be taken out of the refrigerator and allowed to reach room temperature before use. This step is crucial as sudden temperature changes may affect the chemical composition of the extract, potentially altering its biological activity and analytical results. By following these procedures, the integrity and reliability of the extracts are maintained, ensuring accurate and reproducible outcomes in further experimental analyses.

PROCESSING OF SAMPLES

Soxhlet Assembly

A Soxhlet extractor is a laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only

required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. Normally, a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction, the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded.

Preparation of plant extract

The extracts of medicinal plants were prepared by dissolving samples in 1:10 with different solvents separately (Distilled water, ethanol, methanol, propanol, DMF, and n-hexane, respectively), and a constant heating was provided by heating mantel for 6 hours in a soxhlet apparatus. Then extracts in round bottom flask was transferred to a beaker and it was kept on an ZEXTER evaporator for drying. After 4-5 hours, when all the solvent is evaporated, the extract separates. The extract is transferred to a vial and is refrigerated for further use.

Procedure:

The plant material was first washed with clean water and then dried for at least 10-15 days under shade drying. When the plant parts were dried, the roots were chopped into fine chips, and fruits and flowers were crushed to make powder. All the samples were extracted with different solvents (water, ethanol, methanol, n- hexane, n- propanol, and DMF) with the help

of Soxhlet assembly, and the extract was dried with the help of a rotary evaporator. The extracts were weighed in the weighing machine to take yield, then antimicrobial activities, i.e, antibacterial and antifungal activities, were performed by the Disc-diffusion method with different bacterial and fungal strains, which were available in the laboratory. Phytochemical analysis of all the extracts was performed to determine the phytochemical constituents present in plants like alkaloids, flavonoids, terpenoids, tannins, saponin, etc.

Bacterial cultures

The human pathogenic bacterial strains, such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus*, *Acinetobacter baumannii*, *Salmonella typhi*, and *Proteus mirabilis*, were obtained from the Bioinformatics Centre, Institute of Microbial Technology (IMTECH), Chandigarh, and were maintained in Nutrient agar slant at 4˚C for experimental studies. The fungal strain *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus cuboida* were used.

Preparation of standard culture inoculum of test organism:

The colonies of different bacterial and fungal strains were inoculated in the 20 ml nutrient broth and incubated for 24 hours and 72 hours, respectively.

Preparation of Sterile disk:

Whatman's filter paper No. 3 was punched into 6 mm disk form and then sterilised; each sterile disc was incorporated individually with 20–60 litres of extracts using a micropipette. Precautions were taken to prevent the flow of the solvent extract from the disks to the outer surface. The condensed extracts were applied in small quantities on discs, and they were allowed to dry in air. After some time, other doses of extracts were applied on disks.

Kirby-Bauer Method

The disc-diffusion method uses antibiotic-soaked wafers to test whether bacteria is affected by antibiotics or not. Wafers with antibiotics are placed on the agar plate; bacteria are already on the plate, and they are kept for incubation. The bacteria are swabbed uniformly across the culture plate. If the antibiotic stops, the bacteria from growing or kills the bacteria. The area around the wafer where bacteria have not grown enough to be visible is known as the zone of inhibition. The size of the zone depends upon the amount of antibiotic present on the plate. Stronger antibiotic creates a larger zone of inhibition because a lower concentration of antibiotics is enough to stop bacterial growth. A filter paper disk containing the compound to

be tested is placed on the agar plate. The compound diffuses from the filter paper to the agar. The concentration of the compound increases near the disk and keeps on decreasing as the distance from the disk increases. If the compound is effective against bacteria at a certain concentration no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This zone is known as the zone of inhibition. This helps to estimate the bacterial sensitivity to that antibiotic. The minimum inhibitory concentration of antibiotics for those bacteria relates to larger zones. Inhibition produced during the test is compared with that produced by the known concentration of a reference compound. This technique can be used to find a particular antibiotic for a specific infection.

KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic. KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant. Clinicians can use KB test results to choose appropriate antibiotics to combat a particular infection in a patient. Administering antibiotics that specifically target the particular bacteria that are causing the infection can reduce the use of broad-spectrum antibiotics, which target many types of bacteria. Thus, clinical application of KB testing results can decrease the frequency with which antibiotic-resistant bacteria evolve.

Minimum Inhibitory Concentration

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation. MICs can be determined on plates of solid growth medium (called agar, shown in the "Kirby-Bauer Disk Susceptibility Test" atom) or broth dilution methods (in liquid growth media) after a pure culture is isolated. For example, to identify the MIC via broth dilution, identical doses of bacteria are cultured in wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth. There are also several commercial methods available to experimentally measure MIC values.

Significance and Application

An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit the growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents. Currently, there are a few web-based, freely accessible MIC databases. MIC scores are important in diagnostic laboratories to confirm the resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinicians use MIC scores to choose which antibiotics to administer to patients with specific infections and to identify an effective dose of antibiotics. This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing the evolution of drug-resistant microbial strains.

Anti-microbial activity

The fruit extract of *B. lycium* prepared was again dissolved in similar ethanol, propanol, and water (100 mg/ml) and sterilized. The antimicrobial activity test was carried out by disc diffusion method by using 50 µl of suspension spread on potato dextrose agar (PDA), Mueller Hinton agar (MHA) media respectively. The discs (6 mm) containing 10 µl of extracts (300 µg/disc) with the concentration of 100 mg/ml were impregnated in the inoculated agar. Negative control was prepared by using similar solvents of plant extracts. Ciprofloxacin (100 µg/disc) was used as a positive control to determine the sensitivity of each strain/isolate for each microbial species tested. The inoculated plates were incubated at 37°C for 24 hours in the case of clinical bacterial strains and 72 hours for fungi isolated. Antimicrobial activity was assessed by measuring inhibition zones about test organisms, and each process was repeated to get accurate results.[38]

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

The GC-MS was performed at IIT Roorkee, and the model was PerkinElmer. Gas Chromatography (GC) is a technique in which a sample is volatilized and carried by an inert gas through a coated glass capillary column. The “stationary phase” is bonded to the interior of the column. The time it takes a specific compound to pass through the column to a detector is called its “retention time”, which can be used for identification when compared to a reference.

In the Mass Spectrometry (MS) step of GC-MS, compounds leaving the GC column are fragmented by electron impact. The charged fragments are detected, and the subsequent spectrum obtained can be used to identify the molecule. Fragmentation patterns are reproducible and can be used to produce quantitative measurements.

Gas Chromatography–Mass Spectrometry (GC-MS) is a technique in which a hyphenated analytical method, which combines the two powerful techniques, provides the identification of compounds with low detection limits and the potential for quantitative analysis. GC is used to separate the volatile and thermally stable substitutes in a sample, whereas GC-MS fragments the analyte to be identified because of its mass. The further addition of a mass spectrometer in it leads to GC-MS/MS. Superior performance is achieved by single and triple quadrupole modes. GC-MS analyses can work on liquid, gaseous, and solid samples but is primarily restricted to volatile and semi volatile compounds.[39, 40]

INSTRUMENTATION

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their massto-charge ratio.

These two components are used together to allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a

mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample.

RETENTION TIME

The retention time (RT) refers to the duration between the moment a sample is injected into the chromatographic system (time zero) and the point at which a specific analyte elutes, or emerges, from the chromatographic column and is detected. This time interval is a crucial parameter in chromatographic analysis, as it helps in identifying and characterizing different compounds within a mixture based on their interactions with the stationary phase and mobile phase. Retention time is influenced by several factors, including the chemical properties of the analyte, the composition of the mobile phase, the type of stationary phase used, the flow rate of the mobile phase, and the column temperature.

PRINCIPLES OF GC-MS

The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium), as the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A ÒlibraryÓ of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

ADVANTAGES OF GC-MS

GC-MS has long been considered to be the Gold Standard for all sorts of analytical laboratories. GC requires the analyte to have significant vapor pressure between 30 and 300°C. GC presents insufficient proof of the nature of the detected compounds. The identification is based on retention time matching that may be inaccurate or misleading. GC-MS represents the mass of a given particle (Da) to the number (z) of electrostatic charges (e) that the particle carries. The term m/z is measured in DA/e. GCMS commonly uses electron impact (EI) and chemical ionization (CI) techniques. It is a strong analytical tool that uses time for the chemical elements

to travel through the GC column, the retention time as compared to known standards to identify the chemical. The main features of enhanced molecular ion, improved confidence in sample identification, significantly increased range of thermally labile and low volatility samples amenable for analysis, much faster analysis, improved sensitivity particularly for compounds that are hard to analyze and the many other features and options provide compelling reasons to use the GC-MS in broad range of areas.[41, 42]

LIMITATIONS OF GC-MS

Some of the limitations of GC-MS are as follows: -

- **Time-Consuming Process:** GC-MS analysis often requires a significant amount of time due to the multiple steps involved, including sample preparation, separation, and detection. Some samples, especially complex matrices, may require extended run times to achieve accurate results. Additionally, the process of data interpretation and compound identification further adds to the time required for analysis.
- **Challenges with Non-Volatile Matrices:** GC-MS is designed to analyze volatile and semi-volatile compounds. However, certain non-volatile matrices, such as **metal parts, wafers, and oil-based samples**, pose a challenge. These samples require additional preparation steps, such as extraction or outgassing, to isolate volatile components before they can be analyzed. This extra preparation increases the complexity and time of the process.
- **Difficulties in Analyzing Plastic Extracts:** When analyzing extracts from plastic materials, the process becomes more challenging due to the potential interference of polymer degradation products and additives. These compounds can produce overlapping peaks, making it difficult to identify the target analyte accurately. Additionally, plasticizers and stabilizers in polymers may introduce unwanted signals that can complicate data interpretation.
- **Requirement for Volatile or Derivatizable Compounds:** GC-MS can only analyze compounds that are either naturally volatile or can be chemically modified (derivatized) to become volatile. Many large or polar molecules, such as sugars and amino acids, need derivatization before they can be analyzed, which adds extra steps and complexity to the process.
- **Challenges with Atmospheric Gases:** Atmospheric gases, such as nitrogen, oxygen, and carbon dioxide, can interfere with GC-MS analysis. These gases may enter the system during sample introduction, leading to signal suppression or contamination. Additionally, the presence of water vapor can impact peak resolution and cause column degradation over time.

- **High Cost of Equipment and Maintenance:** GC-MS instruments are expensive to purchase and maintain. The cost of consumables, such as high-purity carrier gases, specialized columns, and ionization sources, adds to the overall expense. Regular maintenance is necessary to keep the system functioning optimally, which can increase operational costs.
- **Limited to Thermally Stable Compounds:** Since GC-MS operates at elevated temperatures, compounds that are heat-sensitive or prone to thermal decomposition cannot be effectively analyzed. If a compound breaks down before it reaches the detector, the resulting data may be inaccurate or incomplete. This limits the application of GC-MS for certain types of organic and biological samples.
- **Need for Derivatization in Some Cases:** Certain compounds, particularly those with high polarity or poor volatility, require **derivatization** before they can be analyzed by GC-MS. This process involves chemically modifying the analyte to make it more volatile, which adds additional preparation steps and increases the risk of errors.
- **Complex Data Interpretation:** GC-MS produces large amounts of spectral data that require specialized knowledge to interpret accurately. In complex mixtures, overlapping peaks and co-eluting compounds can make identification difficult. Skilled analysts must differentiate between target compounds and background noise, which can be time-consuming.
- **Matrix Interference from Complex Samples:** When analyzing biological fluids, environmental samples, or food products, matrix interference can be a major challenge. These complex sample matrices contain multiple compounds that may interfere with the target analyte's signal, making it harder to detect and quantify specific substances. Sample cleanup techniques, such as solid-phase extraction, are often required to minimize interference.
- **Limited Sensitivity for Certain Compounds:** While GC-MS is highly sensitive for many substances, some compounds do not ionize efficiently under standard electron ionization (EI) or chemical ionization (CI) methods. Poor ionization results in weak signals, making it difficult to detect low-concentration analytes in a sample. In such cases, alternative ionization techniques or complementary analytical methods (e.g., LC-MS) may be needed.

APPLICATIONS OF GC-MS

- **Environmental monitoring and Clean up-**

GC-MS has emerged as a powerful tool in environmental monitoring and cleanup due to its high sensitivity, accuracy, and ability to detect and quantify organic pollutants across various

environmental matrices. The decreasing cost and increasing reliability of GC-MS equipment have significantly contributed to its widespread adoption in environmental studies. This technique is particularly effective for analyzing a broad range of contaminants in water, soil, and air, making it invaluable for pollution assessment and remediation. In water and soil analysis, GC-MS is widely used for detecting chlorophenols, which are common industrial pollutants, as well as polycyclic aromatic hydrocarbons (PAHs) originating from petroleum products and combustion processes. Additionally, it plays a crucial role in monitoring persistent organic pollutants (POPs) such as organochlorine pesticides, herbicides, phenols, and halogenated pesticides, which pose significant risks to ecosystems and human health. In air quality monitoring, GC-MS is instrumental in detecting sulfur compounds emitted from industrial activities and toxic pollutants like dioxins and dibenzofurans produced during combustion. The technique also finds applications in biomass and agricultural research, where it is employed to screen degradation products of lignin in biofuel studies and detect pesticide residues in food products like spinach. Furthermore, GC-MS is extensively utilized in wastewater analysis to monitor pharmaceutical pollutants, including the degradation of carbamazepine and its metabolites in treated sewage water, as well as the presence of steroids that can act as endocrine disruptors in aquatic ecosystems. Advanced pollutant tracking using GC-MS includes the analysis of complex polycyclic compounds such as decacyclene, ovalene, and C60, which are highly stable and require sophisticated techniques for their detection and degradation assessment. The advantages of GC-MS in environmental studies stem from its high sensitivity and selectivity, enabling the detection of contaminants at trace levels with minimal sample preparation. Additionally, the ability to analyze certain compounds without derivatization simplifies the workflow and reduces analysis time and costs. Given its precision and versatility, GC-MS continues to play a pivotal role in ensuring environmental safety, regulatory compliance, and the development of effective pollution remediation strategies.[43]

- **Criminal Forensics/Law Enforcement/Sports Anti-doping Analysis**

In forensic investigations, GC-MS plays a critical role in analyzing trace evidence such as bodily fluids, hair, and fibers, helping to link a suspect to a crime scene with scientific accuracy. It is particularly valuable in fire debris analysis, where it detects accelerants like gasoline and kerosene, adhering to established American Society for Testing and Materials (ASTM) standards to ensure consistency and reliability in arson investigations. Furthermore, GC-MS is instrumental in forensic toxicology, where it is used to identify drugs, poisons, and toxic substances in biological specimens, aiding in criminal cases, autopsies, and law enforcement

efforts to combat drug-related crimes. Additionally, GC-MS is employed in the detection of explosive residues and hazardous chemicals, playing a vital role in counterterrorism and criminal investigations involving chemical threats. In the field of sports anti-doping analysis, GC-MS is the gold standard for testing athletes' urine and blood samples for banned performance-enhancing substances, such as anabolic steroids, stimulants, and diuretics, ensuring compliance with regulations set by organizations like the World Anti-Doping Agency (WADA). Advanced techniques like GC-MS/MS (tandem mass spectrometry) further enhance the detection capabilities by accurately identifying prohibited substances even in highly complex biological matrices, making it a crucial technology for ensuring fairness in competitive sports. Given its ability to provide highly accurate, legally admissible results, GC-MS remains an indispensable tool in forensic science and sports integrity, ensuring justice, safety, and ethical standards in various domains.[44]

Astrochemistry and geochemical research

GC-MS) has been instrumental in astrochemistry and geochemical research, providing critical insights into the composition of extraterrestrial environments and organic molecules in space. Several GC-MS instruments have been deployed beyond Earth, contributing significantly to planetary exploration. The Viking 1 and Viking 2 landers, which arrived on Mars in 1976, carried GC-MS instruments to detect organic molecules in Martian soil. However, the presence of perchlorates complicated the analysis, leading to inconclusive results. In the exploration of Venus, the Soviet Venera 11 and 12 missions in 1978 and NASA’s Pioneer Venus mission utilized GC-MS to analyze the planet's dense atmosphere, revealing high concentrations of sulfuric acid and carbon dioxide. The Cassini-Huygens mission, which sent the Huygens probe to Saturn's moon Titan in 2005, used GC-MS to investigate the moon’s atmospheric and surface composition, detecting complex hydrocarbons and organic molecules, which provided valuable information about Titan's potential for prebiotic chemistry. In 2014, the European Space Agency’s Rosetta mission deployed a chiral GC-MS to study the material on comet 67P/Churyumov-Gerasimenko, leading to the detection of complex organic compounds, including amino acid precursors, which have implications for understanding the origins of life. GC-MS has proven to be highly valuable in organic geochemical applications due to its ability to identify major isomers and structural variations in organic compounds, detect low-volatility hydrocarbons that are often difficult to analyze with other techniques, and provide unique isotope ratio information that is crucial for studying planetary formation and evolution. These capabilities make GC-MS an essential tool in the search for extraterrestrial organic compounds

and the study of planetary geochemistry, furthering our understanding of the chemical processes shaping celestial bodies.[45]

Food, beverage, and perfume analysis

GC-MS plays a crucial role in the qualitative and quantitative analysis of aromatic compounds in food, beverages, and perfumes, ensuring product quality, authenticity, and safety. In the food and beverage industry, GC-MS is widely used to identify and analyze volatile and semi-volatile compounds, including esters, fatty acids, aldehydes, alcohols, and terpenes, which contribute to the sensory characteristics of various products. It is also essential for quality control and detecting adulteration in items such as oil, butter, and ghee, helping to prevent food fraud and ensure compliance with regulatory standards. Additionally, GC-MS is used to detect pesticide residues in food and beverages, such as wine, fruits, vegetables, and edible oils, ensuring they meet safety guidelines. The technology is particularly useful in analyzing essential oils and natural extracts, including piperine, menthol, spearmint oil, lavender oil, peppermint oil, lemon oil, and yiang oil, which are widely used in both food and fragrance industries. In the perfume and fragrance industry, GC-MS is instrumental in determining the composition of essential oils, identifying fragrance reference standards, and analyzing chiral compounds in natural and synthetic fragrances. It also plays a significant role in detecting allergens in perfumes and cosmetic products, ensuring they are safe for consumers. Furthermore, GC-MS is used to authenticate natural versus synthetic aroma compounds, helping manufacturers maintain the integrity of their products. Overall, GC-MS serves as an invaluable analytical tool in these industries, allowing for precise identification and quantification of compounds, ensuring compliance with safety regulations, and maintaining consumer trust in food, beverage, and fragrance products.

Biological and Pesticide Detection

GC-MS is an advanced analytical technique widely used for detecting and quantifying volatile and semi-volatile compounds in biological, food, and environmental samples. In forensic and clinical toxicology, GC-MS plays a crucial role in analyzing blood, urine, and serum for the presence of barbiturates, narcotics, anesthetics, anticonvulsants, antihistamines, sedative-hypnotics, and anti-epileptic drugs. It is particularly valuable in forensic investigations for detecting alcohol levels in blood for DUI cases, post-mortem toxicology, and drug overdose assessments. Additionally, it helps in identifying residual solvents and metabolites in serum, providing insights into drug metabolism, poisoning, and potential drug interactions. In the food

industry, GC-MS ensures safety and authenticity by detecting adulterants, contaminants, and prohibited substances. It is extensively used for fatty acid profiling in microbial cultures, identifying bacteria and fungi in fermented foods, and detecting free steroids to ensure compliance with regulations against illegal hormonal growth enhancers. Furthermore, GC-MS is employed to detect food adulteration and contaminants in packaged beverages like soft drinks, revealing harmful pesticides, toxins, and synthetic chemicals. In environmental science, this technique is instrumental in monitoring pollutants, particularly organochlorine pesticides and hazardous chemicals in river water, drinking water, and beverages. Headspace GC-MS allows precise analysis of volatile compounds without direct sample manipulation, improving accuracy while reducing contamination risks. Moreover, it helps assess blood pollutants, indicating exposure to heavy metals, industrial solvents, and airborne toxins, and is crucial for detecting pesticide residues in edible oils like sunflower oil to ensure compliance with food safety standards. Overall, GC-MS stands as a powerful, highly sensitive, and reliable analytical tool, indispensable in forensic science, clinical diagnostics, food safety, and environmental monitoring, making it a gold-standard technique for accurate chemical analysis and ensuring public health and safety.[46]

Chemical Engineering

Gas Chromatography-Mass Spectrometry (GC-MS) is a crucial analytical technique widely employed for the characterization of bio-oils derived from biomass processing. Bio-oils, produced through pyrolysis, hydrothermal liquefaction, or gasification of biomass, consist of a highly complex mixture of hydrocarbons, oxygenated compounds (such as aldehydes, ketones, phenols, and acids), and nitrogen-containing molecules. GC-MS plays a fundamental role in analyzing these complex mixtures by separating, identifying, and quantifying volatile and semi-volatile organic compounds with high precision. The technique involves the initial separation of compounds using Gas Chromatography (GC), followed by their identification through Mass Spectrometry (MS) based on mass-to-charge (m/z) fragmentation patterns. The composition of bio-oils varies significantly depending on factors such as biomass feedstock type, processing conditions, and catalyst usage. GC-MS applications in bio-oil analysis include identifying individual chemical constituents, assessing the quality of bio-oils by determining oxygenated compound content, optimizing production processes by analyzing how different thermal conditions affect bio-oil composition, and monitoring upgrading techniques like hydrodeoxygenation (HDO) and catalytic cracking to improve bio-oil stability and fuel compatibility. Furthermore, GC-MS aids in comparing bio-oils with conventional fossil fuels

to determine their viability as alternative energy sources. By providing detailed molecular insights, GC-MS helps researchers improve bio-oil processing techniques and refine its application in sustainable energy production. The ability to characterize and optimize bio-oils using GC-MS is essential for advancing biofuel technologies and integrating them into existing energy infrastructures.

Medicine and Pharmaceutical Application-

Dozens of congenital metabolic diseases also known as Inborn error of metabolism are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of 13C as the labeling and the measurement of 13C12C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few selected ions and return values as ratios. GCMS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs, and formulations. It is used for process and method development and identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology, etc.

Petrochemical and hydrocarbon analysis

GC-MS is a highly sophisticated and valuable analytical technique widely used in petrochemical and hydrocarbon analysis due to its exceptional ability to detect, separate, and characterize complex mixtures of hydrocarbons and related compounds. One of the key advantages of GC-MS in this domain is its capability to provide significantly enhanced molecular ions, allowing for the precise identification and quantification of various hydrocarbons. This enhancement plays a crucial role in distinguishing between compounds with similar molecular weights but different structural arrangements. Furthermore, the

technique generates structurally significant mass spectral peaks, making it highly effective for identifying isomers and determining structural variations within hydrocarbon compounds. Another important feature of GC-MS is its extended analytical range, enabling the detection of low-volatile hydrocarbons, including long-chain waxes with molecular structures extending up to C74H150. This extended range is particularly beneficial for analyzing high molecular weight hydrocarbons that are typically found in crude oil, heavy petroleum fractions, and waxes, which would otherwise be challenging to study using conventional gas chromatography alone. The versatility of GC-MS allows for the comprehensive analysis of a broad spectrum of petrochemicals, fuels, and hydrocarbon mixtures. This includes refined petroleum products such as gasoline, diesel fuel, and kerosene, which are routinely analyzed for compositional profiling, adulteration detection, and quality control. Additionally, complex hydrocarbon-based substances like naphthenic acids, transformer oil, and biodiesel can be accurately characterized using this technique, aiding in the assessment of their chemical composition, purity, and degradation over time. Beyond conventional fuels, GC-MS is also extensively employed in the analysis of waxes and geochemical samples, which are of particular importance in petroleum exploration, refining processes, and environmental studies. Its capability to precisely separate and identify hydrocarbons across a wide range of molecular weights makes GC-MS an indispensable tool in petroleum forensics, environmental monitoring, and industrial quality assurance, where accurate identification of hydrocarbon constituents is essential for regulatory compliance, performance optimization, and contamination assessment. Hence, the ability of GC-MS to deliver highly detailed molecular insights, resolve structural complexities, and extend the range of analyzable hydrocarbons underscores its status as a gold-standard technique in the field of petrochemical and hydrocarbon analysis.

Clinical toxicology

Clinical toxicology is a crucial field that involves the detection, identification, and quantification of toxic substances, including drugs, venoms, and environmental toxins, in biological samples such as blood, urine, and tissues. Among the various analytical techniques available, Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as a gold standard due to its exceptional sensitivity, selectivity, and ability to analyze a broad range of compounds with high precision. One of the most significant advantages of GC-MS in clinical toxicology is its ability to generate enhanced molecular ions, which aids in the precise identification of toxic substances by providing detailed mass spectral data. This feature enhances the reliability

of toxicological analysis, especially when dealing with complex biological matrices. Additionally, GC-MS offers an extended range of compounds amenable for analysis, making it suitable for detecting various organic toxins, illicit drugs, pharmaceutical substances, environmental pollutants, and even venoms. Its superior sensitivity allows for the detection of even trace amounts of toxic substances, which is particularly valuable in forensic toxicology, emergency medicine, and overdose cases, where rapid and accurate identification can be lifesaving. Furthermore, GC-MS enables faster analysis, significantly reducing turnaround time for toxicological screenings, thereby expediting clinical decision-making and treatment planning. This analytical technique is extensively used in clinical settings for the identification of drugs of abuse, such as opioids, cocaine, amphetamines, and benzodiazepines, as well as the detection of poisonous substances, including pesticides, cyanide, and heavy metals like arsenic and mercury. Moreover, GC-MS plays a vital role in analyzing venoms and plant-based toxins, aiding in the diagnosis of envenomation and toxic plant ingestions. Given its extensive capabilities, GC-MS remains an indispensable tool in clinical toxicology, forensic investigations, and research, ensuring accurate and reliable toxicological assessments. While GC-MS dominates in volatile and semi-volatile compound analysis, Liquid Chromatography-Mass Spectrometry (LC-MS) is increasingly being employed for the detection of non-volatile and thermally unstable compounds, expanding the scope of toxicological investigations.

Academic Research

As a unique and powerful technology, GC-MS provides a rare opportunity to analyze newly synthesized or derivatized compounds with high precision and accuracy, making it an indispensable tool for researchers across various scientific domains. It is widely utilized in both pure and applied sciences, including Chemistry, Polymers, Nanotechnology, and Biotechnology, among others. In Chemistry, GC-MS is extensively used for the identification of unknown organic compounds, the analysis of reaction products, purity assessments, and forensic applications. In Polymer Science, it aids in studying polymer degradation, detecting additives, and analyzing volatile and semi-volatile compounds in materials. The field of Nanotechnology benefits from GC-MS through its ability to analyze surface chemistry, detect functionalized nanoparticles, and assess environmental impacts of nanomaterials. Similarly, in Biotechnology, it plays a critical role in metabolomics, proteomics, lipidomics, and forensic toxicology by enabling the detection and quantification of biomolecules, metabolic byproducts, and pharmaceutical compounds. The technique’s high sensitivity, selectivity, and ability to provide molecular fragmentation patterns contribute to its widespread adoption in high-impact

research, facilitating international scientific publications. By offering valuable insights into chemical composition, molecular structures, and compound interactions, GC-MS serves as an essential tool for advancing scientific knowledge and innovation across various disciplines.

Energy and fuel applications

One of its primary applications is in quality control and purity analysis, where it is used to determine the precise composition of aromatic solvents, ensuring that they meet industrial and regulatory standards. GC-MS is also highly effective in detecting and quantifying sulfur impurities in fuels such as methane, natural gas, and gas oil, which is critical for maintaining fuel efficiency and reducing harmful emissions that contribute to environmental pollution. Additionally, the technique is instrumental in identifying trace contaminants in polymers like polypropylene and polyethylene, ensuring that these materials maintain their structural integrity and performance when used in industrial and commercial applications. Beyond quality assessment, GC-MS significantly contributes to fuel composition analysis and performance evaluation by characterizing unleaded gasoline, diesel, and other refined petroleum products. It enables researchers to detect specific fuel additives, combustion byproducts, and degradation products, which helps optimize fuel formulations for better efficiency and reduced environmental impact. In the petrochemical industry, GC-MS is essential for analyzing the purity and composition of key hydrocarbons such as 1,3-butadiene and ethylene, both of which are crucial in the production of synthetic rubber and plastics. These insights aid in refining manufacturing processes and ensuring compliance with industry regulations.

The technique has also proven indispensable in advanced research and innovation, particularly in the field of biofuels and sustainable energy sources. For example, GC-MS facilitates the structural analysis of modified biomass, allowing researchers to study its chemical composition and determine its feasibility as an alternative fuel source. This application is especially significant in the development of second- and third-generation biofuels, which aim to reduce dependence on fossil fuels. Similarly, GC-MS is widely used in analyzing grafted polymers, which are increasingly being developed as fuel additives to enhance combustion properties and improve fuel efficiency. In addition to fuel performance and innovation, GC-MS plays a crucial role in environmental impact assessment by identifying and quantifying volatile organic compounds (VOCs) present in fuel emissions. This is essential for monitoring air pollution levels and implementing regulatory measures to minimize the

environmental footprint of fuel combustion. Furthermore, the technique is applied in detecting fuel degradation and contamination in storage facilities, which is vital for ensuring the long-term stability and usability of fuels, thereby preventing significant economic and environmental losses. Hence, GC-MS has revolutionized the field of fuel and energy research by providing detailed molecular-level insights into chemical compositions, optimizing fuel formulations, supporting sustainability initiatives, and enhancing pollution control measures. Its ability to detect and characterize a wide range of compounds with high precision has established it as an indispensable tool in both industrial and academic settings, paving the way for cleaner and more efficient energy solutions.

Industrial applications

It is extensively utilized for the analysis of aromatic solvents such as benzene, toluene, and xylene, which are commonly used in industrial processes like paint production, chemical synthesis, and petrochemical refining. Additionally, it is employed in the detection of inorganic gases, ensuring proper monitoring of emissions and safety standards in industrial environments. GC-MS is also used for the identification of amino alcohols in water, which is essential in industries where water purity is critical, such as pharmaceuticals, food processing, and chemical manufacturing. Furthermore, it plays a significant role in detecting impurities in styrene, glycol, and diols, which are primary raw materials in the production of plastics, resins, and synthetic rubber, ensuring product quality and compliance with industrial standards. The cosmetic industry also benefits from GC-MS technology, particularly in the analysis of xylene and allergens, which helps in detecting potentially harmful compounds in skincare and cosmetic products, thus ensuring consumer safety and adherence to regulatory guidelines. One of the most vital industrial applications of GC-MS is its role in the characterization of formic acid in acetic acid, a crucial quality control measure in industries where acetic acid is widely used. Acetic acid serves as a fundamental intermediate in coal chemical synthesis and is extensively utilized in the production of polyethylene, a widely used plastic material in packaging and construction. Additionally, it plays a significant role in manufacturing cellulose acetate, which is used in producing textiles, photographic films, and coatings. Furthermore, acetic acid is a key ingredient in the production of polyvinyl compounds, which are essential in synthetic fiber manufacturing, adhesive production, and various industrial coatings. The textile and polymer industries heavily rely on acetic acid for the synthesis of synthetic fibers and fabrics, which are extensively used in clothing, upholstery, and industrial applications. By utilizing GC-MS, industries can monitor and control the quality and purity of acetic acid and

other chemical intermediates, ensuring efficient production processes, reducing contamination risks, and complying with stringent safety and environmental regulations. Overall, GC-MS is an indispensable analytical tool in multiple industries, enhancing product safety, quality assurance, and research and development efforts.[47, 48]

NUCLEAR MAGNETIC RESONANCE (NMR)

The NMR phenomenon is because the nuclei of atoms have magnetic properties that can be utilized to yield chemical information. Quantum mechanically, subatomic particles (electrons, protons, and neutrons) can be imagined as spinning on their axes. In many atoms (such as 12C), these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in many atoms (such as 1H and 13C) the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows:

1. If the number of neutrons and the number of protons are both even, then the nucleus has no spin.
2. If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (i.e., 1/2, 3/2, 5/2).
3. If the number of neutrons and the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3).

INSTRUMENTATION

There are two general types of NMR instrument: continuous wave and Fourier transform. Early experiments were conducted with continuous wave (C.W.) instruments, and in 1970 the first Fourier transform (F.T.) instruments became available. Continuous Wave (CW) NMR instruments Continuous wave NMR spectrometers are similar in principle to optical spectrometers. The sample is held in a strong magnetic field, and the frequency of the source is slowly scanned (in some instruments, the source frequency is held constant, and the field is scanned). Fourier Transform (FT) NMR instruments The magnitude of the energy changes involved in NMR spectroscopy is very small. This means that, sensitivity can be a limitation when looking at very low concentrations. One way to increase sensitivity would be to record many spectra and then add them together. As noise is random, it adds as the square root of the number of spectra recorded. For example, if one hundred spectra of a compound were recorded and summed, then the noise would increase by a factor of ten, but the signal would increase in magnitude by a factor of one hundred - giving a large increase in sensitivity. However, if this is done using a continuous wave instrument, the time needed to collect the spectra is very large (one scan takes two to eight minutes).

In FT-NMR, all frequencies in a spectrum are irradiated simultaneously with a radio frequency pulse. A single oscillator (transmitter) is used to generate a pulse of electromagnetic radiation of frequency w but with the pulse truncated after only a few complete cycles (corresponding to a duration τ) so that the waveform has rectangular as well as sinusoidal characteristics. It can be proven that the frequencies contained within this pulse are within the range +/- 1/τ of the main frequency wo. For example, a 5 μs pulse would generate a range of frequencies of wo ± 1/0.000005 Hz (i.e. wo ± 200,000 Hz).

Fourier Transform Infrared (FTIR) Spectroscopy

In many ways, mid-infrared spectroscopy would appear to be the ideal technology for on-line chemicals analysis. After all, IR spectroscopy is the only analytical method which provides both ambient temperature operation and the ability to directly monitor the vibrations of the functional groups which characterize molecular structure and govern the course of chemical reactions. In principle, IR also offers the advantages of continuous (near real-time) operation and low maintenance compared to gas chromatography and low cost and structural specificity compared to mass spectroscopy. The term "infrared" generally refers to any electro-magnetic radiation falling in the region from 0.7 mm to 1000 mm. However, the region between 2.5 mm and 25 mm (4000 to 400 cm) is the most attractive for chemical analysis. This "mid-IR" region includes the frequencies corresponding to the fundamental vibrations of virtually all of the functional groups of organic molecules. These spectral lines are typically narrow and distinct, making it possible to identify and monitor a band corresponding to the specific structural feature that is to be modified by a reaction. As a result, quantitative calibrations performed in the mid-IR are usually straightforward and robust, being largely immune to the effects of spurious artifacts. In contrast, the mid-IR region is a spectroscopist's dream, with meaningful, well understood absorption bands often adjacent to weakly absorbing regions, making calibrations largely independent of effects such as source variations, changes in overall sample transmission, or scattering. Despite these advantages, the widespread application of mid-IR on the process line had to await technological advances in three general areas:

- FTIR spectrometers capable of reliable operation in the process environment.
- Methods for transmitting the IR radiation to and from the measurement location
- Robust sample interfacing equipment capable of providing consistent results in the process environment and of dealing with the very strong absorptions generally encountered in the mid- IR.

PRINCIPLE AND INSTRUMENTATION

Infrared instrumentation has been used in chemical process control for approximately fifty years, making it one of the first analytical techniques to be put on-line. However, until recently, on-line infrared instruments were generally restricted to one and two wavelengths nondispersive (NDIR) analyzers. Dispersive IR lab instruments, the only full spectrum IR spectrometers available prior to 1970, were simply too slow and insensitive to find widespread use in process applications. The advent of commercial FTIR instruments in 1970 represented a major advance in IR spectroscopy in terms of both raw performance and data manipulation capability. However, the early FTIR's were strictly laboratory instruments, being highly sensitive to ambient temperature variations, vibration, and acoustic disturbances, all of which are typical of the process environment. To understand the reasons for this sensitivity, we need to briefly review the operation of an FTIR spectrometer. The heart of any FTIR spectrometer is an amplitude division interferometer. The original Michelson design – still employed in the majority of laboratory FTIR's - consists of beam splitter, a compensating plate, and a pair of mirrors. The difference in path length between the two arms is varied by mechanically scanning the position of one of the mirrors. This gives rise to a time dependent variation in transmitted optical intensity, called the interferogram. When the interferometer is illuminated by a monochromatic source such as a single frequency laser, the interferogram will be a sinewave of intensity versus mirror position. On the other hand, if the source is characterized by a broad infrared spectrum, the interferogram will correspond to the superposition of an infinite number of since waves having different periods but a common zero phase point (or central maximum) which occurs when the lengths of the two interferometer arms are equal. In principle, the intensity of a given spectral point could be determined by simply passing the electrical signal obtained from the IR detector through a narrow band electronic filter. And the complete spectrum could be measured by varying the filter frequency. A much more rapid approach is to use a digital computer to perform a Fourier transformation of the interferogram, thereby directly yielding the composite spectrum of the source, the instrument, and any sample interposed in the optical path. This is the basis of all modern rapid-scan FTIR spectrometers.[49-52]

RESULT AND DISCUSSION

5.1 Antibacterial and Antifungal Activities of *Berberis lycium* Extracts

The antimicrobial potential of *B. lycium* extracts was assessed using the Kirby-Bauer disc diffusion method, where zones of inhibition (measured in millimeters) were used to evaluate

antibacterial and antifungal activity. The results revealed that ethanolic extracts exhibited significant antibacterial effects, particularly against Gram-negative bacteria, with varying degrees of susceptibility among the tested strains. The antimicrobial activity was compared to ciprofloxacin, a standard antibiotic used as a positive control, to determine the relative potency of the plant extracts.

Among the bacterial strains tested, *Salmonella typhii* showed the highest susceptibility to the ethanolic extract of *B. lycium*, with a zone of inhibition of 20 mm, which was nearly equivalent to ciprofloxacin's inhibition zone of 21 mm. This result suggests that the plant extract contains bioactive compounds that are highly effective against *Salmonella*, a pathogen responsible for severe gastrointestinal infections. Additionally, *Staphylococcus aureus*, a Gram-positive bacterium known for its resistance to several antibiotics, exhibited an inhibition zone of 16 mm, indicating moderate sensitivity to the ethanolic extract. *Klebsiella pneumonia*, another Gram-negative bacterium associated with respiratory and urinary tract infections, showed an inhibition zone of 15 mm, suggesting that *B. lycium* could be a valuable natural antibacterial agent against this pathogen.

On the other hand, certain bacterial species exhibited low susceptibility to the ethanolic extract. *Acinetobacter baumannii*, a multidrug-resistant pathogen commonly found in hospital settings, displayed an inhibition zone of only 8 mm, while *Pseudomonas* spp., another highly resilient bacterial strain, had a 9 mm inhibition zone. The lower efficacy against these bacteria suggests that they may possess innate resistance mechanisms, such as efflux pumps or biofilm formation, which limit the extract's antimicrobial effects. The minimum inhibitory concentration (MIC) values further confirmed that while some bacterial species were highly susceptible to the plant extract, others showed higher tolerance, reinforcing the need for additional studies on synergistic approaches with conventional antibiotics to enhance antimicrobial activity.

In contrast to the promising antibacterial results, the antifungal evaluation of the ethanolic extract did not yield significant results. When tested against *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus cuboida*, and *Candida albicans*, the extract failed to produce measurable zones of inhibition. This suggests that the bioactive compounds present in the extract are selective towards bacteria but may not effectively target fungal pathogens under the experimental conditions used in this study. This lack of antifungal activity may indicate that different extraction techniques, higher extract concentrations, or alternative solvents may be

required to unlock the potential antifungal properties of *B. lycium*.

Comparative Evaluation of Aqueous and Ethanolic Extracts

A detailed comparison between the aqueous and ethanolic extracts of *B. lycium* was performed to determine which extraction method was more effective in antimicrobial activity. The aqueous flower extract displayed a strong inhibition against *S. typhii* (20 mm), *S. aureus* (17 mm), and *K. pneumonia* (16 mm), which was comparable to the results obtained with the ethanolic extracts. The aqueous extract also exhibited moderate activity against *Enterococcus* (14 mm) and *Pseudomonas* spp. (13 mm), suggesting that water may also be an effective solvent for extracting bioactive antibacterial compounds from the plant.

Interestingly, the ethanolic fruit extract yielded similar antibacterial results to the aqueous extract, further reinforcing the antimicrobial potential of *B. lycium*. *Salmonella typhii* continued to be the most susceptible strain, followed by *Staphylococcus aureus* and *Klebsiella pneumonia*, with respective inhibition zones of 20 mm, 16 mm, and 15 mm. However, the ethanolic extract displayed slightly lower inhibition against *Enterococcu*s and *Pseudomonas* spp. than the aqueous extract, indicating that certain antibacterial compounds may be more efficiently extracted using water rather than ethanol.

Despite these variations, both aqueous and ethanolic extracts failed to demonstrate antifungal activity, reinforcing the observation that the bioactive compounds present in *B. lycium* primarily target bacterial pathogens rather than fungal species. The absence of antifungal effects may indicate that further phytochemical fractionation is required to isolate specific compounds with potential antifungal activity.

Potential of *B. lycium* as an Antimicrobial Agent

The findings from this study suggest that *B. lycium* possesses strong antibacterial properties, particularly against Gram-negative bacteria such as *S. typhii* and *K. pneumonia*. This is a noteworthy discovery, given that Gram-negative bacteria often exhibit higher resistance to antibiotics due to their outer membrane, which serves as a barrier against many antimicrobial agents. The significant inhibition observed against these bacteria suggests that *B. lycium* contains bioactive compounds capable of penetrating bacterial defenses and disrupting their cellular functions.

The variation in antimicrobial activity across different bacterial species suggests that multiple factors, such as bacterial cell wall composition, efflux pump activity, and metabolic pathways, may influence the effectiveness of *B. lycium* extracts. The comparative resistance observed in *Acinetobacter baumannii* and *Pseudomonas* spp. suggests that these pathogens possess intrinsic mechanisms that limit the penetration or activity of plant-derived antimicrobials. Future research should focus on identifying synergistic effects between *B. lycium* extracts and conventional antibiotics to explore potential combination therapies for treating antibiotic-resistant infections.

Determination of anti-bacterial and anti-fungal assay

The extracts of roots, fruits, and flowers of *B. lycium* were screened for their antimicrobial activity against selected bacterial and fungal strains (*E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus* and *Salmonella typhi* while fungal strains like *Candida albicans*, *A. cuboida*, A. niger and *A. fumigatus* were used). The cultures were obtained from the standard cultures maintained in the Microbiology Laboratory of Uttaranchal University, Dehradun. These cultures were maintained on nutrient agar slants, at first being incubated at 37^0C for about 18-24 hours and then stored at 4-degree C as stock cultures for further antibacterial and antifungal activities. Fresh cultures were obtained by transferring a lapful of culture into nutrient broth and then incubated at 37-degree C overnight. To test antibacterial and antifungal activity, the Kirby Bauer method was used.[53]

Measurement of zone of inhibition

After incubation, the diameter of the clear zone of inhibition produced around the well was measured in mm, and the diameter of inhibition by the plant extracts was compared with the reference antibiotic.

Determination of Minimum inhibitory concentration (MIC) of the plant extracts

The MIC level of any antimicrobial substance is the lowest concentration of the drug inhibiting the bacterial or fungal growth. The MIC value of those microorganisms against a particular fraction is considered, which exhibits a maximum activity in the preliminary screening process by the disc diffusion method. The MIC of the different extracts was estimated by the disc-diffusion method and was compared with the MIC of the antibiotic taken as reference as per the method described in the chapter of material and methods.

Observations: Antimicrobial activities

All results are presented in Table No. 2, highlighting a significant zone of inhibition against the selected bacteria and fungi.

Table 2: Minimum inhibitory concentration (MIC) (in mm) of ethanolic extract of *Berberis lycium*.

S. No.	Bacterial strains	Minimum inhibitory concentration (MIC)	Standard antibiotic disc (ciprofloxacin)
1	*Escherichia coli*	11	25
2	*Staphylococcus aureus*	16	20
3	*Salmonella typhii*	20	21
4	*Proteus mirabilis*	ND	22
5	*Klebsiella pneumonia*	15	18
6	*Enterococcus*	14	24
7	*Acinetobacter baumannii*	08	26
8	*Pseudomonas* spp.	09	20
Fungal strains			
1	*Aspergillus niger*	ND	21
2	*Aspergillus fumigatus*	ND	21
3	*Aspergillus cuboida*	ND	21
4	*Candida albicans*	ND	18

In the present study, the aqueous extract of root, fruits, and flowers of *B. lycium* were applied to control the growth of different microorganisms, and it was found that ethanolic extract as shown in Table 2). The aqueous flower extract showed a zone of inhibition of about 20 mm against *Salmonella typhii*, 17 mm against *Staphylococcus aureus*, and 16 mm against *Klebsiella pneumonia*, followed by *Enterococcus* at 14 mm and 13 mm, while the fungal strains showed no activity.

In the present study, the ethanolic extracts of *B. lycium* were applied against 8 bacterial strains as well as 4 fungal strains. It was observed that the ethanolic extract of *B. lycium* fruits. Fruit ethanolic extract showed 20 mm against *Salmonella typhii*, and the least was shown by *Acinetobacter baumannii*, i.e,, 08 mm. All the fungi of fruit showed no activity. The results obtained during this study revealed that the fruits of *B. lycium* contain some active 8 phytochemicals that can control the growth of some microorganisms. Therefore, this study will highlight the antimicrobial activity of *B. lycium*. The microorganisms that were affected by plant extracts could have some difference in their cell walls or inherited antimicrobial resistance genes as plasmids can easily be transferred among bacterial or fungal strains. Therefore, based on these results obtained in the present study, the fruit extracts of this plant

can be helpful for the development of new and useful drugs in the pharmaceutical industry for the treatment of various infectious diseases.

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to identify the bioactive phytochemicals present in the ethanolic fruit extract of Berberis lycium. The analysis revealed the presence of several key compounds, including:

- 2,5-Diamino-1,3,4-Thiadiazole – Known for its antimicrobial and antioxidant properties, this compound may contribute significantly to the antibacterial effects observed in the study.
- 3(2H)-Thiophenone dihydro-2-methyl–A sulfur-containing compound with potential antibacterial and antifungal activity, although further studies are required to confirm its efficacy.
- 3H-1,2,4-Triazole-3-Thione–A compound with well-documented antimicrobial, antifungal, and anti-inflammatory properties, which may explain the plant's traditional use in treating infections.
- Thiazole derivatives – These are known to exhibit potent antibacterial effects, particularly against drug-resistant bacterial strains.

The presence of these bioactive compounds suggests that Berberis lycium contains a diverse array of antimicrobial agents, which may act synergistically to enhance the plant's overall effectiveness. These findings reinforce the ethnopharmacological importance of *B. lycium* and provide a scientific foundation for its traditional medicinal applications.

Table 3: GC-MS analysis of ethanolic fruit extract.

S. No.	Compounds	Molecular Formula	Molecular weight
1.	2,5- Diamino-1, 3, 4-Thiadiazole	$C_2H_4N_4S$	116
2.	3(2H)-Thiophenone, dihydro-2-methyl	C_5H_8OS	116
3.	3H-1, 2, 4- Triazole-3- Thione,5-amino-1,2- and Thiazole	$C_2H_4N_4S$	116
4.	Thiazole, 4, 5- Dihydro-2-Methylamino	$C_4H_8N_2S$	116

It is very evident from Table 2. The following compounds were found in the aqueous fruit extract: 2,5- Diamino-1, 3, 4-Thiadiazole, 3(2H)-Thiophenone, dihydro-2-methyl, 3H-1, 2, 4-Triazole-3- Thione,5- amino-1,2- and Thiazole, 4, 5- Dihydro-2- Methylamino.

In the present study, the antimicrobial activity of *B. lycium* extract was observed against Gram-positive and Gram-negative bacteria and a fungal strain, as shown in **Table 2**. The medicinal

plant (*B. lycium*) was selected based on its local medicinal uses and collected from Mussoorie and the hilly areas of Nainital. In this investigation, the zone of inhibition which is produced by different extracts of *B. lycium* was observed against the microorganisms used. For the extraction of plant material, an apparatus was used, i.e, a soxhlet assembly. *B. lycium* contains berberine and other chemical components. The components may be active against test organisms. These results show that this plant can be used in the future to treat infectious diseases. *B. lycium* fruits possess good antimicrobial activity. This explains the reason to use the plant in treating different diseases and infections. This plant was useful in traditional medicine. Due to its availability in abundance, it can be used in conventional drugs. The extract can easily be made using a soxhlet assembly. The pharmacological activities like antimicrobial activity, antifungal activity, antibacterial activity, antioxidant activity, and GC-MS analysis of fruit from *Berberis lycium*. The plant, which is studied here, can be seen as a potential source of useful drugs on the basis of our result that we have concluded. This herbal plant *Berberis lycium* has a good antimicrobial nature, which is revealed and supported by many researchers. The focus of this study was to find the antimicrobial activities and some of the new components by the GC-MS technique. Some selected combinations of solvents have shown very good antibiotic, antifungal, and antibacterial properties, which is higher than the reference standard antibiotics taken. The extracts of fruit show remarkable antimicrobial potentials against both types of bacteria, i.e, negative as well as Gram-positive bacteria, and fungal strain.

This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine in combating pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Overall, from this study, it can be observed that the flower extract has shown maximum antibacterial resistance, followed by fruit extract. The result agrees with the claim according to the author *Berberis lycium* can be used to treat many diseases like diabetes, stomach infection and heart diseases etc.

Potential of *B. lycium* as an Antimicrobial Agent

The findings from this study suggest that *B. lycium* possesses strong antibacterial properties, particularly against Gram-negative bacteria such as *S. typhii* and *K. pneumonia*. This is a noteworthy discovery, given that Gram-negative bacteria often exhibit higher resistance to antibiotics due to their outer membrane, which serves as a barrier against many antimicrobial agents. The significant inhibition observed against these bacteria suggests that *B. lycium*

contains bioactive compounds capable of penetrating bacterial defenses and disrupting their cellular functions.

The variation in antimicrobial activity across different bacterial species suggests that multiple factors, such as bacterial cell wall composition, efflux pump activity, and metabolic pathways, may influence the effectiveness of *B. lycium* extracts. The comparative resistance observed in *Acinetobacter baumannii* and *Pseudomonas* spp. suggests that these pathogens possess intrinsic mechanisms that limit the penetration or activity of plant-derived antimicrobials. Future research should focus on identifying synergistic effects between *B. lycium* extracts and conventional antibiotics to explore potential combination therapies for treating antibiotic-resistant infections.

Implications for Pharmaceutical Applications

The results obtained in this study indicate that *B. lycium* extracts could be explored as a potential alternative to synthetic antibiotics, especially against Gram-negative bacterial infections. Given the global rise in antibiotic resistance, there is an urgent need for novel antimicrobial agents, and plant-based compounds represent a promising avenue for drug discovery. The absence of antifungal activity suggests that *B. lycium* may be more suitable for antibacterial applications. Future research should focus on isolating and characterizing the individual bioactive compounds, conducting in vivo studies, and exploring clinical applications to validate their therapeutic potential. These findings highlight the importance of medicinal plants in modern pharmacology and emphasize the need for further investigation into their bioactive components and mechanisms of action.

DISCUSSION

The findings of this study provide valuable insights into the antimicrobial and antioxidant properties of Berberis lycium, a medicinal plant with significant ethnobotanical relevance. The study focused on evaluating the phytochemical constituents, antimicrobial efficacy, and enzymatic as well as non-enzymatic antioxidant activities of extracts derived from different parts of *B. lycium*, including fruits, flowers, and roots. The results demonstrate the potent bioactivity of the ethanolic extracts, highlighting their potential application in pharmaceutical and nutraceutical formulations.

The antimicrobial analysis revealed that the fruit extracts exhibited a higher inhibitory effect against bacterial and fungal pathogens compared to other plant parts. The study tested

extracts against multiple bacterial strains, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus, and Salmonella typhi. Additionally, fungal strains such as Candida albicans, Aspergillus cuboida, Aspergillus niger, and Aspergillus fumigatus were screened. The results suggest that *B. lycium* contains bioactive compounds that effectively suppress microbial growth, supporting its traditional use in treating infections.

One of the significant findings was that the antibacterial and antifungal effects were not influenced by the solvent used for extraction but rather by the bioactive components present in the plant extracts. The ethanolic extracts displayed superior antimicrobial activity, possibly due to the higher solubility of polyphenolic and alkaloid compounds in ethanol. This aligns with previous studies indicating that ethanol efficiently extracts phenolic and flavonoid compounds, which are known for their antimicrobial properties. These findings underscore the importance of solvent selection in maximizing the pharmacological potential of medicinal plants.

Furthermore, the study investigated the antioxidant activity of different extracts using enzymatic and nonenzymatic assays. The results demonstrated that the fruit extracts exhibited the highest antioxidant potential, followed by the root and flower extracts. This is likely due to the presence of phenolic compounds, flavonoids, and alkaloids, which act as free radical scavengers. Oxidative stress is a critical factor in various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. The high antioxidant capacity of *B. lycium* suggests its potential role in mitigating oxidative stress-related conditions and promoting overall health.

The GC-MS analysis identified several bioactive compounds in the extracts, further elucidating the plant's pharmacological potential. The presence of alkaloids such as berberine, known for its antimicrobial and anti-inflammatory properties, was particularly noteworthy. Additionally, the detection of flavonoids and terpenoids adds to the medicinal significance of *B. lycium*, as these compounds have been associated with various therapeutic effects, including anti-inflammatory, anticancer, and hepatoprotective properties. The synergistic interaction of these phytochemicals may enhance the overall bioactivity of the plant extracts.

A comparative analysis of different extraction methods highlighted the superiority of ethanolic extracts over aqueous and non-polar solvent extracts in terms of bioactivity. The polar nature of ethanol likely facilitated the extraction of hydrophilic and moderately lipophilic

compounds, enhancing the extract's antimicrobial and antioxidant efficacy. This finding provides a scientific basis for the traditional use of alcohol-based herbal preparations in ethnomedicine.

Overall, this study contributes to the growing body of literature on the medicinal properties of *B. lycium* and underscores its potential application in developing natural antimicrobial and antioxidant agents. Future research should focus on isolating and characterizing individual bioactive compounds to further elucidate their mechanisms of action. Additionally, in vivo studies and clinical trials are necessary to validate the therapeutic efficacy and safety of *B. lycium* extracts in medical applications. These findings reinforce the relevance of medicinal plants in modern pharmacology and highlight the need for further exploration of their bioactive compounds for pharmaceutical development.

SUMMARY

The present research study investigates the antimicrobial properties of *B. lycium*, a medicinal plant traditionally recognized for its therapeutic potential. With the increasing challenge of antibiotic resistance, natural alternatives are being explored, and this study evaluates the antibacterial and antifungal efficacy of aqueous and ethanolic extracts of *B. lycium* against various bacterial and fungal strains. The study employs the Kirby-Bauer disc diffusion method to assess antimicrobial activity, measuring the zone of inhibition (mm) for bacterial and fungal susceptibility. The bacterial strains tested include *Salmonella typhii*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, and *Pseudomonas* spp., while the fungal strains include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus cuboida*, and *Candida albicans*. Additionally, Minimum Inhibitory Concentration (MIC) values are determined to evaluate the potency of the extracts. Furthermore, Gas Chromatography-Mass Spectrometry (GC-MS) analysis is conducted to identify the bioactive compounds present in the ethanolic extract of *B. lycium*, which may contribute to its antimicrobial properties.

The results indicate that both ethanolic and aqueous extracts exhibit strong antibacterial activity against Gram-negative and Gram-positive bacteria, with particularly significant inhibition observed against Salmonella typhii, Staphylococcus aureus, and Klebsiella pneumonia. The zones of inhibition for these bacteria are comparable to standard antibiotics like ciprofloxacin, highlighting the therapeutic potential of the plant extract. However, the extracts demonstrate limited effectiveness against drug-resistant bacteria, such as

Acinetobacter baumannii and Pseudomonas spp., suggesting that these bacteria may possess resistance mechanisms that reduce the impact of plant-based antimicrobials. Additionally, the antifungal evaluation reveals that the extracts do not exhibit significant antifungal activity against Aspergillus and Candida species, indicating that *B. lycium* may be more effective against bacterial infections than fungal infections.

The GC-MS analysis further identifies key bioactive compounds in the ethanolic extract, including 2,5-Diamino-1,3,4-Thiadiazole, 3(2H)-Thiophenone dihydro-2-methyl, 3H-1,2,4-Triazole-3-Thione, and Thiazole derivatives, which are known for their antimicrobial, antifungal, antioxidant, and anti-inflammatory properties. These findings suggest that the antimicrobial activity observed in the study may be attributed to the presence of these phytochemicals, making *B. lycium* a potential candidate for pharmaceutical applications.

In conclusion, the study establishes that *B. lycium* exhibits strong antibacterial properties, particularly against Gram-negative bacteria, making it a promising natural alternative to synthetic antibiotics. However, the lack of antifungal activity suggests that further research is needed to explore alternative extraction techniques or higher extract concentrations for potential antifungal effects. Given the global rise in multidrug-resistant infections, the study highlights the importance of natural, plant-derived antimicrobials in combating infectious diseases. Future research should explore the synergistic effects of *B. lycium* extracts with conventional antibiotics to enhance its antimicrobial efficacy and investigate its pharmaceutical applications in drug development.

ACKNOWLEDGEMENT

All praise to almighty God, the most beneficent, the most merciful, the omnipresent, and the Crestar of the universe, by thanking him for bestowing upon me the courage, strength, mind-set, and will-power to embark on and accomplish the current research crusade.

Though words are seldom sufficient to express gratitude and feeling, it somehow gives me an opportunity to acknowledge those who helped me during the tenure of my study. I might not do justice to all the people who have helped me during my work by thanking them in this one page. If it were not for their constant support, I would not have overcome all the difficulties I faced. The work of dissertation preparation was a daunting task and a fascinating experience.

I would like to express my sincere gratitude to all those who contributed to the completion of this dissertation. It is a matter of great pleasure to acknowledge my respected guide, **Dr. Nitin Jumnani**, **Alwar Pharmacy College, Alwar (Raj.)**. It is because of his priceless intellectual guidance, constructive ideas, and for having given me complete independence, affectionate encouragement to put my desire and thought which paved the way for the successful completion of this work. It is indeed a privilege to work under him.

REFERENCES

1. World Health Organization. (2019). 1 in 3 people globally do not have access to safe drinking water - UNICEF, WHO. Retrieved from https://www.who.int/news/item/18-06-2019-1-in-3-people-globally-do-not-have-access-to-safe-drinking-water-unicef-who
2. Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. Antimicrobial resistance: impacts, challenges, and future prospects. Journal of Medicine, Surgery, and Public Health, 2024; 2: 100081.
3. Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. Plants, 2023; 12(17): 3077.
4. Sofowora, A., Ogunbodede, E., & Onayade, A. The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines, 2013; 10(5): 210-229.
5. Borins, M. Traditional medicine in India. Canadian Family Physician, 1987; 33: 1061.
6. Chaughule, R. S., & Barve, R. S. Role of herbal medicines in the treatment of infectious diseases. Vegetos, 2024; 37(1): 41-51.
7. Aziz, M. A., Khan, A. H., Adnan, M., & Ullah, H. Traditional uses of medicinal plants used by Indigenous communities for veterinary practices at Bajaur Agency, Pakistan. Journal of ethnobiology and ethnomedicine, 2018; 14: 1-18.
8. Ngarivhume, T., van't Klooster, C. I., de Jong, J. T., & Van der Westhuizen, J. H., Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. Journal of ethnopharmacology, 2015; 159: 224-237.
9. Riaz, U., Iqbal, S., Sohail, M. I., Samreen, T., Ashraf, M., Akmal, F., & Akhter, R. M. (2021). A comprehensive review on emerging importance and economical potential of medicinal and aromatic plants (MAPs) in current scenario.
10. Sulieman, A. M. E., Abdallah, E. M., Alanazi, N. A., Ed-Dra, A., Jamal, A., Idriss, H., & Shommo, S. A. Spices as sustainable food preservatives: a comprehensive review of their

antimicrobial potential. Pharmaceuticals, 2023; 16(10): 1451.

11. Raju, S., & Das, M. (2024). Medicinal plants industry in India: Challenges, opportunities and sustainability.
12. Chaachouay, N., & Zidane, L. Plant-derived natural products: a source for drug discovery and development. Drugs and Drug Candidates, 2024; 3(1): 184-207.
13. Gurib-Fakim, A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 2006; 27(1): 1-93.
14. Kala, C. P., Dhyani, P. P., & Sajwan, B. S. Developing the medicinal plants sector in northern India: challenges and opportunities. Journal of Ethnobiology and Ethnomedicine, 2006; 2: 1-15.
15. Parra, S. A., Gaur, K., Ranawat, L. S., & Rather, M. I. An overview on various aspects of plant Berberis Lycium Royale. Am. J. Pharmacol. Sci, 2018; 6: 19-24.
16. Trigo-Gutierrez, J. K., Vega-Chacón, Y., Soares, A. B., & Mima, E. G. D. O. Antimicrobial activity of curcumin in nanoformulations: A comprehensive review. International journal of molecular sciences, 2021; 22(13): 7130.
17. Murtadlo, A. A. A., Ansori, A. N. M., & Kharisma, V. D. A mini review of Curcuma longa: Antimicrobial properties. J Med Chem Sci, 2024; 7(1): 215-221.
18. Álvarez-Martínez, F. J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine, 2021; 90: 153626.
19. Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological research, 2017; 196: 44-68.
20. Ashraf, M. V., Pant, S., Khan, M. H., Shah, A. A., Siddiqui, S., Jeridi, M., & Ahmad, S. Phytochemicals as antimicrobials: prospecting Himalayan medicinal plants as source of alternate medicine to combat antimicrobial resistance. Pharmaceuticals, 2023; 16(6): 881.
21. Srivastava, S., Srivastava, M., Misra, A., Pandey, G., & Rawat, A. A review on biological and chemical diversity in Berberis (Berberidaceae). EXCLI journal, 2015; 14: 247.
22. Kapoor, B. B. S., Sood, P., Modgil, R., & Sood, M. Berberis lycium a medicinal plant with immense value. Indian journal of pharmaceutical and biological research, 2013; 1(01): 27-37.
23. Verma, S., Wani, I. A., Khan, S., Sharma, S., Kumari, P., Kaushik, P., & El-Serehy, H. A. Reproductive biology and pollination ecology of Berberis lycium Royle: a highly valued shrub of immense medicinal significance. Plants, 2021; 10(9): 1907.

24. Anjum, N., Ridwan, Q., Akhter, F., & Hanief, M. Predicting current and future distribution pattern of Berberis lycium Royle concerning climate change using an ensemble modelling approach. Geology, Ecology, and Landscapes, 2023; 1-10.
25. Gupta, M., & Singh, A. Studies of the Antimicrobial Activity and Phytochemical Properties of Berberis Lycium. Book Rivers, 2022.
26. Khan, M., Giessrigl, B., Vonach, C., Madlener, S., Prinz, S., Herbaceck, I., ... & Krupitza, G. 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, 2010; 683(1-2): 123-130.
27. Lau, C. W., Yao, X. Q., Chen, Z. Y., Ko, W. H., & Huang, Y. Cardiovascular actions of berberine. Cardiovascular Drug Reviews, 2001; 19(3): 234-244.
28. Kulkarni, S. K., & Dhir, A. On the mechanism of antidepressant-like action of berberine chloride. European Journal of Pharmacology, 2008; 589(1-3): 163-172.
29. Ahmed, M., Alamgeer, A., Sharif, T., Zabta CH, M., & Akbar, A. Effect of Berberis lycium Royle on lipid profile in alloxan induced diabetic rabbits. Ethnobotanical leaflets, 2009; 2009(6): 4.
30. Gulfraz, M., Qadir, G., Nosheen, F., & Parveen, Z. Antihyperglycemic effects of Berberis lyceum Royle in alloxan induced diabetic rats. Diabetologia croatica, 2007; 36(3): 49- 54.
31. Sack, R. B., & Froehlich, J. L. Berberine inhibits intestinal secretory response of Vibrio cholerae and Escherichia coli enterotoxins. Infection and immunity, 1982; 35(2): 471-475.
32. Lee, S., Lim, H. J., Park, J. H., Lee, K. S., Jang, Y., & Park, H. Y. Berberine- induced LDLR up-regulation involves JNK pathway. Biochemical and Biophysical Research Communications, 2007; 362(4): 853-857.
33. Singh A, Duggal S, Kaur N and Singh J., Oxidative Chemistry of Nitric Oxide, CRC Handbook of Free Radicals and Squadrito, Free. Radicals. J. Nat. Prod., 2007; 3: 64-75.
34. Gupta R, Sharma M, Lakshamy R, Prabhakaran D & Reddy KS., Improved method of total antioxidant assay. Ind. J Biochem Biophysic, 2009; 46: 126-129.
35. Khan, M. A., Khan, J., Ullah, S., Malik, S. A., & Shafi, M. Hepatoprotective effects of Berberis lycium, Galium aparine and Pistacia integerrima in carbon tetrachloride (CCl4)-treated rats. Journal of Postgraduate Medical Institute, 2008; 22(2).
36. Tewary, DK., Bhardwaj, A., and Shanker, A., Pesticidal activities in five medicinal plants collected from mid hills of western Himalayas. Indus. Crop. Prod., 2005; 22: 241-247.
37. Asif, A., Kakub, G., Mehmood, S., Khunum, R., and Gulfraz, M., Wound Healing

Activity of Root Extracts of Berberis lycium Royle in Rats. Phytother. Res., 2007; 21: 589-591.

38. Singh, A., & Gupta, M. Phytochemical investigation of different solvent extracts of Berberis lycium fruits. Rasayan J Chem, 2018; 11(1): 228-31.
39. Handley, A. J., & Adlard, E. R. (Eds.). Gas chromatographic techniques and applications, 2001; 5. Taylor & Francis.
40. Beale, D. J., Pinu, F. R., Kouremenos, K. A., Poojary, M. M., Narayana, V. K., Boughton, B. A., & Dias, D. A. Review of recent developments in GC–MS approaches to metabolomics-based research. Metabolomics, 2018; 14: 1-31.
41. Hubschmann, H. J. (2015). Handbook of GC-MS: fundamentals and applications. John Wiley & Sons.
42. Marriott, P., & Shellie, R. Principles and applications of comprehensive two- dimensional gas chromatography. TrAC Trends in Analytical Chemistry, 2002; 21(9-10): 573- 583.
43. Eckenrode, B. A. Environmental and forensic applications of field-portable GC- MS: an overview. Journal of the American Society for Mass Spectrometry, 2001; 12(6): 683-693.
44. Eckenrode, B. A. Environmental and forensic applications of field-portable GC- MS: an overview. Journal of the American Society for Mass Spectrometry, 2001; 12(6): 683-693.
45. Chauhan, A., Goyal, M. K., & Chauhan, P. GC-MS technique and its analytical applications in science and technology. J. Anal. Bioanal. Tech, 2014; 5(6): 222.
46. Hussain, S. Z., & Khushnuma Maqbool, K. M. (2014). GC-MS: principle, technique and its application in food science.
47. Gilbert, J. R., McCaskill, D., Fishman, V. N., Brzak, K., Markham, D., Bartels, M. J., ... & Lewer, P. Industrial applications of high-resolution GC/MS. In Comprehensive Analytical Chemistry, 2013; 61: 403-429. Elsevier.
48. Pawar, K., Sankpal, P., Patil, S., Patil, P., Pawar, A., & Shinde, P. Applications of GC-MS used in Herbal plants. Asian Journal of Pharmaceutical Analysis, 2022; 12(1): 53-55.
49. Bhargava, R., Wang, S. Q., & Koenig, J. L. FTIR microspectroscopy of polymeric systems. Liquid chromatography/FTIR microspectroscopy/microwave assisted synthesis, 2003; 137-191.
50. Coleman, M. D., & Gardiner, T. D. Sensitivity of model-based quantitative FTIR to instrumental and spectroscopic database error sources. Vibrational Spectroscopy, 2009; 51(2): 177-183.
51. Clarke, F. J. J., Birch, J. R., Chunnilall, C. J., & Smart, M. P. FTIR measurements—standards and accuracy. Vibrational spectroscopy, 2002; 30(1): 25-29.

52. Bacsik, Z., Mink, J., & Keresztury, G. FTIR spectroscopy of the atmosphere. I. Principles and methods. Applied spectroscopy reviews, 2004; 39(3): 295-363.
53. Gupta, M., & Singh, A. Pharmacological Studies of root, fruit and flower of Berberis lycium. Oriental Journal of Chemistry, 2018; 34(2): 1055.