Nepal J Biotechnol. 2024 Jul; 12 (1):67-73 Research article DOI: https://doi.org/10.54796/njb.v12i1.305

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Investigation of Phytochemicals, Antimicrobial and Antioxidant Properties of *Taxus wallichiana* Found in Different Altitudes of Nepal

Samikshya Kafle[1], Saugat R. C.[1], Sahana Thapa[1], Sabin Pokharel[1], Anon Chaulagain[1], Anil S. Basnyat[2]

[1] Department of Biotechnology, Himalayan White House International College, 44600, Nepal.
[2] Research Department, National Ayurveda Research and Training Center, 44600, Nepal.

Received: 15 Mar 2024; Revised: 30 Jun 2024; Accepted: 02 Jul 2024; Published online: 31 Jul 2024

Abstract

Indigenous people of hilly region have been using *Taxus* as a medicinal source for various diseases including cancer. Taxol, one of its main constituents, has been recognized and backed by scientific research; it has been used as a cancer cell suppressing biomolecule in chemo therapies. This study was carried out to collect data that would provide information on the influence of altitude on the production of metabolites and to investigate the phytochemicals, antimicrobial, and antioxidant properties present in *Taxus wallichiana Zucc.* found in Nepal. Needles were used for phytochemical extraction by Soxhlet method where methanol was used as a solvent. Some of the primary metabolites including carbohydrate and secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, coumarin & steroid were present. Quantitative estimation of the total flavonoid and phenol content of plant extracts were determined. TPC of the plant extract from Dolakha and Jajarkot were 68.745 ± 0.70 and 21.323 ± 1.15GAE/g in 100% concentration respectively. Similarly, TFC from Dolakha and Jajarkot were found to be 5.904 ± 0.024 and 6.275 ± 0.54 Quercetin equivalent per gram. Furthermore, their antioxidant (DPPH) and antimicrobial property was studied. The IC50 value for extract of *T. wallichiana* from Dolakha was 94.72 µg/mL and Jajarkot was 129 µg/mL, which was comparatively higher than the IC50 (14.54 µg/mL) of Quercetin. Additionally, the plant extract of concentrations of 700 and 950 mg/mL exhibited promising antibacterial properties in *E. coli* and *S. aureus,* except *K. pneumonia*. This study indicates that there is no significant role of altitude in metabolite production of *T. wallichiana zucc*. Nevertheless, it could be the potential drug due to considerable amounts of phenol and flavonoid contents including natural oxidant and antimicrobial properties.

Keywords: Phytochemical, Antioxidant, Antimicrobial, Total Phenolic Content, Total Flavonoid Content

Corresponding author, email: saugatrc01@hotmail.com

Introduction

Nepal has a diverse array of medicinal plants, and combining this with local knowledge of aromatic and medicinal plants offers a compelling strategy for the creation of novel e.g.: anti-infective medications [1]. Himalayan yew has long been used to treat inflammatory diseases that cause pain and high fever. For the treatments of colds, coughs, respiratory infections, dyspepsia, and epilepsy, it is ingested as herbal tea, and juice[2].

The Himalayan yew (*Taxus wallichiana*) is a small to medium-sized evergreen tree that may be found between 1800 and 3100 meters above sea level in the hills and temperate Himalayas. The genus *Taxus* has received the most attention in terms of the existence of Toxoid. Studying the needles, stem bark, heartwood, and seeds of *T. wallichiana* separately led to the creation of a number of structurally unique Taxoid varieties[2]. Although there is a long history of using medicinal plants, ethnology-pharmacology as a well- defined *field* of study is relatively new, having only been around for 50 years. Testing randomly chosen plants is not as effective as using ethnology pharmacological selection along with testing on a variety of diseases (viruses, bacteria, fungi, etc). Multi-drug resistance has arisen from indiscriminate use of over-the-counter antibiotics commonly used to treat infections. In addition to this problem, antibiotics have a negative effect on immunosuppression, hypersensitivity, and allergic reactions[3]. For these reasons, scientists are producing alternative antimicrobial from medicinal plants to treat infections.

Due to varied climatic and altitude variations in Nepal, it offers greater possibilities for the survival of plants with high medicinal values. However, the lack of adequate research and studies on them has limited their use in therapeutics. This warrants the need for further research and development on plant species and their natural products with a focus on naturally derived compounds to be used as anticancer drugs[4]. The main objective of this research is to investigate the Phytochemicals, antimicrobial and antioxidant

properties of *Taxus wallichiana* found in Dolakha (1900m) and Jajarkot (3100m) districts of Nepal.

Materials and Methods

Qualitative and quantitative research methods were applied. Analytical study was performed on the needles of *T. wallichiana*. At first, needles (leaves) of *T. wallichiana* were collected from Dolakha and Jajarkot districts of Nepal. The altitude of collection site is 1900 (Latitude: 27° 38.269' N Longitude: 86° 9.777' E) and 3100 (Latitude: 28° 57.992' N Longitude: 82° 7.248' E) meters in Baitheswor-1, Dolakha and Paink-9, Jajarkot respectively. The leaves were sampled in early July 2021, and they were young at the time of collection. The samples were collected in bulk; however, due to inadequate transportation to the sample sites, replication for each district could not be performed. To identify the study plant, the herbarium of each plant was submitted to the Department of Plant Resources, National Herbarium and Plant Laboratories (KATH). The needles along with the twigs were shade dried for a week. Subsequently, the needles were separated from the twigs to make a fine powder.

In the Soxhlet extractor, 25g of powered needle was placed on a thimble and extracted with 80% methanol solvent droplets from a distillation flask. The extraction process was repeated until the sample extract became colorless. The extract was concentrated using rotary evaporator in which a plant extract was placed in a 250 mL round bottom flask [5].The Rotary Evaporator (Bio Base Re 2000 B) was run for 45 minutes and the final extract was weighed at 14 grams. Finally, the extract was stored in a refrigerator at 4 °C for further experimental work[6].

The phytochemical investigation was carried out according the methods described on the paper with few modifications[7].

Tannin Test

0.28 g of the extract was added to 5 mL of distilled water and mixed it properly. Then, 1 mL of 1% Ferric chloride solution was added. The dark blue color indicated the presence of tannin**.**

Saponin Test:

For the detection of saponins in the extract, 0.1 g of plant extract was added to 2 mL of distilled water. The extract was further diluted up to 6 mL by adding 4 mL of distilled water. The mixture was then shaken vigorously and foam was observed. A constant foam indicated the presence of saponins.

Flavonoid Test

For the detection of flavonoids in the extract, the plant extract solution was prepared by mixing 0.25 g of the extract in 5 mL of distilled water. Then 5 mL of diluted ammonia was added to a 5 mL plant extract solution. Finally, 5 mL of sulfuric acid was added to the test tube containing plant extract and treated with a diluted ammonia solution. The orange color indicated the presence of flavonoid.

Alkaloid Test

For the detection of alkaloids in the extract, 0.36 g of plant extract was added to 6 mL of distilled water. The solution was then treated with 6 mL of 1 % HCl. Then, it was filtered and 1 mL of Wagner's reagent was added drop-wise. The orange-brown precipitate indicated the presence of an alkaloid.

Glycoside Test

0.5 g of plant extract was added to 2 mL of chloroform to which 1 mL of Concentrated Sulfuric acid was added drop-wise. The pink color indicated the presence of Glycosides.

Anthraquinonoid Test

For the detection of anthraquinonoid in the extract, 1 g of plant extract was added to the 4 mL of concentrated sulfuric acid. The solution was then filtered. The filtrate was treated with 1 mL chloroform. After which the chloroform layer was pipetted out. 1 mL of 10% diluted ammonia was added to the pipetted chloroform. The formation of a blue color indicated the presence of anthraquinonoid.

Steroid Test

For the detection of steroids in the extract, 1 mL of plant extract was prepared to which 1 mL of chloroform was added. The solution was then treated with concentrated Sulfuric acid (drop-wise). The red color indicated the presence of steroid.

Coumarin Test

For the detection of coumarin in the extract, a 2N NaOH (Sodium Hydroxide) solution was prepared by adding 0.8 grams of NaOH to 10 mL of distilled water. Then, 1 mL of plant extract was treated with 2N NaOH drop-wise. The yellow color indicated the presence of coumarin.

Terpenoid Test

0.5 g of plant extract was dissolved in 2 mL of chloroform. The solution was then treated with 1 mL of concentrated Sulphur acid. To, the formation of reddish

brown color at chloroform inter phase indicated the presence of Terpenoid.

Phenols Test

0.3 g of plant extract was dissolved in 6 mL of distilled water. Then, a few drops of 10% $FeCl_3$ were added. The blue-green color indicated the presence of phenol.

Reducing Sugar Test

1 mL of plant extract was mixed with 10 mL of distilled water in a test tube. Then it was treated with 1 mL of each of the Fehling solution A and Fehling's solution B. The brisk red color indicated the presence of reducing sugar.

Carbohydrate Test

2 mL of plant extract was treated with a few drops of alpha-napthanol. Then, a few drops of concentrated sulfuric acid were added drop-wise. The formation of a violet ring indicated the presence of carbohydrates.

The estimation of Total Phenolic Content and Total Flavonoid Content was carried out according the methods mentioned in the paper with few modifications [8].

Preparation of a standard solution for TPCs

0.03 grams of Gallic acid was dissolved in 30 mL of 80% methanol. Then a serial dilution was performed to obtain concentrations of (10, 25, 50, 75 and 100) mg/mL using 80% methanol as a diluting solution.

Preparation of 10% FC reagent solution:

8 mL of FC reagent was added to 80 mL of distilled water to get a 10% (v/v) FC reagent.

Preparation of 7.5% sodium carbonate solution:

5.25 g of sodium carbonate (Na_2CO_3) was dissolved in 70 mL of distilled water to obtain 7.5% sodium carbonate.

Preparation of blank

Blank was prepared by adding 1 mL of 80% methanol in a test tube containing 5 mL of 10 % FC reagent and 4 mL of 7.5% sodium carbonate solution.

Sample preparation

The sample was prepared by adding 0.03 g of powdered plant extract to 30 mL of 80% methanol. Then, the absorbance was measured against the blank at 510nm using a UV-vis spectrophotometer.

The serial dilution was performed using 1 mL of stock solution for each concentration to obtain a final concentration of 100%, 75%, 50%, 25% and 10%.

The total phenol content (TPC) is calculated using:

$$TPC = \frac{x \times v}{m}$$

Whereas, x= concentration of Gallic acid (mg/mL)

v= volume of extract used

m= mass of extraction used

Preparation of standard solution for TFCs

0.02 g of Quercetin acid was dissolved in 20 mL of absolute methanol. A serial dilution was performed and concentrations of 10, 25, 50, 75, and 100 mg/mL were made using absolute methanol as a diluting solution.

Preparation of 5% sodium nitrate and 10% aluminum chloride

0.5 g of sodium nitrate ($NaNO_3$) was added to 10 mL of distilled water to obtain a solution of sodium nitrate of 5% (w / v). 1 g of aluminum chloride ($AlCl_3$) was added to 10 mL of distilled water to obtain 10% (w / v) aluminum chloride of 10% (w / v).

Preparation of blank

To prepare a blank, 1 mL of absolute methanol was added to a test tube containing 4 mL of distilled water, 0.3 mL of 10% aluminum chloride and 0.3 mL of 5% sodium nitrate solution.

Sample preparation

0.03g of powered needle extract was dissolved in 30 mL of absolute methanol. Then, the absorbance was measured against the blank at 510nm using a UV-Vis spectrophotometer.

The total flavonoid content (TFC) is calculated using;

$$TFC = \frac{x \times v}{m}$$

However, x = concentration of Quercetin (mg/mL)

v= volume of extract used

m= mass of extract used

Microbial Test

To standardize microbial testing, a McFarland standard was used. When adjusting the turbidity of the liquid or bacterial suspension in the vial or tube in the microbiology laboratory, McFarland standards were used as the reference [9].

Disc Diffusion Method

The most widely used agar diffusion technique, the Kirby-Bauer disk diffusion method, was used. The filter paper discs of 6 mm were made by paper stapler and sterilized using autoclave. The diameter of the zone of impeded growth surrounding the disk is measured after overnight incubation. The antimicrobial test has been carried out in the ATCC 700603 culture of *Klebsiella pneumonia* and the ATCC 25923 culture of *Staphylococcus aureus*. It has been carried out on clinical samples of *E.*

coli. Similarly, for the susceptibility test, the bacteria were cultured under those conditions similar to those mentioned in the antimicrobial test. A 30 mcg disc of Tetracyclin (TE), 30mcg of Nalidixic Acid (NA), 30/10 mcg of Clavulanic acid (CAC), and 20/10 mcg of Amoxicillin clavulanic acid (AMC) was used in each culture plate and the inhibition zone was calculated using mm ruler.

Agar Well Method

To perform an antimicrobial test, the bacteria were first cultured in liquid broth for 14 hours. The bacteria were then sub-cultured on Mueller Hinton Agar plates with the help of a sterile cotton swab. Finally, the different concentration of plant extract from 500, 600, 700, 800 and 950 mg/mL was made respectively and the disc diffusion method was employed to detect antimicrobial property. A sterile borer was used to create a hole on the surface of the agar where extract samples were placed. The broth culture was incubated for 14 hours followed by 14 hours of agar plate incubation. After which, the zone of inhibition was measured using an mm ruler[10].

DPPH Assay

The sample extracts were sent to the Department of Plant Resources, Thapathali-Kathmandu, Nepal, to assess the antioxidant property.

Data Analysis

The data obtained were analyzed using MS excel and IBM SPSS Statistics version: 29.0.0.0 (241) application.

Results

Phytochemical screening results

After screening the extraction, phytochemical analysis was performed. The phytochemical results were as shown in **Table 1.**

Table 1. Result of phytochemical screening (Primary Metabolites)

Plants	Phytochemicals	
	Carbohydrates	Reducing sugar
T. wallichiana (J)	+	+
T. wallichiana (D)	+	+

(The '+' sign indicates the presence of phytochemicals, while the '-' sign indicates the absence of phytochemicals, J = Jajarkot D= Dolakha)

Table 2. Result of phytochemical screening (Secondary Metabolites)

Plants	Phytochemicals									
	Terpenoids	Flavonoids	Tannins	Alkaloids	Saponins	Anthraquinones	Phenols	Glycosides	Steroids	Coumarins
T. wallichiana (J)	+	+	+	+	+	-	+	-	+	+
T. wallichiana (D)	+	+	+	+	+	-	+	-	+	+

Quantification of total phenolic content

The folic acid-ciocalteu method was used to calculate the total phenol content in the needle extracts of *T. wallichiana* from Jajarkot and Dolakha, while standard Gallic acid was used. From regression equation of calibration curve (Y=0.03x; R^2=0.985), the total phenol content was calculated and expressed as mg Gallic Acid Equivalents (GAE) per gram of sample in dry weight (mg/g).

The Phenol Content at the extract in 100, 75, 50, 25 and 10 % concentration was found to be 68.745 ± 0.70 mg GAE/g, 29.187 ± 0.75 mg GAE/g, 18.347, 5.777 ± 0.491 mg GAE/g and 0.833 ± 0.3 mg GAE/g respectively for the Dolakha sample while for the sample from Jajarkot, it was found to be 21.323± 1.15 mg GAE/g, 8.054 ± 0.1 mg GAE/g, 4.235 ± 0.35 mg GAE/g, 1.198 ± 0.45 mg GAE / g, and 0.056 ± 0.70 mg GAE/g respectively.

The absorbance for each concentration was recorded in three repetitions and the mean TPC value was calculated.

Table 3. Total phenol content of different extracts of T. *wallichiana* (GAE/g)

Concentration (%)	Mean TPC value (GAE/g)	
	T.wallichiana (Dolakha)	*T. wallichiana* (Jajarkot)
100	68.745 ± 0.70	21.323± 1.15
75	29.187 ± 0.75	8.054 ± 0.10
50	18.347± 0.68	4.235 ± 0.35
25	5.777 ± 0.491	1.198 ± 0.45
10	0.833 ± 0.30	0.056 ± 0.70

Quantification of total flavonoid content

The total flavonoid content was quantified from the regression equation of the calibration curve (Y=0.03x; R^2=0.987) and expressed as mg equivalents of quercetin (QE) per gram of sample in dry weight (mg/g).

TFC in the extract from Dolakha and Jajarkot was found to be 5.904 ± 0.024 and 6.275 ± 0.54 QE/g respectively.

Table 4 was formulated using the absorbance for each concentration of extracts and the mean TFC was calculated. The samples were prepared in triplicates for each analysis.

The polarity of the solvent used for extraction determines the concentration of phenols and flavonoids[11].

Table 4. Total Flavonoid Contents in Different Extracts of *T. wallichiana* (QE/g)

	Mean TFC value(QE/g)
T. wallichiana (Dolakha)	5.904 ± 0.024
T. wallichiana (Jajarkot)	6.275 ± 0.54

Table 5. IC_{50} Value of extracts

Pass No.	Name of sample	IC_{50} Value (µg/mL)
Standard	Quercetin	14.54
1	*T. wallichiana* (D)	94.72
2	*T. wallichiana* (J)	129

Antimicrobial test

For the susceptibility test, the bacteria were cultured and different classes of antibiotic drugs such as a 30 mg disc of Tetracycline (TE), Nalidixic acid (NA), Clavulanic acid (CAC) and Amoxicillin clavulanic acid (AMC) were used in each culture plate and zone of inhibition was calculated using a mm ruler. For negative control 80% methanol was used. In the *K. pneumonia* plate, the antibiotic AMC showed the highest zone of inhibition, while TE showed the lowest zone of inhibition. On *E. coli* (clinical) plate, antibiotic NA showed the highest zone of inhibition i.e. 9.2 mm whereas TE showed the lowest zone of inhibition i.e. 6.3 mm. on *S. aureus* plate, antibiotic NA showed the highest zone of inhibition i.e. 9.4 mm whereas, TE showed the lowest zone of inhibition i.e. 6.3 mm. The Jajarkot sample extract showed the maximum zone of inhibition zone, i.e. 12 mm on *S. aureus*. Similarly, the Dolakha sample extract showed the maximum on *E. coli* plate, that is, 12 mm in a disc diffused plate of 700 mg/mL concentration.

The Jajarkot plant extract showed the maximum zone of inhibition i.e. 12mm on *S. aureus.* Similarly, the extract from Dolakha showed the maximum zone of inhibition i.e. 13 mm on *E . coli* urine disc diffused plate. Moreover, in *E. coli* blood sample Jajarkot sample showed no zone of inhibition (nil).

In agar well plates at 700 mg/mL concentration, the zone of inhibition was not obtained on *S. aureus* and *K. pneumonia* plates. However, in *E. coli* urine plates both extracts showed the same length of inhibition zone, that is, 9mm. Furthermore, the extract from Jajarkot *E. coli* blood plates showed the maximum zone of inhibition, that is, 9 mm.

In agar well plates at concentrations of 950 mg / mL, no zone of inhibition was observed in plates of *S. aureus* and *K. pneumonia.* On the contrary, the extract from the Jajarkot sample showed the maximum zone of inhibition, i.e., 9 mm *in the E. coli* plate.

Antioxidant activity result by DPPH

According to the Department of Plant Resources (DPR), the antioxidant properties of plant extracts were determined by calculating the IC50 value as given below

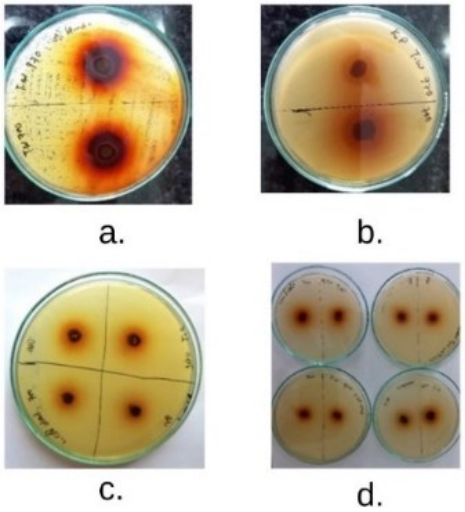

Figure 1. (a) ZOI seen in *E. coli* blood culture using *T. wallichiana* (Dolakha), (b) ZOI seen in *K. pneumoniae* culture using *T. wallichiana* (Dolakha), (c) ZOI on E. coli blood culture using *T. wallichiana* (Jajarkot), (d) ZOI seen on *E. coli* (Urine), S. aureus, and *E. coli* (Blood) using T. *wallichiana* (Dolakha) extract and on S. aureus using *T. wallichiana* extract (Jajarkot). [Serially from left to right]

Discussion

By conducting a series of biochemical tests, including the chloroform test, ammonia test, Molish test, Buiret test, sulphuric test, Wagner's test, and ferric chloride test, we confirmed the presence of metabolites such as carbohydrates, alkaloids, Flavonoids, tannins, phenol, saponins, steroids, and coumarin. We also confirmed the absence of metabolites like anthraquinone, glycosides, and protein. The presence of alkaloids suggests the potential for anti-cancer molecules such as taxol [12].

Prior studies focused on *Taxus wallichiana Zucc.*'s anti-cancer properties, with recent research in Nepal revealing its antibacterial and antioxidant potential. A new study delves into phytochemicals and biological activities of *T. wallichiana* from Jiri, Dolakha, evaluating its antimicrobial and antioxidant strengths in leaves and stems. Using the FRAP assay, researchers aim to enhance understanding of the plant's antioxidant capabilities. The study underscores the impact of geography and environment on *T. wallichiana*'s bioactive compounds, emphasizing its potential as a natural source of antioxidants and antimicrobials for innovative therapeutic uses [13].

According to [14], their research on *T. wallichiana* found metabolites similar to our study, including alkaloids, coumarin, glycosides, tannins, phenol, reducing sugars, Flavonoids, and steroids. However, they reported the absence of saponins, which were present in our extracts.

The estimation of phenol and flavonoid content was performed using Gallic acid and Quercetin as standards, respectively. Our results indicate that a 100% concentration of the Dolakha sample extract contained 68.745 ± 0.70 (GAE/g), whereas the Jajarkot sample contained 21.323 (GAE/g) of phenol. The flavonoid content was 5.904 ± 0.024 (QE/g) in the Dolakha sample and 6.275 ± 0.54 (QE/g) in the Jajarkot sample, showing higher flavonoid content per gram extract in *T. wallichiana* from Jajarkot compared to Dolakha.

According to [15], the total phenol and flavonoid contents of *T. wallichiana* were significantly affected by altitude variations, with the highest phenol (92.67±0.68 GAE mg/g DW) and flavonoid (84.66±0.52 QE mg/g DW) contents observed. Similarly, [16] demonstrated in their study on grape extracts that elevated UV-B radiation at higher altitudes increases the synthesis of anthocyanins, flavonoids, and tannins.

The Department of Plant Reports provided significant insights into the antioxidant properties of the extracts. According to their report, the IC_{50} value of extract TC-1 (Dolakha sample) was 94.72, while the IC_{50} value of extract TC-2 (Jajarkot sample) was 129.0. A lower IC_{50} value indicates higher antioxidant properties, suggesting that *T. wallichiana* from Dolakha has more potent antioxidant molecules than those from Jajarkot. The presence of flavonoids in the extracts, such as quercetin and its derivatives, supports these findings, as quercetin is known for its antioxidant activity[17].

The antioxidant activity was expressed as the inhibitory concentration (IC_{50}), which is the quantity of extract required to reduce 50% of the initial concentration of free radicals. In the concentration range of 40–60 g/mL, the extracts were found to be strong antioxidants, capable of scavenging and neutralizing 50% of free radicals. This range was comparable to the antioxidant activity observed in *Auricularia* and *Termitomyces spp*. Extracts[18]. Methanol macerated extracts showed an IC50 of 38.59 ± 1.74 g/mL, while aqueous macerated extracts demonstrated the most potent anti-radical action with an IC50 of 2.78 ± 1.03 g/mL [19]. These results suggest that *Atractylis gummifera* macerates can enhance the antioxidant enzyme defense system and reduce oxidative stress-related tissue damage in diabetic mice.

Quercetin was used to estimate the antioxidant properties of *T. wallichiana* extracts. Quercetin is known to promote p53-independent cell death in numerous cancer cell lines and encourages lysosomal-dependent ferrite breakdown and free iron release. It has various cancer-inhibiting capabilities alongside its well-studied antioxidant, anti-inflammatory, anti-fibrotic, and antiviral properties [20].

Triplicates of the antimicrobial test per sample were done. For the investigation of the antimicrobial property, two effective methods were used: the disc diffusion method and the agar well diffusion method. The antibiotic susceptibility test showed that all tested microbes, having zones of inhibition ranging from 10 to 12 mm, were resistant to all antibiotics used. Plant extracts at concentrations of 950 and 700 mg/mL were prepared, and the zones of inhibition were measured. In the disc diffusion method, the extract exhibited antibacterial effects on all sample strains except for *E. coli* from the *T. wallichiana* (Jajarkot) sample. In the agar well diffusion method, proper zones of inhibition were observed on plates with *E. coli* samples, but the extract was found to be ineffective against *K. pneumonia* and *S. aureus.* These results indicate that the needle extract from both samples possesses antimicrobial properties.

According to [15], qualitative estimations showed that crude extracts of plant parts exhibited inhibitory effects on both gram-positive and gram-negative bacteria as well as fungi. The inhibitory effects were visible on various bacteria using our plant extracts.

The variation in the antibacterial effects of *T. wallichiana* extracts on different bacterial strains can be attributed to factors such as bacterial resistance mechanisms, specific bio active compounds in the extracts, and the interaction between these compounds and bacterial cells. Bacterial resistance mechanisms like efflux pumps, enzyme production, and alterations in target sites can influence susceptibility to plant extracts, explaining why the *E. coli* strain from Jajarkot showed resistance in the disc diffusion method but inhibition in the agar well diffusion method. Variations in bio active compound concentrations and their effectiveness against specific bacteria, as well as differences in solvent diffusion rates and local extract concentrations between the two methods, also contribute to this variability. In contrast, the *T. wallichiana* extract from Dolakha exhibited broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria in both testing methods, indicating a consistent and potent antimicrobial profile, likely due to a higher or more

effective concentration of bio-active compounds compared to the Jajarkot sample.

Conclusion

Himalayan yew, also known as *Taxus wallichiana*, is a threatened healing plant that has sparked research curiosity due to its potent anticancer effects. Our research examined the species in Nepal at different elevations, with a focus on its phytochemical, antimicrobial, and antioxidant characteristics. Needle samplings in Dolakha and Jajarkot districts showed the existence of important phytochemicals, such as alkaloids, flavonoids, tannins, and others. These compounds showed antibacterial effects on gram-positive and gram-negative bacteria, suggesting promise as potential new antibiotics.

The study enhances the scientific knowledge of *T. wallichiana*'s chemical makeup and emphasizes its potential for medical purposes. Significantly, the anticancer alkaloids are in line with worldwide emphasis on natural cancer therapies. The existence of phenols and flavonoids in the antioxidant properties indicates possible advantages in fighting diseases caused by oxidative stress. Surprisingly, altitude did not significantly affect the phytochemical levels and antimicrobial effectiveness, suggesting that *T. wallichiana* can thrive at different altitudes without compromising its medicinal properties. These results back up the need for the creation of plans to protect and utilize *T. wallichiana* in Nepal for medicinal purposes, encouraging more studies to maximize its advantages.

Data availability

All the data generated during this study are presented in this article.

Conflict of interest

The authors declare no conflicts of interest.

Funding Statement

This work was solely supported by the Himalayan WhiteHouse International College, Putalisadak-Kathmandu, Nepal.

Acknowledgement

The authors express their deepest gratitude to Himalayan White House International College, National Ayurveda Research Center for enabling us successfully complete the project.

References

1. Joshi B, Panda, Kumar S, Jouneghani, Saleh R, Liu M, et al. Medicinal Plants of Nepal Selected Based on Ethnobotanical Evidence. Evidence-Based Complement Altern Med. 2020;2020:1–14.
2. Juyal D, Thawani V, Thaledi S, Joshi M. Ethnomedical properties of Taxus wallichiana Zucc. (Himalayan yew). J Tradit Complement Med. 2014;4(3):159–61.
3. Kazmi N, Hassan W, Hussain S, Khan K, Amir S, Rehman HU, et al. Estimation of phytochemicals, inorganic profile and antimicrobial activity of Taxus baccata shoots. J Pure Appl Microbiol. 2015;9(Special Edition 1):375–82.
4. Greenwell M, Rahman PKSM. Medicinal Plants: Their Use in Anticancer Treatment. Int J Pharm Sci Res [Internet]. 2015;6(10):4103–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26594645%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4650206
5. López-Bascón-Bascon MA, Luque de Castro MD. Soxhlet extraction. Liq Extr. 2019;327–54.
6. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020;12(1):1–10.
7. Harris KK, Verma D, Sahu M. Phytochemical Analysis of Helianthus Annus Lin., (Angiosperms: Asteraceae Phytochemical Analysis of Helianthus Annus Lin., (Angiosperms: Asteraceae). World J Pharm Pharm Sci . 2017;6(3):825–46.
8. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. In Vitro Antibacterial Activities of Methanolic Extracts of Fruits, Seeds, and Bark of Zanthoxylum armatum DC. J Trop Med. 2020;2020.
9. Mcfarland J. mouths of the blanching an instrument for estimating the number of bac-teria in suspensions used for calculating the opsonic index and for vaccines. Those who have used the method of Leishman or the later method of Wright for estimating the phagocytic or. J Am Med Assoc. 1907;49(15):1176–8.
10. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9.
11. Jing L, Ma H, Fan P, Gao R, Jia Z. Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. BMC Complement Altern Med [Internet]. 2015;15(1):1–12. Available from: http://dx.doi.org/10.1186/s12906-015-0820-3
12. Giupponi L, Leoni V, Pavlovic R, Giorgi A. Influence of altitude on phytochemical composition of hemp inflorescence: A metabolomic approach. Molecules. 2020;25(6).
13. Subba B. Analysis of Phytochemical Constituents and Biological Activity of Taxus Wallichiana Zucc. Dolakha District of Nepal. Int J Appl Sci Biotechnol. 2018;6(2):110–4.
14. Adhikari P, Joshi K, Singh M, Pandey A. Influence of altitude on secondary metabolites, antioxidants, and antimicrobial activities of Himalayan yew (Taxus wallichiana). Plant Biosyst [Internet]. 2022;156(1):187–95. Available from: http://dx.doi.org/10.1080/11263504.2020.1845845
15. Adhikari P, Pandey A. Taxus wallichiana zucc . (Himalayan Yew) in antimicrobial perspective. Adv Biotech Micro. 2017;4(5):10–3.
16. Mansour G, Ghanem C, Mercenaro L, Nassif N, Hassoun G, Del Caro A. Effects of altitude on the chemical composition of grapes and wine: a review. Oeno One. 2022;56(1):227–39.
17. de Lima Paula P, de Oliveira Lemos AS, Campos LM, Ferreira TG, Freitas de Souza T, Queiroz LS, et al. Pharmacological investigation of antioxidant and anti-inflammatory activities of leaves and branches extracts from Plinia cauliflora (Jaboticaba). J Ethnopharmacol. 2021;280(May).
18. Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Investigation of Antioxidant and Antimicrobial Activities of Different Extracts of Auricularia and Termitomyces Species of Mushrooms. Sci World J. 2019;2019.
19. Bouabid K, Lamchouri F, Toufik H, Faouzi MEA. Phytochemical investigation, in vitro and in vivo antioxidant properties of aqueous and organic extracts of toxic plant: Atractylis gummifera L. J Ethnopharmacol. 2020;253(January).
20. Wang ZX, Ma J, Li XY, Wu Y, Shi H, Chen Y, et al. Quercetin induces p53-independent cancer cell death through lysosome activation by the transcription factor EB and Reactive Oxygen Species-dependent ferroptosis. Br J Pharmacol. 2021;178(5):1133–48.