Journal of Institute of Science and Technology, 29(2), 183-191 (2024)
ISSN: 2467-9062 (print), e-ISSN: 2467-9240
https://doi.org/10.3126/jist.v29i2.71359

Research Article

PHYTOCHEMICAL ANALYSIS AND EVALUATION OF BIOACTIVITIES OF *Artemisia vulgaris* SOLVENT EXTRACTS

Sijan Gautam, Manisha Bhusal, Akash Budha Magar, Ram Chandra Basnyat, Niranjan Parajuli, Khaga Raj Sharma*
Central Department of Chemistry, Institute of Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: khaga.sharma@cdc.tu.edu.np*

(Received:** November 08, 2024; **Final Revision:** December 27, 2024; **Accepted:** December 31, 2024**)

ABSTRACT
Artemisia vulgaris, a prominent medicinal species within the genus *Artemisia*, has drawn significant scientific interest due to its rich composition of biologically active secondary metabolites. This study evaluates the phenolic and flavonoid content, antioxidant, α-glucosidase inhibitory, and antimicrobial activities of *Artemisia vulgaris*, sourced from Khaptad National Park at an elevation of 2907 meters. Different solvent fractions, including water, methanol, dichloromethane (DCM), ethyl acetate + hexane, and hexane, were used for the extraction of phytochemicals as secondary metabolites. Methanol and water extracts exhibited the highest total phenolic content (TPC), with values of 54.40 ± 1.55 mg GAE/g and 50.29 ± 1.69 mg GAE/g, respectively. The highest total flavonoid content (TFC) was observed in methanol and DCM extracts, measuring 42.31 ± 1.83 mg QE/g and 19.96 ± 0.89 mg QE/g, respectively. Methanol extracts demonstrated the strongest antioxidant activity, with a DPPH radical scavenging IC_{50} of 19.00 ± 0.81μg/mL, followed by DCM extracts at 25.90 ± 0.76 μg/mL. The methanol extract also exhibited the most potent α-glucosidase inhibition, with an IC_{50} of 63.23 ± 0.20 μg/mL, while DCM, water, and ethyl acetate + hexane extracts showed lower activity. Antimicrobial analysis revealed inhibition of *Staphylococcus aureus* with zones of inhibition (ZOI) of 9 mm for methanol and 5 mm for DCM extracts, and a ZOI of 4 mm for *Escherichia coli* with methanol extract. Results suggest that *Artemisia vulgaris* imparts the potential effect, particularly in antioxidant and antibacterial activities, underscoring its therapeutic potential. The plant could be used as a source of natural antioxidant and antibacterial agents for isolating the target compounds in the future drug development process.

Keywords: *Artemisia vulgaris*, bioactivities, DPPH, flavonoid content, phenolic content, α-glucosidase

INTRODUCTION
Nepal's rich biodiversity, supported by a wide range of altitudes from tropical to alpine zones, includes an estimated 7000 species of flowering plants and ferns, over 700 of which are recognized for their medicinal value (Rajbhandary *et al.*, 2020). Known locally as *Jadibuti*, these medicinal plants have long been used in traditional medicine to treat and prevent diseases. Despite this, Nepal has not fully capitalized on the potential of these plants for health and economic benefits, largely due to insufficient policy support and limited scientific research (Devkota *et al.*, 2021). With 80% of Nepal's population living in rural areas where healthcare facilities are scarce, people heavily rely on traditional healing practices, drawing on knowledge passed down through generations (Ambu *et al.*, 2020). This traditional knowledge, deeply embedded in Nepal's multiethnic culture, spans folk, shamanistic, and Ayurvedic medicine.

Medicinal plants are central to drug discovery, as exemplified by the natural compounds used to create widely recognized drugs like Taxol and Doxorubicin, effective in treating cancers by inhibiting enzymes critical for cancer cell growth (Heirangkhongjam & Ngaseppam, 2018). *Artemisia vulgaris* is a widely distributed species known for its various applications in traditional and modern medicinal practices. The plant, commonly referred to as *Titepati* in Nepal, is valued for its anti-inflammatory, hepatoprotective, antispasmodic, and antioxidant properties, attributed to its rich composition of essential oils, flavonoids, and other bioactive compounds (Temraz & El-Tantawy, 2008a). This versatile plant, part of the *Asteraceae* family, is not only important in traditional Nepali medicine but also has culinary and therapeutic uses worldwide (Erel *et al.*, 2011).

The genus *Artemisia*, comprising over 500 species, thrives globally, especially in temperate zones. Its adaptability allows it to grow across varied environments and altitudes, showing a wide range of morphological and phytochemical diversity (Abad *et al.*, 2012). Cytogenetic studies indicate that *Artemisia* species possess distinct chromosomal counts, with variations linked to polyploidy and dysploidy, contributing to their evolutionary adaptability (Vallès *et al.*, 2011). *A. vulgaris*, known for its aromatic qualities, has been used historically as a medicinal herb, valued for its digestive and gynecological benefits in traditional European, Chinese, and Hindu medicine (Ekiert *et al.*, 2020a). It also shows promise as a raw material in homeopathy and as a potential therapeutic agent in modern pharmacology due to its complex phytochemistry.

Phytochemical studies reveal that *A. vulgaris* is rich in essential oils, particularly monoterpenoids, and sesquiterpenoids, and contains compounds like 1,8-cineole, α-thujone, and camphor, which contribute to its therapeutic properties (Blagojević *et al*., 2006). Research on Nepalese specimens of *A. vulgaris* demonstrates significant antioxidant activity and phenolic content, highlighting its potential as a natural antioxidant and antimicrobial agent. Local studies in Nepal have also shown that *A. vulgaris* extracts exhibit notable DPPH radical scavenging abilities and antibacterial activity, underscoring its relevance in herbal medicine (Chaudhary *et al*., 2021).

Artemisia vulgaris, commonly known as mugwort, has garnered significant attention in the fields of herbal medicine, pharmacology, and immunology due to its diverse therapeutic properties. Widely distributed across various geographical regions, including the high-altitude areas of Nepal, this plant is renowned for its antioxidant and antibacterial activities. Research has highlighted the variation in its bioactive properties, influenced by geographical location and extraction methods (Sharma and Adhikari, 2023). The current study focuses on evaluating the antibacterial, antidiabetic, and antioxidant activities of *A. vulgaris* collected from high-altitude regions of Nepal. By analyzing its biological activities, active compounds, and underlying mechanisms, this research aims to expand the scientific understanding of *A. vulgaris* as a valuable medicinal resource. This knowledge can contribute to the exploration of its potential therapeutic applications and its integration into modern medicine.

MATERIALS AND METHODS

Chemicals

In this study, common analytical-grade solvents were used, including water, methanol, ethyl acetate, dichloromethane (DCM), dimethyl sulfoxide (DMSO), and hexane sourced from Merck and Scientific Fischer. Distilled water was obtained from a local vendor. For the α-glucosidase enzyme inhibition assay, α-glucosidase derived from *Saccharomyces cerevisiae* was used as the enzyme, and p-nitrophenyl-α-D-glucopyranoside (p-NPG) was employed as the substrate, both supplied by Sigma-Aldrich.

Plant Sample Collection, Identification, and Solvent Extraction

Plant samples were collected from Khaptad National Park in Nepal at an altitude of 2907 meters (latitude N 29°23.2731′, longitude E 081°08.6694′). The soil in the collection area had a pH of 6.72, and the ambient temperature was 13.4 °C. The selection of plants was based on ethnobotanical value, informed by literature and consultations with local people. The samples were identified at the National Herbarium and Plant Laboratories (NHPL), Godavari, Nepal, of voucher specimen number KHP14. After collection, the plant materials were washed, cut into small pieces, shade-dried, and ground into powder (pulverization). The powdered samples were stored in waterproof bags in a cool, dry place.

Extraction of plant secondary metabolites, including alkaloids, glycosides, phenolics, terpenoids, and flavonoids, was conducted using different solvents based on polarities like water, methanol, dichloromethane (DCM), a mixture of ethyl acetate + hexane, and hexane through maceration. Approximately 40 grams of the powdered plant material was soaked in 200 mL of each solvent for 24 hours, then filtered. The resulting filtrates were collected and concentrated using a rotary evaporator under a vacuum at 40 ^{o}C.

Phytochemical Analysis of Plant Extracts

A colorimetric reaction was employed for the qualitative phytochemical analysis of different extracts of *Artemisia vulgaris*. This technique helps in identifying the presence of various bioactive compounds, including alkaloids, flavonoids, phenolics, terpenoids, and glycosides. By observing color changes upon the addition of specific reagents, the presence of these phytochemicals was qualitatively assessed in each extract (Mallikharjuna *et al*., 2007).

Estimation of Total Phenolic and Total Flavonoid Content

A 1M sodium carbonate solution was prepared by dissolving 10.6 g of sodium carbonate in 100 mL of distilled water, and a 1:9 v/v Folin-Ciocalteu reagent was made by diluting 2 mL of the commercially available reagent in 18 mL of distilled water. Stock solutions for total phenolic content were created by dissolving 10.0 mg of gallic acid in 10 mL of ethanol to achieve a concentration of 1000 µg/mL, from which dilutions were made to obtain final concentrations of 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL. For plant extracts, 5 mg of plant material from different solvents was dissolved in 50% DMSO to form a 5 mg/mL stock solution. The total phenolic content was measured using the Folin-Ciocalteu reagent via a modified 96-well plate method (Lu *et al*., 2011), where 20 µL of each standard concentration of gallic acid (10-100 µg/mL) and 20 µL of the plant sample (5000 µg/mL) were added to the wells. After adding 100 µL of Folin-Ciocalteu reagent and 80 µL of sodium carbonate, the plate was incubated in the dark for 30 minutes before absorbance was measured at 765 nm using a microplate reader (Epoch2, BioTek, Instruments, Inc., USA). The total polyphenolic content in the extracts was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) using the standard curve. For total flavonoid content, a 10% aluminum trichloride solution was prepared by dissolving 1.0 g of $AlCl_3$ in 10 mL of distilled water, and a 1 M sodium acetate solution was made by dissolving 1360.8 mg of sodium acetate trihydrate in 10 mL of distilled water. A stock solution of quercetin (7.7 mg in 5 mL of ethanol) was diluted to prepare final concentrations of 10-100 µg/mL. The total flavonoid content was assessed using a modified 96-well plate method (Chang *et al*., 2002), where 130 µL of each quercetin standard concentration and 20 µL of the plant

sample (5000 µg/mL) were loaded into the wells, followed by 110 µL of distilled water. In each well, 60 µL of ethanol, 5 µL of $AlCl_3$, and 5 µL of sodium acetate were added, and after a 30-minute incubation in the dark, absorbance was measured at 415 nm.

Antioxidant Activity

A 0.2 mM DPPH solution was prepared by dissolving 3.9 mg of DPPH in 50 mL of methanol in a volumetric flask covered with aluminum foil. The stock solution of quercetin was made by dissolving 1.0 mg of quercetin in 1 mL of methanol, followed by the preparation of final concentrations 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0.07 µg/mL through dilution. Plant extracts (5 mg) from different solvents were also diluted in a 50% DMSO solution to achieve the same final concentrations. The antioxidant activity of the extracts was assessed using a modified 96-well plate method (Sabudak *et al.*, 2013; Subedi *et al.*, 2014). For the DPPH test, quercetin served as the positive control, while 50% DMSO acted as the negative control. A total of 100 µL of the positive control, negative control, and plant samples were added to the 96-well plates in triplicate, followed by the addition of 100 µL of DPPH reagent to each well. The plates were incubated in the dark for 30 minutes, after which absorbance was measured at 517 nm using a microplate reader. The capability to scavenge the DPPH radical was calculated using a specified equation.

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

α- Glucosidase Inhibition

To prepare the buffer solution, 3.12 g of sodium dihydrogen orthophosphate and 3.56 g of disodium hydrogen orthophosphate were weighed and dissolved in 100 mL and 200 mL of distilled water, respectively, using volumetric flasks. A 100 mM buffer solution was created by mixing 98 mL of disodium hydrogen orthophosphate with 102 mL of sodium dihydrogen orthophosphate, with the pH adjusted to 6.8 by adding acid or base, and 29.25 g of NaCl was incorporated to achieve a final concentration of 50 mM. Plant extracts were prepared at a concentration of 5 mg/mL in a 50% DMSO solution. The α-glucosidase activity of the plant extracts was determined using a method adapted from Fouotsa *et al.* (2012). Specifically, at a final concentration of 0.5 units/mL, 20 µL of α-glucosidase was mixed with 20 µL of the plant extract at varying concentrations and 120 µL of 50 mM phosphate buffer saline solution (PBSS) at pH 6.8. Following this, 40 µL of p-nitrophenyl α-D-glucopyranoside (PNPG) solution was added as a substrate. The reaction mixture was pre-incubated for 15 minutes at 37 °C. The α-glucosidase activity was quantified by measuring the release of p-nitrophenyl from the hydrolysis of PNPG at 410 nm. Acarbose was used as a positive control, while 30% DMSO served as the negative control. All experiments were performed in triplicate with a final volume of 200 µL using a microplate reader (Epoch2, BioTek, Instruments, Inc., USA). The percentage of α-glucosidase activity was calculated using a specific formula.

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Data Analysis

The results were processed using Gen5 Microplate Data Collection & Analysis Software, followed by analysis with MS Excel (open source). The IC_{50} value, representing the concentration required to inhibit 50% of the enzymatic hydrolysis of the substrates p-nitrophenyl α-D-glucopyranoside (PNPG) and 4-nitrophenyl β-D-glucopyranoside (CNPG3), was calculated using GraphPad Prism software version 8. All values were expressed as the mean ± standard error of the mean from triplicate measurements.

Antimicrobial Activity

The antimicrobial assay of crude extracts from different solvents was conducted using the agar well diffusion method on Mueller Hinton Agar (MHA) plates (Bolouri Moghaddam *et al.*, 2016). Test organisms were inoculated into Mueller Hinton broth and incubated at 37 °C until the turbidity matched 0.5 McFarland standards, yielding a final inoculum of 1.5×10^8 CFU/mL. The MHA plates were lawn cultured with the prepared microbial inoculum. Plant extracts at a concentration of 50 mg/mL were prepared in 50% DMSO. Using a sterile cork borer, six wells of 6 mm diameter were created in the cultured lawn media, and each well was filled with 50 µL of extract from different plants, along with positive control (neomycin at 1 mg/mL) and negative control (50% DMSO) for each plate. The plates were allowed to diffuse for about 15 minutes at room temperature before being incubated for 18-24 hours at 37 °C. After incubation, the presence of a clear zone of inhibition (ZOI) around the wells was observed and measured in millimeters.

RESULTS AND DISCUSSION

Phytochemical Analysis

Phytochemical screening of the extract revealed the presence of several bioactive compounds. Alkaloids were confirmed in all solvent fractions via Meyer's and Dragendorff's reagent tests. Terpenoids were identified by a reddish-brown color formation at the interface with chloroform and concentrated sulfuric acid. Flavonoids showed positive results through Shinoda's and Lead acetate tests, evidenced by orange coloration and white precipitate in all fractions. The presence of phenols was indicated by greenish-blue coloration with ferric chloride in water-methanol and DCM extracts. Saponins were confirmed by foaming after shaking with distilled water. Carbohydrates were detected through Molisch's test, resulting in a violet ring formation at the interface. Protein presence was indicated by yellow and blue/purple colors in Xanthoproteic and Ninhydrin tests in methanol and DCM extracts. Finally, glycosides were confirmed via Modified Borntrager's and Sulphuric acid tests, resulting in rose-pink and reddish color

formations. These findings highlight the extract's rich phytochemical profile, suggesting potential therapeutic properties.

Total Phenolics and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of the crude extract of *Artemisia vulgaris* were quantified using gallic acid and quercetin as standards, respectively. The TPC was determined by constructing a calibration curve with various concentrations of gallic acid (0, 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL), yielding a regression equation of R^2 = 0.9894. The TPC values obtained from the crude extract in different solvents were as follows: 50.29 ± 1.69 mg GAE/g for water, 54.40 ± 1.55 mg GAE/g for methanol, 30.43 ± 3.61 mg GAE/g for dichloromethane (DCM), 11.85 ± 1.55 mg GAE/g for hexane, and 27.84 ± 0.39 mg GAE/g for ethyl acetate + hexane.

Similarly, the TFC was assessed by constructing a calibration curve using quercetin at concentrations ranging from 0 to 80 µg/mL, resulting in a regression equation of R^2 = 0.9923. The TFC values for the crude extract of *Artemisia vulgaris* were determined as follows: 6.17 ± 1.11 mg QE/g for water, 42.31 ± 1.83 mg QE/g for methanol, 19.96 ± 0.89 mg QE/g for DCM, 14.52 ± 2.979 mg QE/g for hexane, and 19.16 ± 3.35 mg QE/g for ethyl acetate + hexane. The calibration curves for both gallic acid and quercetin are depicted in Figures 1 (a) and (b), respectively, while Table 1 presents a summary of the TPC and TFC values across all extracts. The results indicate a substantial variation in both phenolic and flavonoid content depending on the extraction solvent used, highlighting the influence of extraction methods on the phytochemical profile of *Artemisia vulgaris*. These findings suggest that optimizing extraction conditions could enhance the recovery of bioactive compounds, which may have implications for the therapeutic potential of this plant.

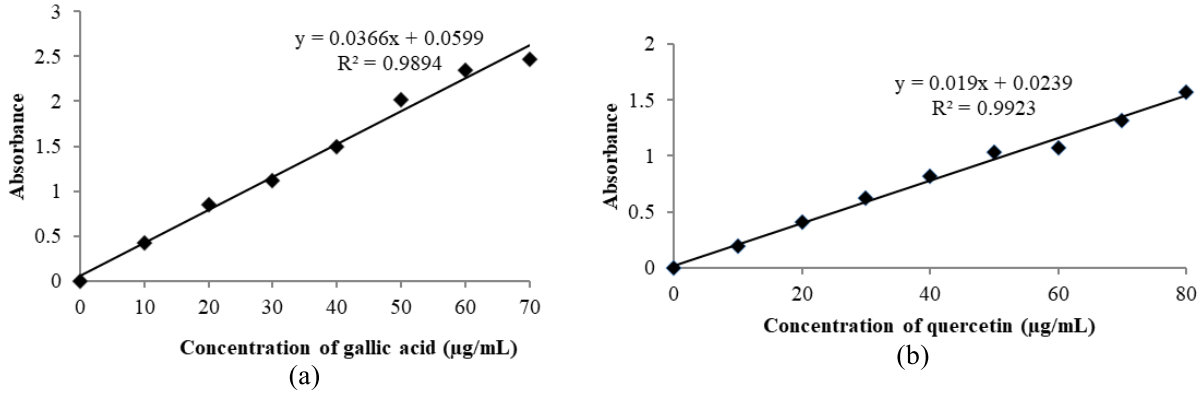

Figure 1. Calibration curve of (a) standard gallic acid (b) standard quercetin

Table 1. TPC and TFC of crude extract of *Artemisia vulgaris* in different solvents

S.N.	Crude plant extracts	TPC mg GAE/g plant extract	TFC mg QE/ g plant extract
1	Water (aqueous)	50.29 ± 1.69	6.17 ± 1.11
2	Methanol	54.40 ± 1.55	42.31 ± 1.83
3	DCM	30.43 ± 3.61	19.96 ± 0.89
4	Hexane	11.85 ± 1.55	14.52 ± 2.97
5	Ethylacetate+Hexane (20:80)	27.84 ± 0.39	19.166 ± 3.35

Antioxidant Potential

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was employed to evaluate the free radical scavenging potential of the plant extract. The DPPH radical, a nitrogen-centered stable free radical, exhibits a maximum absorbance at 517 nm and undergoes a color change from violet to yellow upon reduction (Abubacker *et al.*, 2013). This reduction occurs when antioxidants donate a hydrogen atom or electron, neutralizing the radical. The free radical inhibition activity of the plant extracts was measured at various concentrations, and a plot of inhibition percentage against concentration was constructed to visualize the scavenging efficiency. The DPPH scavenging activity was quantified as the IC_{50} value, representing the concentration of the extract required to inhibit 50% of DPPH radicals. IC_{50} values of the plant extracts were compared with quercetin, a known antioxidant standard. A standard curve for quercetin was generated by plotting the inhibition percentages at final concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.312 µg/mL, yielding a non-linear relationship. This comparison allows for the assessment of the extract's antioxidant capacity relative to quercetin, providing insights into its potential as a free radical scavenger Figure 2 and Figure 3.

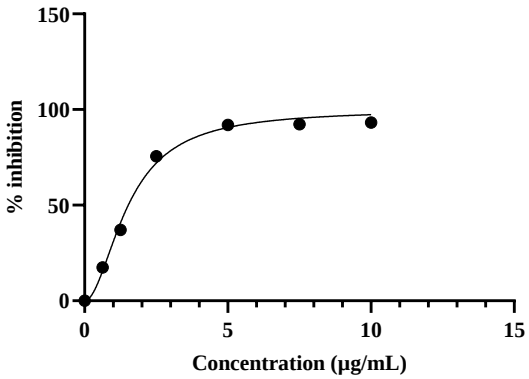

Figure 2. Standard curve of DPPH inhibition shown by quercetin as standard

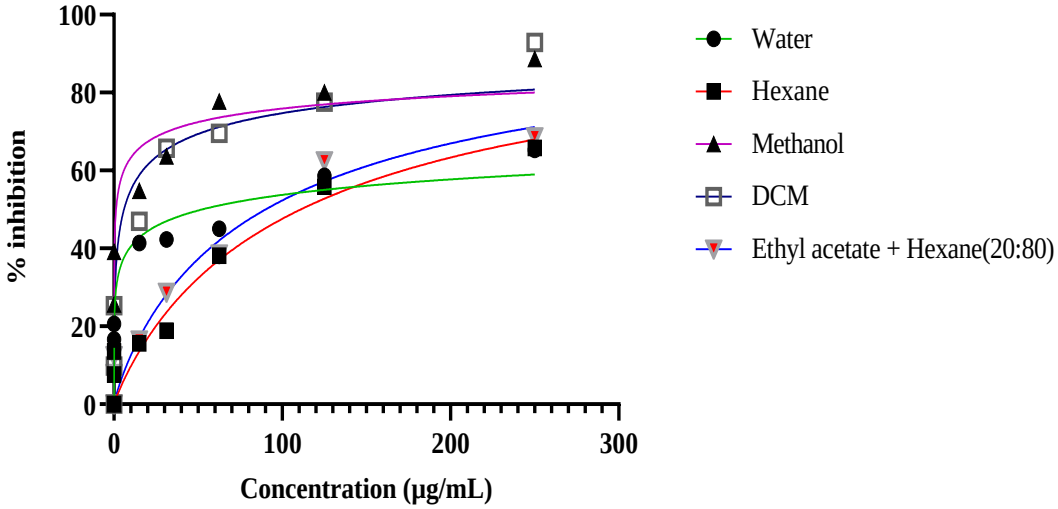

Figure 3. DPPH inhibition activity shown by extract of *Artemisia vulgaris* in different solvents

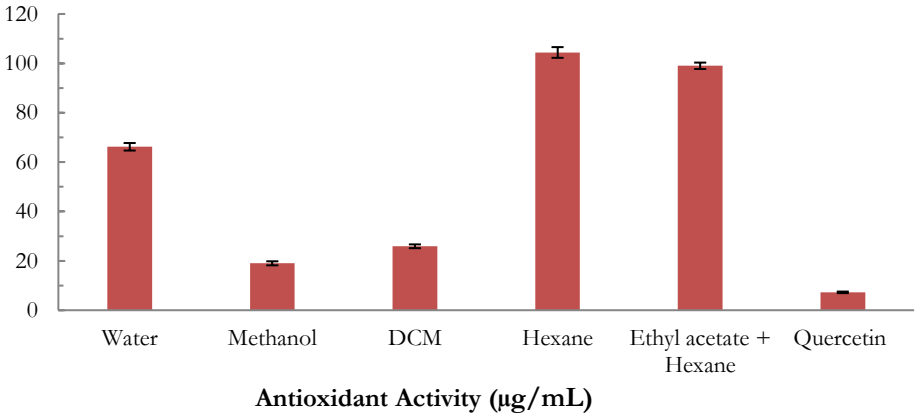

Figure 4. Bar diagram showing IC_{50} of antioxidant activity exhibited by the different fractions of the plant (µg/mL)

The DPPH radical scavenging activity of *Artemisia vulgaris* extracts was evaluated and expressed as the IC_{50} value, which represents the concentration required to achieve 50% inhibition of the DPPH radical. The extracts were assessed in comparison to quercetin, a standard antioxidant with an IC_{50} of 7.24 µg/mL. The IC_{50} values for the extracts indicated varying levels of free radical scavenging activity, with the order of potency observed as follows: Methanol extract (19.00 ± 0.81 µg/mL) > DCM extract (25.90 ± 0.76 µg/mL) > water (aqueous) extract (66.20 ± 1.52 µg/mL) > ethyl acetate + hexane extract (99.0 ± 1.30 µg/mL) > hexane extract (104.40 ± 2.17 µg/mL). The results demonstrate that the methanol fraction of *A. vulgaris* exhibits the highest free radical scavenging activity among the extracts tested, closely followed by the DCM extract, while the hexane

extract showed minimal inhibitory activity. Figure 4 illustrates the IC_{50} values with standard error mean (SEM) for each extract and the quercetin standard, highlighting the potent antioxidant capacity of the methanol extract relative to other fractions.

Alpha-glucosidase Inhibition Activity

The α-glucosidase inhibitory activity of various fractions of *Artemisia vulgaris* crude extracts was evaluated to assess their potential as antidiabetic agents. Initial screenings were conducted at a concentration of 500 µg/mL, with samples exhibiting more than 50% inhibition further diluted to determine the IC_{50} value. Acarbose, a known α-glucosidase inhibitor, served as the standard with an IC_{50} value of 3.55 ± 0.10 µg/mL. However, none of the *A. vulgaris* fractions showed inhibition greater than 50% at the tested concentration, indicating a lack of significant α-glucosidase inhibitory activity, Table 2. These results suggest that the crude extracts of *Artemisia vulgaris* do not exhibit substantial inhibitory effects on α-glucosidase and may have limited application in managing conditions related to this enzyme's activity, such as diabetes Figure 5 and Figure 6.

Table 2. α-glucosidase inhibition assay of the solvent extracts of *Artemisia vulgaris*

Solvent extracts	% inhibition
Water (Aqueous)	20.32
Methanol	29.50
DCM	23.45
Hexane	14.43
Ethyl acetate + Hexane (20:80)	16.43
Acarbose	83.93

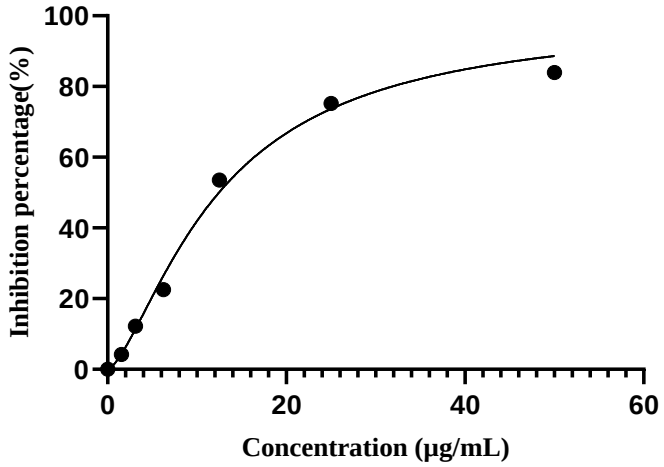

Figure 5. α-Glucosidase inhibition activity shown by acarbose

Among the various solvent extracts of *Artemisia vulgaris*, the methanol extract exhibited the strongest α-glucosidase inhibitory activity with an IC_{50} value of 63.23 ± 0.20 µg/mL, followed by the DCM extract (68.68 ± 0.34 µg/mL), water extract (93.73 ± 0.90 µg/mL), ethyl acetate + hexane extract (20:80) (99.11 ± 0.89 µg/mL), and hexane extract (11.35 ± 0.91 µg/mL) Figure 7. These findings indicate that the methanol extract, among the tested fractions, shows the highest inhibitory potential against α-glucosidase, albeit significantly lower than the standard acarbose (IC_{50} = 3.55 ± 0.10 µg/mL). The results suggest that *A. vulgaris* extracts, particularly the methanol fraction, possess moderate α-glucosidase inhibitory activity, which may contribute to its potential antidiabetic properties.

***In-vitro* Antibacterial Potential**

The study of *Artemisia vulgaris* extracts revealed its antimicrobial and phytochemical properties, with methanol and DCM extracts showing zones of inhibition (ZOI) of 9 mm and 5 mm, respectively, against *Staphylococcus aureus* (ATCC 43300), while the methanol extract showed a 4 mm ZOI against *Escherichia coli* (ATCC 2591) and no inhibition for *Salmonella typhi* (ATCC 14028) Table 3, Figure 8, 9 and 10. *A. vulgaris* from high altitudes was found to contain higher levels of polyphenols and flavonoids compared to low-altitude plants. These compounds, particularly flavonoids, are essential for antioxidant activity, plant defense, and growth. Phytochemical analysis of the leaves and stems indicated the presence of alkaloids, flavonoids, saponins, quinones, sterols, tannins, and reducing sugars, which contribute to its diverse biological activities and medicinal uses. Flavonoids and polyphenols have drawn scientific interest for their strong reducing and antioxidant properties, highlighting the potential of *A. vulgaris* as a source of bioactive compounds influenced by altitude.

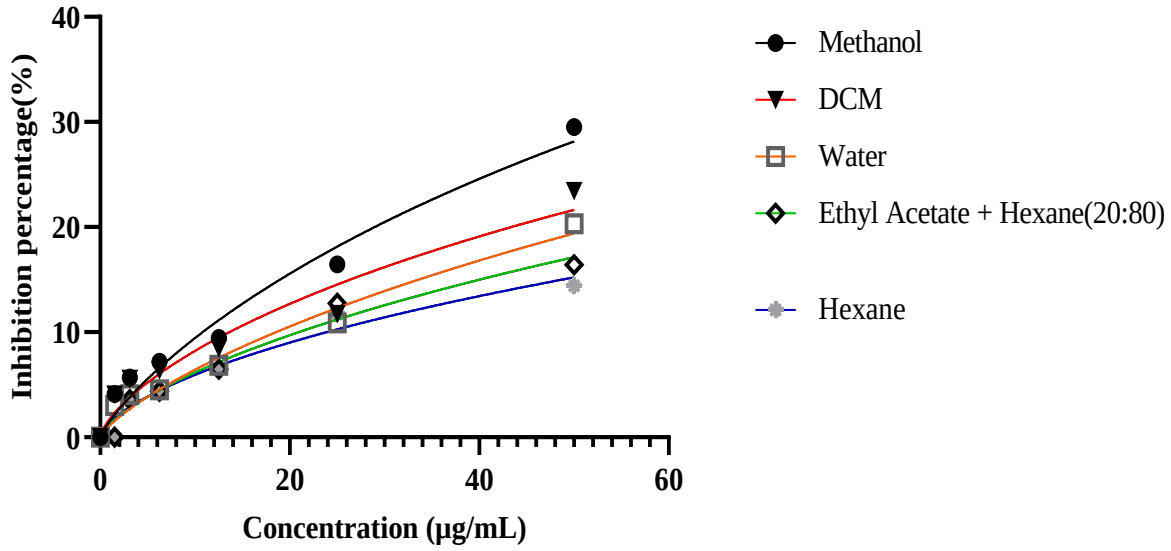

Figure 6. α- Glucosidase inhibition activity shown by different fractions from *A. vulgaris*

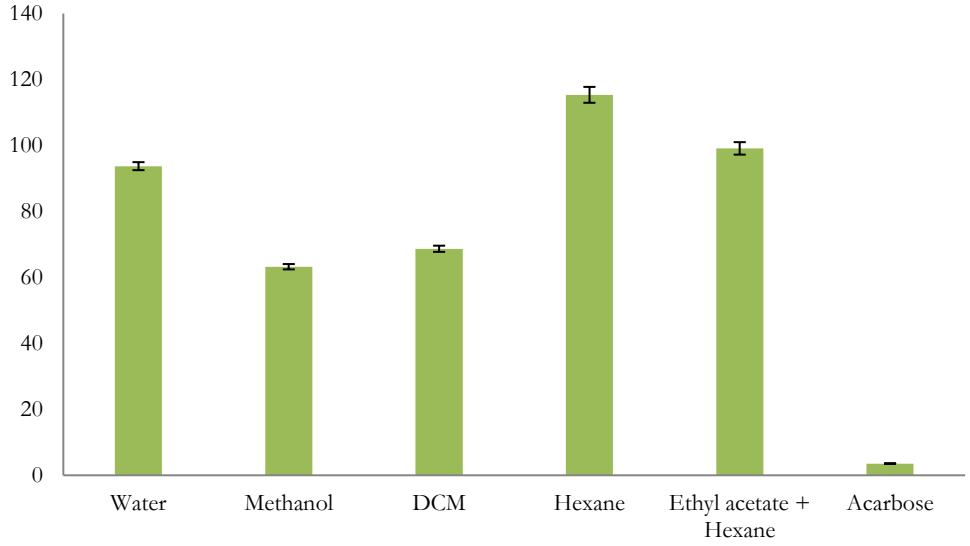

Figure 7. Showing antidiabetic activity in IC_{50} value of different solvent extracts of *Artemisia vulgaris*

Table 3. Antimicrobial activity of ZOI against the bacterial strains

Crude plant extracts	*Salmonella typhi* (ZOI mm)	*Escherichia coli* (ZOI mm)	*Staphylococcus aureus* (ZOI mm)
Water (Aqueous)	-	-	-
Methanol	-	4	9
DCM	-	-	5
Hexane	-	-	-
Ethyl acetate + Hexane	-	-	-
Positive control (Neomycin)	22	21	21

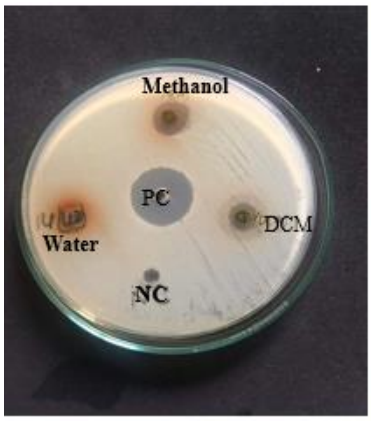

Figure 8. (ZOI shown by *Staphylococcus aureus,* ATCC 43300)

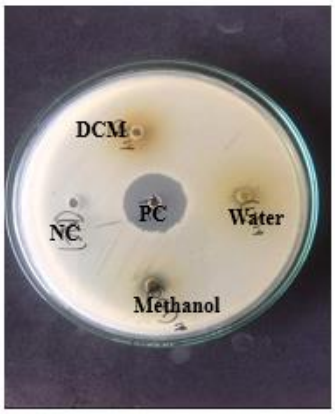

Figure 9. (ZOI shown by *Escherichia coli,* ATCC 2591*)*

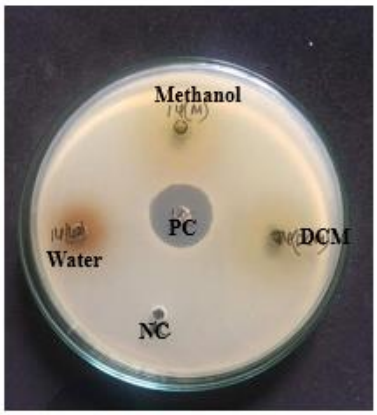

Figure 10. (ZOI shown by *Salmonella typhi,* ATCC 14028*)*

In this study, methanol, DCM, and water extracts of plant *Artemesia Vulgaris* were found to exhibit superior α-glucosidase enzyme inhibitory activities due to the rich content of polyphenols and flavonoids with significant free radical scavenging abilities. Moreover, methanol and DCM extracts of *Artemisia vulgaris* showed the inhibition of *Staphylococcus aureus* and *Escherichia coli*. So, the choice of solvent can play a vital role in different bioactivities and extraction yield. Compounds such as sabinene, β-thujone, chrysanthenone, camphor, borneol, and germacrene D extracted from *Artemisia vulgaris* are responsible for the plant's high antioxidant properties. The chemical composition of these compounds was studied by using GC, GC-MS, and ^{13}C NMR analysis (Blagojević *et al*., 2006).

CONCLUSIONS

Natural product-based pharmaceuticals for treating a variety of disorders are of interest to researchers since they have little or no side effects. Results showed that matured *A. vulgaris* at a height of 2907 meters is rich in polyphenols and flavonoids. Plant growth and development, as well as defense against pests and pathogens, depend heavily on flavonoids. Methanol and DCM were determined to be the most effective extracting solvents. DPPH tests were used to examine the extract's antioxidant activity, and the results showed that it had a high antioxidant potential when compared to information previously reported for other *Artemisia* genus species. As a result of a variety of factors, including temperature and climatic variations, altitude may have an impact on chemical composition and biological activity. However, plants growing at higher elevations produced more biologically active material, have greater antibacterial, and anthelmintic effects, and superior free radical scavenging abilities (Singh *et al*., 2017). In light of this, the plant's intended function should be taken into account while determining its growth height. Additional research on samples obtained from more altitudes is required. This research should also examine the impact of various environmental and genetic factors at higher altitudes, including temperature, humidity, rainfall, sunlight, plant variety, collection season, and even collection timing throughout the day, as well as plant drying and extraction techniques, further research on action mechanism, clinical trials on toxicity and its adverse side effect need to be known.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Central Department of Chemistry, Tribhuvan University for Providing laboratory facilities and the University Grants Commission Nepal for providing partial funding to support this research work.

AUTHOR CONTRIBUTIONS

SG: collected samples, conducted laboratory work, analyzed data, and prepared a manuscript draft; MB, ABM: assisted in preparing the manuscript; RCB, NP: supervised the research work, analyzed data and results; KRS: analyzed data, wrote the paper, and finalized the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

REFERENCES

Abad, M.J., Bedoya, L.M., Apaza, L., & Bermejo, P. (2012). The *Artemisia* L. Genus: A review of bioactive essential oils. *Molecules*, *17*, https://doi.org/10.3390/molecules17032542.

Abubacker, M.N., Sathya, C., & Prabakaran, R. (2013). In vitro antifungal potentials of *Hamelia patens* Jacq. (Rubiaceae) aqueous extracts of leaves, flowers and fruits. *Biosciences Biotechnology Research Asia*, *10*, 699-704. https://doi. org/10.13005/bbra/1183.

Ambu, G., Chaudhary, R.P., Mariotti, M., & Cornara, L. (2020). Traditional uses of medicinal plants by ethnic people in the Kavrepalanchok District, Central Nepal. *Plants*, *9*, https://doi.org/10.3390/plants9060759.

Blagojević, P., Radulović, N., Palić, R., & Stojanović, G. (2006). Chemical composition of the essential oils of

Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. *Journal of Agricultural and Food Chemistry*, *54*, 4780–4789. https://doi.org/10.1021/jf060123o.

Bolouri Moghaddam, M.R., Vilcinskas, A., & Rahnamaeian, M. (2016). Cooperative interaction of antimicrobial peptides with the interrelated immune pathways in plants: AMPs in plant immunity. *Molecular Plant Pathology*, *17*, 464-471, https://doi.org/10.1111/mpp.12299.

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*, 5. https://doi.org/10.38212/2224-6614.2748.

Chaudhary, R., Maharjan, B., Bharati, S., Shrestha, T., Mishra, P.K., Karanjit, S., Bhattarai, D.P., Homagai, P.L., & Shrestha, R.L.S. (2021). Phytochemical screening, GC-MS analysis and biological activities of extracts of *Artemisia vulgaris* Linn. *Amrit Research Journal*, *2*(1), 83-92. https://doi.org/10.3126/arjv2i01. 40743.

Devkota, H.P., Devkota, A.A., Kaundinnyayana, A., & Bhandari, D.R., (2021). Strengthening traditional medicine systems in Nepal through chemical and pharmacological research. *Annapurna Journal of Health Sciences*, *1*(2), 66-72. https://doi.org/10.52910/ajhs.48.

Ekiert, H., Pajor, J., Klin, P., Rzepiela, A., Ślesak, H., & Szopa, A. (2020). Significance of *Artemisia vulgaris* L. (Common Mugwort) in the history of medicine and its possible contemporary applications substantiated by phytochemical and pharmacological studies. *Molecules*, *25*, https://doi. org/10.3390/molecules25194415.

Erel, Ş.B., Şenol, S.G., Köse, F.A., & Ballar, P. (2011). In vitro cytotoxic properties of six *Artemisia* L. species. *Turkey Journal of Pharmaceutical Sciences, 8(3)*, 247-252.

Fouotsa, H., Lannang, A.M., Mbazoa, C.D., Rasheed, S., Marasini, B.P., Ali, Z., Devkota, K.P., Kengfack, A.E., Shaheen, F., Choudhary, M.I., & Sewald, N. (2012). Xanthones inhibitors of α-glucosidase and glycation from *Garcinia nobilis*. *Phytochemistry Letters*, *5*, 236-239. https://doi.org/10.1016/j.phytol. 2012.01.002.

Heirangkhongjam, M.D., & Ngaseppam, I.S. (2018). Traditional medicinal uses and pharmacological properties of *Rhus chinensis* Mill.: A systematic review. *European Journal of Integrative Medicine*, *21*, 43-49. https://doi.org/10. 1016/j.eujim.2018.06.011.

Lu, X., Ross, C.F., Powers, J.R., Aston, D.E., & Rasco, B.A. (2011). Determination of total phenolic content and antioxidant activity of garlic (*Allium sativum*) and elephant garlic (*Allium ampeloprasum*) by attenuated total reflectance–fourier transformed infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, *59*, 5215-5221. https://doi.org/10.1021/jf 201254f.

Mallikharjuna, P.B., Rajanna, L.N., Seetharam, Y.N., & Sharanabasappa, G.K. (2007). Phytochemical studies of *Strychnos potatorum* L.f.- A medicinal plant. *E-Journal of Chemistry, 4*, 510-518

Rajbhandary, S., Siwakoti, M., Rai, S.K., & Jha, P.K. (2020). An overview of plant diversity in Nepal (pp. 1-15). In Siwakoti, M., Jha, P.K., Rajbhandary, S., & Rai, S.K. (Eds.), *Plant Diversity in Nepal*. Botanical Society of Nepal.

Sabudak, T., Demirkiran, O., Ozturk, M., & Topcu, G. (2013). Phenolic compounds from *Trifolium echinatum* Bieb. and investigation of their tyrosinase inhibitory and antioxidant activities. *Phytochemistry*, *96*, 305-311. https://doi.org/10. 1016/j.phytochem.2013.08.014.

Sharma, K.R., & Adhikari, S. (2023). Phytochemical analysis and biological activities of *Artemisia vulgaris* grown in different altitudes of Nepal. *International Journal of Food Properties*, *26*, 414-427, doi:10. 1080/10942912 .2023.2166954.

Singh, M., Pandey, N., Agnihotri, V., Singh, K.K., & Pandey, A. (2017). Antioxidant, antimicrobial activity and bioactive compounds of *Bergenia ciliata* Sternb.: A valuable medicinal herb of Sikkim Himalaya. *Journal of Traditional and Complementary Medicine*, 7, 152-157, 152e157. https://doi.org/10.1016/j.jtcme.2016.04.002.

Subedi, L., Timalsena, S., Duwadi, P., Thapa, R., Paudel, A., & Parajuli, K. (2014). Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. *Journal of Traditional Chinese Medicine*, *34*, 584-590. https://doi.org/10.1016/S0254-6272(15)30067-4.

Temraz, A., & El-Tantawy, W. (2008). Characterization of antioxidant activity of extract from *Artemisia vulgaris*. *Pakistan Journal of Pharmaceutical Sciences*, *21*, 321-326.

Vallès, J., Garcia, S., Hidalgo, O., Martín, J., Pellicer, J., Sanz, M., & Garnatje, T. (2011). Biology, Genome Evolution, Biotechnological Issues and Research Including Applied Perspectives in *Artemisia* (Asteraceae). *Advances in Botanical Research*, 60, 349-419, https://doi.org/10.1016/B978-0-12-385851-1.00015-9.