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PACLITAXEL AS ANTICANCER AGENT: ISOLATION, ACTIVITY, SYNTHESIS AND STABILITY

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ABSTRACT: Paclitaxel, a potent anticancer agent, was first isolated from the bark of *Taxus brevifolia* in the 1960s and has since become a cornerstone in chemotherapy. Its anticancer efficacy stems from its ability to stabilize microtubules, preventing their disassembly, which inhibits mitosis and ultimately induces apoptosis in cancer cells. This mechanism of action makes paclitaxel particularly effective against a variety of cancers, including ovarian, breast, and non-small cell lung cancer. Despite its clinical success, the isolation of paclitaxel from the *Taxus* species is limited by low yields and environmental sustainability concerns, prompting the development of alternative synthetic routes. The synthesis of paclitaxel has posed significant challenges due to its complex structure, particularly the C-ring, which is pivotal for its biological activity. Over the years, total syntheses have been achieved using both chemical and semi-synthetic approaches, with the latter being more commercially viable. One notable method involves converting baccatin III, a compound found in *Taxus*, into paclitaxel *via* chemical modifications. Stability issues are another hurdle for paclitaxel's therapeutic use, as its formulation requires careful handling to avoid degradation. It is typically delivered in a solvent formulation, which can sometimes lead to hypersensitivity reactions. Research into enhancing the stability and solubility of paclitaxel formulations is ongoing, including the development of nanoparticle-based drug delivery systems.

INTRODUCTION:

Nature Sources of Paclitaxel:

The National Cancer Institute associate degree alysed the content of an extract from a bark of western yew (*Taxus brevifolia*), 1st isolated from a stuff in 1963 [1].

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The western yew may be a rare, low-growing evergreen, locating within the previous forests of the north-western Pacific. Preclinical- studies of the extract showed that it had a cytostatic activity on many varieties of tumours [2]. In 1971, paclitaxel was known as an energetic constituent of this extract.

Development of paclitaxel for healthful use proceeded slowly despite its antineoplastic activity as a result of aggregation decent quantity needed a lot of time and material (for regarding one kilo yield, it's necessary to isolate the extract from three, 000 of yew trees).

Interest during this substance was aroused in 1979, once its mechanism of action as Associate in nursing antineoplastic drug was known [3]. Paclitaxel is additionally contained in gymnosperm genus yannanensis, and has been obtained from *Taxus baccata* by semi-synthesis [4]. known the plant of Taxomyces and reanae that produces paclitaxel. though the yield of paclitaxel was little (24–50 ng/dm3), this discovery was of major interest to scientists [7]. Many procedures are advised for isolation from the endophytic plant [6], showing that organism of gymnosperm genus species will turn out paclitaxel.

Therefore, the method of fermentation employing a paclitaxel-produced microorganism- philosophical system represents another procedure for paclitaxel production. The most important issues concerned in flora fermentation are the terribly little yield of paclitaxel and its instability: the yields ranged from twenty-four metric weight unit to seventy µg per cubic decimetre of the investigated flora [8]. The spore of Pestalotiopsis CP-4 [8] made. Paclitaxel from fifty to 1487 ng/dm^3. The goal of the current analysis is discovering the best fermentation conditions for gymnosperm genus cell cultures. Though the quantity of the made paclitaxel from flora was under people who were obtained from yews, the shorter production time and high rate of flora growth have had a vital role in any studies. Once virtually 2 years of analysis, scientists have isolated many endophytic funguses from gymnosperm genus chinensis power unit. Maier and gymnosperm genus yunnanensis. Species of Ozonium BT2 can also produce paclitaxel and taxane baccatin III (intermediates in the production of paclitaxel). Production of paclitaxel by a microbiological pathway is also possible using a pure culture of Actinomycetes [11]. Microorganisms from the Actinomycetes group usually belong to the suprageneric group of Streptomyces- cetes, Actinoplanetes, Maduromycetes, Thermomonos- poras or Nocardioforms, but more often to the Strep- tomyces, Actinoplanes, Nocardiopsis, Micromonospora, Actinomadura or Kitasatosporia genus, and mostly to the *Kitasato sporia* genus. Among them, the most significant is Kitasatosporia sp. CECT 4991.

General Characteristics of Paclitaxel: The IUPAC name for paclitaxel is (2α,4α, 5β, 7β, 10β, 13α)- 4, 10-bis (acetyloxy)-13-{[(2R,3S)-3-(benzoylamino)- 2-hydroxy – 3 - phenylpropanoyl] oxy}-1,7-dihydroxy-9- oxo-5,20- epoxytax-11-en-2-yl benzoate. Its structural formula is shown at **Fig. 1**, and **Table 1** lists its char- acteristics. Paclitaxel is soluble in dimethyl sulfoxide (DMSO) (50 mg/cm^3) [3]. A 0.01 mol/dm3 solution of paclitaxel in DMSO has been stored as aliquots until use and fur- ther diluted to 10-10 mol/dm^3 with medium [12]. Paclitaxel is also soluble in methanol (50 mg/cm^3). It undergoes hydrolysis and transesterification to ~30% of the peak signal at 227 nm by a high-performance liquid chromatography method (HPLC) after two weeks at room temperature. Paclitaxel is rapidly destroyed in weakly alkaline, methanolic solutions and in strongly acidic methanolic Ssolutions (1:1 of methanol: concentrated HCl). A sample with 0.1% acetic acid added to methanol showed no signs of degradation for seven days at room temperature or three months at 4°C (presumably due to the ability of the acetic acid to neutralize traces of alkali in the methanol) [13]. Paclitaxel is also soluble in ethanol and acetonitrile [14].

Paclitaxel has low solubility in water and is rapidly destroyed in weakly alkaline aqueous solutions [13]. The lowest amount of degradation in aqueous paclitaxel solutions occurs in pH 3 to 5. Paclitaxel solutions at 0.1 and 1 mg/cm3 in 5% dextrose injection or 0.9% sodium chloride injection remained active for at least three days at 4, 22, or 32°C [Xu Q *et al*].

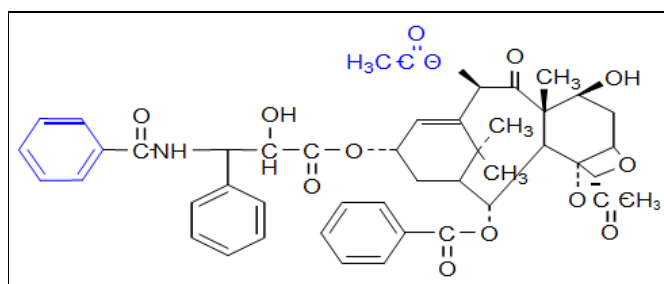

FIG. 1: STRUCTURAL FORMULA OF PACLITAXEL

Paclitaxel Procedures of Isolation from Natural Sources: The first step involves washing raw plant material using deionized water [16]. The temperature of water is in the range of 20 to 25°C, and duration of the process is 3 h. Then, the water is removed along with all water-soluble impurities. The second step is extraction with an organic solvent. The adequate solvents for this extraction are alcohols

(methanol), ketones (acetone) and their mixtures. In cases where a mixture is used, the volume ratio in the mixture is about 1:1. The obtained extract is filtered to remove deposits, and then transferred to a double walled tank, where the water temperature is in the range of 65–70°C. The organic solvent is then removed by distillation. The remaining solution is drained into another tank. Because there is residual water, that solution is a non-concentrated extract. Biomass from the solution obtained in the previous step.

The extract is diluted in methanol and water and then salted out to precipitate the biomass. Sodium chloride is quickly added to the extract under heavy stirring. The formed biomass is separated from the solution by filtration or by centrifugation. The separated biomass, which is wet, can be dried by ventilation or lyophilization. The fourth step removes the organic compound and natural pigments by treating the dried biomass with mixture of solvent and solvent. An even higher procedure is to place the dried biomass into answer by initial adding the mixture of solvent and solvent and adding one. additional volumes of pure hexane.

The final quantitative relation of solvent and solvent is 1:4. Then, pure water is added to the obtained solution to form a paclitaxel- enriched oily phase, which is then transferred into decanting desk. The oily phase at the bottom of the flask contains paclitaxel and other taxanes. The fifth and final step is natural process purification of solution| of the paclitaxel-enriched oily part and chemical phenomenon the pure solution obtained by action a minimum of once. In this procedure, the paclitaxel-enriched oily phase is first mixed with silica gel and dried under ventilation. The colloid coated with the oily part is loaded onto a natural process column containing identical form of gel. The purification is conducted using the mixture comprising 35% of acetone and about 65% hexane. The fractions containing paclitaxel obtained by chromatography in the preceding step are evaporated to dryness and put back in an acetone solution. Then, the paclitaxel is crystallized by adding from three to four volumes of methane series to the ketone solution. Acetone as an appropriate solvent for isolation of paclitaxel from plant material [S.S.K *et al*]. Acetone/water precipitation provides a really pure paclitaxel, which is useful for industrial scale isolation and normal-phase silica chromatography. Accelerated solvent extraction (ASE) has been investigated beneath whole totally different conditions for isolation of paclitaxel and connected analogue compounds from the bark of yew (Japanese yew) [18].

This method uses more pressure in the cells to keep the solvent warm during the extraction. In this procedure, the time of extraction is shorter and the amount of desired product is higher. The conditions under which the obtained yield of paclitaxel is the highest are the following: solvent, MeOH: H2O (90:10 v/v); temperature, 150°C; and pressure, 10.13 MPa (the yield after drying the sample was 0.128% m/m). ASE does not require a chlorinated solvent. It reduces the use of solvent, because of higher dissolving power. The obtained amount of paclitaxel and related compounds during ASE is higher even when water is used as the solvent as compared the yield from other methods.

Semi-synthesis of Paclitaxel: An initial substance for semi-synthesis of paclitaxel is 10-deacetylbaccatin-III (10-DAB), which can be isolated from needles and wattles of the European yew (*Taxus baccata*) [20]. There are two procedures for synthesis, both of which start with 7-O-TES-baccatin-III obtained by selective silylation and acetylation of 10-DAB [21]. In the first procedure developed and published in the patent literature by Holton [22], lithium ions are reacted with β- lactam to introduce a desirable amino acid chain at the 13-position. Bristol-

Myers Squibb Co [23], and also [24] presented the second procedure for synthesis in the patent literature. 7-O-TES-baccatin-III was reacted with oxazolinecarboxylic acid ((4S-trans)-4,5-dihydro-2,4-di- phenil-5-oxazolecarboxylic acid) using dicyclohexylcar- bodiimide (DCC) or a similar dehydrating agent. A third synthetic procedure is based on coupling 7-O-Troc- baccatin-III with the protected β-phenylisoserine using DCC [25].

Total Synthesis of Paclitaxel: The structural elements of paclitaxel **Fig. 1**, in addition to the main A, B and C rings, include an oxetan ring (D-ring), an N-benzoyphenylisoserine side chain appended to C-13 of the A ring and the benzoate

group at C-2 of the B ring [7]. Two groups of scientists [Nicolaou K.C *et al*] [27] have published procedures of total synthesis of paclitaxel with low yields at 2.7% and 0.07%, respectively. Nicolaou's group started first with construction of the A and C rings separately and then coupling the two molecules using a Shapiro and McMurry coupling to form the B ring. Further reactions were carried out to produce the final product of Paclitaxel [26]. started with (-)- borneol, which they converted to an unsaturated ketone through 13 synthetic steps. The method used the Wieland-Miescher-ovim ketone as a starting material, which was then converted to a complex enol triflate containing an olefin on the C ring that allowed for the development of paclitaxel *via* an intramolecular Heck reaction.

Morihira and coworkers have published yet another procedure for paclitaxel synthesis [29]. These procedures represent great achievements in the scientific community, but still can- not be used for industrial production, because in several synthetic steps the yields of intermediates are very low. Therefore, these total synthesis methods of paclitaxel production are too expensive and too complex.

Complexes of Taxol: Pharmaceutical substances are usually insoluble in water; therefore, increased solubility is important for a drug to be administered intravenously. Different types of carriers that increase solubility, such as cyclodextrins [30] oligosaccharides (dextrane, pullulan) [33], liposomes, and polymers, have been tested. These substances provide better absorption of the active substance during a treatment of a disorder. Coupling a bioactive compound to a biocompatible chemical compound offers, in general, several benefits, like higher drug solubilization, higher stabilization, specific localization, and controlled release. Cyclodextrins (CyD) are complexing agents that can increase the solubility and stability of poorly soluble ac- tive substances [35]. Studies have been done on the different types of β- and γ- CyDs. (Hydroxypropyl)-(HP β-CyD), (hydroxyethyl)-(HE β-CyD) and dimethyl-(DM β-CyD) β-CyD belong to this group; they increase the solubility of paclitaxel by a factor of 2,000 without chang- ing the cytostatic activity of paclitaxel *in-vitro*. The amount of dissolved active substance increased with increasing cyclodextrin concentration. In some cases, the precipita- tion of cyclodextrins in solution was observed. Thermal and spectroscopic analyses (fluorescence, IR, NMR and circular dichroism) confirmed the formation of complexes, which were more stable in the solid form than in the solu- tion. This can be explaine the occurrence of precipitate in the solution. DM β- CyD solutions of ≤ 3.7 mol% (mol paclitaxel/mol cyclodextrin) showed no precipitation upon dilution, nor did HP β-CyD solutions of ≤ 0.14 mol%.

The solubility and bioactivity of inclusion complexes of paclitaxel using 11 different cyclodextrins, (α-CyD, β-CyD, γ-CyD, mono-6-O-maltosyl α-CyD (G2-α-CyD), mono-6-O-maltosyl β-CyD (G2- β-CyD), mono-6-O-maltosyl γ-CyD (G2-γ-CyD), heptakis-(2,6-di-O-methyl) α-CyD (DM-α-CyD), heptakis-(2,6-di-O-methyl) β-CyD (DM-β-CyD), heptakis-(2,3,6-tri-O-methyl) β-CyD (TM- β- CyD), hydroxyethyl β-CyD (HE-β-CyD), hydroxypro- pyl β-CyD (HP-β-CyD)), was studied by Hamada and coworkers [36]. From this cluster of cyclodextrins, 2,6-di- methyl β-CyD was shown to be the most efficient, with the solubility of paclitaxel at 2.3 mmol/dm3 in a cyclodex- trin solution of 0.1 mmol/dm^3. This inclusion advanced of paclitaxel has one. [23] times higher chemical change activity than paclitaxel in tubulin.

Today, the formulation on the basis of the inclusion complexes of cyclodextrin and paclitaxel is used [35], most commonly with a paclitaxel to cyclodextrin ratio of 1:10 to 1:150. Different types of cyclodextrins, such as hydroxypropyl – sulphobutyl – 7 – β - CyD, sulphobutylether- 7-β-CyD, acetyl-γ- CyD, hydroxypropyl-β-CyD, hydroxypropyl-γ-CyD, bis(β-CyD), γ-CyD, succinyl- methyl-β-CyD, anionic-β-CyD, as well as their mixtures, can be used for making these formulations. The stability constant of these inclusion complexes lie in the range K = 5.396 – 1.412 dm3/mol. Preparing the formulations is finished within the following manner: (a) resolution| the answer} of cyclodextrin is additional dropwise to the fermentation alcohol solution of paclitaxel. (b) After the paclitaxel dissolves, the obtained mixture is filtered through a microporous membrane (0.2–0.4 µm) (c) then, ethanol is removed at low pressure, giving a liquid inclusion that contains less than 2% ethanol. The residual water is also removed in the same way.

Paclitaxel (20 mg/cm3) remains more active in cyclodextrin solutions (10%–20%) than in buffer solutions of comparable pH [37]: There was less than 1% decomposition of paclitaxel in the cyclodextrin solutions stored for one month at 37°C.

The incorporation of paclitaxel into hydrotropic poly- meric micelles, with a shell of poly (ethylene glycol) (PEG), and a core of poly(2-(4-vinylbenzyloxy)-N, N-di- ethylnicotinamide) (P(VBODENA), has been described [38]. This procedure improved the solubility and stability of paclitaxel. To study the stability, a control was used in which paclitaxel was incorporated in polymeric micelles of poly(ethylene glycol)-b-P(D,L-lactide) (PEG-b-PLA). The maximal amount of paclitaxel incorporated in the micelles of PEG-b-P(VBODENA) was higher than 37.4 mass%, compared with the 27.6 mass% in the micelles of PEG-b-PLA. Above 30 mass% of paclitaxel was located inside polymeric micelles in a molecularly dispersed amorphous state, as confirmed by thermal analysis. Hydrotropic polymer micelles can remain stable for a few weeks, while micelles of PEG-b-PLA precipitate after a few days. Hydrotropic polymer micelles are also more efficient than PEG-PLA micelle formulations in inhibiting the proliferation of human cancer cells. Paclitaxel in the form of hydrotropic polymer micelles has been administered to rats orally (3.8 mg/kg), intravenously (2.5 mg/ kg), or through the portal vein (2.5 mg/kg). The results of research showed that the polymer micelles with ahydrotropic structure were the better carriers, because of their high solubility and long-term stability.

Paclitaxel-loaded poly(ε-caprolactone) (PCL)/ pluronic F68 (F68) nanoparticles were spherical with a porous surface [39]. F68 was incorporated in the matrix of PCL. These substances are usually used as an agent for formation of pores. Paclitaxel-loaded PCL/F68 nanoparticles were shown to be more efficient than conventional injections of paclitaxel. Therefore, paclitaxel-loaded PCL/F68 nanoparticles can effectively prevent tumor cell growth. These data suggest an eventual possible use of this preparation for treatment of breast cancer. Nanoparticles were formulated with pluronic F-68 and liquid PEG (Mr 400) using a rapid, simple, and continuous procedure without presence of solvents [40]. Paclitaxel was contained in these nanoparticles **Fig. 2.** The liquid PEG was used as a solubilizer of paclitaxel. A liquid polymer mixture was formulated from the emulsion of PEG, paclitaxel and pluronic F-68. On a nanometer scale, PEG/paclitaxel was incorporated in pluronic F-68 by cooling at 0°C until the appearance of pluronic nanoparticles. The morphology and size of the pluronic nanoparticles formed can be observed using FE-SEM and TEM. These studies were done on tumor mice to investigate a use of pluronic nanoparticles as a system for controlled release of active substance in tumor treatment. Also, the time-dependent excretion profile, *in-vivo* biodistribution, and circulation time was evaluated. The paclitaxel-loaded was rapidly released in the first 7 h. Up to 48 h, the paclitaxel release remained constant. The paclitaxel loaded pluronic nanoparticles were more efficient compared with paclitaxel formulated in Chromophore EL.

Phospholipids in combination with aqueous solvent or hydrophilic polymers, as PEG, were used as a matrix for incorporation of paclitaxel molecules [41]. The par- ticles formed had an average size of 1000 nm. Conjugates on the basis of linear biodegradable copolymers of N-(2- hydroxypropyl) metha-croylamide (HPMA) and units of methacryloylated hydrazones of amino acids and oligopeptides with paclitaxel have been described in the literature [42]. The research was also moved in the direction of obtaining a polymer analog. Hyaluronic–paclitaxel hydrosoluble bioconjugates appear promising as cancer therapy. Their cytotoxicity against various cancer cell lines is, in fact, comparable to that of free paclitaxel, and the systemic toxicity is reduced owing to selective targeting of cancer cells [43].

Application of paclitaxel in the form of intravenous infusion required lecithin as a carrier. A paclitaxel concentration of 1 mg/cm^3 was achieved in 24 h by making micelles. Aqueous dispersions of egg or soya lecithin (water-lecithin dispersion, WLD), and mixed micellar (MM) solutions of lecithin from egg and sodium deoxycholate were investigated. Also, a new formulation with lecithin, co-surfactants and co-solvents poloxamer, polysorbate, Span, benzalkonium chloride, and macrogol were developed. The amorphous paclitaxel was prepared by lyophilization. Unlike crystalline paclitaxel, the drug in an amorphous form is easily soluble in a

1% to 5% (w/w) WLD or MM. The highest solubility (up to 5.70 mg/cm^3) was achieved in 5% WLD. Dissolved paclitaxel precipitated in all investigated formulations after 24 h. Otherwise, paclitaxel concentration of 1 mg/cm-3 after 24 h in 5% egg WLD, 1% to 5% soya WLD, and in 5% MM (lecithin: deoxycholate ratio 1: 1 w/w). After 24 h, precipitation of paclitaxel was not noted for different batches of 5% WLD. That formulation was adequate for further *in-vivo* studies. The solubility of paclitaxel in lecithin is not increased by addition of surfactants and co-lyophilization.

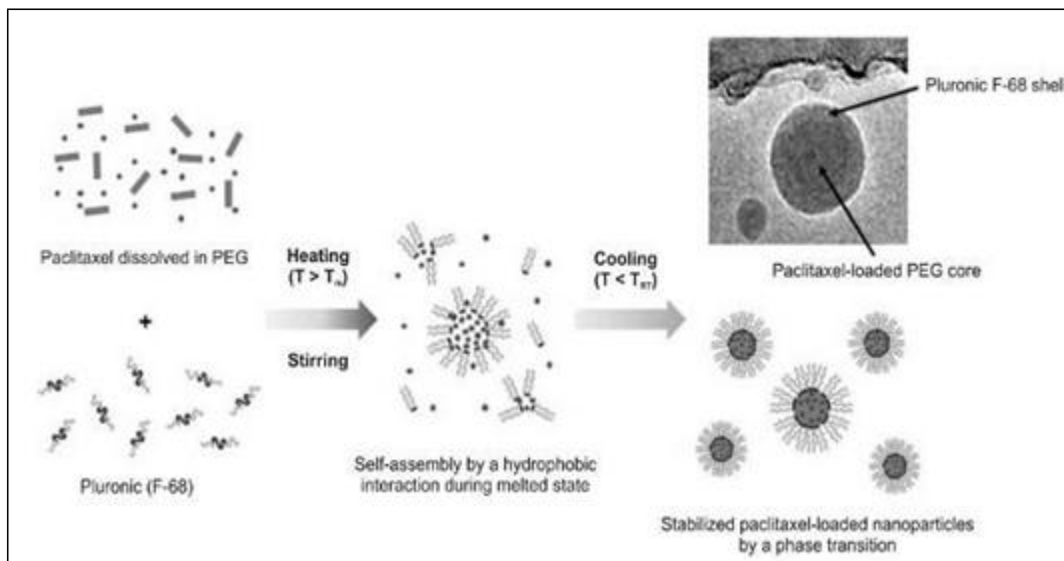

FIG. 2: PROCEDURE FOR MAKING NANOPARTICLES OF PACLITAXEL WITH PLURONIC F-68 AND LIQUID PEG

Methods for Isolation and Purification of Paclitaxel from Plant Materials: Paclitaxel was isolated from a crude plant extract by normal phase chromatography [45]. Polyamide was used as the stationary phase, with a mixture of dialkyl ketone and weakly polar solvent as the mobile phase. Acetone and methyl isobutyl ketone are dialkyl ketones, while (C5-C8) aliphatic hydrocarbons, (C6-C8) aromatic hydrogen carbons, (C1-C4) dialkyl ether to weakly polar co-solvents. The obtained chromatogram at exposed conditions is shown as **Fig. 3.**

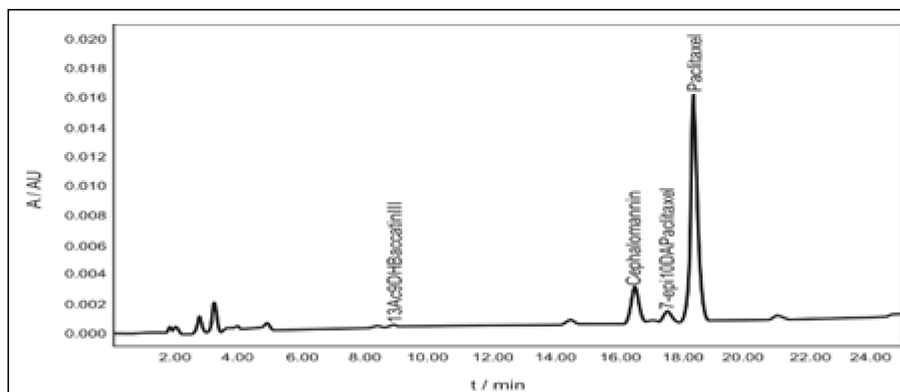

FIG. 3: CHROMATOGRAM OF CRUDE PLANT EXTRACT

An improved method for separation of paclitaxel and its analogues from *Taxus* species includes treating the extract by reverse-phase liquid chromatography on the adsorbent [46]. First, paclitaxel and its analogs are adsorbed on the adsorbent and then eluted. The compounds, which are isolated in pure form, are paclitaxel, paclitaxel-7-xyloside, 10-deacetylpaclitaxel, 10- deacetyl Paclitaxel - 7 - xyloside, cephalomannine, cephalo-mannine-7-xylo- side, 10- deacetylce phalomannine – 7 - xyloside, baccatin III, 10-deacetylbaccatin III, baccatin VI, brevitaxane A and taxiflorine. The simple and efficient procedure for isolation of paclitaxel from the bark of *Taxus brevifolia* is described in literature [47]; this method uses a reverse phase C18 column.

Paclitaxel Mechanism of Action: Paclitaxel is a complex diterpene with antitumor activity against ovarian, breast, lung, and prostate cancer [48], and acts as a promoter of tubulin polymerization and stabilizes microtubules to depolymerization by different agents, both *in-vitro* and *in-vivo* [49]. Paclitaxel alters the normal equilibrium between tubulin dimmers and microtubules, and, therefore, disrupts cell division [51]. The paclitaxel-stabilized microtubules are resistant to depolymerization upon exposure to calcium ions and cold temperatures, and do not require the presence of guanosine triphosphate (GTP) [50]. Unlike other spindle poisons, which prevent polymerization of the monomer, paclitaxel has a binding site on the micro- tubule [52].

Paclitaxel in concentrations as low as 0.05 μmol/ dm3 promoted microtubule assembly *in-vitro,* even in the absence of GTP or microtubule-associated proteins (MAPs) [50]. Studies of HeLa cells and fibroblasts treated with paclitaxel (0.25 or 10 μmol/dm^3) show that paclitaxel blocks cells in the G2 and M phase of the cell cycle [61]. More than 90% of the cells treated with 10 μmo1/dm^3 taxol for 22 h at 37°C displayed bundles of microtubules that appeared to radiate from a common site (or sites), in addition to their cytoplasmic microtubules.

Untreated cells that were kept in the cold (4°C) for 16 h lost their microtubules, whereas cells that were pretreated with taxol for 22 h at 37°C continued to display their microtubules and bundles of microtubules in the cold. HeLa (human) cells, strain S3, were grown in sus- pension culture in Joklik's modified Eagle's minimal es- sential medium supplemented with 5% fetal calf serum and 1% glutamine. A primary cell line of male BALB/c mouse fibroblasts (provided by Susie Chen) and Swiss 3T3 mouse fibroblasts were grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Fibroblast cells used in the experiments were no older than 20 passages. In non-malignant cells, paclitaxel inhibits specific functions such as chemotaxis, migration, and cell spreading. It inhibits the slow transportation of tubulin, actin, and polypeptides in axons [27] and the proliferation of stimulated lymphocytes, decreased tumor necro- sis factor-α (TNF-α) receptors, induced (at 1–30 mmol/ dm^3) murine macrophages to express TNF-α mRNA and genes associated with the LPS-induced macrophage activation, and induces protein tyrosine phosphorylation [64]. Paclitaxel inhibits secretory functions of specialized cells, catecholamine from adrenal medullar cells, plasma proteins from rat liver cells, and prothrombinase from platelets [27].

The Side Effects of Paclitaxel: Common side effects include nausea and vomiting, loss of appetite, change in taste, thinned or brittle hair, pain in the joints of the arms or legs lasting two to three days, changes in the color of the nails, and tingling in the hands or toes [65]. More serious side effects such as unusual bruising or bleeding, change in normal bow- el habits for more than two days, fever, chills, cough, sore throat, difficulty swallowing, dizziness, shortness of breath, severe exhaustion, skin rash, facial flushing, female infertility by ovarian damage, and chest pain can also occur. A number of these side effects are the results of other excipients in the applied preparation.

Methods for Monitoring of Paclitaxel Concentration: S.C *et al,* [66] and is adequate for routine determination in pharmaceutical dosage form. Separation of the analyzed samples was done on US C18 column, where the mobile phase is a methanol and aqueous solution of potassium dihydrogen phosphate of 0.02 mol/dm^3 ratio 80:20 v/v (pH of 2.5c was adjusted with phosphoric acid). The flow rate was 1 cm^3/min1, and wavelength, 225 nm. Retention time of paclitaxel was 4.978 min under these conditions. The detector response was linear in the range 15–180 μg/cm^3. HPLC method for quantitative determination of paclitaxel in plasma, tissue and tumor mice. Samples of liver, kidney, spleen, lung, heart, and tumor tissues were separately homogenized in bovine serum albumin (BSA, 40 g/dm3. Homogenates of plasma or tissue (0.1 cm^3), containing paclitaxel and inter standard (dimethyl- 4,4'-dimethoxy-5,6,5',6'- dimethilen dioxy biphenyl-2,2' dicarboxilate), were extracted with ethyl acetate (10 cm^3). An ODS column was used for separation of components from the biological samples. The samples were recorded at 227 nm. A gradient system containing acetonitrile and deionized water was used for quantification of paclitaxel.

This HPLC method has been successfully used for determination of paclitaxel for pharmacokinetic and biodistribution studies. Monitoring of paclitaxel concentration andverapamil in rats blood plasma is possible using a HPLC method with isocratic mode and UV detection [67]. First, the plasma samples were exposed to one-step liquid-liquid extraction by t-buthyl methyl ether. Paclitaxel, vera- pamil and n-buthyl p-hydroxy benzoate, as inter standard, were separated very well on an ODS column. As the mobile phase, this method used a mixture of ammonium acetate buffer (50 mmol/dm3, pH 6.0) and acetonitrile (54:46, v/v). Another HPLC method has been applied for rapid and simple determination of paclitaxel, without prior sample preparation, in the presence of polyoxyl castor oil [68].

To lower the administered doses of paclitaxel to re- duce its side effects, the ability to measure lower concentrations of paclitaxel is important [Baldery *et al.*] [68] have developed two methods, one using HPLC-UV and the other, LC-MS-MS [68]. In the HPLC-UV method, developed to analyze the paclitaxel in dog toxicokinetic studies, paclitaxel was extracted from human plasma by a simple solid phase extraction on cyano cartridges. For analysis, the volume of plasma between 0.5 and 1.0 cm^3 is necessary, which can sometimes be a problem for toxicology studies in small animals. The compound was eluted from the cartridges with a mixture of acetonitrile and triethylamine (1000:1, v/v). The eluate was evaporated and the compound was dissolved in the mobile phase; the sample was then injected onto an Apex-Octyl column (150×4.6 mm). The mobile phase of acetonitrile: methanol: ammonium acetate (0.02 mol/dm^3, pH 5) in a proportion of 4:1:5 (v/v/v) was run at 1 cm^3/min.

The samples were detected at 227 nm. The LC-MS-MS method used human plasma (0.1 cm^3). The cleanup of the samples was performed by an L-L extraction using diethyl ether to extract paclitaxel from plasma at pH 4 (by addition of ammonium acetate). The sample tubes were rotary mixed and centrifuged. The organic layer was transferred and blown to dryness. The compound was dissolved in a mobile phase and then injected onto a Hypersil C1 column (100×2.1 mm). The mobile phase was mixture of acetonitrile and aqueous formic acid (0.1%, v/v) in a proportion 1:1 (v/v).

CONCLUSION: Paclitaxel, as anticancer drug, can be isolated from *Taxus* species. It occurs as a metabolite of a special fungus. The microbiological route characteristically involves a shorter time for paclitaxel production compared with synthetic procedures. Its main disadvantage is a low yield of the bioactive substance. In medicine, paclitaxel is usually used for treatment of ovarian, breast, lung, and prostate cancer. Different types of carriers are used to improving its solubility and stability. Analytical methods have been developed and validated for monitoring the paclitaxel content in pharmaceutical formulation.

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CONFLICT OF INTEREST: Nil

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