Taxol Extract from Lauth Sala (West Himalayan Yew) for Cancer Therapy

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Abstract

Thirty-one years ago, promising new chemotherapy medications were introduced. Taxol or paclitaxel was extremely potent. Paclitaxel disrupts microtubule dynamics, causing tubulin polymerization and cell death. Membrane phosphor-glycoprotein drug-efflux pumps and tubulin structural alterations generate paclitaxel resistance. Taxaceae contains Taxus contorta, the West Himalayan yew. A medicinal tree from the Himalayas. This evergreen tree grows from 1700–2600 MSL in Nepal. Native civilizations use it to treat fever, coughing, colds, and discomfort. Cancer medicine paclitaxel derives from West Himalayan yew tree bark or leaves. Taxol's increased demand threatens yew trees. Researchers are employing Lauth Sala (West Himalayan Yew) as a Taxol substitute to remedy this. In this study, Lauth Sala bark or leaves were ground, soaked in ethanol, then separated and purified using column chromatography to produce Taxol. TLC determined taxol purity. Bark provided 0.11% pure dry Taxol, whereas leaves yielded 0.39%, according to the study. Bark provided less pure dry Taxol than leaves from the West Himalayan yew. According to this study, Lauth Sala may replace Taxol. Paclitaxel produces neurotoxicity, hypersensitivity, and haematological damage. Schedule and dose determine toxicity. Paclitaxel cures malignancies of the breast, ovary, lung, head, and neck.

Keywords: Taxol extraction; Lauth Sala (Taxus contorta); Taxus species; Anticancer compounds; Natural product isolation; Chemotherapy alternatives; Sustainable drug sources

1. INTRODUCTION

The Taxanes are a novel class of drugs with a distinct chemical makeup and mode of action. The US National Cancer Institute initiative, which examined thousands of plants for potential anticancer properties, led to the discovery of paclitaxel. In preclinical research, it was discovered in 1963 that a crude extract from the bark of the Pacific Yew, Taxus brevifolia, a rare and slowly growing evergreen found in the old-growth woods of the Pacific Northwest, has cytotoxic effect against various tumors. In 1971, paclitaxel was discovered to be the extract's active ingredient. The limited availability of drugs initially hampered the development of paclitaxel. The semi-synthetic production of 10-deacetylbaccatin III and other inactive precursor taxanes found in the needles and other parts of more prevalent Taxus species, such as the European Yew, later took the role of the natural supply (Taxus baccata). When paclitaxel's distinct mode of action as an antitumor medication was discovered and outstanding activity was shown in the National Cancer Institute cancer screening in 1979[1]-[3], interest was sparked. As a result of the early quest for taxanes, docetaxel, which is likewise made from 10-deacetylbaccatin III, was also developed. Docetaxel is a more effective drug against acellular tubulin systems and is somewhat more water-soluble. The clinical advantages of these variations are not yet obvious, though Many of the therapeutic plants found in the Himalayan mountains haven't even been subjected to formal scientific research. Yews have medicinal benefits for a variety of illnesses, but their ability to prevent cancer is what makes them so highly prized. Taxol, a substance found in its leaves, bark, and trunks, has been shown to prevent the development of new cancer cells. One of the three Taxus species present in Nepal is the Maire's Yew. While Taxus wallichiana (Himalayan yew) is found in central to eastern Nepal,

Taxus contorta (West Himalayan yew) is found from west to central Nepal. The most uncommon Taxus mairei, however, is only found in the mountainous regions close to Kathmandu Valley in the districts of Kavre, Makwanpur, and Sindhuli. The Himalayan yew tree is a Nepalese native that grows between the elevations of 2300 and 3400 meters. Himalayan yew and West Himalayan yew are known in Nepal as lauth Sala and belong to the Taxaceae family [4]-[7].

West Himalayan yew has been used for generations in traditional medicine to treat a variety of illnesses. The creation of Taxol, a potent chemotherapy medication used to treat many forms of cancer, by Lauth Sala is particularly notable. The Pacific yew tree's bark was originally used to extract Taxol, but there was a limited supply of this species, and harvesting it created environmental risks. This prompted a search for alternative sources of Taxol, and Lauth Sala was identified as a strong contender. Although the process of extracting and purifying Taxol from Lauth Sala is difficult, research in this field is still underway because of the possible advantages it could provide for cancer patients. Lauth Sala is prized for its wood and decorative features in addition to its therapeutic benefits. Taxus contorta is a coniferous tree that can grow up to 20 meters in height, with a diameter of up to 300 centimeters at breast height. It has many branches and a crown that is either spherical or pyramidal. The bark exfoliates in big flakes and is thin and reddish-brown. Although they are organized in a V shape, the leaves are inserted spirally. They are linear, 15–40 mm long, 1.5–2.5 mm wide, and slightly twisted at the short-petiolate base. The stomata are dispersed irregularly, and the leaves are dark green with two pale yellow stomatal bands on the bottom surface. The tree is dioecious, which means that there are distinct male and female trees. In contrast to the female trees, which produce tiny spherical cones with a diameter of 1.5–3 cm, the male trees produce tiny yellow flowers. Axial, solitary, and ovoid in shape, the pollen cones are 6–8 mm in diameter, become pink when mature, and then turn brown. The axillary, single-seed cones feature a thin, green aril that ripens to a succulent crimson, and they are also axillary. They are 7-9 mm wide and range in length from 9 to 12 mm. The oblong, somewhat flattened seeds have dimensions of 6-7 mm in length and 4-5 mm in breadth. They start green and eventually turn dark brown as they get older. The West Himalayan Yew is a widespread tree that grows in forests at elevations between 1,700 and 3,400 meters in the western Himalayas, from Afghanistan to western Nepal. It has a moderate growth rate and an approximate 1,000-year lifespan. Compared to European and American yew, the Himalayan yew has a higher purity of the drug called Taxol. Deacetylbaccatin, a substance with strong anticancer effects, is present in Himalayan yew. A similar situation arises with other Taxus species as well, such as Taxus chinensis. According to the report from CEC China Pharmaceuticals Ltd 10 thousand kilograms (kg) of bark from this species are required to isolate 1 kg of paclitaxel.



Figure 1. West Himalayan yew

2. CHEMICAL STRUCTURE

A four-member oxetane ring connected to a taxane ring structure at locations C-4 and -5 makes up the alkaloid ester paclitaxel (Figure). Paclitaxel and docetaxel's taxane rings are connected to an ester at position C-13, whereas 10-deacetylbaccatin III is not [3].

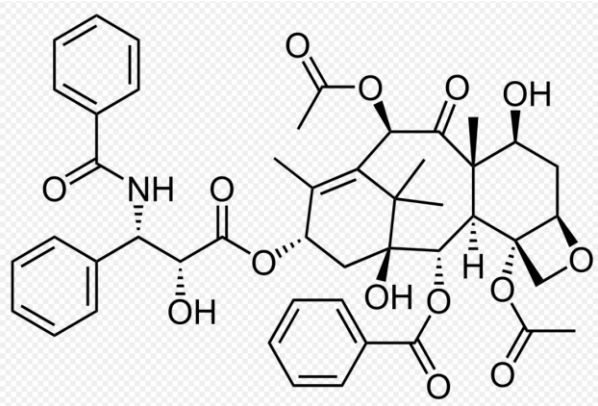


Figure 2. Chemical Structure of Taxol

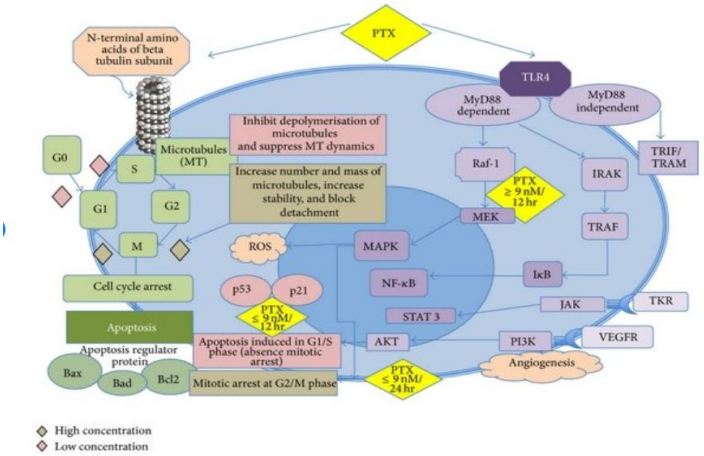


Fig.3 Mechanism of Taxol

3. MECHANISM

In a dynamic equilibrium with tubulin heterodimers made up of α- and β-protein subunits, microtubules are polymers of tubulin. Microtubules play numerous crucial interphase roles, including maintaining cell shape, motility, signal transduction, and intracellular transport, in addition to their fundamental role in the creation of the mitotic spindle during cell division. Paclitaxel encourages the polymerization of tubulin, in contrast to other antimicrotubular targeted drugs like vinca alkaloids, which cause the disassembly of microtubules. Due to the abnormally stable and dysfunctional microtubule dynamics that are essential for cell division and interphase events, paclitaxel-induced microtubule formation results in cell death [8]-[10].

Additionally, taxanes cause cellular actions that result in programmed cell death, or apoptosis. Even at concentrations that do not cause tubulin polymerization, cells undergo taxane therapy and exit mitosis without proliferating further. In contrast, significant DNA fragmentation suggestive of apoptosis is observed, and cell death takes place in 2–3 days. Although it is not yet clear how exactly mitotic arrest and apoptosis are related, taxanes have been found to interact with a variety of regulatory molecules and oncogenes that bind to the mitotic apparatus.

Although structure-activity investigations show that these activities are unrelated to paclitaxel's effects on microtubule assembly, the question of what role these cytokines play in paclitaxel's antitumor action remains. Paclitaxel also stimulates TNF α- gene expression. In contrast, docetaxel and other taxanes do not have this impact. The clinical consequences of these differences are unknown.

Mechanism of action of paclitaxel. Paclitaxel targets microtubules. At high concentrations, PTX causes mitotic arrest at the G2/M phase whereas at low concentrations, apoptosis is induced at G0 and G1/S phase either via Raf-1 kinase activation or p53/p21 depending on the dose concentration. Even at a lower dose but with exposure beyond 24 hours, paclitaxel can cause mitotic arrest. Paclitaxel also activates multiple signaling pathways to exert proapoptotic activity as well as immunomodulatory effects. Paclitaxel also develops resistance via these signaling pathways. PTX: Paclitaxel, TLR4: Toll-like receptor 4, G0: resting phase, G1; cells enlarge and make new protein, S phase: DNA replication, G2: preparation for division, M phase: cell division/mitosis, Raf-1: Raf kinase family, MEK/MAPK: mitogen-activated protein kinase, IRAK: IL-1 receptor-associated kinase, TRAF: TNFR associated factor, NF-κB: nuclear factor kappa B, TRIF/TRAM: TIR-domain-containing adapter-inducing interferon-β, TKR: tyrosine kinase receptor, VEGFR: vascular endothelial growth receptor, PI3K: phosphoinositide 3-kinase, JAK: Janus kinase, STAT: signal transducer and activator of transcription factor[24],[25].

4. MECHANISM RESISTANCE

There are two distinct pathways of acquired resistance to taxanes. First off, certain tumors have tubulin that is unable to polymerize into microtubules and has a naturally sluggish rate of microtubule assembly that

taxanes can normalize. It has been demonstrated that a variety of cell lines that are resistant to the taxanes and other tubulin binding agents exhibit changes in their tubulin content, expression of tubulin isotypes, dynamics of tubulin polymerization, or tubulin isotype content. In several individuals with non-small cell lung cancer, it has been observed that paclitaxel resistance is strongly influenced by taxane-resistant cells and -tubulin mutations. The expansion of membrane phospho-glycoproteins, which serve as drug-efflux pumps, constitutes a second mechanism. The multi-drug resistant (MDR) phenotype of tumor cells confers several structurally bulky natural compounds, such as anthracyclines, etoposide, vinca alkaloids, colchicine, and taxanes, with varied degrees of cross-resistance. Many classes of medications, such as calcium channel blockers, tamoxifen, cyclosporin A, antiarrhythmic agents, and the main ingredients of the drugs used to make paclitaxel and docetaxel (polyoxymethylene castor oil [Cremophor EL]), can reverse resistance to paclitaxel and docetaxel (referred to as MDR-1) (polysorbate 80).

The effectiveness of various types of MDR modulators in the clinic in reversing taxane resistance is being studied.

The regulation of apoptosis during development and tumorigenesis is governed by several mechanisms that have recently been identified. Numerous genes, such as the proto-oncogene bcl-2, the related gene bcl-x, and the tumor suppressor (proapoptotic) p53 gene, have been identified as being crucial in the control of these pathways. The antiapoptotic properties of bcl-2 and bcl-x have been demonstrated to be influenced by post-translational phosphorylation, which is hypothesized to be caused by medications that target microtubules, such as paclitaxel. Anti-tumor medicines' ability to induce apoptosis may need the increased expression of wild-type p53, as well as a decrease in the expression of bcl-2 and bcl-x and/or an increase in the expression of a gene associated with apoptosis called bax[28]-[30].

5. MATERIALS AND METHODS

5.1. Chemical and reagent

The required materials for the extraction of Taxol from yew tree bark or yew tree leaves include ethanol (95%), methanol (99%), dichloromethane, hexane, acetic acid, water, sodium hydroxide, chloroform, separatory funnel, rotary evaporator, Buchner funnel, filter paper, and various glassware such as flasks, beakers, pipettes, etc. Additionally, procedures like HPLC, column chromatography, HPTLC, and TLC may also be used.

5.2. Procedure

Harvest West Himalayan yew bark or leaves from the tree using a sharp knife or pruning shearsand make them dried and powdered.

↓

Weigh out 100 g of the powdered yew bark or leaves and place it into a large flask

↓

Add 500 mL of 95% ethanol to the flask and stir the mixture vigorously for 24 hours to extract theTaxol and other plant compounds.

↓

Filter the mixture through a Buchner funnel and filter paper to remove any solid particles.

↓

Pour the filtered mixture into a rotary evaporator and evaporate the ethanol under reduced pressure at a temperature of 40-50°C.

↓

Dissolve the remaining residue in 500 mL of methanol and add 100 mL of dichloromethane to themixture.

↓

Add 1 mL of acetic acid to the mixture and stir it for 15 minutes.

↓

Filter the mixture through a Buchner funnel and filter paper to remove any solid particles.

↓

Pour the filtered mixture into a separatory funnel and add 500 mL of water.

↓

Shake the funnel vigorously for several minutes to allow the two layers to separate.

↓

Drain the lower layer (methanol-dichloromethane) into a flask and discard the upper layer (water).

↓

Add 10 mL of 10% sodium hydroxide solution to the flask and shake it for 10 minutes.

↓

Add 500 mL of chloroform to the flask and shake it for 10 minutes.

↓

Drain the lower layer (chloroform) into a separate flask and discard the upper layer (aqueous layer).

↓

Pour the chloroform extract into a rotary evaporator and evaporate the chloroform under reducedpressure at a temperature of 40-50°C.

↓

The remaining residue will contain Taxol and other plant compounds. Dissolve this residue in a small amount of methanol or ethanol and store it in a tightly sealed container.

↓

Purify the crude extract using column chromatography or other separation techniques to isolate Taxol fromother plant compounds.

↓

Analyze the purity and quality of the Taxol using analytical instrument TLC.

6.Discussion

The species Taxus contorta, which is native to Nepal and goes by the local name Lauth Sala, is a member of the family Taxaceae. Its range extends from western to central Nepal. To harvest the barks and leaves of west Himalayan yew, we traveled to the Humala district, which is situated in the western region of Nepal. We used a sharp knife or pruning shears to remove the West Himalayan yew bark or leaves off the tree, and then we dried them in the sun for a week. Utilizing a mortar and pestle, the dried leaves or barks were ground into a powder. After determining how much-powdered yew bark or leaves you need, weigh it out and put it into a big flask. To extract Taxol and the other plant chemicals, we poured 500 milliliters of methanol into the flask and swirled the contents of the flask vigorously for twenty-four hours. To extract Taxol in the laboratory, methanol was used as a solvent since it is a polar solvent that was able to successfully remove taxes, including Taxol, from plant material. Because its boiling point is lower than that of water, methanol is more readily extracted with the use of a rotary evaporator. To eliminate any solid particles, we filtered the mixture using a Buchner funnel and filter paper (Whatman filter paper). After passing the extraction mixture through a filter, we transferred it to a rotary evaporator for the next step in the processing. The rotary evaporator implemented the process of evaporation under decreasing pressure so that it could successfully separate the ethanol solvent from the mixture. The filtered mixture was then transferred into a flask that had a circular bottom and was then coupled to the rotary evaporator. The pressure inside the system was reduced, and the flask was placed in a bath of hot water with only a portion of its circumference exposed. The ethanol vaporized and produced a cloud of vapor when the flask was rotated; this cloud was later cooled, collected, and stored in a different flask. This method was carried out at a controlled temperature of 40-50 degrees Celsius and speed ranging from 100 to 110 revolutions per minute to facilitate efficient solvent evaporation while simultaneously minimizing Taxol loss. The methanol evaporated after we had collected the dry residue that had been left over from the extraction mixture. After the residue had dried, it was weighed, and a calculation was made to determine the percentage yield of plant components that contained crude Taxol. We were able to obtain a yield of nine percent of a plant extract compound from the bark of West Himalayan yew that included crude Taxol. In addition, we calculated the percentage yield of plant compounds using the crude Taxol extracted from the leaves, which came to 15.65%. These calculations provided helpful information on the efficiency of the extraction process as well as the quantity of the necessary compounds that were present in the plant material.

For barks

The initial weight of bark powder = 100 gm
The final weight of extract dried residue = 9.41gm

The percentage yield of plant components containing crude Taxol from the bark of west Himalayan yew =

$$\frac{\text{The initial weight of bark powder}}{\text{The Final weight of extract dried residue}} * 100$$

= (9.41/100) *100 %
= 9 .41%

For leaves
The initial weight of leaves powder = 100 gm
The final weight of extract dried residue = 9.41gm

The percentage yield of plant components containing crude Taxol from the leaves of west Himalayan yew

$$\frac{\text{The initial weight of leaves powder}}{\text{The final weight of extract dried residue}} *100$$

= (15.65/100) *100 %
= 15.65%

The leftover extract residue was dissolved in 500 mL of methanol, and then 100 mL of dichloromethane was added to the mixture after it had been stirred. It is permissible to make use of dichloromethane in the Taxol extraction procedure due to the dichloromethane's compatibility with the solvent and its capacity to partition. The polarity of methanol makes it possible for it to dissolve a broad variety of polar and nonpolar compounds, including Taxol. On the other hand, the lower polarity of dichloromethane allows it to selectively extract Taxol and separate it from molecules that may interfere with its functioning. This particular mix of solvents enhances the selectivity of the Taxol extraction process, making it more likely that the Taxol will be isolated from any unwanted components that may be present in the extract. The exact amounts are most likely chosen based on previous research or processes to maximize the effectiveness of Taxol extraction from the yew plant material and the amount of Taxol that is obtained. After the addition of 1 mL of acetic acid to the liquid that was being used for the Taxol extraction and 15 minutes of laboratory stirring, several objectives were accomplished. The procedure of extracting Taxol required the use of acetic acid since it helped adjust the pH of the combination, increased the solubility of Taxol and other compounds of interest, aided in the process of those chemicals being partitioned between organic and aqueous phases, and stopped the degradation of Taxol. The use of acetic acid enhanced the extraction of Taxol by optimizing the pH and solubility, and the procedure of stirring ensured that all of the components were well mixed together and interacted with one another. In the end, this step helped significantly in the successful extraction and storage of Taxol during the laboratory operation. Following this, we repeated the process of filtering the mixture using a Buchner funnel and some filter paper to remove any remaining solid particles. To successfully separate and purify the extracted taxol, it was necessary to pour the filtered liquid into a separatory funnel and then add 500 mL of water at various points throughout the process of taxol extraction. This strategy proved helpful in improving the separation of the organic and aqueous phases that took place inside the separatory funnel. As a result of its enhanced solubility in the organic phase, taxol partitioned preferentially into the organic layer, whereas water-soluble pollutants and interfering compounds remained in the aqueous layer. This was due to Taxol's greater solubility in the organic phase. Water played an important role in the process of removing polar impurities and increasing the overall quality of the Taxol extract by helping to separate the two phases. When water was added to the separatory funnel, it helped the process of purification go more smoothly. To assist in the process of separating the layers, we violently shook the funnel for some minutes. In the case of the leaves extract complex, three layers appeared, and we discarded the upper (water) and lower (impurities) layers after we drained the center layer (methanol-dichloromethane) into a flask. The middle layer included methanol and dichloromethane. For the bark extract compound, we obtained two layers and discarded the top layer, which included water, before draining the bottom layer, which contained methanol and dichloromethane, into a flask. Using this method of separation, we were able to successfully separate the target layer that contained the taxol and remove any undesired components or impurities. After stirring the Taxol extraction mixture for ten minutes, we added 10 milliliters (mL) of a sodium hydroxide (NaOH) solution that was 10% strength. In the process of purification, an alkaline solution known as sodium hydroxide can be utilized in many different ways. In the first place, it facilitates the alkaline hydrolysis of the combination that already contains Taxol, which in turn breaks down other acidic functional groups. This technique simplifies difficult Taxol-related compounds into forms that may be further refined. It does this by

converting various Taxol derivatives into their respective salts. Second, sodium hydroxide contributes to the process of moving the pH of the mixture into the basic range. It is necessary to maintain a certain pH range to facilitate the solubility and selective precipitation of Taxol-related compounds. By controlling the pH and eliminating any pollutants that are present, it is possible to make a solution that contains more pure Taxol.

The selective precipitation of several Taxol-related compounds also requires the presence of sodium hydroxide as an ingredient. Because the necessary components will preferentially precipitate as a result of the adjustment to the pH, the Taxol molecule that is supposed to be produced may continue to exist in the solution. The solubility of Taxol in the solution is improved by the addition of sodium hydroxide, which in turn makes it easier to isolate Taxol from the other components during the subsequent purification steps. Because sodium hydroxide acts as a neutralizing agent, it converts any acidic substances that are present in the mixture used for the Taxol extraction into their corresponding salts, which are then very easy to separate. It is vital for dissolving difficult compounds, controlling pH, and precipitating undesirable chemicals during the process of purifying Taxol. It is important to go through this process to obtain a more refined and pure form of Taxol from the bark of the West Himalayan yew. Following the addition of 500 mL of chloroform to the flask, we swirled the mixture for ten minutes. The Taxol extraction method called for the use of chloroform as a solvent to successfully separate the Taxol from the mixture of methanol and dichloromethane. When compared to polar solvents like water or methanol, the nonpolar solvent chloroform, which has a low polarity, is more successful in dissolving the drug Taxol. This is because chloroform has a lower polarity. Following the addition of sodium hydroxide, the pH of the solution changed to a more basic state, which led to an increase in the solubility of the Taxol in the nonpolar chloroform solvent. During the shaking process, the Taxol was able to dissolve in the chloroform layer, whereas the polar impurities were able to stay put in the aqueous layer. In addition, chloroform was selected because it has a low boiling point, which made it simple to remove chloroform from the Taxol-chloroform solution via rotating evaporation while maintaining a low temperature. Taking this preventative measure decreased the likelihood that any taxol would be degraded or lost in the course of the extraction process. The entire liquid mixture was poured into the separating funnel until it was full. After then, the funnel was violently shaken for a good number of minutes to separate the two layers. The bottom layer, which contained the chloroform, was transferred to a new flask, while the top layer, which contained the aqueous solution, was discarded. After collecting the chloroform extract, the chloroform solvent was evaporated in a rotary evaporator at temperatures between 40 and 50 degrees Celsius and under reduced pressure. In the residue that was left over, Taxol and some other plant compounds were discovered. After being dissolved in a little amount of methanol, this residue was placed in a container that had its lid securely fastened to it. During the process of extracting Taxol, rotary evaporation played an essential role since it made it possible to concentrate the extract by eliminating the solvent and so increased the amount of Taxol as well as other beneficial components. The rotary evaporation method enabled good temperature control, which prevented heat-sensitive Taxol molecules from deteriorating or disappearing altogether throughout the process. The huge surface area of the rotary evaporator combined with the low-pressure circumstances, led to the effective evaporation of the solvent, resulting in the quick evaporation of the solvent. In addition, the procedure was effective in removing volatile contaminants and residual solvents, which contributed to the purification of the Taxol extract. Following the collection of the dry residue left over from the extraction mixture, the chloroform was allowed to evaporate. The weight of the dried residue was then used to calculate the proportion of crude Taxol that had been produced. From the bark of the West Himalayan yew, we were able to obtain 0.47% of a plant extract called crude Taxol. In addition, the crude Taxol produced a percentage yield of 1.7% from the leaves of the Lauth Sala plant. These calculations provided valuable information regarding the effectiveness and operation of the extraction process.

For bark
The initial weight of bark powder = 100 gm
The final weight of extract dried residue = 0.47gm

The percentage yield of crude Taxol from the bark of west Himalayan yew

$$= \frac{\text{The initial weight of bark powder}}{\text{The final weight of extract dried residue}} *100$$

= (0.47/100) *100 %
= 0 .47%

For leaves
The initial weight of leaves powder = 100gm
The final weight of extract dried residue = 1.7gm

The percentage yield of crude Taxol from the leaves of west Himalayan yew

$$= \frac{\text{The initial weight of leaves powder}}{\text{The Final weight of extract dried residue}} *100$$

= (1.7/100) *100 %
= 1.7%

The crude extract was purified using column chromatography after Taxol was extracted from other plant components. A stationary phase of silica gel was packed into a column 60 cm long and 2.5 cm in diameter. The column's bed length was 30 cm, and 73.63 g of silica gel powder with a density of 0.5 g/cm3 was employed. The calculated mass of silica gel powder for the column was determined as follows:
Since
the bed length of the column = 30 cm
the density of the silica gel = 0.5 g/cm3
the volume of the silica gel bed =?
Volume = Bed length × π × (Column diameter/2)2
= 30 cm × 3.14 × (2.5 cm/2)2
= 30 cm × 3.14 × 0.625 cm2
= 147.2621 cm3
Now, we can calculate the mass of silica gel powder needed based on its density: Mass = Volume × Density
Mass
= 147.2621 cm3 × 0.5 g/cm3
= 73.63 g
Therefore, the calculated mass of silica gel powder required for the column was approximately 73.63 g.
The dry procedure was used to prepare the column, which began with a layer of cotton at the bottom and was followed by the addition of silica gel powder. The column was then rinsed with chloroform as a buffer solution to ensure that any air bubbles in `the silica gel bed were removed. To begin the chromatographic separation, a 9.5:0.5 chloroform: methanol solvent solution was placed into the column and allowed to flow down and fill the bed. The crude Taxol sample was put to the top of the column, followed by the addition of the solvent to enhance molecular migration. Because the crude Taxol sample contains both polar and nonpolar components, a polar-to-nonpolar solvent system was used. Because chloroform is a nonpolar solvent, it elutes nonpolar molecules initially, whereas methanol, a polar solvent, elutes less polar ones later. The eluted fractions were separated into test tubes based on the varied color bands detected on the column bed.
Three elution samples were taken from the crude Taxol isolated from the bark of the West Himalayan yew in this process. Similarly, four elution samples of crude Taxol isolated from West Himalayan yew were obtained. To protect the samples from light and deterioration, they were housed in glass jars with tight seals in a dark environment. Column chromatography with silica gel as the stationary phase is widely used for chemical purification and separation. The solvent solution used is critical in ensuring successful separation based on the polarity of the target molecules. Purified fractions enriched with the target drug, such as Taxol, can be obtained by carefully adjusting the solvent flow rate and elution conditions. The presence and purity of Taxol may be verified in the obtained fractions utilizing further analysis and characterization methods, such as TLC. To ensure that Taxol was present and pure, further testing was done on the fractions extracted from the West Himalayan yew's bark and leaves. The bark provided three fractions, whereas the leaves produced four fractions on the TLC plate. The West Himalayan yew was used to produce pure Taxol, which was then refined using a rotary evaporator. According to research, the yield of pure dry Taxol from the bark was found to be 0.11%, compared to the yield from the leaves, which was found to be 0.39%. 100 grams of bark powder and 100 grams of leaf powder were used in the first extraction. In the end, 0.11 grams of pure dry Taxol were recovered from the bark, and 0.39 grams from the leaves. According to these findings, the West Himalayan yew's leaves produced a larger proportion of pure dry Taxol than the tree's bark.

For barks
Initial weight of bark powder = 100 gm
Final extract weight in purified dry Taxol = 0.11 gm

The percentage yield of Final extract weight in purified dry Taxol from the bark of west Himalayan yew

$$\frac{\text{Initial weight of bark powder}}{\text{Final extract weight in purified dry Taxol}} *100$$

$$= (0.11/100) *100 \%$$
$$= 0.11\%$$

For leaves
The initial weight of leaves powder = 100 gm
The final extract weight in purified dry Taxol = 0.39gm

The percentage yield of Final extract weight in purified dry Taxol from the leaves of west Himalayan yew

$$\frac{\text{The initial weight of leaves powder}}{\text{The Final extract weight in purified dry Taxol}} *100$$

$$= (0.39/100) *100 \%$$
$$= 0.39\%$$

The collected fractions can be further analyzed and characterized to confirm the presence and purity of Taxol using analytical techniques TLC.

7. Result

Analyzed the purity of Taxol using TLC analytical techniques; In the research investigation, TLC (Thin-Layer Chromatography) analytical methods were used to assess the purity of Taxol. To enable compound separation based on their rates of migration, a variety of materials and instruments, including TLC plates covered with a layer of adsorbent material, such as silica gel, were used. Based on the polarity of the chemicals being studied, a 7:3 ratio of chloroform to methanol was chosen as the solvent solution to be employed. A standard Taxol sample was received from Sisco Research Laboratories Pvt. Ltd. to start the analysis. The proper mixture of methanol and chloroform, the standard solvent, was made into a solution. Following the cutting of the TLC plate to the necessary size, capillary tubes were used to precisely place the standard sample, together with the bark and leaf samples, onto the plate. The developing chamber was closed to prevent solvent evaporation, and the TLC plate, which served as the stationary phase, was put within. A lower solvent solution was used to fill the chamber, which allowed the solvent to move up the plate and help the Taxol molecules separate. The plate was taken out of the chamber after a certain amount of time. Vanillin-sulfuric acid and iodine vapors were used as detection reagents to make the separated chemicals visible. The TLC plate was treated with chemicals such as vanillin sulfuric acid before being heated in a hot air furnace for 10 minutes at 110 degrees Celsius.

Two techniques were used in the research study to find Taxol in the West Himalayan yew's bark and leaves. A dark purple color was created by the vanillin-sulfuric acid reagent, which included vanillin, sulfuric acid, and methanol, and indicated the presence of Taxol. When water was used in place of methanol in another iteration of the vanillin-sulfuric acid approach, Taxol was likewise detected as a dark blue-gray color. The iodine vapor approach also produced a light-yellow color as proof that Taxol was present. These color changes served as qualitative proof that Taxol was present in the samples, confirming the effective extraction of Taxol from the bark and leaves of West Himalayan yew. The migration distances (Rf values) of the Taxol spots on the TLC plate were measured, and a recorded value of 9.2 was achieved. the West Himalayan yew's leaves samples, the Rf value was similarly found to be 9.2, demonstrating Taxol's existence in both the bark and leaves. These results show the efficiency of TLC analysis in identifying Taxol components and assessing their purity while offering useful insights into the purity of Taxol isolated from Lauth Sala. Future research and development initiatives in the field of cancer treatment will be made easier by the thorough methodology and outcomes of the experiment, which contribute to a greater knowledge of Taxol extraction and purification procedures

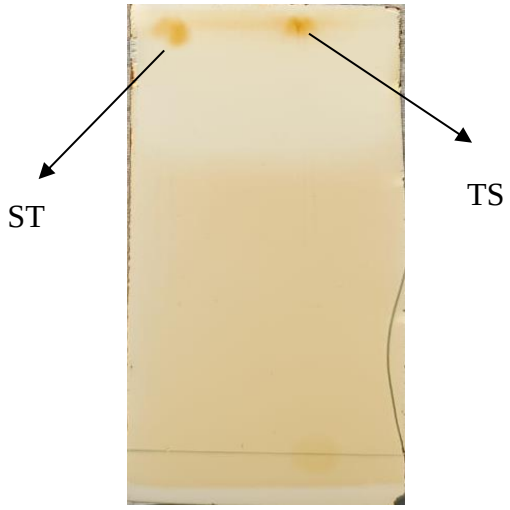


Fig 4. The iodine vapour approach also produced a light-yellow colour as a proof that Taxol was present. The first spot is the standard Taxol while the second spot is the extract sample from west Himalayan yew.

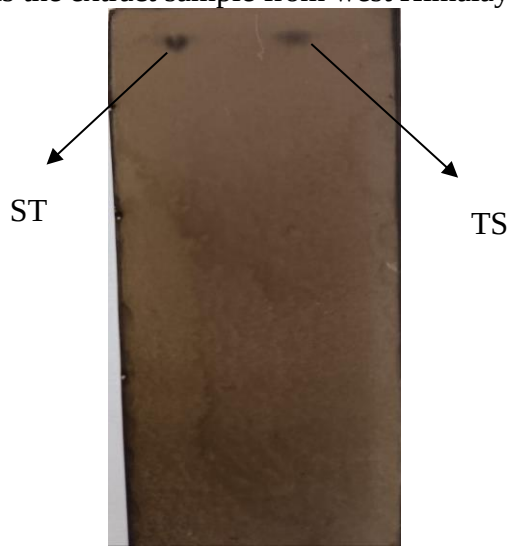


Fig 6. A dark purple colour was created by the vanillin-sulfuric acid reagent, which included vanillin, sulfuric acid, and methanol and indicated the presence of Taxol. The first spot is the standard Taxol while the second spot is the extract sample from west Himalayan yew.

8. USES IN TRADITIONAL MEDICINE

Traditional medicine in the Himalayan region has traditionally utilized the West Himalayan yew. Many traditional and alternative medical systems, including Amchi, Ayurveda, Han Chinese, and Unani medicine, make use of it. A number of treatments for a range of disorders employ the tree's bark, leaves, and shoots. Traditional Western uses of Himalayan yew in medicine include:

- Treatment for inflammatory illnesses that raise the body's temperature and create pain.
- Treatment for epilepsy, dyspepsia, respiratory infections, and colds
- Use as a poultice to burns and infected wounds.
- Fractures and headaches can be treated with a bark-based paste.
- Used to cure rheumatism in steam rooms.
- Extracts from the tree are used to make therapeutic hair oils.
- Treating TB by using the stem as a decoction.
- Making Zarnab, a medication from the bark and leaves that is used in Unani medicine as a sedative, aphrodisiac, and treatment for bronchitis, asthma, epilepsy, snake bites, and scorpion stings.
- Young shoots are used in Ayurveda to prepare a tincture that is used to treat severe biliousness, giddiness, a weak and falling pulse, coldness in the extremities, headaches, and diarrhea.

9. USES IN MODEN MEDICINE

Modern medicine has found several applications for the Taxol extract from Lauth Sala (West Himalayan Yew). As examples of its uses, consider the following:

- Cancer Treatment: Taxol, which is isolated from Lauth Sala, has undergone significant research and is used to treat a variety of cancers. It has shown promise in the fight against Kaposi's sarcoma, ovarian cancer, breast cancer, lung cancer, head and neck cancer, esophageal cancer, gastric cancer, bladder cancer, and prostate cancer. Taxol disrupts the microtubule dynamics of cancer cells, preventing them from proliferating and encouraging cell death.Taxol is frequently used

as a chemotherapy treatment, either alone or in combination with other anticancer medications. It is used in many treatment plans and is essential for limiting tumor development and enhancing patient outcomes.

- Adjuvant Therapy: In some instances, Taxol is given as adjuvant therapy following the surgical excision of tumors to further lower the chance of cancer recurrence. It aids in the eradication of any cancer cells still present and inhibits the development of fresh tumors.

- Combination therapy: To increase the effectiveness of the treatment, Taxol is sometimes used in conjunction with radiation therapy or other chemotherapy drugs. These combinations offer a thorough strategy for targeting cancer cells from several directions, increasing the likelihood of a successful cure.

- Research and Development: Taxol from Lauth Sala extract is still a topic that is being studied. Researchers are looking at new delivery systems, dose optimization, and potential interactions with other medications as they consider the potential uses of this substance in a variety of cancer types.

- Investigational Uses: In addition to the treatment of cancer, Taxol may also be used to treat several other illnesses, according to ongoing research. It has shown promise in the treatment of neurodegenerative diseases including Parkinson's and Alzheimer's. Its anti-inflammatory capabilities and prospective uses in autoimmune illnesses are also being researched.

10. TOXICITY OF TAXOL

- Hypersensitivity responses: Taxol has a history of causing hypersensitivity responses, especially during the first infusions. The symptoms of these responses, which can be mild to severe, include rash, itching, fever, dyspnea, bronchospasm, and hypotension. They may be handled with premedication and supportive measures, and they are often seen during the first 10 minutes of therapy. These symptoms are likely to be caused by histamine release from polyoxyethylene castor oil, a component of Taxol formulation.

- Hematological Toxicity: Neutropenia, which commonly appears between days 8 and 10 after therapy, is the major side effect of Taxol. Hematopoietic cells in development do not sustain long-term harm since it is reversible. Especially in individuals with a history of past chemotherapy, the administration of granulocyte colony-stimulating factor (G-CSF) may be required to treat neutropenia. Taxol's unusual side effects include anemia and thrombocytopenia.
- Neurotoxicity: Taxol can result in sensory symptoms including numbness and paraesthesia in a glove-and-stocking distribution, which is known as peripheral neuropathy. The risk of neurotoxicity is increased by greater dosages and more frequent infusion regimens. The strong neurotoxic side effects of Taxol prevent, its long-term usage. Motor and autonomic disorders are also possible in certain individuals. It is unclear exactly how neurotoxicity works.

- Muscular Toxicity: Taxol usually causes transient myalgia, especially at dosages of more than 170 mg/m2. Myalgia can be treated and prevented by the prophylactic administration of opioids or antihistamines.

- Cardiac Toxicity: Taxol can disrupt the heart's rhythm; asymptomatic bradycardia is the most frequent cardiac side effect; severe bradyarrhythmias, such as third-degree heart block, have been reported but are uncommon; continuous cardiac monitoring is advised for patients with pre-existing cardiac conduction abnormalities or ventricular dysfunction; and congestive heart failure has been noticed when Taxol is combined with specific other chemotherapeutic agents.

11. CONCLUSION

The goal of the research was to identify a reliable alternative supply of Taxol, a valuable molecule used in cancer therapy, and to extract and purify it from Lauth Sala's bark and leaves. Taxol, which is produced from the Taxus contorta plant, has demonstrated tremendous promise as a powerful chemotherapeutic agent, suppressing cell proliferation and encouraging cell death in a variety of cancers. Yew trees have been overharvested as a result of the rising demand for Taxol, jeopardizing their ability to survive. So, it becomes essential to look at different sources like Lauth Sala. Grinding the dried bark or leaves of Lauth Sala, soaking them in ethanol, then isolating and purifying them using column chromatography were the steps in the extraction procedure. To determine the purity of Taxol, the resultant fractions were then subjected to TLC analytical methods. The findings showed that Taxol was present in both the bark and leaf samples, with the leaves showing a greater percentage yield of dry, pure Taxol. Taxol has had a big influence on oncology since it is a crucial part of cancer treatment. Its FDA clearance in 1992 revolutionized the way that different forms of cancer were treated, improving patient quality of life and survival rates. Drug discovery and development have advanced thanks to the extraction and synthesis of Taxol from Taxus contorta, enabling more study and comprehension of its biological and ecological relevance. The results of this study's investigation shed light on Lauth Sala as a viable source of Taxol in place of overharvesting yew trees. This research adds to the body of knowledge for the next projects aimed at developing and researching cancer therapies by offering insightful information on the extraction and purifying methods of Taxol from Lauth Sala. It stimulates more research into the effectiveness and prospective uses of Taxol in the treatment of cancer and opens the door for investigating other medicinal plant-based medicines.

REFERENCES:

1. WITHERUP, KEITH M et al. "Taxus spp. needles contain amounts of taxol comparable to the bark of Taxus brevifolia: analysis and isolation." Journal of Natural Products 53.5 (1990): 1249-1255.

2. SUBEDI, T." Phytochemical Studies of Taxus Species and Their Uses in Cancer Treatment". Janapriya Journal of Interdisciplinary 6 (2018) 160-171.

3. TAREK M MIKHAIL & MAURIE MARKMAN "Paclitaxel Drugs for Cancer Therapy "USA, Expert Opin. Pharmacother. (2002) 3(6):755-766.

4. POUDEL, RAM C., et al. "Genetic diversity, demographical history and conservation aspects of the endangered yew tree Taxus contorta (s yn. Taxus fauna) in Pakistan." Tree Genetics & Genomes 10 (2014): 653-665.

5. UNIYAL, SANJAY KR. "Bark removal and population structure of Taxus wallichiana Zucc. in a temperate mixed conifer forest of western Himalaya." Environmental monitoring and assessment 185 (2013): 2921-2928.

6. GAIRE, D., et al. "Predicting habitat suitability and threats assessment of Himalayan yew (Taxus wallichiana zucc.) in Nepal." Applied ecology & environmental research 21.3 (2023).

7. EISENHAUER, ELIZABETH A, AND JAN B VERMORKEN "The taxoids: comparative clinical pharmacology and therapeutic potential." Drugs 55 (1998): 5-30.

8. SCHIFF, PETER B, AND SUSAN BAND HORWITZ. "Taxol stabilizes microtubules in mouse fibroblast cells." Proceedings of the National Academy of Sciences 77.3 (1980): 1561-1565.

9. DUMONET, CHARLES, and BRANIMIR I. SIKIC. "Mechanisms of action of and resistance to anti-tubulin agents: microtubule dynamics, drug transport, and cell death."

Journal of Clinical Oncology 17.3 (1999): 1061-1061.

10. ROWINSKY, ERIC K., LORRANIE A. CAZENAVE, and Ross C. DONEHOWER. "Taxol: a novel investigational antimicrotubule agent." JNCI: Journal of the National Cancer institute 82.15 (1990): 1247-1259.

11. WANI MC, TAYLOR HL, WALL ME, et al.: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J. Am. Chem. Soc. (1971) 93:2325-2327.

12. M. NISAR, I. KHAN, S.U. SIMJEE, A.H. GILANIil, OBAIDULLAH, H. PERVEEN Anticonvulsant, analgesic, and antipyretic activities of Taxus wallichiana ZuccJ Ethnopharmacol, 116 (2008), pp. 490-494.

13. WANI MC, TAYLOR HL, WALL ME, et al.: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J. Am. Chem. Soc. (1971) 93:2325-2327.
14. M. KHAN, S.C. VERMA, S.K. SRIVASTAVA, A.S. SHAWL, K.V. SYAMSUNDAR, S.P. KHANUJA, et al. The essential oil composition of Taxus wallichiana Zucc. from the Northern Himalayan region of India Flavour Frag J, 21 (2006), pp. 772-775.

15. M. NISAR, I. KHAN, S.U. SIMJEE, A.H. GILANIil, OBAIDULLAH, PERVEEN H. Anticonvulsant, analgesic, and antipyretic activities of Taxus wallichiana Zucc J Ethnopharmacol, 116 (2008), pp. 490-494.

16. P. KOVACS, G. CSABA, E. PALLINGER, R. CZAKER. Effects of Taxol treatment on the microtubular system and mitochondria of Tetra hymena Cell Biol Int, 31 (2007), pp. 724-732.

17. J.F. BISHOP, J. DEWAR, G.C. TONER, M.H. TATTERSALL, I.N. OLVER, S. ACKLAND, et al. Paclitaxel as first-line treatment for metastatic breast cancer. The Taxol Investigational Trials Group, Australia and New Zealand.

18. M. NISAR, I. KKAN, B. AHMAD, I. ALI, W. AHMAD, M.I. CHOUDHARY. Antifungal and antibacterial activities of Taxus wallichiana Zucc J Enzyme Inhib Med Chem, 23 (2008), pp. 256-260.

19. S. ASHRAFI, M.R. MOFID, M. OTROSHI, M. EBRAHIMI, M. KHOSROSHAHLI Effects of plant growth regulators on the callogenesis and Taxol production in cell suspension of Taxus baccata Trakia J Sci, 8 (2010), pp. 36-43.

20. MCGUIRE WP, ROWINSKY EK, ROSENSHEIN NB et al.: Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Intern. Med. (1989) 111:273-279.

21. KOHN EC, SAROSY G, BICHER A, et al.: Dose-intense Taxol: high response rate in patients with platinum-resistant recurrent ovarian cancer. J. Natl. Cancer Inst. (1994) 86:18-24.

22. BENASSO M, NUMICO G, ROSSO R et al.: Chemotherapy for relapsed head and neck cancer: paclitaxel, cisplatin and 5- fluorouracil in chemotherapy-naive patients. A dose-finding study. Semin. Oncol. (1997) 24: S19-46-S19-50.

23. FOUNTZILAS G, SKARLOS D, ATHANASSIADES A, et al.: Paclitaxel by three-hour infusion and carboplatin in advanced carcinoma of nasopharynx and other sites of the head and neck. A Phase II study conducted by the Hellenic Cooperative Oncology Group. Ann. Oncol. (1997) 8:451-455.

24. SCHIFF PB, FANT J, HORWITZ SB: Promotion of microtubule assembly in vitro by taxol. Nature (1979) 277:665-667.

25. ROWINSKY EK, CAZENAVE LA, DONEHOWER RC: Taxol: a novel

investigational antimicrotubule agent. J. Natl. Cancer Inst. (1990) 82:1247-1259.

26. WANG LG, LIU XM, KREIS W, et al.: The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. Cancer Chemother. Pharmacol. (1999) 44:355-361.

27. BHALLA K, IBRADO AM, TOURKINA E, et al.: Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukaemia (1993) 7:563- 568.

28. BLAGOSKLONNY MV, GIANNAKAKOU P, EL-DEIRY WS, et al.: Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res. (1997) 57:130-135.

29. HALDAR S, CHINTAPALLI J, CROCE CM: Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res. (1996) 56:1253-1255.

30. STROBEL T, KRAEFT SK, CHEN LB, et al.: BAX expression is associated with enhanced intracellular accumulation of paclitaxel: a novel role for BAX during chemotherapy-induced cell death. Cancer Res. (1998) 58:4776-4781.

31. PEEREBOOM DM, DONEHOWER RC, EISENHAUER EA, et al.: Successful retreatment with taxol after major hypersensitivity reactions. J. Clin. Oncol. (1993) 11:885-890.

32. ROWINSKY EK, EISENHAUER EA, CHAUDHRY V, et al.: Clinical toxicities encountered with paclitaxel (Taxol). Semin. Oncol. (1993) 20:1-15.

33. HOFSTRA LS, DE VRIES EG, WILLEMSE PH: Ophthalmic toxicity following paclitaxel infusion. Ann. Oncol.(1997) 8:1053.

34. MARTONI A, ZAMAGNI C, GHEKA A, et al.: Antihistamines in the treatment of taxol-induced paroxystic pain syndrome. J. Natl. Cancer Inst. (1993) 85:676.

35. ROWINSKY EK, MCGUIRE WP, GUARNIERI T, et al.: Cardiac disturbances during the administration of taxol. J. Clin. Oncol. (1991) 9:1704-1712.

36. ARBUCK SG, STRAUSS H, ROWINSKY E, et al.: A reassessment of cardiac toxicity associated with Taxol. J. Natl. Cancer Inst. Monogr. (1993) 15:117-130.

37. MARKMAN M, KENNEDY A, WEBSTER K, et al.: Paclitaxel administration to gynecologic cancer patients with major cardiac risk factors. J.

38. HUIZING MT, KEUNG ACF, ROSING H. et al.: Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. J. Clin. Oncol. (1993) 11:2127-2135