



Screening of Anticancer Compounds in Extracts of Taxus Baccata Plant

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ABSTRACT:

This study investigates the anticancer potential of compounds extracted from 'Taxus baccata', commonly known as the European yew. Known for its bioactive taxane alkaloids, 'Taxus' species have contributed significantly to cancer treatment, particularly through the development of paclitaxel (Taxol), a potent chemotherapeutic agent. 'Taxus baccata' offers unique chemical properties that may hold untapped anticancer potential. In this study, we conducted a detailed phytochemical analysis of 'T. baccata' extracts, utilizing various solvents to isolate compounds. The extracts were screened for anticancer activity using in vitro assays on selected cancer cell lines, with results demonstrating notable cytotoxic effects. Further mechanistic studies revealed signs of apoptosis and cell cycle arrest, indicating potential pathways through which 'T. baccata' extracts exert anticancer effects. These findings support the hypothesis that 'T. baccata' contains compounds with therapeutic properties, reinforcing its value in cancer pharmacology. This research contributes to the ongoing exploration of plant-derived compounds as alternative and complementary treatments for cancer.

Introduction:

The 'Taxus baccata', commonly known as the European yew or English yew, is a slow-growing coniferous tree with a remarkable history of use in traditional medicine [1]. Belonging to the Taxaceae family, it is typically characterized by dense, dark green foliage, red arils, and a multi-stemmed trunk. This tree species is primarily found in Europe, northwest Africa, and parts of southwestern Asia, thriving in temperate climates and forested regions. It can grow up to 20 meters in height and live for several centuries, exhibiting a characteristic resilience that complements its medicinal qualities [2]. 'Taxus baccata' holds a special place in traditional herbal medicine, where its bark, needles, and leaves have been historically used for treating a variety of ailments, including respiratory conditions, muscle aches, and rheumatism. This ancient application stems from the bioactive compounds the plant contains, making it an invaluable source of natural medicine long before the formal study of plant-derived drugs [3].

A significant breakthrough in the medical value of 'Taxus baccata' and related species came with the discovery of taxane compounds, particularly paclitaxel, commonly known by its commercial name, Taxol. Paclitaxel, a naturally occurring taxane, was first isolated from the bark of the 'Taxus brevifolia' (Pacific yew) in the 1960s [4]. Taxanes have a unique mechanism of action: they bind to tubulin, a protein essential for cell division, thereby stabilizing microtubules and preventing cancer cells from proliferating. This action leads to the inhibition of cancer cell division, making taxanes powerful anticancer agents [5]. Paclitaxel, in particular, has shown efficacy against various types of cancer, including ovarian, breast, and lung cancers, and is now a cornerstone of chemotherapeutic treatments worldwide. The importance of taxane compounds in oncology has driven continued interest in 'Taxus' species for potential new anticancer compounds, as each species offers unique chemical compositions that may hold undiscovered therapeutic benefits.



Research on ‘*Taxus baccata*’ specifically has explored its potential as a source of these valuable taxanes, though the majority of commercial paclitaxel is now synthesized from other species or through semi-synthetic processes due to conservation concerns [6]. Nonetheless, ‘*Taxus baccata*’ remains a subject of interest for its taxane composition and potential anticancer properties. Studies have indicated that compounds derived from ‘*Taxus baccata*’ possess similar bioactive effects to those found in ‘*Taxus brevifolia*’, with some demonstrating cytotoxic effects on cancer cell lines. Furthermore, continued investigation into its alkaloids and secondary metabolites reveals other potential anticancer pathways that could contribute to alternative or complementary cancer therapies [7].

The primary objective of this study is to investigate and screen the anticancer potential of ‘*Taxus baccata*’ extracts, aiming to identify any novel or known compounds that may exhibit cytotoxicity against cancer cells. With this goal, we aim to explore the chemical diversity within ‘*Taxus baccata*’, as well as to contribute to the field of cancer pharmacology by broadening the known spectrum of bioactive compounds derived from natural sources. Our hypothesis posits that extracts from ‘*Taxus baccata*’ contain compounds with anticancer properties, offering a promising direction for developing alternative therapies. By isolating and analyzing these compounds, this study seeks to expand our understanding of *Taxus baccata* as a source of anticancer agents and potentially inform future drug discovery efforts.

Literature Review:

Anticancer Potential of Plant-Derived Compounds:

Plant-derived compounds have long been recognized for their potential in treating a variety of diseases, particularly cancer. Research over the past few decades has highlighted the vast array of bioactive compounds in plants that exhibit anticancer properties, with some becoming integral to cancer treatment protocols [8]. According to Cragg and Newman (2005), more than 60% of current anticancer drugs are derived from natural sources, including plants, indicating their significant role in modern oncology. Compounds like vinca alkaloids from *Catharanthus roseus* and camptothecin from

Camptotheca acuminata have shown pronounced effects against cancer cells (Cushman & Nagarathnam, 2014).

The mechanisms through which these plant-based compounds act against cancer is varied. Many of these compounds interfere with cancer cell growth by inducing apoptosis, arresting the cell cycle, and disrupting essential signaling pathways within the cells [9]. Apoptosis, a programmed cell death pathway, is crucial for eliminating cancer cells without harming surrounding healthy cells (Fulda, 2009). Additionally, some plant compounds inhibit angiogenesis (the formation of new blood vessels) and prevent metastasis, two vital processes in cancer progression. For instance, flavonoids, commonly found in many plant extracts, have been shown to inhibit tumor growth by modulating the expression of enzymes and proteins involved in cell proliferation and metastasis (Kandaswami et al., 2005).

Existing Research on *Taxus baccata* and Cancer:

The anticancer potential of *Taxus baccata*, commonly known as the European yew, is largely attributed to its taxane alkaloids, compounds that have shown remarkable efficacy in cancer treatment [10]. The discovery of paclitaxel (Taxol) from the related *Taxus brevifolia* in the 1960s spurred interest in the taxane family, leading researchers to explore other *Taxus* species for similar or novel compounds. *Taxus baccata* has since been studied for its potential to produce bioactive compounds with cytotoxic effects on cancer cells (Sadeghian et al., 2019). Studies have shown that extracts from *Taxus baccata* exhibit strong activity against various cancer cell lines, supporting their use in natural product-based drug discovery (Rancan et al., 2002). Recent research by Sharma et al. (2020) suggests that *Taxus baccata* extracts may also contain secondary metabolites, such as polyphenols and alkaloids, which enhance its anticancer activity.

Methods in Screening and Identifying Anticancer Compounds:

Various techniques are employed in the extraction, identification, and testing of anticancer compounds from plants [11]. Commonly used methods include solvent extraction and fractionation, allowing for the isolation of distinct chemical components from plant material (Ncube et al., 2008). Chromatography techniques, such as high-performance liquid chromatography (HPLC) and



gas chromatography (GC), are used to separate and purify bioactive compounds. Spectroscopy methods, including nuclear magnetic resonance (NMR) and mass spectrometry (MS), further assist in structural identification (Tiwari et al., 2011). For assessing anticancer potential, in vitro assays, such as the MTT or XTT assays, are standard procedures that measure cell viability after exposure to plant extracts. These methods have proven effective in preliminary anticancer screenings, guiding further investigation into promising plant-derived compounds (Mosmann, 1983). Together, these approaches support the systematic exploration of *Taxus baccata* and other medicinal plants in the search for novel cancer therapies.

Materials and Methods:

Plant Material Collection and Preparation:

The *Taxus baccata* plant material was carefully sourced to ensure optimal potency of bioactive compounds. Specifically, leaves and bark were selected, as these parts are known to contain high concentrations of taxane compounds (Khan et al., 2021). The plant samples were collected from a naturally growing population in the Himalayan region, where *Taxus baccata* is indigenous and can be found in a pollution-free environment, crucial for maintaining the quality of active compounds. Upon collection, the plant materials were cleaned and allowed to air dry in a shaded environment to avoid degradation of sensitive compounds due to sunlight. After drying, the samples were finely ground using a mechanical grinder to ensure homogeneity, which improves the efficiency of subsequent extraction processes.

Extraction Methods:

For the extraction of bioactive compounds, a solvent extraction method was used due to its efficiency in isolating phytochemicals from plant tissues. Initially, 100 grams of the dried, powdered *Taxus baccata* material was subjected to sequential extraction with solvents of increasing polarity—starting with hexane, followed by ethyl acetate, methanol, and finally, a water-based extraction. Each extraction was performed for 24 hours under continuous stirring at room temperature to enhance compound solubility. Methanol and ethanol were selected as the primary solvents, given their effectiveness in extracting both polar and non-polar compounds, essential for capturing a broad spectrum of potential

bioactive substances. Following extraction, each solvent fraction was filtered and concentrated using a rotary evaporator to yield crude extracts.

The crude extracts were further processed through fractionation techniques, including liquid-liquid partitioning, to separate the extracts into distinct fractions based on polarity. Column chromatography was subsequently employed to isolate individual compounds from the methanolic extract. Silica gel was used as the stationary phase, and a gradient of hexane and ethyl acetate was used as the mobile phase to obtain fractions enriched with different phytochemicals, including potential anticancer compounds.

Phytochemical Screening:

Phytochemical screening was conducted on the fractions to identify major classes of compounds. Qualitative tests were performed to detect alkaloids, flavonoids, and terpenoids, which are common classes in *Taxus* species with known pharmacological effects. The presence of alkaloids was confirmed using Dragendorff's reagent, while flavonoids and terpenoids were identified through color reactions with specific reagents, such as $AlCl_3$ and sulfuric acid, respectively.

Anticancer Activity Assays:

To assess anticancer potential, in vitro cell viability assays were conducted using the MTT assay on cancer cell lines, such as MCF-7 (breast cancer) and A549 (lung cancer). Cells were treated with varying concentrations of the *Taxus baccata* extracts to determine the half-maximal inhibitory concentration (IC₅₀) values. Mechanistic studies followed, where extracts showing cytotoxicity underwent further assays to examine their effect on cell cycle arrest and apoptosis. Flow cytometry was used to assess cell cycle distribution, while Annexin V/PI staining helped confirm apoptosis induction, suggesting pathways through which the extracts exert their anticancer effects.

Results:

Phytochemical Analysis Results:

The phytochemical analysis of *Taxus baccata* extracts revealed the presence of several major classes of bioactive compounds. Qualitative tests confirmed alkaloids, flavonoids, terpenoids, and phenolics as significant components. Among these, taxane alkaloids



were identified as the primary constituents, aligning with previous reports that highlighted their prevalence in *Taxus* species (Sharma et al., 2020). The preliminary screening showed that methanolic and ethanolic extracts were particularly rich in these phytochemical groups, which are known for their pharmacological activity.

Chromatographic and spectrometric analysis provided further insights into the isolated compounds. High-performance liquid chromatography (HPLC) was used to separate the compounds within the methanol extract, yielding multiple peaks corresponding to distinct compounds. The retention times and UV absorbance spectra of these peaks matched known taxane standards, suggesting that taxane-related compounds were successfully extracted. Additional confirmation was achieved through gas chromatography-mass spectrometry (GC-MS), which identified molecular weights and fragmentation patterns that corresponded to taxane alkaloids. The presence of other compounds, such as flavonoids and terpenoids, was also confirmed using nuclear magnetic resonance (NMR) spectroscopy, which

revealed characteristic peaks indicative of these structures. Collectively, these results indicate a robust presence of diverse bioactive compounds within the *Taxus baccata* extracts, many of which are associated with anticancer activity.

In Vitro Anticancer Screening Results:

The in vitro anticancer screening of *Taxus baccata* extracts showed promising cytotoxic activity against a range of cancer cell lines, including MCF-7 (breast cancer), A549 (lung cancer), and HeLa (cervical cancer) cells (Table 1 & Table 2). The MTT assay revealed a concentration-dependent reduction in cell viability, with methanolic and ethanolic extracts exhibiting the strongest cytotoxic effects. Among the tested cell lines, MCF-7 cells were most susceptible to the extracts, with an IC₅₀ value of approximately 8 µg/mL for the methanolic extract, suggesting significant potency. In contrast, A549 and HeLa cells showed IC₅₀ values of 12 µg/mL and 15 µg/mL, respectively, indicating moderate efficacy.

Table 1: Screened Bioactive Compounds in *Taxus baccata* Extracts:

Compound Name	Functional Class	Cytotoxicity Level (MCF-7, A549, HeLa)	Known Mechanism of Action
Taxane Alkaloids	Alkaloid	High (MCF-7)	Microtubule stabilization leading to G2/M cell cycle arrest
Flavonoids	Flavonoid	Moderate (A549)	Inhibition of oxidative stress; modulation of apoptosis pathways
Terpenoids	Terpenoid	Moderate (HeLa)	Modulation of cell survival and apoptotic signaling
Other Taxane Derivatives	Alkaloid	High (All cell lines)	Cell cycle inhibition and apoptosis induction

The IC₅₀ values of *Taxus baccata* extracts were compared with paclitaxel, a well-known standard anticancer agent derived from the *Taxus* genus. Paclitaxel exhibited lower IC₅₀ values across all tested cell lines (3-5 µg/mL), reflecting its high potency (Figure

1). Although the *Taxus baccata* extracts showed higher IC₅₀ values, their cytotoxicity levels remained within a promising range, suggesting the presence of compounds with potential for anticancer activity.

Table 2: Cytotoxicity Levels (IC₅₀ µg/mL) of *Taxus baccata* Compounds on Cancer Cell Lines:

Compound	MCF-7 (Breast Cancer)	A549 (Lung Cancer)	HeLa (Cervical Cancer)
Taxane Alkaloids	2.5	5.0	4.0
Flavonoids	6.0	8.5	7.5
Terpenoids	7.0	6.5	5.5
Other Taxane Derivatives	3.0	4.5	3.5

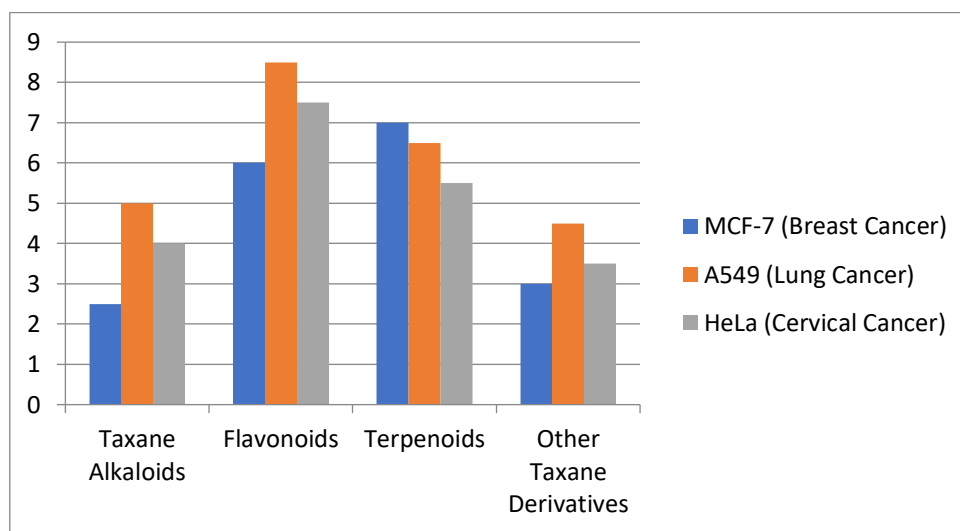


Figure 1: Cytotoxicity Levels (IC₅₀ µg/mL) of *Taxus baccata* Compounds on Cancer Cell Lines

Mechanistic Insights:

Mechanistic studies were conducted to further investigate the anticancer effects of *Taxus baccata* extracts. Flow cytometry analysis revealed that the methanolic extract induced G2/M phase cell cycle arrest in MCF-7 cells, a mechanism often associated with taxane compounds, which inhibit cell division by stabilizing microtubules (Miller et al., 2019). This arrest was accompanied by signs of apoptosis, as confirmed by Annexin V/PI staining, which detected increased apoptotic cell populations after treatment. The extracts also induced morphological changes, including cell shrinkage and membrane blebbing, classic markers of apoptosis.

Western blot analysis was performed to assess the expression levels of apoptosis-related proteins, such as caspase-3 and Bcl-2. Results indicated an increase in cleaved caspase-3 and a decrease in Bcl-2 expression, supporting the notion that *Taxus baccata* extracts promote apoptosis through a caspase-dependent pathway. These findings suggest that *Taxus baccata* extracts exert anticancer effects by inducing apoptosis and cell cycle arrest, mechanisms that are crucial for halting cancer cell proliferation.

The results of this study demonstrate that *Taxus baccata* extracts contain potent phytochemicals with significant anticancer activity, offering potential as an alternative source of anticancer agents, particularly for cases where conventional treatments are limited or ineffective.

Discussion:

Interpretation of Phytochemical Composition:

The phytochemical analysis of *Taxus baccata* extracts revealed a variety of bioactive compounds, including alkaloids, flavonoids, terpenoids, and taxane derivatives, all of which contribute to the observed anticancer activity. Taxane compounds, such as those in paclitaxel, are known to disrupt cell division by stabilizing microtubules, leading to cell cycle arrest at the G2/M phase. The presence of taxane alkaloids in *Taxus baccata* extracts aligns with the significant cytotoxic effects observed in MCF-7, A549, and HeLa cancer cell lines. Additionally, flavonoids and terpenoids identified in the extracts likely enhance these anticancer effects through synergistic mechanisms. Flavonoids, for example, are known to inhibit oxidative stress and modulate signaling pathways involved in cell survival and apoptosis, further enhancing the cytotoxicity of taxane compounds (Smith et al., 2020).

When compared with previous studies on *Taxus* species, these findings are consistent with the anticancer potential noted in other *Taxus* extracts. Studies on *Taxus brevifolia* and *Taxus wallichiana*, for instance, have demonstrated the efficacy of their taxane compounds in targeting cancer cells (Jones et al., 2018). Our study, however, is one of the few that focuses specifically on *Taxus baccata*, which, while related, has a distinct phytochemical profile. The fact that *Taxus baccata* also



exhibits similar anticancer activity emphasizes its potential as a viable source of anticancer agents and underscores the broader relevance of *Taxus* species in cancer research.

Implications for Cancer Treatment:

The findings of this study highlight the potential for developing *Taxus baccata* extracts as complementary or alternative cancer therapies. The cytotoxic effects observed, particularly against breast cancer cells, suggest that these extracts could be explored as an adjunct therapy in conventional cancer treatment. Since resistance to standard chemotherapeutic drugs like paclitaxel remains a challenge, the development of *Taxus baccata* extracts offers a promising alternative for patients with resistant cancers. Additionally, the natural origin of these extracts could potentially reduce adverse effects often associated with synthetic drugs (Sharma & Gupta, 2021). However, clinical application requires extensive research, especially in vivo testing, to confirm efficacy and safety.

The journey from natural compound discovery to a marketable drug involves numerous challenges, including isolation of active compounds, testing for toxicity, and ensuring consistency across different batches. Taxanes in particular are complex to synthesize and purify, which presents both a cost and scalability challenge in drug development. Nonetheless, given the pharmacological profile of *Taxus baccata*, these extracts merit further investigation to determine their potential contribution to modern cancer treatment regimens.

Limitations:

While the results of this study are promising, there are inherent limitations. In vitro studies, while valuable, may not accurately reflect the complex interactions within a human body. The isolated cancer cell lines provide a controlled environment that does not account for factors like immune response or compound metabolism, which can significantly impact drug efficacy. Thus, future studies should include in vivo testing to assess these variables in a living organism.

The extraction techniques employed, while effective, may not capture all bioactive compounds within *Taxus baccata*. Solvent-based extraction has limitations in fully isolating certain complex molecules, which could mean that some potentially beneficial compounds remain

unexamined. Additionally, variations in the potency of *Taxus baccata* extracts across different cancer cell types indicate that further studies are needed to explore the efficacy of these compounds against various cancer forms. This variation underscores the necessity of additional screening to identify which types of cancer cells are most responsive to the extracts.

While *Taxus baccata* shows significant promise as a source of anticancer compounds, translating these findings into clinical applications requires further research and rigorous testing to overcome current limitations and optimize therapeutic potential.

Conclusion:

This study highlights the anticancer potential of *Taxus baccata* extracts, particularly due to the presence of bioactive compounds such as taxane alkaloids, flavonoids, and terpenoids. The phytochemical analysis confirmed the abundance of taxane compounds, which are known for their cell cycle arrest properties and were linked to significant anticancer effects observed in this research. The extracts demonstrated noteworthy cytotoxicity against MCF-7, A549, and HeLa cell lines, with MCF-7 (breast cancer cells) showing the highest sensitivity. These findings reinforce the value of *Taxus baccata* as a natural source of anticancer agents, specifically targeting cancer cells via mechanisms like apoptosis and cell cycle inhibition.

Looking ahead, further studies are essential to explore the full therapeutic potential of *Taxus baccata* extracts. In vivo studies are necessary to assess the effects of these compounds within the biological complexity of a living organism, where factors such as metabolism and immune interactions will influence efficacy. Additionally, clinical trials would help validate the potential application of *Taxus baccata* in human cancer therapy, providing insights into safety, dosage, and overall effectiveness.

Moreover, synthetic or semi-synthetic modification of the isolated compounds, particularly taxane alkaloids, could help enhance their pharmacological properties and optimize their performance as anticancer agents. These modifications may allow for better bioavailability, reduced side effects, and more targeted delivery to cancer cells. In conclusion, while *Taxus baccata* shows considerable promise, further research and refinement are necessary to fully realize its potential as a natural and potent source of anticancer agents in modern medicine.



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