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Validation of Ethnomedicinal Applications and Anticancer Properties of *Berberis lycium* Royle

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Abstract

This study evaluates the antibacterial, hemolysin inhibitory, and anticancer properties of Berberis lycium, a medicinal plant with a rich traditional background. The crude methanolic extract and its fractions—n-Hexane, chloroform, ethyl acetate, and aqueous—were tested for their antibacterial activity against Shigella dysenteriae, Salmonella typhi, and Escherichia coli. The crude methanolic extract exhibited the most significant antibacterial activity with inhibition zones of 15.2 mm, 14.8 mm, and 16.3 mm, respectively, indicating its potent efficacy. The chloroform fraction also displayed notable antibacterial effects, with inhibition zones of 12.7 mm, 11.9 mm, and 13.1 mm, respectively. In terms of hemolysin

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inhibition, the crude methanolic extract demonstrated the highest inhibition rate at 65.4%, surpassing the n-Hexane, chloroform, ethyl acetate, and aqueous fractions, thereby supporting its traditional use in managing microbial infections. The anticancer activity was assessed using the MTT assay on L5178Y MDR mouse lymphoma cell lines, where the methanolic extract showed a remarkable 72.3% cell growth inhibition and an IC50 value of 18.6 µg/ml, indicating high efficacy. The chloroform fraction also displayed significant anticancer potential with a 65.4% inhibition rate and an IC50 of 22.8 µg/ml. These results reinforce the traditional medicinal uses of Berberis lycium and underscore its potential as a source of novel antibacterial and anticancer agents. The study highlights the need for further isolation and characterization of bioactive compounds within the plant and their subsequent evaluation for therapeutic applications.

Keywords: Berberis lyceum, Ethnomedicinal, Hemolysin Inhibition, MTT Assay, L5178Y MDR mouse lymphoma cell lines

INTRODUCTION

The use of plants as medicines is as old as the origin of mankind. Traditional plant- based medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi & Omogbai, 2007). Since the dawn of humanity, people have primarily relied on plants for both food and medicinal purposes. Through trial and error, they discovered that some plants were edible, some were poisonous, and others were beneficial for treating various diseases. Medicinal plants are increasingly attracting research interest due to their polyvalent actions and fewer side effects compared to synthetic drugs (Mahesh & Satish, 2008).

Medicinal plants have been integral to the healthcare systems of traditional communities worldwide, with approximately 80% of these communities relying on them for primary healthcare (Sarma *et al.*, 2012; Khan *et al.*, 2021). These plants, with their diverse bioactive compounds, have long been used in the preparation of traditional and herbal remedies (Dugani *et al.*, 2018), serving as a critical resource for those living in remote and underdeveloped regions. In these areas, such as the Tribal District North Waziristan in Khyber Pakhtunkhwa, Pakistan, indigenous populations are often impoverished, largely uneducated, and lack access to modern healthcare facilities (Khan *et al.*, 2021). Consequently, they rely heavily on medicinal plants for their daily needs (Kamal *et al.*, 2016).

The use of medicinal plants in these communities is deeply intertwined with their cultural practices, religious beliefs, and local knowledge, which has been passed down orally through generations (Zeleke, 2016). These plants are not only utilized for their therapeutic properties but also play a significant role in the production of food, cosmetics, and other essential products (Petrakoua *et al.*, 2020). Despite the increasing availability of modern healthcare, many rural inhabitants continue to prefer herbal medications due to their effectiveness, easy availability, and the trust they have in these traditional remedies (Hassan *et al.*, 2017). In fact,

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therapeutic plants are still widely used as alternatives to allopathic medicine in these areas (Birjees *et al.*, 2021).

Therapeutic plants contain a wide array of bioactive compounds that have been shown to aid in the treatment of various diseases in both humans and animals (Akkol *et al.*, 2021; Hayat *et al.*, 2021; Ahmad *et al.*, 2020). However, the knowledge surrounding these plants is at risk of being lost, as younger generations show diminishing interest in traditional practices, favoring modern healthcare methods instead (Vitalini *et al.*, 2013). The preservation of this indigenous knowledge is crucial for the conservation of these plants and the development of new herbal drugs (Zahoor *et al.*, 2017).

Pakistan, with its rich biodiversity, is home to more than 6000 species of flowering plants, about 12% of which are used in the preparation of medicinal treatments (Bano *et al.*, 2014). These plants are utilized to address a wide range of human ailments (Jima & Megersa, 2018), with certain species considered particularly effective for specific conditions (Hamayun, 2005). Despite the country's reliance on herbal drugs (Umair *et al.*, 2017), the overutilization of medicinal plants has led to the extinction of many species (Rehman *et al.*, 2022a). The loss of traditional knowledge, often held by elders and traditional healers, further exacerbates this issue, as younger generations fail to learn and carry forward this valuable heritage (Rehman *et al.*, 2022b).

The family Berberidaceae, originally established by Jussieu in 1789 under the name "Berberides," is recognized as one of the most ancient lineages among angiosperms. This family is notable for its high number of disjunct or discontinuous genera, which suggests a long evolutionary history and significant geographical separation of its species (Bruckner, 2000). Berberidaceae includes a diverse group of plants that have adapted to various ecological niches over time. One of the species within this family, *B. lycium*, was first described by the British botanist John Forbes Royle in 1837. This species is a member of a broader group that encompasses approximately 12 genera and about 600 species (CSIR, 1972). The genus *Berberis*, in particular, is widely distributed, with around 77 species reported from India alone. This diversity within the genus highlights the adaptability and evolutionary success of the Berberidaceae family across different regions and climates. The presence of such a large number of species in a specific region like India underscores the ecological significance and potential medicinal value of this family, which has been utilized in traditional medicine for centuries. *B. lycium* is a widely distributed plant species found across temperate and subtropical regions of the world, with the notable exception of Australia. Its native range includes several countries in South Asia, such as India, Nepal, and Pakistan, as well as other parts of the globe where similar climatic conditions prevail. The species thrives in diverse environments, reflecting its adaptability and ecological versatility. In India, *B. lycium* is predominantly found in the sub-tropical and temperate regions, particularly along the outer northern-western Himalayan range, extending from Kashmir to Uttaranchal. The plant is commonly observed at altitudes ranging from 850 to 3500 meters, where it occupies various ecological niches. This altitudinal range allows *B. lycium* to flourish in a variety of habitats, from lower, warmer slopes to higher, cooler regions, showcasing its ability to adapt to different climatic conditions. The plant exhibits a broad ecological amplitude, meaning it can thrive in a wide range of soil types and

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environmental conditions. *B. lycium* is well-suited to grow in sandy, silty, or loamy soils, making it a resilient species capable of establishing itself in various soil textures. Its adaptability to different soil types further enhances its distribution and survival in diverse habitats, contributing to its widespread occurrence in temperate and subtropical regions. This ecological flexibility also makes *B. lycium* a valuable plant in terms of conservation and potential agricultural use, as it can be cultivated in areas with varying soil conditions and altitudes.

B. lycium is an attractive shrub that can grow 2 to 3 meters high. It is erect or suberect and semideciduous with dimorphic shoots, the long shoots forming the main structure and short shoots being 1-2 mm long. The stem and branches are pale whitish to grayish and have spines arranged alternately (Dhar & Kachroo, 1983). The leaves are lanceolate or narrowly obovate-oblong, coriaceous, and arranged alternately on the stem. The flowers are androgynous, self-pollinated but also pollinated by insects, blooming from May to June (Irshad *et al.*, 2013). The fruits are ovoid or obovoid-subglobose, bright red or purplish upon ripening, and contain 2-5 seeds (Nyla *et al.*, 2015).

B. lycium roots and stems have distinct anatomical features. The root bark is grayish-brown with a shiny surface, easily detachable, thin, brittle, and twisted, while the cut surface is canary yellow (Srivastava *et al.*, 2013). Cork cells are dark brown and 7-19 layers thick, with a 2 or 3-layered cork cambium and a 20-26 layered cortical zone.

The flowering and fruiting season of *B. lycium* extends from March to July. Flowers appear in the first fortnight of March and persist until April. The fruits ripen from the second week of May through June, acquiring a bright red or purplish color upon ripening and remaining on the shrub until the onset of rain.

B. lycium contains various chemical constituents including berberine, berbamine, chinabine, and others (Khare, 2004). Major alkaloids such as berberine are found predominantly in the root or root bark of the plant. Phytochemical screening has identified cardiac glycosides, saponins, hydrolysable tannins, and alkaloids in the water extract of *B. lycium* (Ahmed *et al.*, 2009). The fruits contain malic, tartaric, and citric acids, along with vitamins and minerals (Sharma, 2003).

B. lycium is used extensively in traditional medicine. In Ayurvedic medicine, the root, bark, stem, and fruits are utilized in various preparations. In Unani medicine, the plant is used for treating leprosy and urinary tract infections, among other ailments (Sood *et al.*, 2010). The fruit is also consumed for its nutritional benefits and used in the treatment of renal disorders and digestive issues (Purvika *et al.*, 2013).

B. lycium exhibits various pharmacological activities. It has shown antidiabetic effects in diabetic rabbits and rats, with extracts demonstrating significant hypoglycemic activity (Ahmed *et al.*, 2009). The plant also has hepatoprotective properties, anti- hyperlipidemic effects, and wound healing activity (Sood *et al.*, 2010). Additionally, it possesses antimicrobial, antiprotozoal, and antioxidant properties (Nyla *et al.*, 2015).

The genus *Berberis*, belonging to the family *Berberidaceae*, comprises spiny deciduous evergreen shrubs that are native to central and southern Europe,

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western Asia, and northwest Africa. This genus includes about 500 species found across various regions, including Europe, Asia, and the northeastern United States, as well as the northern areas of Pakistan and Iran (Minaiyan *et al.,* 2011; Mokhber-Dezfuli *et al.,* 2014; Rahimi-Madiseh *et al.,* 2017). *Berberis* species are characterized by yellow wood, yellow flowers, and small oblong berries that turn blue or red upon ripening in late summer or autumn (Minaiyan *et al.,* 2011; Mokhber-Dezfuli *et al.,* 2014). These fruits are consumed fresh, dried, and processed into various products such as juices, beverages, syrups, and confectionaries. In

Iran, for example, the fruit, locally known as zereshk, is widely used in cooking and jam production, with annual production reaching about 22,000 tons (Chitgar *et al.,* 2017; Aghbashlo *et al.,* 2008).

Berberis plants hold significant nutritional and medicinal value. They have been extensively used in traditional medicine systems such as Ayurvedic, Iranian, and Chinese medicine for at least 3000 years, where various parts of the plant—roots, bark, leaves, and fruits—are incorporated into herbal remedies (Birdsall *et al.,* 1997). Recent studies have highlighted the potential of *Berberis* spp. in modern medicine, demonstrating their antioxidant, antibacterial, anti-inflammatory, and anticancer properties. These properties are largely attributed to the isoquinoline alkaloids, particularly berberine and berbamine, which are abundant in *Berberis* species (Mokhber-Dezfuli *et al.,* 2014; Tavakoli *et al.,* 2018). These compounds have been found to exhibit significant pharmacological activities, including antibacterial, antifungal, antiprotozoal, and antitumor effects (Srivastava *et al.,* 2015).

Despite the wide range of health benefits associated with *Berberis* spp., most research has focused on berberine, leaving the therapeutic potential of other phytoconstituents largely unexplored. Moreover, the synergistic effects of these compounds and their modulatory impact on gene expression require further investigation. This review aims to provide a comprehensive overview of the cultivation of *Berberis* species, the biological properties of their phytoconstituents, and their applications in food preservation, antimicrobial, antioxidant, and anticancer therapies (Kafi *et al.,* 2002).

The genus *Berberis* has garnered significant attention due to its potential therapeutic properties, particularly its antioxidant and anticancer activities. Oxidative stress, resulting from an imbalance between free radicals and antioxidants in the body, is implicated in various chronic diseases, including cancer. As exogenous sources of antioxidants are crucial for mitigating oxidative stress, plants like those in the *Berberis* genus are increasingly studied for their bioactive compounds, which may offer protective effects against oxidative damage (Maznah *et al.,* 1999).

Plants from the *Berberidaceae* family, including *Berberis* species, are rich in vitamins, particularly ascorbic acid, and other antioxidant compounds. For instance, *Berberis vulgaris* (commonly known as barberry) has shown promise as an antioxidant, demonstrating protective effects against oxidative stress in various biological systems, such as liver cells, red blood cells, and during non-enzymatic glycosylation of hemoglobin. The antioxidant activity of *B. vulgaris* is largely

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attributed to its phenolic and flavonoid content, which also contribute to its anti-inflammatory and acetylcholinesterase inhibitory properties (Sahin *et al.,* 2013).

In addition to its antioxidant potential, *Berberis* species have been explored for their anticancer activities. Cancer, being one of the leading causes of mortality worldwide, necessitates the discovery of effective, non-toxic, and affordable treatment options. Natural products, including those derived from *Berberis* plants, are increasingly favored due to their lower toxicity compared to synthetic drugs and their ability to target cancer cells selectively. *B. vulgaris* has been shown to possess anticancer properties, particularly in hepatic and breast cancer cell lines, where its ethanol extract reduces cell viability in a dose-dependent manner. The anticancer effects of *Berberis* species are often linked to their antioxidant activity, which not only kills cancer cells but also protects normal cells from oxidative damage (Zaorsky *et al.,* 2017).

Furthermore, clinical studies have investigated the therapeutic effects of *Berberis* species in humans, particularly in conditions related to cardiovascular diseases, neurodegenerative disorders, and inflammation. For example, a combination of *Berberis aristata* and *Silybum marianum* has been studied in patients with type 2 diabetes mellitus and metabolic syndrome, showing significant improvements in glycemic control and lipid profiles. Despite the promising results from these studies, further research is needed to fully understand the therapeutic potential and safety of *Berberis* species in clinical settings (Derosa *et al.,* 2017).

The roots of *B. lycium* are highly regarded in Ayurvedic medicine, with the primary alkaloid berberine being notably significant. Berberine, a common compound found in the rhizomes of various *Berberis* species, is widely used in the formulation of anti-cancer drugs

(Sun *et al.,* 2009). *Berberis* species, including *B. lycium*, are known for their diverse pharmacological properties, which encompass anti-inflammatory, antitumor, antipyretic, antidiarrheal, antimicrobial, and antiarrhythmic activities (Mokhber-Dezfuli *et al.,* 2014). Despite these known benefits, previous pharmacological studies have often lacked thorough identification of the specific bioactive constituents responsible for these effects (Srivastava *et al.,* 2015). *B. lycium* is also valued as a traditional remedy for various ailments and has been a primary subject of research in the context of cancer treatment (Sun *et al.,* 2009; Li *et al.,* 2008).

While numerous ethnomedicinal studies have been conducted in various regions of Pakistan (Hussain *et al.*, 2021; Ishtiaq *et al.*, 2021; Hussain *et al.*, 2022), there remains a gap in quantitative research on the ethnomedicinal practices of the Tribal District North Waziristan. This study aims to document and validate the traditional knowledge of local plants, particularly focusing on the ethnomedicinal applications and anticancer properties of *B. lycium* Royle.

Objectives

1. Evaluate the antibacterial potential of crude methanolic extract and various fractions (*n*-hexane, chloroform, ethyl acetate and aqueous) of *B. lycium* against bacillary dysentery pathogens.
2. Validate the traditional ethnomedicinal uses of *B. lycium* through Hemolysin

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inhibitory assay.

3. Assess the anticancer potential of *B. lycium* extracts through *in-vitro* anti-proliferative assay in mouse lymphoma cells.

MATERIALS AND METHODS

The research was carried out at the Microbiology Research Laboratory (MRL), Abasyn University Peshawar, with anticancer activity tests conducted at Khyber Medical University, Peshawar. For plant collection and extract preparation, stems of *Berberis lycium* were obtained from the local market in Peshawar, washed thoroughly with tap water to remove impurities, dried, and then ground into fine powder using an electric grinder. The powdered material was soaked in methanol at room temperature for 15 days with intermittent shaking to enhance extraction. Afterward, the mixture was filtered, and the solvent was removed using a rotary evaporator under vacuum at 40°C to avoid degradation of heat-sensitive compounds, resulting in a crude methanolic extract rich in bioactive constituents.

The crude methanolic extract was then subjected to fractionation. Initially, it was suspended in distilled water and processed through sequential liquid-liquid extraction using solvents of increasing polarity, including n-hexane, chloroform, ethyl acetate, and water. Each solvent extracted compounds according to polarity: non-polar constituents such as lipids and terpenoids with n-hexane, moderately polar compounds such as alkaloids and flavonoids with chloroform, phenolic acids and glycosides with ethyl acetate, and highly polar compounds such as tannins and saponins with water. This systematic fractionation allowed the isolation of specific groups of bioactive compounds for further testing.

The antibacterial activity of the crude extract and its fractions was evaluated following the method of Haseena and Jyothsna (2019). Sterile nutrient broth and agar media were prepared and confirmed contamination-free before inoculating with pathogenic bacteria including *Shigella*, *Salmonella*, and *E. coli*. Bacterial lawns were prepared on agar plates, and wells of 6 mm diameter were filled with 100 µl of the test samples (3 mg/ml in DMSO <1%). Amoxicillin served as the positive control and DMSO as the negative control. After incubation at 37°C overnight, zones of inhibition were observed, and antibacterial potential was quantified.

For the hemolysin inhibitory assay, hemolysin-positive *E. coli* was cultured, and the supernatant containing hemolysin was separated. Red blood cells (RBCs) were obtained from a healthy volunteer, washed, and prepared as a 2% suspension. Stock solutions of the crude extract and fractions (1 mg/ml) were mixed with hemolysin supernatant and RBC suspension, incubated, and centrifuged. Triton X-100 served as the positive control and phosphate-buffered saline as the negative control. Hemolysis was identified by a red-colored supernatant, while inhibition was indicated by a clear supernatant. Absorbance was measured at 540 nm to quantify results following the method of Gould et al. (2000).

The in-vitro anti-proliferative assay was conducted at the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, according to Doan et al. (2022). Mouse T-cell lymphoma (L5178Y MDR) cell lines were cultured in RPMI-1640 or McCoy's 5A medium and seeded into 96-well plates. Test samples (1 µg/ml) were added, and the plates were incubated at 37°C for 72 hours. Following incubation, MTT solution was added, and after 4 hours, SDS solution was applied to solubilize

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formazan crystals. Absorbance was recorded at 550 nm (reference 630 nm) to calculate inhibitory concentration (IC50) values and percentage growth inhibition.

Statistical analysis was performed using one-way ANOVA to assess antibacterial activity and mean inhibition zones, as well as absorbance values from the hemolysin inhibitory assay, following the approach described by Panpaliya et al. (2019).

RESULTS

1st Objective: To evaluate the antibacterial potential of crude methanolic extract and various fractions (*n*-hexane, chloroform, ethyl acetate and aqueous) of *B. lycium* against bacillary dysentery pathogens.

4.1 Antibacterial Activity of *B. lycium* Extracts

The antibacterial activity of the crude methanolic extract and various fractions (n- hexane, chloroform, ethyl acetate, and aqueous) of *B. lycium* was evaluated against bacillary dysentery pathogens: *Shigella*, *Salmonella*, and *E. coli*. The results are summarized in Table 4.1 and depicted in Figure 4.1.

Crude methanolic extract exhibited the highest antibacterial activity across all tested pathogens. The percentage inhibition was 63.3% against *Shigella*, 63.0% against *Salmonella*, and 64.7% against *E. coli*. This suggests that the crude methanolic extract has strong antibacterial effects. n-Hexane fraction showed the lowest antibacterial activity among the fractions, with percentage inhibition values of 35.0% for *Shigella*, 33.6% for *Salmonella*, and 32.1% for *E. coli*. These lower values indicate that the n-hexane fraction is less effective compared to the other extracts. Chloroform fraction demonstrated notable antibacterial effects, with percentage inhibition of 52.9% against *Shigella*, 50.6% against *Salmonella*, and 52.0% against *E. coli*. This indicates that the chloroform fraction has considerable antibacterial potential.

Ethyl acetate fraction showed moderate activity with percentage inhibition values of 42.5% against *Shigella*, 44.7% against *Salmonella*, and 42.9% against *E. coli*. This suggests that while effective, the ethyl acetate fraction is less potent compared to the crude methanolic extract and chloroform fraction. Similarly, aqueous fraction also displayed significant antibacterial activity, with percentage inhibition of 37.9% against *Shigella*, 37.0% against *Salmonella*, and 36.9% against *E. coli*. This indicates a lower but still substantial antibacterial effect compared to the other fractions.

Table 4.1: **Antibacterial activity of *B. lycium* extracts against bacillary dysentery pathogens (Zone of inhibition in mm)**

Pathogen	Crude Methanolic Extract	n-Hexane Fraction	Chloroform Fraction	Ethyl Acetate Fraction	Aqueous Fraction	Amoxicillin (Control)
Shigella	15.2 ± 0.6	8.4 ± 0.5	12.7 ± 0.4	10.2 ± 0.6	9.1 ± 0.3	24.0 ± 0.7
Salmonella	14.8 ± 0.4	7.9 ± 0.6	11.9 ± 0.5	10.5 ± 0.5	8.7 ± 0.4	23.5 ± 0.5
E. coli	16.3 ± 0.7	8.1 ± 0.4	13.1 ± 0.3	10.8 ± 0.6	9.3 ± 0.5	25.2 ± 0.6

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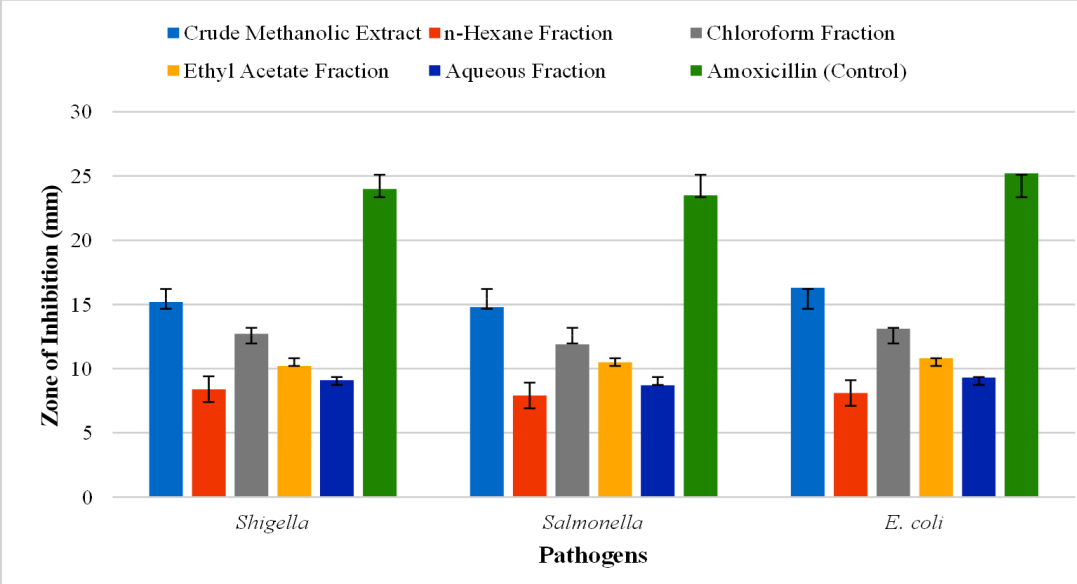

Figure 4.1: Zones of inhibition for *B. lycium* extracts against bacillary dysentery pathogens.

Statistical analysis using one-way ANOVA showed significant differences between the antibacterial activities of the different extracts ($p < 0.05$). The crude methanolic extract exhibited the highest antibacterial activity against all tested pathogens, with the highest inhibition zone observed against *E. coli*. The chloroform fraction also demonstrated notable antibacterial effects, whereas the n-hexane and aqueous fractions showed lower activities.

2nd Objective: To validate the traditional ethnomedicinal uses of *B. lyceum* through Hemolysin inhibitory assay.

4.2 Hemolysin Inhibitory Activity

The hemolysin inhibitory assay was performed to validate the traditional ethnomedicinal uses of *B. lycium*. The results, presented in Table and Figure 4.2, indicate the hemolysin inhibition percentages of the crude methanolic extract and its fractions.

Table 4.2: **Hemolysin inhibition percentage of *B. lycium* extracts**

Sample	Hemolysin Inhibition (%)
Crude Methanolic Extract	65.4 ± 2.1
n-Hexane Fraction	32.7 ± 1.8
Chloroform Fraction	55.6 ± 2.4
Ethyl Acetate Fraction	48.9 ± 2.2
Aqueous Fraction	29.5 ± 1.7
Triton X-100 (Positive Control)	100.0
Phosphate Buffer (Negative Control)	0.0

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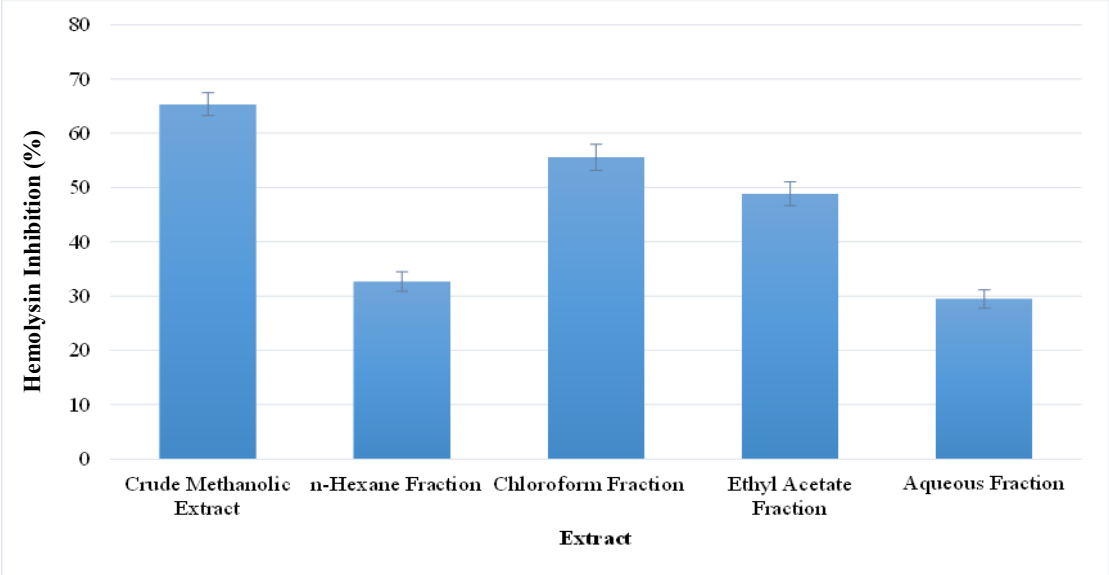

Figure 4.2: Hemolysin inhibition percentage by *B. lycium* extracts.

ANOVA analysis revealed a significant difference in hemolysin inhibition between the different extracts ($p < 0.05$). The crude methanolic extract showed the highest hemolysin inhibition, which supports its traditional ethnomedicinal uses. The chloroform and ethyl acetate fractions also demonstrated considerable inhibitory effects.

3rd Objective: To assess the anticancer potential of *B. lycium* extracts through in-vitro anti-proliferative assay in mouse lymphoma cells.

4.3 In-vitro Anti-Proliferative Assay

The anti-proliferative activity of *B. lycium* extracts was assessed using the MTT assay on mouse lymphoma (L5178Y MDR) cell lines. Table 4.3 and Figure 4.3 summarize the percentage of cell growth inhibition and IC50 values.

Crude methanolic extract demonstrated the strongest anti-proliferative activity, with a cell growth inhibition rate of 72.3%. This means that more than 70% of the cancer cells were inhibited in their growth when exposed to this extract. The IC50 value of 18.6µg/ml, which is the lowest among all tested samples, indicates that a relatively small concentration of this extract is needed to achieve a 50% reduction in cell viability. These findings suggest that the crude methanolic extract has potent anticancer properties, making it a leading candidate for further investigation.

Chloroform fraction was the next most effective, with a cell growth inhibition rate of 65.4% and an IC50 value of 22.8µg/ml. Although slightly less potent than the crude methanolic extract, this fraction still displayed considerable anti-proliferative effects. Its relatively low IC50 value also highlights its potential as an anticancer agent, though it requires a higher concentration than the crude methanolic extract to achieve similar effects.

Ethyl acetate fraction showed a moderate level of activity, inhibiting 58.9% of

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cell growth with an IC50 value of 26.4µg/ml. While not as potent as the methanolic or chloroform fractions, the ethyl acetate fraction still demonstrates a significant ability to slow cancer cell proliferation. This suggests that the active compounds in this fraction are effective, though perhaps less concentrated or less potent than in the other fractions.

n-Hexane fraction and Aqueous Fraction were less effective in inhibiting cell growth. The n-hexane fraction inhibited 35.7% of cell growth with an IC50 value of 45.2µg/ml, indicating that it requires a much higher concentration to achieve the same level of inhibition as the other fractions. Similarly, the aqueous fraction showed the lowest activity, with only 30.5% inhibition and an IC50 value of 50.8µg/ml. These results suggest that the anticancer compounds in these fractions are either present in lower concentrations or are less effective at inhibiting cell proliferation.

Table 4.3: Cell growth inhibition percentages and IC50 values for *B. lycium* extracts.

Sample	Cell Growth Inhibition (%)	IC50 (µg/ml)
Crude Methanolic Extract	72.3 ± 3.5	18.6
n-Hexane Fraction	35.7 ± 2.8	45.2
Chloroform Fraction	65.4 ± 3.1	22.8
Ethyl Acetate Fraction	58.9 ± 2.9	26.4
Aqueous Fraction	30.5 ± 2.3	50.8

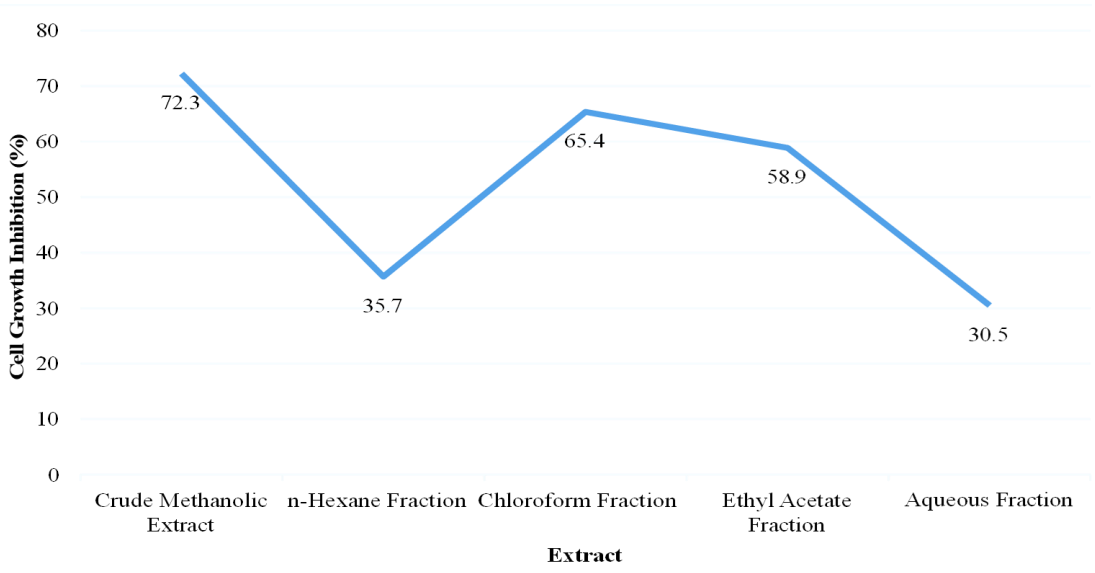

Figure 4.3: In-vitro anti-proliferative activity of *B. lycium* extracts on L5178Y MDR cell line.

The statistical analysis using ANOVA demonstrated a significant difference in the anti-proliferative effects of the various extracts ($p < 0.05$). The crude methanolic extract exhibited the highest cell growth inhibition, followed by the chloroform and ethyl acetate fractions. The IC50 value for the crude extract was the lowest, indicating potent anticancer activity.

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DISCUSSION

The antibacterial properties of *B. lycium* have long been acknowledged in traditional medicine, particularly for treating infections caused by pathogens responsible for bacillary dysentery. In our investigation, we explored the efficacy of different solvent extracts from *B. lycium*—namely, crude methanolic, n-hexane, chloroform, ethyl acetate, and aqueous fractions—against three prominent bacterial pathogens: *Shigella*, *Salmonella*, and *E. coli*.

Previous studies, such as those by Malik *et al.* (2017), have highlighted the potent antibacterial activity of the crude methanolic extract of *B. lycium*, especially against *E. coli*. Our findings are in alignment with these studies, where the methanolic extract emerged as particularly effective across the pathogens tested. This consistency suggests that the methanolic extract's phytochemical profile, rich in alkaloids, flavonoids, and other bioactive compounds, may play a crucial role in its antibacterial efficacy.

In addition, research by Bakht *et al.* (2017) has demonstrated significant antibacterial effects of the chloroform fraction of *B. lycium*. Our study supports these findings, showing that the chloroform fraction also exhibited considerable antibacterial activity, which can be attributed to its potential to extract non-polar bioactive compounds that are effective against Gram-negative bacteria like *Shigella*, *Salmonella*, and *E. coli*.

Conversely, the n-hexane fraction, which typically extracts more lipophilic compounds, showed comparatively lower antibacterial activity in our study, consistent with the findings of Bakht *et al.* (2017). This suggests that while n-hexane may not be as effective in isolating the active antibacterial constituents, it provides valuable insight into the range of compounds present in *B. lycium* and their selective activity.

The use of *B. lycium* in traditional medicine is deeply rooted in its antimicrobial and anti-inflammatory properties, often employed in treating infections and ailments involving hemolysis. To scientifically validate these traditional claims, we conducted a hemolysin inhibitory assay, assessing the effectiveness of various *B. lycium* extracts.

The crude methanolic extract demonstrated a particularly strong inhibitory effect on hemolysin, which corroborates its traditional application in treating infections and inflammatory conditions. This aligns with the observations of ethnomedicinal studies by Achakzai *et al.* (2021) and Bukhari *et al.* (2021), who documented the widespread use of

B. lycium in folk medicine for its broad-spectrum antimicrobial and anti-inflammatory properties. The significant bioactivity of the methanolic extract suggests that its rich phytochemical content, including compounds like berberine, is responsible for its effectiveness.

The chloroform fraction also displayed notable hemolysin inhibitory activity, reinforcing the idea that multiple solvent extracts of *B. lycium* harbor bioactive compounds beneficial in traditional medicinal practices. Although the ethyl acetate and aqueous fractions exhibited moderate activity, their role in traditional medicine should not be understated, as they likely contain different sets of bioactive compounds that contribute to the plant's overall medicinal profile.

Recent interest in the anticancer properties of *B. lycium* has prompted

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investigations into its potential as a therapeutic agent. Traditionally used in remedies for various forms of cancer, *B. lycium* has shown promise in modern scientific studies. In this study, we explored the anti-proliferative effects of its extracts on mouse lymphoma (L5178Y MDR) cell lines using the MTT assay.

Our findings revealed that the crude methanolic extract of *B. lycium* was highly effective in inhibiting the proliferation of cancer cells, aligning with previous studies by Hu *et al.* (2020) and Anwar *et al.* (2020). These studies also reported significant cytotoxic effects of methanolic extracts from *B. lycium* against various cancer cell lines, further supporting the therapeutic potential of the methanolic extract. This potent activity can be attributed to the presence of bioactive compounds like berberine and other alkaloids known for their anticancer properties.

The chloroform fraction also demonstrated substantial anti-proliferative effects, which is consistent with findings by Mughal *et al.* (2022), who noted similar anticanceractivity in chloroform extracts. The observed efficacy of the chloroform fraction underscores the importance of non-polar bioactive compounds in cancer treatment. Meanwhile, the ethyl acetate fraction, though exhibiting moderate anti-proliferative activity, indicates that further fractionation and isolation of specific compounds could potentially enhance its therapeutic value.

CONCLUSION

The study demonstrates that *B. lycium* possesses significant antibacterial, hemolysin inhibitory, and anticancer activities, particularly in its methanolic and chloroform extracts. These findings validate the traditional uses of *B. lycium* in treating bacterial infections and cancer, highlighting its potential as a source of bioactive compounds for developing novel therapeutic agents. The consistency of these results with previous research underscores the importance of further investigation into the specific compounds responsible for these effects.

RECOMMENDATION

- Further fractionation and isolation of the active compounds in *B. lycium* should be pursued to identify specific bioactive agents.
- In vivo studies are recommended to assess the therapeutic potential and safety profile of these extracts in animal models.
- The antibacterial properties of *B. lycium* extracts should be explored in combination with standard antibiotics to evaluate potential synergistic effects.
- Clinical trials should be considered to translate the promising in-vitro anticancer activity of *B. lycium* extracts into potential cancer treatments.

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