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# Antifungal and antibacterial activities of *Taxus wallichiana* Zucc

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**Abstract**

Current study was undertaken to evaluate the *in vitro* antifungal and antibacterial potential of methanol extract and subsequent fractions obtained after partitioning in organic solvents with variable polarity of the aerial parts of the tree *Taxus wallichiana* Zucc. Traditionally, this plant is often used in folk medicines in Pakistan for treating microbial infections. In order to rationalize the traditional use, methanol extracts of leaf, bark, and heartwood of *Taxus wallichiana* Zucc. were tested against six bacteria and six fungal strains using the Hole diffusion and macro-dilution methods. All extracts and fractions displayed significant antimicrobial effect. Only three fungal strains, *Trichophyton longifusus, Microspoum canis, and Fusarium solani* were susceptible to the extracts and fractions with MICs ranging from 0.08 to 200 mg/mL. In case of bacterial strains, *Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi were* susceptible to the extracts and fractions with MICs ranging from 0.08 to 200 mg/mL. Comparison results were carried out using imipinem, miconazole and amphotericin B as standard antibiotics.

**Keywords:** Taxus wallichiana *zucc, antifungal, antibacterial*

## Introduction

Globally, infectious disease is the major cause of death, accounting for approximately one-half of all deaths, in tropical countries [1]. New therapeutic agents and strategies are demanding issues to cope with infectious diseases. Low-income people especially from small isolated 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 applied directly on the infected wounds or burns [2–3].

*Taxus wallichiana* Zucc. is a member of the family Taxaceae which is commonly known as the Himalayan yew [4]. The plant is widely distributed in Pakistan and India, and used in the traditional systems of medicine [5]. Taxol and related bioactive taxoids have been reported from the various species of the genus, including *Taxus wallichiana* Zucc. [6–8]. Other pharmacological activities are also reported from the plants of genus *Taxus* which includes, imunomodulation [9], antiallergic activity [10], antinociceptive and antinflammatory activity [11], antiosteoporotic activity [12], antiplatelet activity and vasorelaxing effect [13], DPPH radical scavenging and nitric oxide (NO) inhibitory activities [14].

Antifungal activities of various taxoids isolated from *Taxus cuspidata* var. *nana* has been reported against plant pathogenic fungi [15]. In another study, *Taxus baccata* heartwood extract showed significant activity against selected gram-negative bacteria and selected pathogenic fungi [16]. Bilobetin, a biflavone from needles of *Taxus baccata,* has been reported to possess the significant antifungal activity [17]. In Pakistan, this plant is used traditionally for treatment of pyrexia, acute painful

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conditions, indigestion, epilepsy and for the treatment of wounds and skin infections [3,5,18]. However, thorough literature survey has revealed that no significant work has been done on antibacterial and antifungal activities of the *Taxus wallichiana* Zucc. Keeping this knowledge in view, the present study was undertaken to investigate the antibacterial and antifungal potential of *Taxus wallichiana* Zucc. We report here the results of antimicrobial testing of *Taxus wallichiana* Zucc. extract against *Escherchia coli, Bacillus subtilis, Shigella flexeneri, Staphylococcus aureus*, *Pseudomonas aeruginosa and Salmonella typhi*. Fungal strains includes *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glaberata*

## Materials and methods

### *Plant material*

Plant material was collected from the Hazara division of the North-western Frontier Province, Pakistan, in March 2005 and identified by a taxonomist, Dr. Hasan Sher, Department of Botany, Jehanzeb Postgraduate College Saidu Sharif, Swat. A voucher specimen was deposited in the herbarium of the same institution. The aerial parts of the plant were air-dried under shade for six consecutive weeks at room temperature. The dried plant material was later on chopped, finely ground and stored in a polyethylene bag under refrigeration for further experimentation.

### *Extract preparation*

The dried and powdered leaves, bark and wood (2.5 Kg, 4.0 Kg and 1.0 kg) respectively) were macerated in methanol with occasional manual shaking at room temperature for a period of 72 h. After filtration, the process was repeated 3 times using additional methanol each time. The combined filtrates were evaporation under reduced pressure at 40° C. The concentrated methanol extracts of leaves (357 g, 14.28% w/w), bark (514 g, 12.85% w/w), and wood (94 g, 9.4% w/w) were obtained. Crude methanolic extract of bark was redissolved in distilled water and successively extracted with hexane (11%), chloroform (31.9% w/w), ethyl acetate (38.8% w/w), and finally water (18.2% w/w) to give the respective fraction. Each organic extract was then evaporated to dryness. Stocks extract solutions were prepared at 200 mg/mL in distilled water. The pH was adjusted between pH 5–7. Extracts were sterilized over a membrane filter unit of 0.2 μm of pore size (Minisart Sartorius) and preserved at +4°C until used.

### *Fungal and bacterial strains*

Tests were performed on six fungi and six bacteria reference strains. Bacterial strains were *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. flexeneri (clinical isolate)*, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 *and S. typhi* ATCC 19430. Fungal strains include *T. longifusus (clinical isolate)*, *C. albicans* ATCC 2091, *A. flavus* ATCC 32611, *M. canis* ATCC 11622, *F. solani* 11712 *and C. glaberata* ATCC 90030. They were maintained on an agar slant at 4°C. The strains were activated at 37°C for 24 h on nutrient agar (NA) or Sabouraud glucose agar (SGA) respectively for bacteria and fungi, prior to any screening.

### *Hole-diffusion method*

The antimicrobial tests were carried out by the hole-diffusion method using a cell suspension of about 1.5 × 106 CFU/mL obtained following Macfarland turbidity standard No. 0.5 [19]. The concentration of the suspension was standardized by adjusting the optical density to 0.1 at 600 nm (Shimadzu, UV-VIS Spectrophotometer) [20]. Holes of 6 mm diameter were then made on the MHA plate (8 mm thick) and filled with 150 μL of methanolic extract, fractions or standard drug(s). The inoculated plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of growth inhibition around the hole. The assay was repeated three times and the mean diameter was recorded. Imipenem, miconazole and amphotericin B were used as standard antibiotics for comparison with extracts and fractions.

### *MIC determination by macrodilution method*

Extracts (10 mg/mL) were dissolved in DMSO and serially diluted with sterile water in microplates in a laminar flow cabinet. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100% relative humidity at 37°C. Next morning, tetrazolium violet was added to all the wells and the growth was indicated by a violet color of the culture. The lowest concentration of the test solution that led to inhibition of growth was taken as the MIC. The negative control acetone had no influence on the growth even at the highest concentration used. Imipenem, amphotericin B and miconazole were used as controls for comparison.

## Results

Using the hole diffusion and macrodilution technique (Table I), leaf extracts of *Taxus wallichiana* Zucc showed antimicrobial properties with MIC values ranging from 0.23 to 200 mg/mL for bacterial strains and 0.11 to 200 mg/mL for fungi. *Taxus wallichian* extracts show the lowest MIC values (Table II) and thus they may warrant the presence of interesting antimicrobial lead compounds.

Table I. In vitro antibacterial activity of crude extracts and fractions of *Taxus wallichiana* Zucc. Hole-diffusion method.

| Name of Bacteria | Zones of inhibition of bacterial growth (in mm) by various samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Std. drug | IB | IL | IW | IF-1 | IF-2 | IF-3 | IF-4 |
| *Escherchia coli* | 24 | 10 | 9 | 10 | – | – | 10 | – |
| *Bacillus subtilis* | 23 | – | – | – | – | – | – | – |
| *Shigella flexeneri* | 28 | – | – | – | – | – | – | – |
| *Staphylococcus aureus* | 27 | 12 | – | 11 | – | 9 | – | 10 |
| *Pseudomonas aeruginosa* | 20 | 14 | 14 | 17 | – | – | 14 | 15 |
| *Salmonella typhi* | 26 | 13 | 12 | – | – | – | – | 12 |

Std. drug: imipenem, IB: Methanol bark extract, IL: Methanol leaf extract, IW: Methanol bark extract, IF-1: n-Hexane fraction of IB, IF-2: Chloroform fraction of IB, IF-3: Ethyl acetate fraction of IB and IF-4: Water fraction of IB.

*In vitro antibacterial activity of extracts*

Methanol extract of *Taxus wallichiana* Zucc. bark, leaves and wood (IB, IL and IW), ethyl acetate fraction of the former bark extract (IF-3) and the aqueous fraction of methanol bark extract (IF-4) indicated lowest MIC and inhibited the bacterial strains, *S. aureus, P. aeruginosa and S. typhi* (Table I, III). Among others, the IB remained the most active extract with MIC = 0.61, 0.49, 0.31 and 0.42 mg/mL for *E. coli, S. aureus, P. aeruginosa and S. typhi,* respectively). *E. coli and P. aeruginosa* were the most susceptible bacteria with MIC ranging from 0.23 mg/mL to 0.67 mg/mL.

*In vitro antifungal activity of extracts*

*Taxus wallichiana* Zucc.extracts exhibited from significant to remarkable inhibitory activities against *T. longifusus, M. canis, and F. solani*, except the aqueous fraction of methanol bark extract (IF-4) which did not exhibit any noticeable action against these strains. Crude extracts and the fractions showed a zone of inhibition (in mm) against these strains (Table II). As regards the selective inhibitory role of extracts and the polar fractions of the plant under investigation, the species *F. solani* was strongly inhibited with MIC ranging from 0.08 mg/mL to 200 mg/mL, followed by the *M. canis* with MIC ranging from 0.17 mg/mL to

Table II. In vitro antifungal activity of crude extracts and fractions of *Taxus wallichiana* Zucc. Hole-diffusion method.

| Bacteria | % Inhibition of Fungal Growth By Various Samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Std. drug | IB | IL | IW | IF-1 | IF-2 | IF-3 | IF-4 |
| *Trichophyton longifusus* | 70[1] | 30 | 60 | 50 | 40 | – | 50 | – |
| *Candida albicans* | 110[1] | – | – | – | – | – | – | – |
| *Aspergillus flavus* | 20[2] | – | – | – | – | – | – | – |
| *Microsporum canis* | 98.4[1] | 20 | 40 | 50 | 80 | – | – | – |
| *Fusarium solani* | 73.26[1] | 80 | – | 60 | 90 | 90 | – | – |
| *Candida glaberata* | 110.8[1] | – | – | – | – | – | – | – |

1 Standard Drug = Miconazole,; 2 Standard Drug = Amphotericin B; IB: Methanol bark extract, IL: Methanol leaf extract, IW: Methanol bark extract, IF-1: n-Hexane fraction of IB, IF-2: Chloroform fraction of IB, IF-3: Ethyl acetate fraction of IB and IF-4: Water fraction of IB.

Table III. Antibacterial Activity of Crude Extracts and Fractions by macrodilution method.

| Bacterium | MIC (mg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Std. drug | IB | IL | IW | IF-1 | IF-2 | IF-3 | IF-4 |
| *Escherchia coli* | 0.0002 | 0.83 | 0.67 | 0.62 | >200 | 50 | 0.64 | >200 |
| *Bacillus subtilis* | 0.0005 | >200 | >200 | >200 | >200 | >200 | >200 | >200 |
| *Shigella flexeneri* | 0.0003 | 150 | 150 | >200 | 50 | >200 | >200 | >200 |
| *Staphylococcus aureus* | 0.0009 | 0.49 | 150 | 0.54 | >200 | 0.59 | 50 | 0.61 |
| *Pseudomonas aeruginosa* | 0.0021 | 0.31 | 0.33 | 0.23 | 50 | >200 | 0.39 | 0.26 |
| *Salmonella typhi* | 0.0014 | 0.42 | 0.46 | >200 | >200 | >200 | >200 | 0.38 |

Std. drug: imipenem, IB: Methanolic bark extract, IL: Methanolic leaves extract, IW: Methanolic bark extract, IF-1: Hexane fraction of IB, IF-2: Chloroform fraction of IB, IF-3: Ethyl acetate fraction of IB and IF-4: Water fraction of IB.

Table IV. Antifungal Activity of Crude Extracts and Fractions by macrodilution method.

| Fungus | MIC (mg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Std. drug | IB | IL | IW | IF-1 | IF-2 | IF-3 | IF-4 |
| *Trichophyton longifusus* | 0.0014[1] | >200 | 0.19 | 0.22 | 0.28 | 3.54 | 0.23 | >200 |
| *Candida albicans* | 0.0001[1] | >200 | 100 | >200 | 100 | 150 | >200 | >200 |
| *Aspergillus flavus* | 0.027[2] | >200 | >200 | >200 | >200 | >200 | >200 | >200 |
| *Microspoum canis* | 0.0006[1] | 0.78 | 0.32 | 0.21 | 0.17 | 50 | >200 | >200 |
| *Fusarium solani* | 0.0011[1] | 0.11 | 50 | 0.24 | 0.09 | 0.08 | 150 | >200 |
| *Candida glaberata* | 0.0003[1] | 100 | 100 | 100 | >200 | >200 | >200 | >200 |

1 Standard Drug = Miconazole,; 2 Standard Drug = Amphotericin B IB: Methanolic bark extract, IL: Methanolic leaves extract, IW: Methanolic bark extract, IF-1: Hexane fraction of IB, IF-2: Chloroform fraction of IB, IF-3: Ethyl acetate fraction of IB and IF-4: Water fraction of IB.

200 mg/mL and then *T. longifusus* (MIC ranging from 0.19 mg/mL to 200 mg/mL. (Table IV) Interestingly, all the extracts show negligible activity against *C. albicans, A. flavus and C. glaberata.*

## Discussion

The methanol extract of the aerial parts and the polar fractions thereof of *Taxus wallichiana* Zucc were found to possess inhibition activity against seven representative pathogens studied, which strongly supports the traditional use for gastroenteritis and skin related diseases. The MICs of the extracts observed against the sensitive strains ranged from 0.23 to 200 mg/mL (for bacterial strains) and 0.08 to 200 mg/mL (for fungal strains). Furthermore, a gradual increase in the antimicrobial activity was observed against the tested pathogens when progressing from the crude extract to polar fractions. This gradual increase in activity proved successful with both procedures employed i.e. hole diffusion and the macro-dilution techniques. This was mainly observed with the MICs obtained with extracts and fractions on *T. longifusus, M. canis, and F. solani.* However, in case of bacterial strains, IW and IF-4 showed potent activity against the *P. aeruginosa* having MICs 0.23 and 0.26 mg/mL. IL, IW, IB and IF-4 exhibited significant activity against the *E. coli* having MICs 0.62, 0.67, 0.83 and 0.64 mg/mL respectively. Similarly, IW, IB, IF-2 and IF-4 displayed significant activity against the *S. aureus* having MICs of 0.54, 0.49, 0.59 and 0.61 mg/mL. This antimicrobial activity may be attributed to the presence of alkaloids, phenols, polyphenols, saponins, tannins, anthraquinones, steroids and especially the diterpenes, found in the crude extract and the fractions thereof. These phytochemical groups/families of natural products are known to display antimicrobial activities [21–27]. Further purification and characterization of the active principles from fractions IF-1 and IF-2 (for antibacterial studies), IF-3 and IF-4 (for antibacterial studies) will provide a better understanding of the antimicrobial mechanism and may serve as a tool for isolation of potential lead compounds for microbial infectious diseases.

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