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Analysis of antibacterial and antioxidant activity of *Taxus baccata* Linn.

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Abstract
The use of plants for treating various diseases is as old as the human civilization. Plant products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from these herbal products. The efficacy and safety of herbal medicines have attracted the major pharmaceutical population towards medicinal plants research. The current study was designed to investigate the leaf extract of *Taxus baccata* Linn. for its antibacterial and antioxidant activity. The antibacterial activity of the acetone, aqueous and methanol leaf extracts was determined *in-vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes* and *Staphylococcus aureus* following agar-well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial species. Methanol leaf extract was found to be more effective against selected pathogenic bacterial species as compared to acetone and aqueous leaf extracts. Further the leaf extract inhibited gram- positive bacteria more efficiently than gram- negative bacteria. The antioxidant capacity of the different extracts (methanol, acetone and aqueous) of *T. baccata* was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and reducing power tests at different concentrations (20-100 µg/mL). The plant exhibited good DPPH radical scavenging and reducing power potential (≥50% at 100 µg/mL). Therefore, the leaf extracts of this plant can be selected for further investigation to determine their therapeutic potential.

Keywords: *Taxus baccata*, leaf extracts, agar-well diffusion, DPPH, reducing power

Introduction

Nature has been a source of medicinal agents for thousands of years and a sufficient number of modern drugs have been derived from natural sources, many of these isolations were based on the use of these agents in traditional medicine [1]. A plant is said to be medicinal if it produces active compounds which are therapeutically more effective [2]. Plants are traditionally being used for medicinal treatment of numerous human disorders including infectious diseases caused by different microorganisms.

Medicinal plant parts are commonly rich in phenolic compounds such as flavonoids, phenols, stilbenes, tannins, coumarins, lignans, lignins etc. These chemical compounds have multiple biological effects including antimicrobial and antioxidant [3]. An antimicrobial is a substance that retards or inhibits the growth of microorganisms such as bacteria, fungi or protozoan. These antimicrobial substances are of natural origin, and it is considered that their influences on the environment are few and can be used as effective biological control agents [4]. The development of continuous bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.

Antioxidants, also called inhibitors of oxidation, are compounds which retard or prevent the oxidation and in general prolong the life of oxidizable matter [5]. The oxidants or free radicals are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, lipids and DNA. Antioxidant-based drug formulations are widely used for the prevention and treatment of complex diseases such as Alzheimer's disease, cancer, atherosclerosis, stroke and diabetes [6]. So the medicinal plants with antioxidant potential are usually employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress [7].

Taxus baccata Linn. is a small to medium-sized evergreen conifer belonging to family Taxaceae native to western, central and southern Europe, northwest Africa, northern Iran and southwest Asia. In India, the plant occurs in the states of Jammu and Kashmir,

Himachal Pradesh, Uttar Pradesh, Sikkim, West Bengal, Arunachal Pradesh, Meghalaya, Nagaland and Manipur [8, 9].
All parts of *Taxus baccata*, except the fleshy fruit, are antispasmodic, cardiotonic, diaphoretic, emmenagogue, expectorant, narcotic and purgative. Plant contains valuable substance "Taxol" having anticancer property. The leaves have been used internally in the treatment of various illnesses like asthma, bronchitis, hiccup, indigestion, rheumatism and epilepsy. Externally, the leaves of this plant have been used in a steam bath as a treatment for rheumatism [10, 11]. In view of its above mentioned useful properties, we planned to analyse *Taxus baccata* for its antibacterial and antioxidant activities.

Materials and methods

Collection of plant material

Leaves of *Taxus baccata* Linn. were plucked and collected from Churdhar area of District Sirmaur, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further analysis.

Procurement of bacteria

Different strains of bacteria (*Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus* and *Yersinia pestis*) have been procured from IGMC, Shimla and Department of Microbiology & Biotechnology, H.P. University Shimla for screening antibacterial properties of different plant extracts.

Revival of pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Maintenance and preservation of pure culture

Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular interval in order to maintain the cultures. Each bacterial species was transferred from parent source to maintain and preserve the cultures.

Processing of plant material

Leaves of *T. baccata* were washed thoroughly under tap water and then with 2% Mercuric chloride. After that the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in an air tight container at room temperature.

Antibacterial activity test

Agar-well diffusion method

Different extracts (acetone, methanol and aqueous) of *Taxus baccata* were screened using agar-well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6ºC for 30 minutes and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate kept as a control contained pure solvent only. The plates were incubated at 37±2ºC for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter using positive control as standard [12].

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \text{x}100$$

Where, Control = average diameter of bacterial colony in control.
Test = average diameter of bacterial colony in treatment sets [13].

Antioxidant activity test

DPPH radical scavenging activity assay

The free radical scavenging activity of plant extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Blois [14]. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant or test extract, 1 mL of DPPH (0.1 Mm in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution (without plant extract) was used as control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample
Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was evaluated using the following equation given below:

$$IC_{50} = \frac{50 - Y\text{−}Intercept}{\text{Slope}}$$

Reducing Power Assay

The reducing power was determined according to the method described by Oyaizu [15]. Different concentrations of plant extracts (20, 40, 60, 80 and 100 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. Higher absorbance of the reaction mixture indicated greater reductive potential. Experiment was performed in triplicates at each concentration to evaluate percent reducing power. The % reducing power (antioxidant activity) was calculated by using the formula:

$$\% \text{ Reducing power} = \left(\frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

Graphs were plotted against percent inhibition v/s conc. of plant extracts and standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value for each extract and ascorbic acid was evaluated.

Results and discussions

Antibacterial activity of *Taxus baccata* extracts was determined by agar-well diffusion method. The results of the antibacterial assay are shown in Table 1. Maximum diameter of zone of inhibition (ZOI), (24.10 mm) was reported for the methanol extract against *Pseudomonas aeruginosa* while for its acetone extract maximum diameter of zone of inhibition was 19.33 mm against *Listeria monocytogenes*. Aqueous extract inhibited all the bacterial species except *E. coli* and *B. cereus*. Methanol and acetone extracts of *Taxus baccata* and *Vitex negundo* showed inhibitory activity against all the tested bacteria. For methanol extract, the diameter of inhibition zone ranged from 10.10 to 24.10 mm whereas for acetone extract, inhibition zone ranged from 8.22 to 19.33 mm. Billore *et al.* [16] reported the antimicrobial effect of leaf extract of *T. baccata* on commonly encountered bacterial pathogens, viz., *E. coli, P. aeruginosa* and *B. Cereus*. The findings of Bernaitis *et al.* [17] also indicated that *Taxus baccata* showed antimicrobial effect preferably against gram-positive bacteria.

In the present study, extracts of *Taxus baccata* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed good potency for scavenging free radicals as shown in Table 2. The extracts were tested on a concentration range (20-100 µg/mL) and it was found that the activity altogether increased with increase in concentration of plant extracts (Fig. 1). Methanol leaf extract showed highest (78.40%) DPPH scavenging activity at a concentration of 100 µg/mL. In all cases, methanol extracts proved to be better antioxidants than the corresponding acetone and aqueous extracts. A pattern of increasing antioxidant activity with increasing polarity has been reported [18]. Milena *et al.* [19] (2015) investigated the antioxidant activity of methanol extract of *T. baccata* by using DPPH assay. DPPH scavenging activity was recorded upto 95.59% while in our study scavenging activity in methanol extract ranged from 21.00-78.40% (lowest IC_{50} = 62.27 µg/mL). Guleria *et al.* [20] also reported the DPPH radical scavenging activity (IC_{50}) of methanol extract of *T. baccata* leaves (5.46% inhibition).

Reducing power experiment is a good reflector of antioxidant activity of the plants. The reducing capacity of compounds serves as an important indicator of their potential antioxidant activity. The plant having high reducing power generally reported to carry high antioxidant potential too [21].

We investigated the reducing capacity of *T. baccata* by measuring Fe^{3+}-Fe^{2+} conversion as given in Table 3 and Fig. 2. In this experiment, Ferric ions reduced to ferrous ions with the colour of the reaction mixture changes from yellow to bluish green. Reducing power potential of extracts increased with the dose, however, plant extracts exhibited low reducing power than that of standard ascorbic acid. The methanol extract showed more reductive ability than the acetone and aqueous extracts, which was capable for neutralizing the free radicals. *T. baccata* showed 82.70%, 68.30% and 61.45% reducing power for methanol, acetone and aqueous extracts respectively at 100 µg/mL. The percent antioxidant activity (reducing power) was maximum for all the three extracts i.e., methanol, acetone and aqueous of *T. baccata* (82.70%, 68.30%, 61.45% respectively) at 100 µg/mL concentration and lowest IC_{50} value of 43.36 µg/mL was recorded in methanol extract. Kucukboyac *et al.* [22] studied *T. baccata* for its antioxidant potential and reported significant reducing capacity particularly at a concentration of 1000 µg/mL.

Table 1: Zones of inhibition produced by leaf extracts of *Taxus baccata* at different concentrations

Plant Extract	Concentration in %	Inhibition zone diameter (in mm)					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanol	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	12.00±0.00	10.10±1.10	13.00±0.00	12.12±0.66	11.00±0.00	12.50±1.00
	50	14.23±0.07	12.30±0.08	15.90±1.15	13.00±0.20	14.80±1.20	15.55±0.70
	75	15.05±0.45	14.68±0.48	18.80±2.00	15.34±0.28	17.70±1.25	18.00±0.00
	100	18.65±0.40	16.00±0.00	24.10±0.70	18.62±0.70	19.30±0.66	22.00±0.55
Acetone	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	8.22±0.04	12.26±2.25	11.00±0.00	10.45±0.80	9.20±0.20	12.00±0.30
	50	9.42±1.77	14.44±0.30	13.22±1.75	11.30±0.08	12.44±0.20	13.70±2.00
	75	11.20±0.60	16.00±0.00	15.67±1.50	14.77±0.88	14.55±1.23	15.00±1.00
	100	12.18±0.05	19.00±0.00	18.00±0.40	17.00±0.00	19.33±2.00	17.00±0.12
Aqueous	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	8.54±1.27	9.10±2.24	0.00±0.00	11.04±0.65	10.00±0.00
	50	0.00±0.00	9.00±0.00	10.33±0.22	0.00±0.00	13.00±0.05	13.00±0.00
	75	0.00±0.00	11.00±0.00	12.15±0.67	0.00±0.00	14.77±0.40	15.00±0.20
	100	0.00±0.00	14.40±0.90	14.08±0.10	0.00±0.00	16.00±0.00	17.00±0.00

Table 2: Free radical (DPPH) scavenging activity (%) of *Taxus baccata* at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	21.00±0.45	15.60±3.05	10.60±0.54	35.24±0.50
40	34.08±2.22	27.10±2.20	22.20±0.35	50.54±0.42
60	47.90±1.32	39.45±1.80	35.00±0.00	62.35±1.20
80	60.87±0.66	49.00±0.00	47.50±1.20	74.14±0.00
100	78.40±2.13	63.30±0.50	59.90±0.80	83.26±2.20
IC_{50} (µg/mL)	62.27	68.88	84.21	41.44

Values are given as mean ± SD

Table 3: Antioxidant activity percentage (%) of *T. baccata* by reducing power method at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	23.10±0.40	18.62±2.25	14.50±0.50	26.55±2.25
40	38.25±2.28	29.10±1.20	21.90±1.15	43.44±0.45
60	57.90±1.30	43.40±0.80	35.30±0.75	59.90±1.20
80	70.50±0.60	59.70±0.40	49.55±1.25	72.15±0.54
100	82.70±2.45	68.30±2.35	61.45±0.40	88.30±1.50
IC_{50} (µg/mL)	54.08	69.49	82.24	49.40

Values are given as mean ± SD

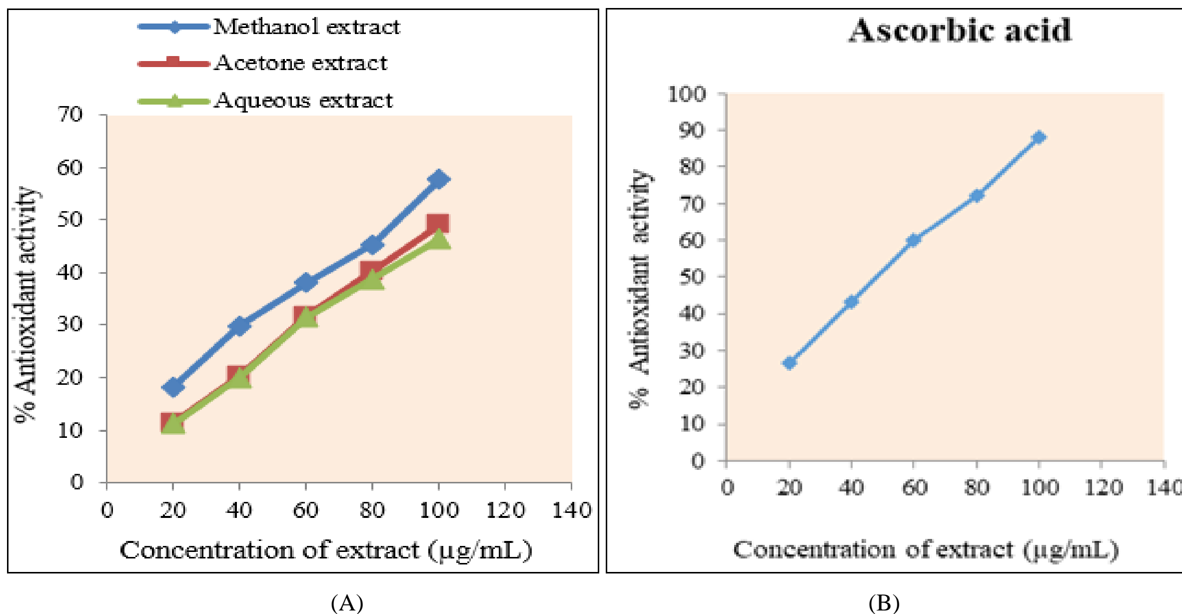

Fig 1: Percent scavenging (DPPH) activity of plant extracts at concentration range of 10-100 µg/mL (A) *T. baccata* and (B) Standard curve of Ascorbic acid

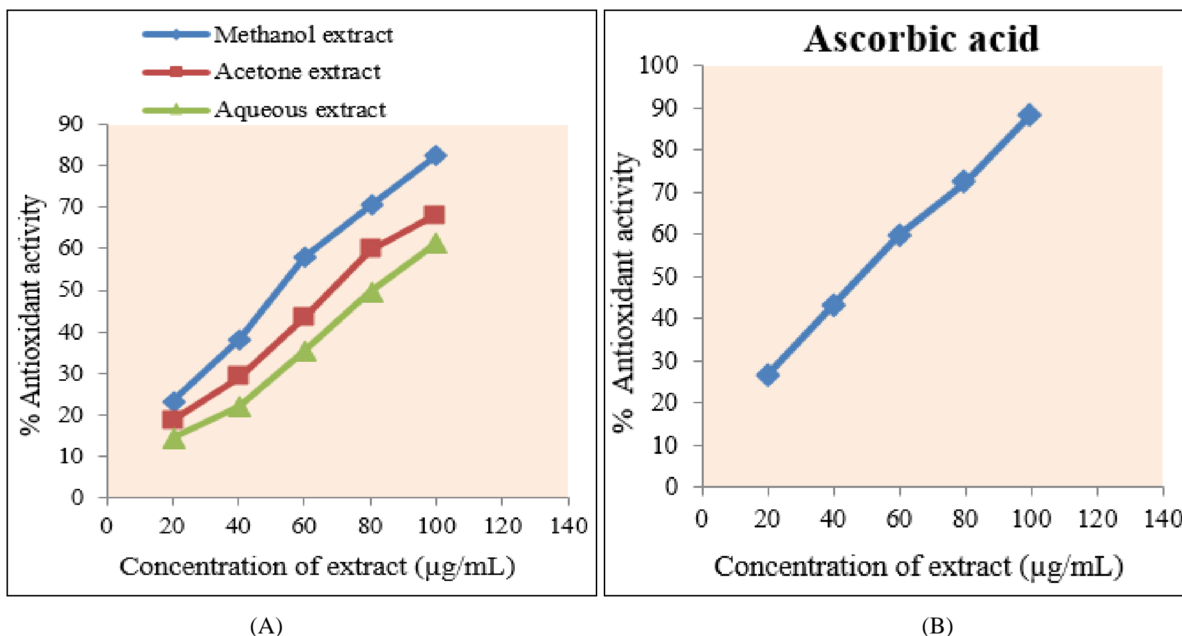

Fig 2: Antioxidant activity percentage (reducing power) of different plant extracts at concentration range of 10-100 µg/mL (A) *T. baccata* (B) Standard curve of Ascorbic acid

Conclusion

It was concluded from the above experimental observations that the plant *Taxus baccata* showed significant antibacterial activity against pathogenic bacterial species considered in this study. The work summarises that the methanol extract showed greater antibacterial activity in comparison to acetone and aqueous extracts. The higher inhibitory activity of methanol and acetone extracts can be attributed to the presence of higher amount of polyphenols as compared to aqueous extracts. Furthermore, Gram-positive bacteria were found to be more sensitive to plant extracts than gram-negative bacteria. These differences are likely to be the result of the differences in the cell wall structure of gram-positive and gram-negative bacteria, with gram-negative bacteria outer membrane acting as a barrier to many environmental substances. In addition to antibacterial activity, plant exhibited good antioxidant potential at different concentrations used. Methanol leaf extract was found to be

more effective followed by acetone and aqueous leaf extracts. This study suggests that the plant extracts possess potent antibacterial and antioxidant activity, which might be helpful in preventing or slowing the progress of various bacterial and oxidative stress-related diseases. This study directs future research in separating the bioactive compounds responsible for this activity. Further investigation on the isolation and identification of antioxidant component (s) in this plant may lead to chemical entities with potential for clinical use. Further

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