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Phytochemistry, Biological Activities, and Chemical Profiling of *Berberis asiatica*

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Abstract

This study focused on chemical profiling and assessed the total phenolic and flavonoid content as well as the antioxidant and antibacterial effect of the medicinal plant Berberis asiatica. The results revealed that this plant has high concentrations of TPC (Total phenol content) and TFC (Total flavonoid content) of 37.686 ± 2.728 *mg GAE/g and* 115.568 ± 8.012 *mg QE/g, respectively. The DPPH free radical scavenging assay demonstrated strong inhibition, with an* IC_{50} *of* $205.7 \pm 5.353 \mu g/mL$*, and also showed robust antibacterial properties against Staphylococcus aureus and Klebsiella pneumoniae with a zone of inhibition (ZOI) of 14 mm and 19 mm, respectively. The extract exhibited an excellent inhibitory potential against S. aureus and K. pneumoniae with a MIC (Minimum inhibitory concentration) of 0.39 mg/mL and 3.125 mg/mL respectively, indicating significant inhibitory action. Furthermore, the MBC (Minimum bactericidal concentration) for both S. aureus and K. pneumoniae was found to be 6.25 mg/mL, emphasizing the extract's consistent bactericidal effectiveness against these bacteria. These findings underscore the potential utility of the methanolic extract of Berberis asiatica as a natural antibacterial agent. GC-MS analysis of hexane fraction indicates the plant is rich in secondary metabolites, specifically 2,2-dimethyl-3-pentanol, 2-methyl-2-pentanol, 2,5-dimethyl-4-hydroxy-3-hexanone, 3-hexanol, 4-methyl-2-pentanol are identified. Overall, this study highlights the importance of plant-based natural products as potential sources of antioxidants and antibacterial agents that contribute to the future drug development process.*

Keywords
Berberis asiatica, antibacterial activity, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), *Staphylococcus aureus*, *Klebsiella pneumoniae*

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1 Introduction

Traditional medicines rely on natural products to treat diseases, and modern pharmaceutical medications are often derived from natural sources [1]. In recent years, there has been an assessment of plant-derived natural products as promising candidates for drug development [2]. The medicinal plant serves as a valuable and organic reservoir of bioactive elements that play a significant role in contemporary medicine [3]. Various plant-derived natural products, including alkaloids, phenols, and flavonoids, have been discovered with capabilities to induce apoptosis, and migration and exhibit potential against cancer, oxidation, diabetes, and inflammation [2, 4]. Renewed interest and focus on medicinal plant research have been triggered by the rise in side effects, the emergence of new intestinal pathogens, and the increasing problem of microbial resistance [5].

In this research, we examined the antioxidant, antibacterial, and alpha-amylase inhibition properties of *Berberis asiatica*, a medicinal plant grown in Nepal belonging to the family Berberidaceae. *Berberis* plants of the Berberidaceae family are extensively distributed across the globe, comprising almost 550 species [6]. Genus *Berberis* is globally recognized in traditional medicine for its potent medicinal properties. Its roots and bark are employed as antipyretic, anti-periodic, and diaphoretic [7]. Barberry fruits, due to their significant richness in anthocyanin content, are consumed as an antidiabetic drug [8]. Among many species of *Berberis*, *Berberis asiatica* is widely used in traditional medicinal practices to cure diabetes and infectious diseases [9].

Berberis asiatica, commonly known as Chutro in Nepali, is a wild shrub found in Nepal between elevations of 1200 to 2500 meters. It thrives in shaded, rocky slopes, north-facing hillsides, and drier regions. This erect, spiny shrub grows up to 4 meters tall, sporting yellow bark. Its leaves are simple, alternate, leathery, dark green on top, and grayish underneath, usually with 2-5 spiny teeth along the margins. The shrub produces pale yellow flowers in flat-topped clusters and dark purple berries with a sweet and sour taste [10, 11]. Traditionally, different parts of *Berberis asiatica* were used for medical purposes. Root and bark have extensive use in indigenous medicine for treating various ailments, including eye and ear diseases, rheumatism, jaundice, diabetes, fever, stomach disorders, skin disease, and malarial fever [9]. These root and stem bark are integral to a renowned Ayurvedic medicine, containing several alkaloids such as berberine, palmatine, jatrorrhizine, columbamine, berbamine, oxyberberine, and oxyacanthine [12]. These secondary metabolites play an important role in treating different diseases and disorders in the human body. It was reported that berberine plays a vital role in controlling diabetes by inhibiting digestive enzymes alpha-amylase and alpha-glucosidase [13].

Oxidation, a fundamental metabolic procedure present in all life forms, holds particular significance in humans as it underpins the production of energy. However, these biochemical reactions create free radicals such as reactive oxygen species (ROS), potentially amplifying oxidative stress and causing potential harm to essential biological components like lipids, proteins, and DNA [14, 15]. As time passes, the harmful effects of ROS become more prominent and are associated with aging and age-related illnesses like heart diseases, cancer, brain disorders, diabetes, and other chronic diseases [16]. Furthermore, diabetes represents a significant metabolic syndrome with abnormally high blood glucose levels (hyperglycemia), and oxidative stress is closely associated with this condition, especially in individuals with type 2 diabetes [17, 18]. Type 2 diabetes mellitus is a multifaceted and diverse collection of metabolic disorders marked by increased serum glucose levels, primarily resulting from deficiencies in both the function of insulin and its secretion [19]. Roughly, 90 to 95 % of diabetes cases are attributed to type 2 diabetes [20]. To managing diabetes, one of the main approaches involves controlling postprandial hyperglycemia through the inhibition of the carbohydrate hydrolyzing enzyme alpha-amylase, responsible for breaking down long-chain carbohydrates like starch, amylase, and amylopectin into glucose, and another is by reducing oxidative stress [14,20,21]. Hence, a novel and promising drug is required in modern times to effectively manage diabetes and oxidative stress. As a result, there has been a concentrated effort to discover novel antidiabetic compounds from plants utilized in traditional medicine [22]. Furthermore, medicinal plants offer a valuable source of antimicrobial agents [23]. Several plants belonging to *Berberis* are renowned for their antibacterial properties [24, 25]. Notably, *Berberis asiatica* has generated particular attention as a source of novel compounds with remarkable antibacterial activity. Taking into account the aforementioned factors, this study was undertaken to explore the antioxidants, antibacterial, and α-amylase inhibition properties using in vitro assays, aiming to contribute significant insights into the holistic understanding of *Berberis asiatica*.

2 Materials and Methods

2.1 Chemicals

All chemicals and reagents utilized in this study were of analytical grade. Gallic acid,

Folin-Ciocalteu's reagent, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acarbose, α-amylase from porcine pancreases, 2-chloro-4-nitrophenyl--D-maltotrioside (CNPG3) were demanded from Sigma-Aldrich.

2.2 Plant Collection and Preparation of Extract

The whole plant of the *Berberis asitica* was collected from the Arghakhanchi district of Nepal growing at an altitude of 1810 m in October 2021 based on ethnobotanical approaches. The plant was verified in the National Herbarium and Plant Laboratories Godavari, Kathmandu, Nepal. To prepare the plant extract, freshly collected whole plant parts (leaves, bark, and roots) were washed with tap water to remove the contaminants, and the collected samples were dried and ground into a fine powder. For the extraction process, methanol was used as a solvent. Due to high volatility, lower boiling point, and strong polarity, methanol is an ideal choice for the extraction process [26,27]. Moreover, methanol is renowned for its ability to yield higher extraction efficiency as compared to other solvents and its efficacy in extracting a diverse range of compounds, including antibacterial compounds, antioxidants, polyphenols, flavonoids, alkaloids, and terpenoids [28–32]. Furthermore, methanol is cost-effective and readily available, making it an easily manageable choice as a preferred extraction solvent.

75 grams of powder was then mixed with 300 mL methanol in a conical flask and meticulously shaken to completely mix the powder in the solvent. Then the conical flask was covered with aluminum foil and left to undergo cold percolation for 72 hours at room temperature. Subsequently, the mixture was filtered using a muslin cloth, and filtrate was collected in a beaker. This process was repeated for subsequent 2 times to get a higher amount of the extract. The filtrate was concentrated using a rotary evaporator at a temperature of 40^0C, and concentrated solution was kept in the water bath for fast evaporation of the solvent. The crude extract was stored in the refrigerator at 4 ^{0}C for the subsequent evaluation of various biological activities, following the standard procedures.

2.3 Phytochemical Analysis

The recently prepared raw extracts of *B. asiatica* were subjected to qualitative tests to analyze the presence or absence of various plant secondary metabolites including alkaloids, flavonoids, steroids, terpenoids, reducing sugars, saponins, tannins, cardiac glycosides, anthraquinones. For qualitative phytochemical analysis, a series of tests were carried out according to the protocols of [33,34].

2.4 Total Phenolic Content (TPC)

To determine the TPC, the Folin-Ciocalteu phenol assay was employed [35,36]. The dry methanolic extract was prepared at a concentration of 5 mg/mL in 30% DMSO. A sample (20 µL) of the plant extract and a standard solution of gallic acid (ranging from 10 to 80 µg/mL) was mixed with Folin-Ciocalteu phenol reagent (100 µL) and sodium carbonate (80 µL). After a 30-minute incubation, the absorbance was measured at 765 nm using a microplate reader (Synergy LX, Bio-Tek, Instruments, Inc., USA). The TPC was calculated using a standard curve and expressed as milligrams of gallic acid equivalent (mg GAE) per gram of plant extract.

2.5 Total Flavonoid Content (TFC)

To estimate the Total Flavonoid Content (TFC), the aluminum chloride ($AlCl_3$) procedure was employed [37]. A 20 μL of the stock solution of the plant extract and 130 µL of the standard quercetin solution (ranging from 10 to 80 µg/mL) were loaded in 96-well plates. The standard quercetin solution was prepared by diluting a stock solution of 0.1 mg/mL. Subsequently, to maintain the volume of 110 µL, distilled water was added to the plant extract in triplicate. Additionally, 60 µL ethanol, 5 µL 10% $AlCl_3$, and 5 µL potassium acetate were added to each well. After a 30-minute incubation at room temperature, the absorbance was measured at 415 nm using a microplate reader (Synergy LX, Bio-Tek Instruments, Inc., USA). The TFC was calculated using a standard curve and expressed as milligrams of quercetin equivalent (mg QE) per gram of plant extract.

2.6 Evaluation of Antioxidant Activity

To evaluate the free radical scavenging activity, the DPPH radical scavenging assay was employed [38,39]. A solution of 0.1 mM DPPH was prepared, and a stock solution of quercetin was also prepared. Quercetin served as the positive control, while 50% DMSO was used as the negative control. The positive control, negative control, and plant samples were loaded into separate wells of a 96-well plate in triplicate. Subsequently, the DPPH reagent was added to each well, followed by a 30-minute incubation period in darkness. After incubation, the absorbance at 517 nm was measured using a microplate reader. Percentage inhibition of the tested sample was calculated using the following formula.

$$\%\text{inhibition} = \frac{[\text{Abs. of control-Abs. of sample}]}{\text{Abs. of control}} \times 100$$

2.7 $\alpha - AmylaseInhibitionActivity$

To assess the inhibitory activity of α-amylase, an *in vitro* assay was conducted using CNPG3 as the substrate [40]. Plant samples of various concentrations (20 L) were mixed with α-amylase (80 L at a final concentration of 1.5 U/mL) and incubated at 37 °C for 15 minutes. The reaction was initiated by adding 100 L of the substrate, CNPG3 (final concentration of 0.5 mM), prepared in the buffer. The experiment was performed in a 50 mM phosphate buffer. After incubation, the absorbance was measured at 405 nm using a microplate reader. DMSO (30%) was used as the negative control, while acarbose served as the standard. The inhibition percentage was calculated using the provided formula.

$$\%\text{inhibition} = \frac{[\text{Abs. of control-Abs. of sample}]}{\text{Abs. of control}} \times 100$$

2.8 Determination of Antibacterial Activity

The antibacterial activity of the extract was evaluated using the agar well diffusion method on Mueller-Hinton Agar (MHA) plates [41]. The test organisms gram-positive *Staphylococcus aureus* (ATCC 25293), and gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella Typhi* (ATCC 14028), and *Klebsiella pneumoniae* (ATCC 13883) were cultured in Mueller-Hinton broth. These tested bacteria *Staphylococcus aureus* and *Escherichia coli* were obtained from clinical isolation whereas *Salmonella typhi* was isolated from the liver of the four-week-old chicken. These cultured bacteria were incubated at 37 ^{0}C until the turbidity reached a standardized 0.5 McFarland unit. The MHA plates were then uniformly inoculated with the microbial suspension. A stock solution of the plant extract at a concentration of 5 mg/mL was prepared. Using a sterile cork borer with an 8 mm diameter, three wells were created on the agar surface. Each well was filled with 50 μL of the plant extract, along with a positive control consisting of neomycin at a concentration of 1 mg/mL, and a negative control consisting of 50% DMSO. These controls were included on each plate. After allowing approximately 15 minutes for the extracts and controls to diffuse into the agar at room temperature, the plates were incubated at 37^{0}C for 18-24 hours. Following the incubation period, the plates were examined for the presence of clear zones of inhibition (ZOI) surrounding the wells. The diameter of the ZOI was measured in millimeters using a ruler, providing a quantitative assessment of the antimicrobial activity of the plant extract.

2.9 Determination of Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration (MIC), which represents the smallest amount of compounds needed to inhibit or kill microorganisms *in vitro*, can be achieved through the broth microdilution method [42]. In this method, the methanolic extract of the plant exhibiting significant antibacterial activity was subjected to a two-fold serial dilution. Under aseptic conditions, a sterile 96-well plate was filled with 100 µL of Mueller Hinton broth in each well. The extract was double diluted by adding equal volumes (100 µl) of the extract to MHB to get a series of concentrations ranging from 25 mg/mL to 0.012 mg/mL in plates. Next, 5 µL of the prepared bacterial inoculums adjusted to 0.5 McFarland Standard was added in each dilution. The microplate was incubated at 37 oC for 12 to 18 hours. After incubation, 10 µL of a 0.003% resazurin solution was added to wells, followed by an additional incubation period of 2 to 3 hours. Any color change from purple/blue to pink or colorless was recorded as a positive result. In this test neomycin solution was taken as positive control and DMSO solution was placed as negative control. The MIC value was determined as the lowest concentration at which no color change occurred, indicating the inhibition of bacterial growth by the plant extract.

2.10 Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by subculturing the MIC cultures on suitable agar plates. The sterile nutrient agar plate was taken in which solution from the microplate was loaded by streaking with the help of an inoculating loop. The solutions of the well showing MIC value and above were loaded in the plate. The plates were incubated at 37^{0}C for 18-24 hours. Then plates were examined for the growth of microorganisms. The tubes with a minimum concentration of extract in which the growth was completely checked were noted as the MBC of the plant extract [10]. MBC is complimentary to MIC and it is the lowest concentration of antibacterial agent that has a reducing capacity of viability of initial bacterial inoculum up to 99.9%.

2.11 GC-MS Profiling of Chemical Compounds

In this study, for the chemical characterization hexane fraction was fractionated from the methanolic extract and this fraction was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent 7890 GC instrument and an

Agilent 5975C inert MSD with Triple-Axis Detector. The analysis employed a gas carrier of helium at a flow rate of 1 mL/min. The sample injection volume was set at 2 μL, and the temperature program consisted of an initial temperature of 32^0C, followed by a ramping rate of 5^0C/min up to 230^0C, with a final hold time of 15 minutes. The auxiliary heater was set to 230^0C to ensure optimal conditions for efficient analysis. These carefully optimized analytical conditions provided valuable insights into the chemical composition of the hexane fraction, facilitating the identification and quantification of its compounds. These findings enhance our understanding of the chemical profile of *B. asiatica* and its potential therapeutic applications.

2.12 Statistical Analysis

All the experiments were performed in triplicate and data were presented in ± standard error of the mean. The TPC, TFC, antioxidant assay, and enzyme inhibition were calculated by Microsoft Excel 2007. The IC_{50} value was calculated using GraphPad Prism software.

3 Results and Discussion

3.1 Percentage Yield

The percentage yield was calculated to measure the abundance of secondary metabolites in the whole plant sample used in the study. The percentage yield of *B. asiatica* was found 10.66%, which is shown in Table 1.

3.2 Phytochemical Screening of Crude Extract

Preliminary phytochemical analysis was performed for the analysis of the presence and absence of different secondary metabolites. The result of the phytochemical screening of the methanolic extract of *Berberis asiatica* is shown in Table 2.

3.3 Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) were estimated by constructing a calibration curve taking different concentrations of gallic acid and quercetin, against the absorbance. TPC was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g), while TFC was expressed as milligrams of quercetin equivalent per gram (mg QE/g). The total phenolic content of the methanolic extract of *B. asiatica* was determined to be 37.686 ± 2.728 mg GAE/g, while the total flavonoid content was estimated as 115.568 ± 8.012 mg QE/g.

3.4 Antioxidant Potential

At a concentration of 1000 g/mL, the crude extracts of *B. asiatica* demonstrated significant inhibitory activity of 89.588% against the DPPH free radical. As a result, further dilution of the samples was performed to determine the IC_{50} value. The methanolic extract of *B. asiatica* exhibited noteworthy radical scavenging activity, with an IC_{50} value of 205.7 ± 5.353 g/mL. This result was compared to the IC_{50} value of the standard quercetin, which was found to be 6.29 ± 1.02 g/mL.

Table 1: Physical characteristics and percentage yield of plant extract.

Plant	Color of Extract	Weight of Dry Sample (g)	Weight of Extract (g)
B. asiatica	Yellow	75	8

Table 2: Phytochemical screening of whole plant extract.

Phytochemicals	Methanolic extract of *Berberis asiatica*
Alkaloids	+
Flavonoids	+
Tannins	-
Saponins	+
Terpenoids	+
Steroids	+
Glycosides	+
Reducing sugars	+
Coumarins	+
Polyphenols	+
Volatile oils	-

where + represents the presence of metabolites and - represents absence.

Table 3: Total phenolic and flavonoid content in methanolic extract of *Berberis asiatica.*

Whole Plant Extracts	TPC (mg GAE/g)	TFC (mg QE/g)
Berberis asiatica	37.686 ± 2.728	115.568 ± 8.012

3.5 *In Vitro* α-Amylase Inhibition Activity

The crude methanolic extracts of *B. asiatica* exhibited an inhibitory activity of 37.732% against α-amylase at a concentration of 500 g/mL. Therefore, additional dilution of the samples was not carried out to determine the IC_{50} value. According to the literature, it has been observed that plants contain secondary metabolites that possess properties similar to metformin, which are known to aid in the control of diabetes [43].

3.6 Antibacterial Activity

The methanolic extract of *B. asiatica*, at a concentration of 50 mg/mL, exhibited varying inhibitory effects against different bacteria. Against *S. aureus*, moderate inhibitory activity was observed, with a zone of inhibition measuring 14 mm. However, no inhibitory effect was detected against *E. coli* and S. typhi. In contrast, the extract displayed a strong inhibitory activity against K. pneumoniae, as evidenced by a substantial zone of inhibition measuring 19 mm. These results indicate that the methanolic extract of *B. asiatica* possesses antibacterial properties, particularly against *S. aureus* and *K. pneumoniae* while showing no inhibitory effect on *E. coli* and *S. typhi.*

3.7 Minimum Inhibitory Concentration

The methanolic extract of *Berberis asiatica* demonstrated significant antibacterial activity against two types of bacteria, namely *S. aureus* (gram-positive) and *K. pneumoniae* (gram-negative). To determine the potency of the extract in inhibiting the growth of these microorganisms in vitro, the Minimum Inhibitory Concentration (MIC) values were determined using the two-fold serial broth microdilution method. MIC is expressed in terms of mg/mL. The MIC shown by the methanolic extract of *Berberis asiatica* and the standard antibiotic neomycin (positive control) against the test bacteria are summarized in Table 6.

The results indicate that the methanolic extract of *Berberis asiatica* exhibited excellent inhibitory activity against S. aureus, with a MIC value of 0.39 mg/mL. Although the methanolic extract also demonstrated inhibitory activity against *K. pneumoniae*, it was less potent than the positive control Neomycin. The MIC value for *K. pneumoniae* was 3.125 mg/mL for the plant extract, whereas it was 0.0019 mg/mL for Neomycin.

3.8 Minimum Bactericidal Concentration

MBC is the lowest concentration of antibacterial agent that has a reducing capacity of viability of initial bacterial inoculum up to 99.9%. MBC is complimentary to MIC. Methanolic extract of *Berberis asiatica* exhibited minimum bactericidal concentration for *S. aureus* was 6.25 mg/mL and for *K. pneumoniae* was 6.25 mg/mL. The results showed that the plant extract exhibited the same MBC for *S. aureus* and *K. pneumoniae.*

3.9 Chemical Constituents Profiling by GC-MS Analysis

The GC-MS analysis of the hexane extract of *Berberis asiatica* revealed the presence of 35 compounds. The GC-MS chromatogram is shown in Figure 2. The NIST08 MS library was employed to compare and identify the chemical constituents. These compounds consist of ketones, alkanes, cycloalkanes, alcohol, and amines along with aromatic compounds. The profiling of chemical compounds in the hexane extract of asiatica with their retention time (RT), molecular weight (MW), and relative area percentage (%) are presented in Table 8.

Table 4: Antioxidant (IC_{50}) of methanolic extract of *Berberis asiatica* and standard quercetin.

Samples	IC_{50} (µg/mL)
Berberis asiatica	205.7 ± 5.353
Quercetin (Standard)	6.29 ± 1.02

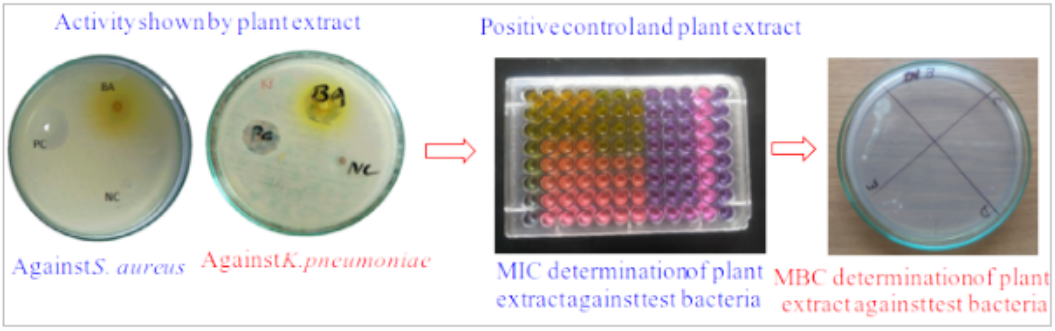

Figure 1: Antibacterial activity, MIC, and MBC determination of plant extract.

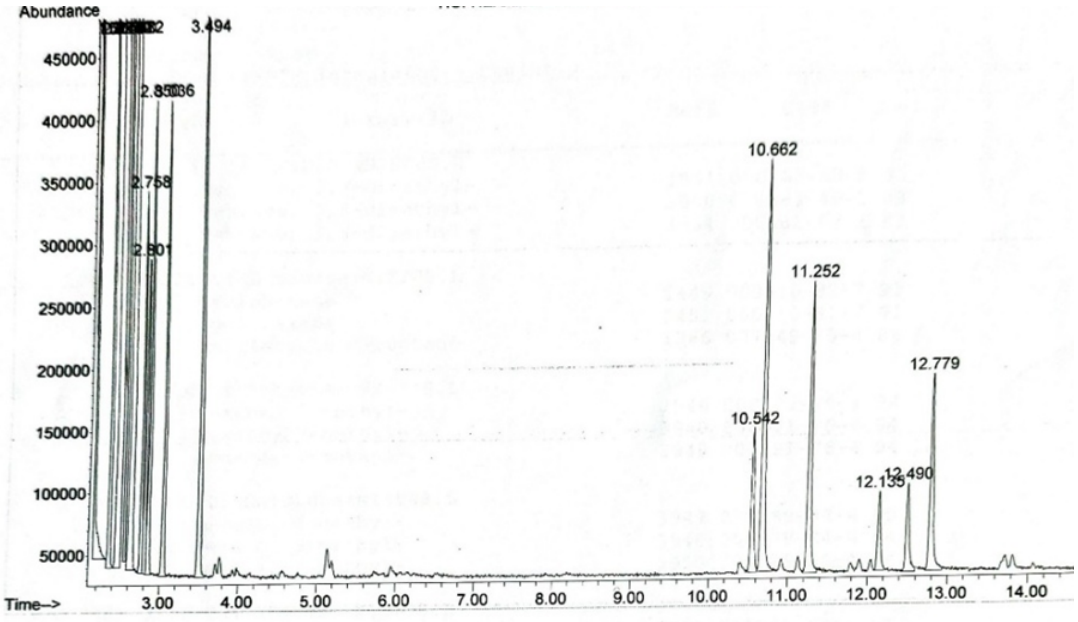

Figure 2: GC-MS chromatogram of hexane fraction of Berberis asiatica.

3.10 Pharmacological Importance of GC-MS Detected Compounds

Among the detected compounds by GC-MS, some of those compounds having the alcohol and ketone functional groups have been reported to possess pharmaceutical significance owing to their biological activities. The compound 2,5-dimethyl-4-hydroxy-3-hexanone is reported to have potential therapeutic effects such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, and also effective against Alzheimer's disease.. The compound 2-methyl-2-pentanol has been demonstrated to induce apoptosis in cancer cells, enhance the immune response to viral infections, and exhibit antimicrobial, antifungal, and antiviral activity. The compound 4-methyl-2-pentanol has been reported to act as an acetylcholinesterase inhibitor and demonstrate antibacterial and antifungal properties. Furthermore, it has shown effectiveness in the treatment of Alzheimer's disease and other neurological disorders. Other identified compounds like 2,2-dimethyl-3-pentanol and 3-hexanol also demonstrated antifungal, antibacterial, and antitumor activities [44]. The compound 3-hexanol can serve as an agonist for the GABA-A receptor, which plays a role in regulating neurotransmitters in the brain. Meanwhile, in vitro studies of 2,2-dimethyl-3-pentanol have shown that this compound can inhibit the growth of various cancer cell lines, including those associated with breast, lung, and colon cancers [44]. The chemical profiling of the present study reported that the plant *B. asiatica* is the source of potential natural compounds that could be used in treating different diseases.

4 Discussion

A limited number of studies have been conducted to explore the bioactive metabolites of plants in Nepal. Being a landlocked country, Nepal boasts remarkable biodiversity in its flora, resulting in a high den-

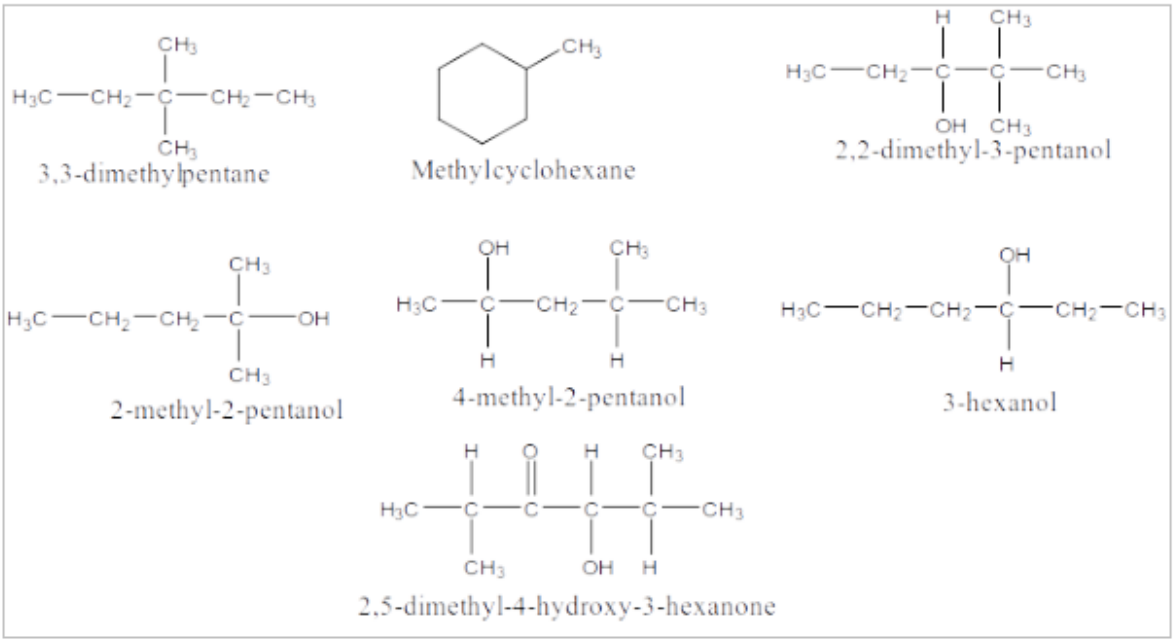

Figure 3: Structures of some chemical compounds detected from GC-MS analysis of hexane fraction of Berberis asiatica.

Table 5: Zone of Inhibition shown by methanolic extract of *Berberis asiatica* against bacterial strain.

Bacterial strain	Zone of inhibition (mm) of plant extract	Zone of inhibition (mm) of positive control
S. aureus	14	13
E. coli	-	16
K. pneumoniae	19	18
S. typhi	-	15

sity and diversity of plant species. For this research, various readily available Berberis species were collected and confirmed to be *Berberis asiatica* . The primary objective of this study was to evaluate the inhibitory effects of *Berberis asiatica* on α-amylase, various Gram-positive and Gram-negative bacteria, as well as the antioxidant properties of plants. In earlier investigations, the methanolic extract of *Berberis asiatica* exhibited a percentage yield of 6.8% [10]. However, in the current study, the percentage yield was found to be higher at 10.66%. The variation in secondary metabolites abundance can be ascribed to the distribution of the selected plant across different geographical locations, thereby affecting its overall availability of these compounds.

In the previous study, the total phenolic content (TPC) and total flavonoid content (TFC) were examined in different parts of *Berberis asiatica* , including leaves, bark, and roots. The TPC values were recorded as 132.63 mg GAE/g, 52.240 $\pm$ 0.119 mg/100g, and 59.076 $\pm$ 0.219 mg/100g for leaves, bark, and roots, respectively [45]. Similarly, the TFC was found to be 31.36 mg QE/g, 1.735 $\pm$ 0.396 mg QE/g, and 1.576 $\pm$ 0.197 mg QE/g for leaves, bark, and roots, respectively [45]. In the present study, a notable difference in TPC and TFC was observed in *Berberis asiatica* compared to the previously reported results. The TPC value for this plant was reported as 37.686 $\pm$ 2.728 mg GAE/g, and the TFC was recorded as 115.568 $\pm$ 8.012 mg QE/g. The plant sample collected in the present study showed higher contents of secondary metabolites which impart a significant effect on the biological activity, particularly in terms of antioxidant and antidiabetic properties.

In previous research, the antioxidant activity of *Berberis asiatica* was investigated, and the IC_{50} value for the root and stem bark extracts was determined to be 102.31 and 120.7 μg/mL, respectively [46]. However, in the current study, a notable difference was observed, showing a significant radical scavenging activity with an IC_{50} of 205.7 $\pm$ 5.353 μg/mL. The variation in the IC_{50} value of *Berberis asiatica* indicates that the antioxidant potential can be influenced by multiple factors, including the extraction methods, a sample collected geographical area, and the timing of sample collection. Apart from exhibiting poor inhibition of

Table 6: Minimum inhibitory concentration of methanolic extract of *Berberis asiatica* against test bacteria.

Bacteria	*Berberis asiatica* MIC (mg/mL)	Positive control (Neomycin) MIC (mg/mL)
S. aureus	0.39	0.0039
K. pneumoniae	3.125	0.0019

Table 7: Minimum bactericidal concentration (MBC) of methanolic extract of *Berberis asiatica* against test bacteria.

Bacterial strain	*Berberis asiatica* MBC (mg/mL)	Positive control (Neomycin) MBC (mg/mL)
S. aureus	6.25	0.015
K. pneumoniae	6.25	0.0078

alpha-amylase in the current study, *Berberis asiatica* is well-documented in some literature for its anti-diabetic properties. Previous research suggests that the plant's blood glucose and lipid regulatory effects can be attributed to berberine, a significant alkaloid present in *Berberis asiatica* . This compound has been found to enhance insulin sensitivity by regulating the secretion of adipokines [47].

The results of the current study demonstrate that the methanolic extract of *Berberis asiatica* exhibited significant inhibition in the growth of two bacterial strains, one gram-positive (*S. aureus*) and one gram-negative (*K. pneumoniae*), with a zone of inhibition (ZOI) of 14 mm and 19 mm, respectively. This finding indicates that the plant extract possesses potent antibacterial properties, likely due to the presence of specific antibacterial agents. However, in the previous study, the crude aqueous extract of *Berberis asiatica* stem bark demonstrated a zone of inhibition in the disk diffusion method. Among the Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella paratyphi-A* exhibited a zone of inhibition of 8 mm [48]. This outcome suggests that our study has exhibited a notably robust antibacterial property when compared to the previous research. The results of the current study revealed that the methanolic extract of B. asiatica displayed a minimum inhibitory concentration (MIC) of 0.39 mg/mL for *S. aureus* and 3.125 mg/mL for *K. pneumoniae*. Notably, the plant extract demonstrated better inhibition for *S. aureus* compared to *K. pneumoniae*. The literature also reported similar findings, where the methanolic stem bark extract of *Berberis asiatica* exhibited a MIC of 312.5 µg/mL for *S. aureus* [10], which aligns with the MIC value obtained in the present study. However, for *K. pneumoniae*, the aqueous stem bark extract of *Berberis asiatica* reported in the literature exhibited a MIC of 2500 µg/mL, which is higher than the MIC value obtained in the present study [48]. Furthermore, the methanolic extract of the *Berberis asiatica* plant exhibited an MBC of 6.25 mg/mL for both *S. aureus* and *K. pneumoniae*, indicating the same bactericidal concentration for both bacterial strains. In comparison, the literature reported a methanolic stem bark extract with an MBC of 0.78125 mg/mL for *S. aureus*, which differs and shows the potential activity of the plant sample used in this study [10]. The difference in the biological activities and the richness of secondary metabolites in the plant may be attributed to the part of the plant sample used and the geographical variation of plant growth.

In the chemical profiling, different secondary metabolites were identified by GC-MS analysis which includes, 3,3-dimethylpentane, cyclohexane, 2-methylhexane, 3-methylhexane, 1,3-dimethylcyclopentane, 1,2-dimethylcyclopentane, Heptane, methylcyclohexane, 2,2-dimethyl-3-pentanol, 2-methyl-2-pentanol, 2,5-dimethyl-4-hydroxy-3-hexanone, 3-hexanol. The previous study reported some of the compounds like berberine, palmatine, jatrorrhirine, oxyacanthine, oxyberberine, tetrahydropalmatine, and columbamine [7]. The GC-MS profiling in this study shows some different compounds with a richness of bioactive compounds like alkaloids, phenolic, and flavonoid compounds highlighting the pharmaceutical potential of the plant and may be a good source of such compounds in the future drug development process. The abundance of secondary metabolites may be affected due to climatic conditions, soil type, temperature, and geographical locations [49]. Although the same plant species with nearby environmental conditions may vary in chemical compositions [50]. Increasing the altitude contribute significantly to chemical composition, at higher altitude plant possess a higher amount of phenolic and flavonoid compounds [41]. However, at lower altitudes, there is a greater abundance of volatile organic compounds in the plants [41]. These variations in chemical composition according to altitude can be ascribed to discrepancies in the biological activity of plant extract. Therefore, current research results show some different potential results as compared to the previous one.

Table 8: Major chemical compounds detected by GC analysis in hexane fraction.

S.N.	Name of Compound	Retention Time (Rt)	Area %	Mol. Wt.
1	3,3-dimethyl pentane	2.354	0.78	100.2
2	Cyclohexane	2.479	86.25	84.16
3	2-methyl hexane	2.518	4.14	100.2
4	3-methyl hexane	2.632	3.45	100.2
5	1,3-dimethyl cyclopentane	2.758	0.40	98.19
6	1,2-dimethyl cyclopentane	2.850	0.53	98.19
7	Heptane	3.036	0.55	100.2
8	Methylcyclohexane	3.494	0.93	98.186
9	2,2-dimethyl-3-pentanol	10.542	0.28	116.2
10	2-methyl-2-pentanol	10.662	0.84	102.17
11	2,5-dimethyl-4-hydroxy-3-hexanone	11.252	0.58	144.21
12	3-hexanol	12.135	0.16	102.17
13	4-methyl-2-pentanol	12.490	0.18	102.174

5 Conclusion

In conclusion, the current study on *Berberis asiatica* from Nepal has provided valuable insights into its bioactive properties. The plant extract demonstrated significant inhibition of α-amylase and exhibited potent antibacterial activity against both gram-positive (*S. aureus*) and gram-negative (*K. pneumoniae*) bacteria. Additionally, the extract showed promising antioxidant potential. Moreover, there were variations in the total phenolic and flavonoid content of the plant when compared to previous findings. These differences could be attributed to various factors, such as plant parts used, extraction methods, and environmental conditions. Chemical profiling revealed the presence of various secondary metabolites in the plant, and some of these compounds were also reported in the literature, further confirming the plant's potential medicinal value. This study highlights the rich biodiversity of Nepal's flora and the importance of exploring and understanding the bioactive properties of its plant species. *Berberis asiatica* emerges as a promising candidate for further research and development of natural remedies and pharmaceutical agents, particularly for its antidiabetic, antibacterial, and antioxidant properties. However, future research should prioritize the bioassay-guided isolation of target bioactive compounds using advanced techniques like LC-HRMS, HPLC, and NMR. Isolation, purification, and identification of potent bioactive components bearing antioxidant, antidiabetic, and antibacterial properties followed by *in vitro* and *in vivo* studies to unveil their mechanisms of action will confirm their potential for inclusion in upcoming drug discovery initiatives.

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