

EVALUATING BIOLOGICAL ACTIVITY AND PHOTOCHEMICAL ANALYSIS OF *TAXUS BACCATA* BARK

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Abstract. *Taxus baccata* is known for its several biological activities, including cytotoxic, antibacterial, antioxidant, etc. In this study, the antibacterial and antioxidant activities of different fractions of *T. baccata* bark have been determined. The antibacterial potential of extracts was determined using the disc diffusion method, whereas the antioxidant activity was evaluated utilizing the 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) test. The other three fractions demonstrated antibacterial activity, with the exception of watery fraction. The highest antibacterial activity was recorded against *Klebsiella pneumonia* (*K. pneumoniae*) with a 11.34 mm zone of inhibition by the ethyl acetate fraction, followed by N-butanol extract against *Klebsiella pneumonia* with a 10.34 mm inhibition zone while against *P. aeruginosa* the recorded zone of inhibition was (9.67 mm) by chloroform fraction. The fractions showed potent antioxidant activities against DPPH free radicals in a dose-dependent manner with IC50 values of 103, 109, 143, and 265 against n-butanol, ethyl acetate, chloroform, and aqueous extracts, respectively. The total flavonoid contents recorded were in order: n-butanol>ethyl acetate>chloroform>aqueous extracts with numerical values; 16050, 5925, 10475, and 14100 mg/100 g respectively. In GC-MS investigation, 17 biologically active phytochemicals were recognized in the methanolic extract of *T. baccata* bark. The extracts exhibited antibacterial and antioxidant potentials, and it was inferred that the observed potential might be at the cost of the reported phytochemicals in the literature. Therefore, this potent medicinal plant needs further investigation to isolate potent antimicrobials and antioxidants in a pure state.

Keywords: *T. baccata*, antibacterial, antioxidant, DPPH, *K. pneumonia*, *P. aeruginosa*, GC-MS

Introduction

Common yew (*Taxus baccata* L) is distinct among all other medicinal plants and has many biological activities (Erdemoglu et al., 2001). There are eight species recognized. The species are highly similar, so they are often easier to be separated geographically than morphologically (Cope, 2001). *T. baccata* is spread all over the moderate regions of the northern hemisphere. It is a small to medium size evergreen tree (Thomas and Polwart, 2003) this tree was primarily known as yew, though with other related trees becoming well-known, it may now be known as European yew or English yew or common yew (Keunecke et al., 2008). *T. baccata* is a conifer native to western, central, northwest Africa, and southern Europe, and southwest Asia (Rushforth, 1999) and it is a

drought- and frost-sensitive species, growing mainly under mild oceanic climates with relatively mild winters, high mist frequency, and abundant rainfall (Thomas and Polwart, 2003).

T. baccata became popular mostly because of its poisonous active ingredients (taxine alkaloids) occurring in varying amounts throughout the plant's sections, with several incidents of poisoning reported in recent years (Zutter et al., 2019). Apart from the fleshy fruit, every portion of *Taxus baccata* has antispasmodic, cardiac tonic, diaphoretic emmenagogue, expectorant, narcotic and purgative properties. A useful chemical called "Taxol" with anticancer property is found in plants. Internal use of the leaves has been used to cure a variety of ailments, including epilepsy, rheumatism, hiccups, asthma and bronchitis. Externally, rheumatism has been treated with the leaves of this plant in a steam bath (Gurbuz et al., 2004). Yew is a drought- and frost-sensitive species, growing mainly under mild oceanic climates with relatively mild winters, abundant rainfall, and high mist frequency (Papadopoulos, 2017). *T. baccata* L. and its varieties are known for their content of taxoids, and diterpenoid compounds which exhibit anticancer properties (Suffness, 1995). Currently, the number of compounds known to have a taxane structure exceeds fifty. The amount of *Taxus* components found in various plant section varied greatly (Teuscher and Lindequist, 2010).

Despite the fact that poison centers are reporting more and more research on yew berries, unintentional intoxication occurrence are uncommon (Pietsch et al., 2008). However, because of many online application guides, using yew plant components for abuse or suicide is becoming increasingly common (Pietsch et al., 2007). Compounds known as antioxidants, or inhibitors of oxidation slow down or stop oxidation and generally extend the life of oxidizable materials (Prakash et al., 2018). The species known as oxidants, or free radicals have a very short half-life, are highly reactive, and can damage macromolecules including DNA, lipids and proteins (Ahmad et al., 2023; Aziz et al., 2024). Drug formulation based on antioxidants are frequently used to prevent and cure complicated conditions such as cancer, diabetes, atherosclerosis, stroke and Alzheimer's disease (Devasagayam et al., 2004). In order to lessen the effect of oxidative-stress related disorders, medicinal plants with antioxidant capacity are typically used as an alternate source of medication (Sharma et al., 2013). *T. baccata* leaves show good potential for decreasing power and scavenging DPPH radical (Prakash et al., 2018).

A material that stops the growth of microorganism like bacteria, fungus or protozoa is known as antimicrobial. These naturally occurring antimicrobial compounds are thought to have little environmental effects and can function as an efficient biological control agents (Lorca et al., 2024). It is now necessary to look for novel antibacterial medicines due to the ongoing evolution of bacterial resistance to already available antibiotics. Gram positive bacteria were suppressed by *T. baccata* leaf extract more effectively than gram negatively bacteria (Prakash et al., 2018). Given its previously indicated beneficial qualities, we intended to do GCMS on the crude extract and analyzed different fractions of *T. baccata* bark for its antibacterial, antioxidant activities, and find total flavonoids contents of in each fraction.

Materials and methods

Plant sample collection

The plant was collected from the hilly area of Dir (U), Khyber Pakhtunkhwa Pakistan. The collected sample was brought to the laboratory for further analysis.

Treating of plant material

Bark of *T. baccata* L were washed thoroughly under tap water and then distilled water to remove any dust or impurities. It was then chopped into pieces so that they would dry quickly. For fifteen to twenty days, cleaned bark was shade dried. With the aid of a grinder, the dried plant material was ground into a fine powder. The 300 g powered sample was dissolved in 900 ml methanol with 20% distal water and kept it for two weeks with constant shaking at different intervals. After two weeks, by using Whatman filter paper, the solution was filtered, and then all the solvent got evaporated with the help of rotary evaporator. After getting crude extract of 60 g, it was then subjected to fractionation by adding 200 ml of distal water. After dissolving in distal water other solvent were added and got four fractions i.e chloroform, ethyl acetate, n-butanol, and aqueous fractions respectively.

Determine the antibacterial activity test via Agar-well diffusion method

Several *Taxus baccata* bark extracts (chloroform, ethyl acetate, n-butanol, and aqueous) were screened using the Agar-well diffusion method. The medium was autoclaved at 121.6°C for 3 hours 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 were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 40, 20, 15 and, 05 µg concentration of prepared extracts. One agar well in each petri plate was taken as a control contained pure solvent only. The plates were incubated at 37±2°C for 24 hours in the incubation chamber. The zone of inhibition was calculated by measuring diameter around the wells including well diameter. The readings were taken in three replicates and the average value were tabulated.

The DPPH method determines free radical scavenging activity

The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) method helped us determine the free radical scavenging ability of each fraction. DPPH produces a deep violet color in methanol in its oxidized state. The electron given by an antioxidant compound to DPPH causes reduction and color changes from deep violet to yellow. A Stock solution of DPPH was prepared by dissolving 11.8296 mg of DPPH in 300 ml of methanol. After that, aluminum foil was used to wrap this stock solution and place it in the dark for twenty-four hours to allow the free radicals to develop. 3 ml were obtained and its absorbance was adjusted at 517 nm and making it the control solution. After that, 10 ml of methanol was dissolved in each 250 mg extract to making it a stock solution. Then using successive dilutions, several diluted solutions including 250, 125, 62.5 µg/ml were created. After combining 2 ml DPPH solution with 50 ml diluted solution was left to stand in the dark for thirty minutes. The percentage inhibition of DPPH by extracts was calculated by using the formula given below.

$$\%inhibition = A-B/A \times 100 \quad (\text{Eq.1})$$

where A is the absorbance of pure DPPH in oxidized state and B is the absorbance of a sample obtained 30 minutes after the DPPH reaction.

Total flavonoids content

The total flavonoids (TFC) content of different fractions such as ethyl acetate, chloroform, N-butanol, and aqueous extract was estimated by making a 2% solution of aluminum chloride. 40 mg of each fraction was dissolved into 10 ml of methanol separately. Then 500 μ l from each fraction was taken in tubes and 500 μ l of 2% AlCl₃ solution was added respectively. The absorbance was measured using a spectrophotometer at 420 nm. The TFC was determined through a formula.

$$\text{TFC} = \text{Conc} * V(\text{ml})/\text{wt} \quad (\text{Eq.2})$$

Investigation of *T. Baccata* by GC-MS technique

The plant extract was subjected to GC-MS analysis utilizing the mass spectrometer model GC: 7890B (Agilent Technologies USA), followed the method of Ihsan et al. (2023). Helium was used as carrier gas with a flow rate of 1.0 ml/min. The injector and detector were operated at 220°C and 290°C respectively. The temperature of oven was set as follows: initially oven temperature was set at 50°C for 3 min and then gradually increased to 250°C at the rate of 10°C/min for 13 min. The results obtained were then tabulated.

Statistical analysis

To statistically analyze the data all experiments were conducted in three replicas. Microsoft excel 2016 and SPSS version 22 was used to calculate mean values and standard deviation of the data. Linear regression to calculate the IC₅₀ values from percent inhibition of DPPH of the different concentration of the plant sample. The percentage inhibition of DPPH by extracts was calculated by using Equation 1.

For total flavonoid contents the absorbance was measured using spectrophotometer at 420 nm. The TFC was determined through Equation 2.

Results

Antibacterial activities

This study evaluated *T. baccata* bark for its antibacterial in the form of extracts. Out of four, three fractions, such as n-butanol, ethyl acetate, and chloroform, showed antibacterial activity and were determined through the disc diffusion method using gram-negative bacteria *K. pneumonia* and *P. Aeruginosa*. Ethyl acetate showed the highest antibacterial activity against *K. pneumonia* with a zone of inhibition of 11.34 mm. In comparison, chloroform fraction was adequate with a high zone of inhibition of 9.67 mm against *P. Aeruginosa* as shown in Table 1.

Table 1. Average zone of inhibition produced by bark extract fractions at different concentrations

Sample	Concentration (mg)	Inhibition zone diameter (mm) (mean±SEM)	
		<i>P. aeruginosa</i>	<i>K. pneumonia</i>
Chloroform	Blank	6.00±0.00	9.00±0.00
	40	7.17±0.76	9.34±0.57
	20	8.33±0.57	9.84±1.00
	10	9.00±1.00	10.00±0.28
	05	9.67±0.57	9.67±0.57
Ethyl acetate	Blank	7.5±0.28	7.00±1.00
	40	8.00±0.00	11.34±0.57
	20	7.00±0.00	7.84±1.00
	10	9.00±1.00	10.17±0.01
	05	9.33±0.57	8.34±0.57
n-butanol	Blank	6.5±0.21	7.00±1.00
	40	8.33±0.57	9.34±0.57
	20	9.17±0.28	10.34±0.57
	10	8.00±0.00	9.00±0.00
	05	10.00±0.00	10.34±0.57
Aqueous	Blank	00±0.00	00±0.00
	40	00±0.00	00±0.00
	20	00±0.00	00±0.00
	10	00±0.00	00±0.00
	05	00±0.00	00±0.00

Blank represents the solvent used for each fraction. Amoxicillin was used as a control which showed 6.5 mm zone of inhibition

Antioxidant activity against DPPH free radical

The fractions also showed potent antioxidant activities against DPPH free radical in a dose-dependent manner with IC₅₀ values respectively against n-butanol, ethyl acetate, chloroform, and aqueous with IC₅₀ values 103, 109, 143, and 265 as shown in Table 2.

Table 2. Percent DPPH Radical Scavenging Potential of Compound

Sample	Concentration (µg/ml)	DPPH percent inhibition (mean ± SEM)	DPPH IC ₅₀ (µg/ml)
Ethyl acetate	250	89.85 ± 0.002	109
	125	83.90 ± 0.001	
	62.5	39.36 ± 0.01	
Chloroform	250	88.09 ± 0.001	143
	125	94.48 ± 0.015	
	62.5	96.03 ± 0.002	
N-butanol	250	80.70 ± 0.001	103
	125	82.13 ± 0.002	
	62.5	91.73 ± 0.002	
Aqueous	250	39.02 ± 0.003	265
	125	87.54 ± 0.002	
	62.5	43.77 ± 0.03	

Total flavonoid contents

The total flavonoid contents of each fraction were determined in order: n-butanol>ethyl acetate>chloroform>aqueous with numerical values 16050, 5925, 10475, and 14100 mg/100 g, respectively. The analysis revealed that the methanolic extract contained mainly 1,2-Cyclohexanedicarboxylic Acid, Bis (2-Ethylhexyl) Ester (43.12%) and 3-Penten-2-one, 4-(dimethylamino)- (12.61%) (Table 3).

Table 3. Total flavonoids contents in each fraction

Sample	Concentration (mg/100g)
Ethyl Acetate	5925
Chloroform	10475
n-butanol	16050
Aqueous	14100

GC-MS characterization of *T. baccata* bark

Identification of Phytochemicals in *T. baccata* bark

The GC-MS result of *T. baccata* bark is tabulated in Table 4 in which the phytochemical peaks along with their retention time (RT), compound name (Compound), molecular formula (Formula), molecular weight (Mol weight) and peak area % are expressed; the chromatogram of the phytochemicals is shown in Figure 1. The results obtained show that 17 of the phytochemicals were eliminated and a total percentage area was determined to be 92.33 % of the total compounds.

Table 4. Identified phytochemicals through GC-MS in methanolic extract of *T. baccata* bark

No.	RT (min)	Name of compound	Molecular formula	Molecular weight(g/mol)	Peak area %
01	2.653	1,3-Dihydroxyacetone dimer	C ₆ H ₁₂ O ₆	180.16	0.93
02	11.817	Benzenemethanamine, N,N,.alpha.-trimethyl-	C ₁₀ H ₁₅ N	149.23	0.32
03	12.417	2,4-Di-tert-butyl-phenol	C ₁₄ H ₂₂ O	206.32	0.35
04	12.985	4-O-Methylmannose	C ₇ H ₁₄ O ₆	194.18	1.31
05	13.091	.alpha.-Methyl mannofuranoside	C ₇ H ₁₄ O ₆	194.18	0.60
06	13.343	3-Hexanol, 2,4-dimethyl-	C ₈ H ₁₈ O	130.229	4.00
07	14.647	4,4-Ethylenedioxy-1-pentylamine	C ₇ H ₁₅ NO ₂	145.2	7.54
08	14.770	1,3-Dioxolane, 2-butyl-2-methyl-	C ₈ H ₁₆ O ₂	144.21	5.76
09	14.815	1-Butene, 1-(methylthio)-, (Z)-	C ₅ H ₁₀ S	102.2	2.41
10	14.979	1,3-Dioxolane, 2-butyl-2-methyl-	C ₈ H ₁₆ O ₂	144.21	6.71
11	17.326	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	0.44
12	19.016	2-(2-Methoxyethoxy)ethanamine, N,N-bis(trimethylsilyl)-	C ₇ H ₁₇ NO ₃	163.21	0.44
13	22.201	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.6	1.02
14	22.677	1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₄₄ O ₄	396.6	43.12

15	23.266	3-Penten-2-one, 4-(dimethylamino)-	C ₇ H ₁₃ NO	127.18	12.61
16	23.854	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester		390.6	4.04
17	27.486	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	C ₁₀ H ₁₀ ClN ₃ C ₂₄ H ₃₈ O	207.66	0.73
Total					92.33

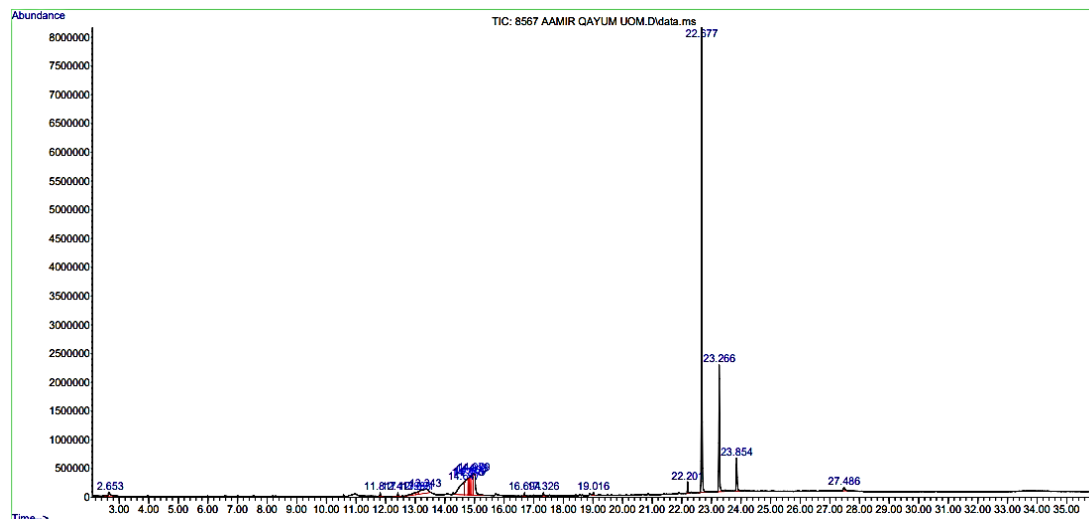


Figure 1. GC-MS chromatogram of methanolic extract of *T. baccata* bark

Discussion

Using the agar-well diffusion method, the antibacterial activity of *T. baccata* extract was ascertained. *Table 1* displays the antimicrobial assay findings. The highest zone of reluctance shown by n-butanol and ethyl acetate. The highest zone of n-butanol is 10 mm at lowest concentration against *P. aeruginosa* while ethyl acetate showed 11.34 mm at high concentration against *K. Pneumonia*. Aqueous extract was inhibited by both the bacterial strains. It is also reported that *T. baccata* leaf extract has antibacterial effects on *E. coli*, and *B. cereus*, two widely found bacterial infections (Patel et al., 2009). Moreover, it was mentioned that *T. baccata* exhibited an antibacterial action, mainly in contrast to gram-positive bacteria (Bernaitis et al., 2013).

The DPPH assay was employed in this study to examine the antioxidant potential of different fractions of *T. baccata* such as ethyl acetate, chloroform, n-butanol, and watery extract. At different concentrations of 250, 125 and 62.5 µg/ml as shown in *Table 2*. The antioxidant test is based on the capacity to donate electron to DPPH, scavenge it and cause its color to change from purple to yellow. The DPPH assay is also used to examine the antioxidant capacity of *T. baccata* methanol extract. There was up to 95.59% DPPH scavenging activity observed (Milutinović et al., 2015) while in our study the free radical scavenging activity ranged from 43.77- 96.03% (lowest IC50 value is 109 µg/ml). It is also shown that the methanol extract of *T. baccata* leaves had 5.46% inhibition in terms of DPPH radical scavenging activity (IC50) (Guleria et al., 2013).

We also determine the Total flavonoids content of methanolic extracts of *T. baccata* bark using aluminum chloride method. The total flavonoid contents of each fraction

were determined in order: n-butanol>ethyl acetate>chloroform>aqueous with numerical values 16050, 5925, 10475, and 14100 mg/100g, respectively which is presented in *Table 3*. The results showed that the *T. baccata* methanolic extract contained a high number of flavonoids. It is also reported that the total flavonoid content of the methanolic extract from the Serbian *T. baccata* was 161.98 ± 1.02 mg Rutin equivalent/g dry extract (Frunzete et al., 2023). Moreover, it is found that total flavonoid content of 48.89 ± 0.76 m quercetin equivalent/g in the ethanolic extract of the Turkish *T. baccata*. Differences in total flavonoid content can be attributed to genetic variation, geographic origins, climatic conditions and plant populations (Senol et al., 2015).

In this study, 17 bioactive phytochemicals of *T. baccata* were identified in the methanolic extract. *Table 4* and *Figure 1* display their retention time (RT), molecular weight (MW), molecular formula (MF), and concentration (peak area %). The GC-MS analysis showed that the primary components extracted from *Taxus baccata* L. were octane (13.36%), 4-methoxycarbonyl-3,5-diphenyl-1 (8.30%), and 9,12,15-Octadecatrienoic acid (10.75%) (Norouzi et al., 2020) while in our study the concentration of 1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester (43.12%) followed by 3-Penten-2-one, 4-(dimethylamino)- which is 12.16%.

Conclusion

From the results, it is concluded that the extracts of different fractions exhibit antibacterial and antioxidant potentials. Several phytochemicals have been reported in this plant, and it is, therefore, a potent candidate for medicinal plant use. The present study will open a new avenue to be investigated in the future.

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