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Taxus wallichiana Zucc. (Himalayan Yew): A Medicinal Plant Exhibiting Antibacterial Properties

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Abstract

Taxus wallichiana Zucc. or the Himalayan yew is a gymnosperm growing along the Himalayan region of India and adjacent countries. Traditionally, this plant was extensively used by indigenous people for folk medicines for treating various diseases such as fever, headache, diarrhoea, fractures, problems of the nervous system etc. It is also practiced in the Unani system of medicine. The plant is rich in various bio-organic compounds and natural products, such as hydrocarbons, glycosides, flavonoids, phenol, tannins, terpenoids etc. In this research work, an effort has been made to highlight the valuable properties of *T. wallichiana*. The present study was undertaken to evaluate the secondary metabolites (flavonoids, glycosides, phenols, saponins, tannins, terpenoids) and antibacterial potential of methanol extracts and the subsequent fractions of the leaves and fruit of *Taxus wallichiana* Zucc. In order to rationalise traditional use, methanol extracts from the leaves and fruit of *Taxus wallichiana Zucc*. were tested against five bacteria using the agar well diffusion method. Ciprofloxacin was used as a standard. All extracts and fractions displayed significant anti-microbial effects. *Taxus wallichiana* leaves and fruit methanolic extracts showed a maximum zone of inhibition with *Bacillus subtilis*, which is 18 ± 0.0 mm, and *Staphylococcus aureus*, 19 ± 0.2 mm. The methanolic extracts of the leaves of *Taxus wallichiana* tested positive for glycosides, flavonoids, phenol, tannins and terpenoids, whereas the *T. wallichiana* fruit tested positive for flavonoids, saponins and terpenoids. According to the research findings, it was identified that the methanol extract of *Taxus wallichiana* exhibited quite high antimicrobial activity as well as secondary metabolites, and with this quality, together with lots of its other values, this plant can very well become a source of medicine for the better management of a large number of diseases, including cancer, and value-added products.

Keywords

Agar well diffusion assay · Antibacterial · Microorganisms · Multidrug resistant · *Taxus wallichiana*

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Abbreviations

ATCC	American Type Culture Collection
C	ciprofloxacin

E	extract
h	hours
MDR	multidrug resistant
SD	standard deviation
T. wallichiana	*Taxus wallichiana*

1 Introduction

Globally, infectious disease is the major cause of death, accounting for approximately one-half of all deaths in tropical countries (Iwu et al. 1999). New therapeutic agents and strategies are demanding issues to cope with infectious diseases. Low-income people, especially from small remote villages and native communities, use folk medicine for the treatment of common infections. These plants are ingested as decoctions, teas and juice preparations to treat respiratory infections or as a poultice and are applied directly on the infected wounds or burns (Gonzalez 1980; Kaul 1997). Exploration in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as a substitute solution to the health problems in cities. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important (Bhardwaj 2022). The Himalayan region has been a rich source of medicinal plants for millions of populations inhabiting the mountain ranges and those around them. The Himalayan region is rich in floral diversity, and plants are extensively used by the local people for their daily needs, such as for thatching and shelter, fuel, fodder, household items and medicines (Rana et al. 2019; Pala et al. 2019).

Taxus wallichiana Zucc. is a member of the family Taxaceae, which is commonly known as the Himalayan yew (Shrestha et al. 1997). *Taxus wallichiana* is one such gymnosperm that grows in the Himalayan region. It is a small to medium-sized evergreen tree with a height of 10–28 m (Juyal et al. 2014). The plant is widely distributed in Asia, and its occurrence spans from Afghanistan to the Philippines and is widely distributed in the Himalayan regions of India and adjacent countries (Hussain et al. 2013). *T. wallichiana* is traditionally used by the local people of the Indian subcontinent for the cure of a number of ailments. In India, tincture prepared from the aerial part of the plant is traditionally used for the treatment of several diseases of the central nervous system, such as hysteria, grittiness, biliousness, epilepsy and nervousness. The plant also forms one of the components of the popular Unani drug ‘Zarnab’, which is known to possess sedative and aphrodisiac properties (Sharma and Garg 2015). *T. wallichiana* is also used indigenously by the people of Nepal for curing respiratory problems, bronchitis and cancer (Gaire and Subedi 2011). The leaves of *T. wallichiana* are also used to prepare herbal tea for the cure of epilepsy and indigestion (Aboutabl 2018). *T. wallichiana* is also reported to have immuno-modulatory, antibacterial, antifungal, analgesic, anti-pyretic and anti-convulsant activities (Rahman et al. 2013).

However, through a literature survey, it was revealed that no significant work has been done on the antibacterial and phytochemical activities of *Taxus wallichiana Zucc*. Keeping this knowledge in view, the present study was undertaken to investigate the antibacterial and secondary metabolite potential of *Taxus wallichiana Zucc.*

Botany and Uses

Taxus wallichiana (Himalayan yew) is the first ever Himalayan species of *Taxus* to be discovered by Joseph Gerhard Zuccarini in 1843, hence giving him the authority of the species and associating the code, Zucc., after his name while naming Taxus wallichiana. Initially, *Taxus baccata* (European yew) was the first *Taxus* species to be identified by Carl Linnaeus in 1753. *Taxus wallichiana* is a dioecious tree species (Yang et al. 2009). The stems are fluted with spreading branches. The barks are thin, reddish brown and scale like (Bhuju and Gauchan 2018). The leaves are dark grey in colour, glossy green above, paler beneath, linear, 2–3.8 × 0.3 cm in length, coriaceous, flattened and arranged in two vertical opposite rows. The cones are

(a) *Taxus wallichiana* leaves

(b) *Taxus wallichiana fruit*

Fig. 1 (**a**) Leaves, (**b**) fruit of *Taxus wallichiana*

axillary and sessile. The male cones are solitary, axillary and sub-globose; their bracts are empty; and they have ten stamens. The female cones are solitary with a few imbricate scales surrounding an erect ovule. The ovules are surrounded at the base by a membranous cup-shaped disc. The fruit has a bright red disc (Fig. 1b) and is succulent, enlarged and 7–8 mm in length. The seeds are olive green in colour, and they are dispersed by birds and animals. The growth of the trees is extremely low, with 12–14 annual rings per 2.5 cm radius and a girth increment of 0.4–1.3 cm per year (ENVIS). Details about the species' outline can be found at http://www.worldbotanical.com/Nomenclature.htm# (Nomenclature, Keys and Descriptions for species of Taxus with discussion and citation of specimens studied).

In our previous study, we analysed the potency of cedar deodar for antibacterial and secondary metabolite potential (Bhardwaj 2022). Also, we investigated that ghaf and mangrove have potential for antioxidant and antimicrobial properties (Bhardwaj 2021a, b, c). In addition, we investigated the nutraceutical and anti-microbial properties of ghaf (Al Ghais et al. 2020a, b, c; Bhardwaj 2021d, e). Therefore, to continue our further research and also meet the increasing demand for anti-microbial agents, we explored natural sources and alternative strategies to search for new anti-microbial agents. Hence, the objective of the study was to seek the anti-microbial activity of the methanolic extract of *T. wallichiana* and also secondary metabolites. This probe was carried out as an awareness of the medicinal value of the plant.

2 Material and Methods

2.1 Plant Material Collection

Samples of the leaves and fruit of the Himalayan yew plant were collected from Chail in Himachal Pradesh, India, in the month of December 2021, at an altitude of 2250 m above sea level, and were placed in plastic bags. The leaves and fruit of *T. wallichiana* were washed with water, dried at 45 °C for 6 h and then crushed into powder with a mixer (Bhardwaj 2021b).

2.2 Preparation of the Extracts

The powdered samples, 5 g, were extracted with 25.0 ml of methanol, followed by a continuous hot extraction method, and were stirred well and kept for incubation in closed containers. The tubes were centrifuged at 4000 rpm for 30 min, then the supernatant extract was transferred for drying for 10 min and, finally, residue of the leave sample was obtained. Weighed accurately 0.1 g of residue of leaves sample in test tube and added 1.0 mL of methanol [10% (w/v) solution]. The final concentration of extracts (residue with methanol) used for further experiment. All the extracts were then stored at 4 °C in a refrigerator for further analysis as crude methanolic extracts (Al Ghais et al. 2020b; Bhardwaj 2021d).

2.3 Chemicals

The chemicals used in the present investigation were of analytical grade and high purity, obtained from Merck and HiMedia. The standard kits and reagents used for analysis were purchased from Germany and the USA.

2.4 Test Organisms

In the present study, the bacterial strains used were *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the American Type Culture Collection (ATCC), to determine their antibacterial activity. The bacterial strains were procured from LTA srl Italia. Pure culture of bacteria was maintained at 4 °C on nutrient agar slants.

2.5 Methodology for the Detection of Antibacterial Activity

2.5.1 Inoculums Preparation

The bacterial pure culture isolates were first grown in 5 ml of nutrient broth in sterile test tubes for 18 h before use.

2.5.2 Agar Well Diffusion Assay

The antibacterial activity of the methanolic extracts of *T. wallichiana* (leaves and fruit) was tested against isolates using the agar-well diffusion method. An aliquot of 100 μl inoculum for each bacterial isolate was evenly spread onto Muller-Hinton agar using a sterile spreader and was allowed to settle at room temperature. A cork borer of 6 mm diameter was used to punch well in agar plates to cut uniform wells. Wells were bored in agar plates. The concentration of the extracts was 10% (w/v), prepared using methanol as solvent. Subsequently, 30 μl of extracts (leaves and fruit) were poured into the wells. Ciprofloxacin 30 μg was used as a positive control. Then the plates were kept at 2–8 °C in a refrigerator to allow diffusion of the extracts into the agar and were further incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured to the nearest millimetre (Sohel 2010; Uddin et al. 2007). The formation of a clear inhibition zone of ≥7 mm diameters around the wells was regarded as a significant susceptibility of the organisms to the extract (Bhardwaj 2022). The effect was compared to those of antibiotic discs. The tests were performed in triplicates, and the mean was taken. The whole experiment was performed under strict aseptic conditions.

2.6 Phytochemical Analysis

Test for Flavonoids (Ammonia Test)
One millilitre of the extract was taken and placed in a test tube, and an ammonia solution was added (1:5), followed by the addition of concentrated sulphuric acid. The appearance of a yellow color and its disappearance on standing indicates a positive test for flavonoids.

Test for Glycosides (Keller-Kiliani Test)
Five millilitres of each extract was added, with 2 ml of glacial acetic acid, which was followed by the addition of a few drops of ferric chloride solution and 1 ml of conc. sulphuric acid. The formation of a brown ring at the interface confirms the presence of glycosides.

Test for Phenols (Ferric Chloride Test)
Next, 0.5 ml of the extract was added, with a few drops of neutral ferric chloride (0.5%) solution. The formation of a dark green color indicates the presence of phenolic compounds.

Test for Saponins (Froth Test)
One millilitre of the extract was taken and placed in a test tube, and distilled water (2 ml) was added to it. The test tube was then kept in a boiling water bath for boiling and was shaken vigorously. The existence of a froth formation during warming confirms the presence of saponins.

Test for Tannins (Ferric Chloride Test)
One millilitre of the extract was added, with 5 ml of distilled water, and kept for boiling in a hot water bath. After boiling, the sample was cooled down, and to this, 0.1% ferric chloride solution

was added. The appearance of a brownish-green or blue-black coloration confirms the presence of tannins.

Test for Terpenoids (Salkowski Test)
Five millilitres of the extract was taken in a test tube, and 2 ml of chloroform was added to it, followed by the addition of 3 ml of conc. sulphuric acid. The formation of a reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

2.7 Statistical Analysis

The tests were performed in triplicates. Data are expressed as mean. Pair-wise comparisons were performed. An experimental error was determined for the triplicate and expressed as standard deviation (SD).

3 Results and Discussion

The objective of this research was to seek the antimicrobial activity of the methanolic extract of *T. wallichiana* and also secondary metabolites. This probe was carried out as an awareness of the medicinal value of *T. wallichiana*, for its activity against selected bacterial pathogens. Three different extracts of different parts of *T. wallichiana* (leaves and fruit) were treated using methanolic extraction. Methanolic extracts were found to be more potent against human pathogens. Similar results were reported by Derwich et al. (2010), who reported that the essential leaves of *Cedrus atlantica* were active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus sphaericus* and *Staphylococcus intermedius*. In the present study, phytochemical analysis of the Himalayan yew extracts was done to explore their composition. The results revealed the presence of terpenoids, flavonoids, glycosides, phenols, saponins and tannins. These results are similar to the results obtained by Devmurari (2010), who reported that phytochemical studies on *Cedrus deodara* revealed the presence of alkaloids, glycosides flavonoids, triterpenoid, tannins, proteins and fixed oil. In particular, terpenoid substances are secondary metabolites that characterise *C. libani*-derived products (Kizil et al. 2002; Yilmaz et al. 2005; Loizzo et al. 2008).

3.1 Antibacterial Activity of *T. wallichiana* Extracts Against Human Pathogenic Bacteria

Table 1 summarises the results of the antibacterial activities of the extracts of *T. wallichiana*, which were evaluated on *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853) by means of the agar well diffusion method. Leaves (Fig. 2) of *T. wallichiana* showed a zone of inhibition with all five strains of microorganisms that were used. The *T. wallichiana* fruit (Fig. 3) methanolic extract showed a maximum zone of inhibition with *Bacillus subtilis*, which is 17 ± 0.5 mm, and the bark showed a maximum zone of inhibition with *Staphylococcus aureus*, 21 ± 0.6 mm (Fig. 3). All the tested extracts of the Himalayan yew fruit showed no activity against *E. coli*, *Salmonella enterica* and *Pseudomonas aeruginosa* (Table 1).

Table 1 Antibacterial activity of the methanolic extracts of *Taxus wallichiana* leaves and *Taxus wallichiana* fruit

SNo.	Microorganisms	*Taxus wallichiana* leaves	*Taxus wallichiana* fruit
1	*Bacillus subtilis* (ATCC 6633)	18 ± 0.0	17 ± 0.5
2	*E. coli* (ATCC 8739)	2 ± 0.5	No zone
3	*Salmonella enterica* (ATCC 14028)	17 ± 0.2	No zone
4	*Staphylococcus aureus* (ATCC 6538)	19 ± 0.2	21 ± 0.6
5	*Pseudomonas aeruginosa* (ATCC 27853)	1 ± 0.1	No zone

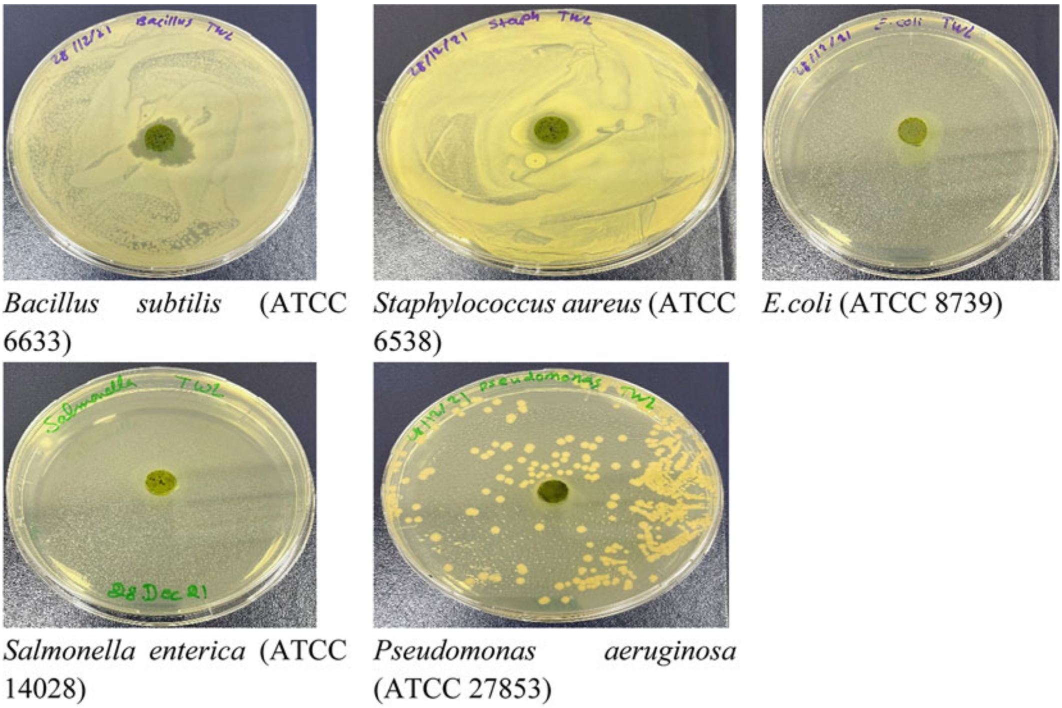
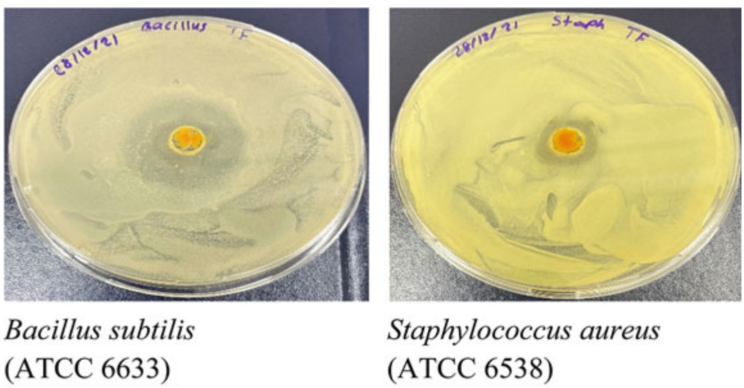


Fig. 2 Extracts of *Taxus wallichiana* leaves showed antibacterial activity, as indicated by the zone of inhibition against different microorganisms' strains

Fig. 3 Extracts of *Taxus wallichiana* fruit showed antibacterial activity, as indicated by the zone of inhibition against different microorganisms' strains

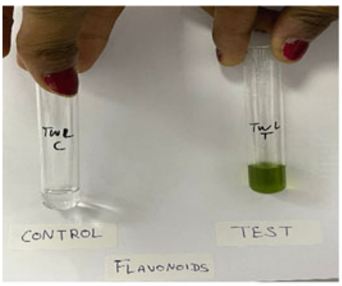


3.2 Phytochemical Screening of *Taxus wallichiana* (Leaves and Fruit)

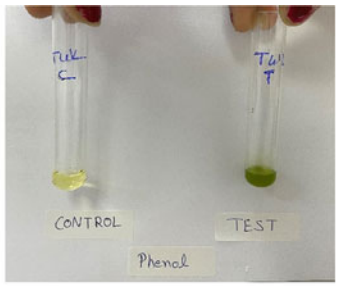
Six phytochemicals were screened for this research work (tannins, phenolic, terpenoids, glycosides, saponins and flavonoids), as seen in Table 2; from the crude extracts obtained from *T. wallichiana* leaves and fruit (Figs. 4 and 5), the methanol crude extracts tested positive for the presence of flavonoids, terpenoids, phenol, tannins and glycosides. Similarly, the *T. wallichiana* fruit (Fig. 5) showed the presence of the phytochemicals flavonoids, saponins and terpenoids.

Table 2 Phytochemicals present in methanolic crude extracts of *Taxus wallichiana* leaves and fruit

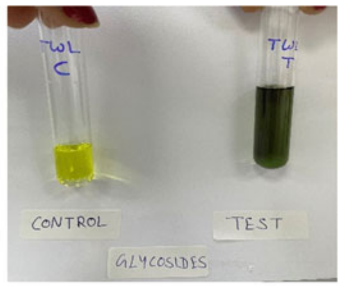
SNo.	Phytochemicals	*Taxus wallichiana* leaves	*Taxus wallichiana* fruit
1	Flavonoids	+	+
2	Glycosides	+	–
3	Phenol	+	–
4	Saponins	–	+
5	Tannins	+	–
6	Terpenoids	+	+



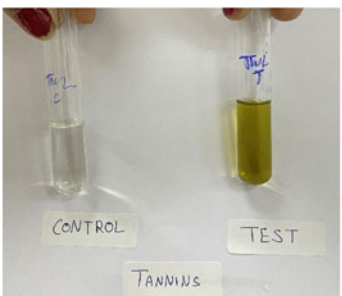
Flavonoids



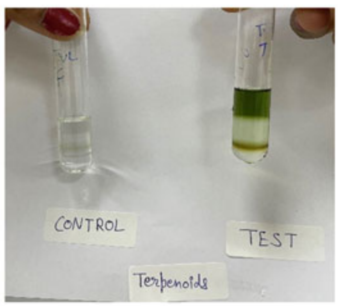
Phenol



Glycosides

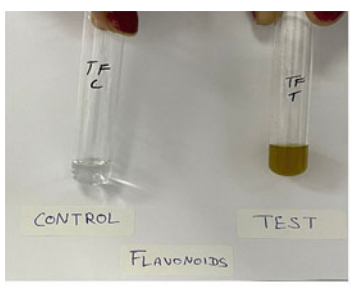


Tannins

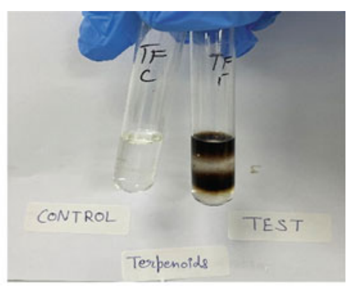


Terpenoids

Fig. 4 Pictures show a confirmation of the phytochemicals present in the crude extracts of *Taxus wallichiana* leaves



Flavonoids



Terpenoides

Fig. 5 Pictures show a confirmation of the phytochemicals present in the crude extracts of the *Taxus wallichiana* fruit

4 Conclusion

This probe was carried out as an awareness of the medicinal value of *T. wallichiana*, for its activity against selected bacterial pathogens. The Himalayan yew extract, particularly the methanolic extract, obtained from different parts of the tree (leaves and fruit) showed an anti-microbial effect against human pathogens, which suggests that it could be considered a safe anti-microbial agent. The broad spectrum of antibacterial activities of the Himalayan yew seems to be due to the presence of terpenes, terpenoids detected in the bioactive fractions. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds as well as secondary metabolites.

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Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Availability of Data and Materials The relevant data and materials are available in the present study.

Competing Interests The authors declare that they have no competing interests. All procedures followed were in accordance with ethical standards (institutional and national).

Authors' Contributions VB performed all the experiments. VB analysed the data and wrote the manuscript.

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