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Study of Phytochemical Constituents and BiologicalActivityof TaxusWallichianaZucc

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Abstract[ZR1]

Abstract

Phytochemical and biological activities of methanolic extract of *Taxus wallichiana* Zucc. (Leaf, stem) were carried out. The brine shrimp bioassay showed *T. wallichiana* is pharmacologically active. The antibacterial potential was studied against one gram positive bacteria (*Staphylococcus aureus*) and one gram negative bacteria (*Escherichia Coli*) using Agar Well Diffusion Method. Stem of *T. wallichiana* showed significant zone of inhibition against gram positive bacteria while the leaf of *T. wallichiana* did not show significant zone of inhibition against both gram positive and gram negative bacteria. Antioxidant activity was evaluated by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity and FRAP assay. Both assay showed that *T. wallichiana* leaves has high antioxidant activities.

Keywords: Antioxidant activity; Agar well diffusion method; Brine shrimp bioassay; DPPH assay;

Full length[ZR2] **article** **Corresponding Author*, e-mail:

1. Introduction

TaxuswallichianaZucc, belongs to the family *Taxaceae* [1]. It is medium sized temperate, himalayan forest tree of medicinal importance [2]. *T. wallichiana*Zucc. (Himalayan Yew) has a remarkable history of medicinal uses in contrast to the other yews. Depending on taxonomic treatment, *T. wallichiana*are found to have a wide growth range in Asia, stretching from Afghanistan through the Himalayas to Philippines. It is found growing in Afghanistan, Bhutan, China, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Philippines and Vietnam at the altitude range of 1500-3500 m asl. The native population for treating common cold, cough, fever and painful inflammatory conditions has used it. The leaves of this plant are used to make herbal tea for indigestion and epilepsy. Traditionally, its bark and leaves are used in steam bath to treat rheumatism and the paste made from its bark is used to treat fractures and headaches [3]. After the novel discovery of anticancer drug taxol from *T. brevifolia*, by Wani and Wall in 1971, tremendous work has been carried out on the chemical investigation of almost all parts (needles, bark, roots, seeds and heartwood) of several yew species, resulting in the isolation and characterization of over 300 taxoids [2], [4-6]. Although its antifungal, antiviral, analgesic, antipyretic, anti-allergic anti-inflammatory and tumor growth inhibitory activity has been reported, most of the research on *Taxus*have been based on its propagation and anti-cancerous properties. Other equally important aspects of this evergreen gymnosperm, such as its antioxidant, antimicrobial potential, still needs advance and focused research efforts as only few has been reported [7]. Studies report that *TaxuswallichianaZucc*. has anticancer activity. However, no antioxidant and antimicrobial activity has yet been reported using *T. wallichiana*Zucc. *T. wallichiana*is renowned for its medicinal potency, but we know that bioactive compounds found in plants may depend on the location where they grow. *T. wallichiana*used in this

study was obtained from Seshadri forest, Tirumala. The findings from this work may add to the overall value of the medicinal potential of *T. wallichiana*.

2. Materials and Methods

2.1. Plant Collection

*Taxuswallichiana's*leaves and stem were collected from Seshadri forest, Tirumala. The plant was identified by K. MADHAVACHETTY, Plant Taxonomist (IAAT: 357), Assistant Professor, Department of Botany, SRI VENKATESWARA UNIVERSITY, TIRUPATI-517502, A.P., INDIA.

2.2. Preparation of Extracts

The collected plants were cleaned, air dried in shade. Exposure to the sunlight is avoided to prevent the loss and transformation of the active components. The completely dried samples were grinded into fine powder. The extraction of chemical constituents of plant material was carried out with methanol by the process of cold percolation. The powdered material was kept in clean and dry conical flask and dipped in methanol. It was left for 2-3 days at room temperature with shaking at intervals. Then it was filtered and filtrate was concentrated using rotator evaporator. This process was repeated for 6-7times. The concentrated filtrate was air dried to obtain solid or semisolid residue. The same process was repeated for all plants. After completely drying they were kept into beaker. The dried extracts were used for different tests.

2.3. Phytochemical Screening

The phytochemical screening of all plant materials were done on the basis of procedure given by Prof. I. Culie (1982).

2.4. DPPH Radical Scavenging Activity (RSA)Assay

The free radical scavenging activity of samples and standard ascorbic acid solution in methanol was determined based on their ability to react with stable 1, 1-diphenyl-2-picrylhyrazyl (DPPH) free radical [8]. The plant samples at various concentrations (15-250 µg/ml) were added to a 100 µM solution of DPPH in ethanol. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517nm. The measurement was performed in triplicates. The antioxidant activity of the samples was expressed as IC_{50} (inhibitory concentration), which was defined as the concentration (in µg/ ml) of sample required to inhibit the formation of DPPH radicals by 50%. Ascorbic acid was used as positive control. Free radical scavenging activity was calculated by using following equation:

$$\%\ of\ free\ radical\ scavenging\ activity = \frac{(A_0 - A_T) \times 100}{A_0}$$

Where, A_O= Absorbance of DPPH solution and AT= Absorbance of test or reference sample. The %scavenging was then plotted against concentrations used and from the graph $IC_{50}$was calculated.

2.5. FRAPAssay

The antioxidant activity by FRAP assay was conducted according to the procedure given by Benzie and Strain with slight modification (Benzie and Strain, 1996). The FRAP reagent was prepared by mixing acetate buffer of pH 3.6 (300mM), TPTZ(tripyridyltriazine) solution of 10mM and ferric chloride solution of 20 mM in the ratio of 10:1:1. Antioxidant activity was calculated with the standard calibration of ferrous sulfate. The leaf extracts (5 mg/ml) was prepared by adding methanol and was used as sample. Finally, absorbance was taken at 593 nm keeping the temperature 37 °C.

2.6. Microorganism

The microorganisms used in this study were identified strains obtained from Central Department of Microbiology, ANU Guntur. In this study one was gram positive (*Staphylococcus aureus*) and one gram negative (*Escherichia coli*) were used.

2.7. Anti-microbialAssay

The anti-microbial activity of the plant extracts were carried by disc diffusion method [9]. A suspension of tested microorganismswas spread on Muller-Hilton Agar (MHA) medium. The sterile filter paper discs (6 mm in diameter) were individually impregnated with different concentration of plant extract prepared in dimethyl sulphoxide (DMSO) and then placed into the agar plates which had previously been inoculated with the tested microorganisms. The plates were subsequently incubated overnight at 37 °C. After incubation, the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For control dimethylsulphoxide (DMSO) discs were used. All tests were performed in triplicate.

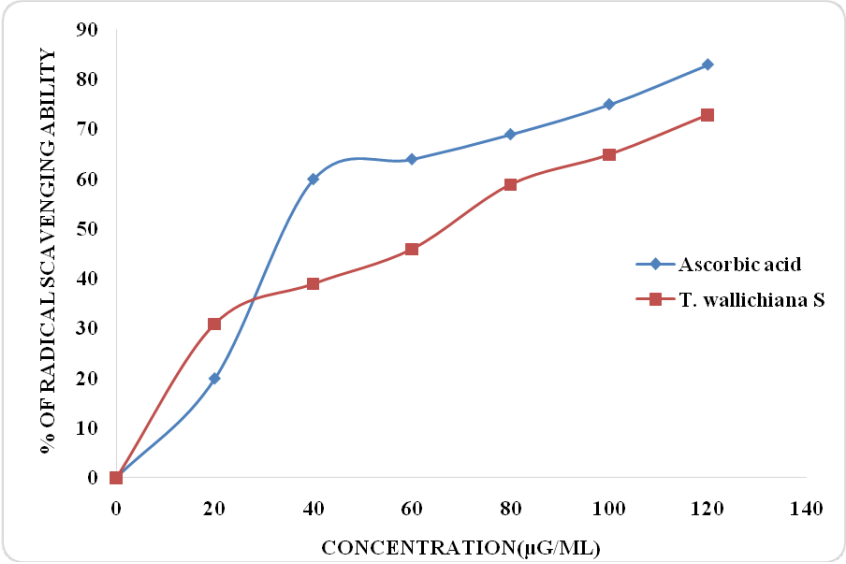
2.8. Brine Shrimp Bioassay

The brine-shrimp toxicity assay for each extract was carried out according to Mayer et al [10]. Briefly, sample solutions were prepared by dissolving 200 mg of each plant extract in DMSO upto the mark in 10ml volumetric flasks. To calculated volume of the sample solution for 10, 100 and 1,000 µg/ml dose levels in three replicates was introduced freshly hatched ten brine-shrimp naupliiin artificial sea water(totalvolume10ml).A control tube for each dose level was also prepared. After 24 h of illumination under a table lamp (60 Watt), the number of survivors was counted. No death was observed in the control tubes. The LC_{50} (Lethal concentration for 50% mortality) values was determined using the probit method (Finney1971), as the measure of toxicity of the extracts.

3. Results and discussion

3.1. Phytochemical Screening

The different phytochemicals in the crude methanol extracts were identified by the color reaction with different reagents. The results of phytochemical screening are shown in Table 1. From the above result, it was observed that polyphenols, terpenoids and flavonoids were present in both extracts, while glycosides were present only in leaves extracts. The alkaloids were absent in both extracts. Similarly, saponins were present only in stem extract.Various studies have revealed phenolic compounds have been reported to possess biological properties such as anticancer, antioxidant, anti-inflammatory, anti-aging etc. Similarly, flavonoids to kill or inhibit many bacterial strains. The glycosides are found antibacterial, antitumor, antioxidant. Likewise, the alkaloids are also found anticancer, antibacterial, analgesic and antimalarial. Further saponins of plant are known to enhance antibody production. The present study insight the presence of various phytochemicals, thus these phytochemical can be attributed to the potential antioxidant and antibacterial properties in the tested sample.



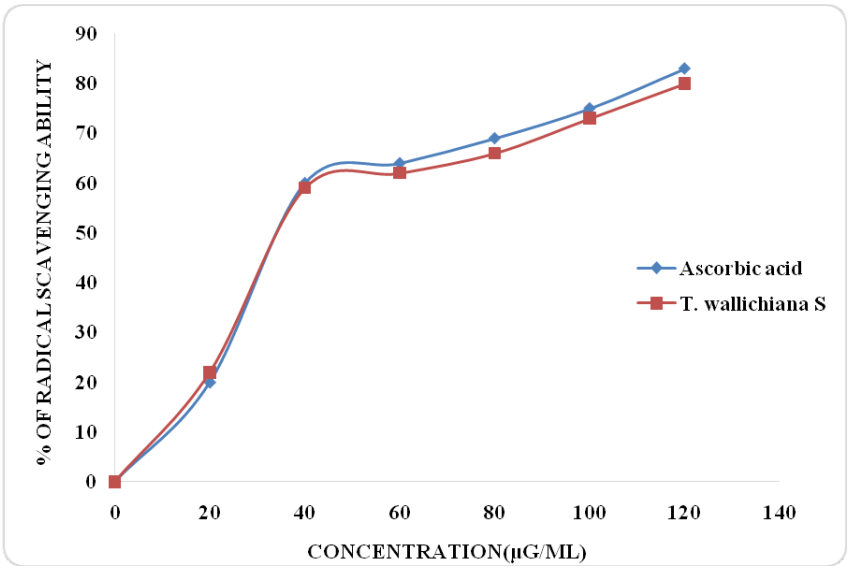


Figure 1. DPPH scavenging activities of the methanolic extracts of *T.wallichiana*

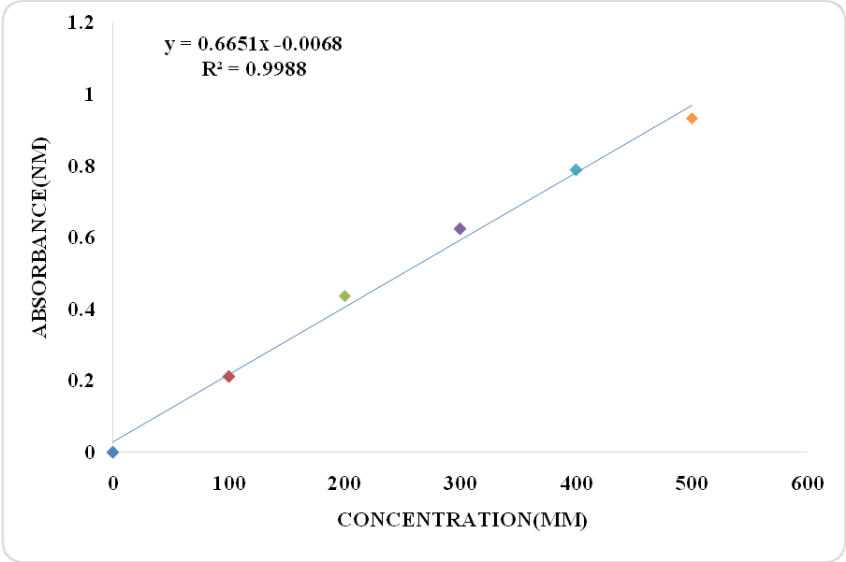


Figure 2. Graph of absorbance vs Concentration of Ferrous sulphate

Table 1. Results of Phytochemical Screening

Plants	Phytochemicals							
	Polyphenols	**Reducing compounds**	**Glycosides**	**Quinones**	**Saponins**	**Basicalkaloids**	**Terpenoids**	**Flavonoids**
T.wallichiana (L)	+	-	+	-	-	-	+	+
T.wallichiana (S)	+	+	-	-	+	-	+	+

Table 2. LC_{50}value of different plant extracts

S.NO	Plant name	LC50(µg/ml)
1	*T.Wallichiana*(L.)	601.17
2	*T.Wallichiana*(S.)	3.56×10^{8}

Table 3. Mean Zone of inhibition (ZOI) shown by *T. wallichiana*stem against testedbacteria

Plantextract	Mean Zone of inhibition(ZOI)							
	Test organisms							
	***S.aureus*(Grampositive)**				***E.coli*(Gramnegative)**			
	Concentration(mg/ml)				Concentration(mg/ml)			
	10	25	50	100	10	25	50	100
***T.wallichiana*(Stem)**	5	8	15	25	0	0	0	0
Neomycin	-	-	-	38	-	-	-	35

Table 4. Antioxidant activities of *T.wallichiana*extracts

S.NO	Plantsname	IC50value
1	*T.wallichiana*leaves	23.18± 6.49
2	*T.wallichiana*stem	56.75± 8.96
3	AscorbicAcid	21.20± 2.52

Table 5. Antioxidant power of methanol extracts of *T. wallichiana*

Botanical name	Concentration (mg/ml)	Antioxidant power [mMFe(II)/L]
T.wallichiana (Leaf)	1	2.25± 0.57
T.wallichiana (Stem)	1	2.23± 0.29

3.2. Biological Screening

3.2.1. Brine-Shrimp Bioassay

The newly hatched brine-shrimp nauplii were exposed to the plant extracts and their biological activities were evaluated on the basis of their toxicity towards the nauplii. The LC_{50}values (µg/ml) for different fractions were determined and those having values less than 1000 are supposed to be pharmacologically active. Results obtained during brine shrimp bioassay are given below in Table 2. The results of brine shrimp bioassay showed that methanolic extract of *T. wallichiana*leaf was pharmacologically active while the stem extract is inactive.

3.2.2. Antibacterial Assay

In biological screening, the ability to kill or inhibit the growth of pathogenic bacteria was evaluated by antibacterial assay of the plant extract. Agar well diffusion method was used in the study of evaluation of antibacterial activity and measured in the form of zone of inhibition (ZOI) given by Dingle *et al* (1953) [11]. Antibacterial activity was shown by the methanolic extract of stem of *T. wallichiana*against gram positive bacteria on the dose dependent manner while no antibacterial activity was observed against *E. coli.* The negative control 5% DMSO did not produce any zone of inhibition where as the positive control neomycin produced zone of inhibition. Results of antibacterial assay are shown in Table 3. Literature survey has revealed that no significant work has been done on the anti-bacterial activities of the *T. wallichiana.* However, Nisar et al, reported the antimicrobial activity of methanol extracts of the leaf, bark and heartwood of *T. wallichiana*against six bacterial and six fungal strains using the hole diffusion and macro-dilution methods [12]. Taxol and related bioactive taxoids from *T. wallichiana*may be responsible for the observed antimicrobial activities [13]. These differences may be due to the altitude variation, time of collection of that plants and laboratory conditions.

3.3. Antioxidant Activity

The antioxidant activity of the methanolic solution of samples were explored by using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay and FRAP (Ferric reducing antioxidant power) assay.

3.3.1. DPPH Assay

The methanol extractives of stem and leaves of *T. wallichiana*were assessed for free radical scavenging activity. The graph of concentration against the corresponding % radical scavenging activity of different samples were plotted (Fig. 1) and concentration providing 50% inhibition was determined, which was shown in the Table 4.IC_{50}value of the standard i.e., Ascorbic acid was found to be 21.20µg/ml.*T.wallichiana*leaves extract has lower IC_{50}value than stem extract. The antioxidant activity of the plant may be due to the phytochemicals like polyphenols, flavonoids and terpenoids etc.

3.3.2. FRAPAssay

The FRAP assay measures the total antioxidant activity on the basis of the ability to reduce a ferric salt Fe(III)(TPTZ)2Cl3 to Fe(II) ions. The FRAP assay was carried out under acidic conditions (pH 3.6) in order to maintain the iron solubility. With reference to the calibration curve obtained at 593 nm for ferrous sulphate solution (R^2 = 0.992) (Fig. 2).The FRAP values of extracts of leaves and stem of *T. wallichiana*were found 2.25 ± 0.57 and 2.23 ± 0.29 mM Fe+2/liter respectively, Table 5. The results obtained from the assay are given below Table 5. The highest antioxidant activity was found for leaves extract in both methods. It becomes evident that the antioxidant activities of the extracts are due to the presence of flavonoids and polyphenols in the plant. Although there is no report on the antioxidant activity of *T.wallichiana*so far, the present study reveals the mild antioxidant and antimicrobial activity of *T. wallichiana.* These results can be correlated with fact that the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants.

4. Conclusion

Phytoconstituents and biological activity of *T. wallichiana*collected from Tirumala hills has been

successfully carried out. A wide range of phytochemicals is present in stem and leaves extract of plant. From Brine shrimp bioassay, leaves of *T. wallichiana*, was found to be pharmacologically toxic against brine shrimp nauplii. The stem of extract also showed significant zone of inhibition against gram positive bacteria. The leaf extract was shown to be inactive based on the antibacterial assay. However, further pharmacological and toxicity studies are necessary to confirm this suggestion. Bioassay guided compound isolation should be carried out to characterize the compounds in *T. wallichiana*that act as antioxidant and antibacterial agents.

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