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Evaluation of Different Brands of Paclitaxel

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ABSTRACT :

A well-known anticancer drug with a distinct mode of action is paclitaxel. It is regarded as one of the most effective all-natural anticancer medications. This paper provides an overview of the most recent developments in our knowledge of the origins, anticancer action, and biosynthesis of paclitaxel. The yield of extracted paclitaxel has greatly increased due to advances in biotechnology, modifications made to endophytic fungal strains, and the application of recombination techniques and microbial fermentation engineering. Paclitaxel has shown remarkable promise in numerous cancer treatments as it has been discovered to play a significant role in tumor immunity. Comparative case studies analyze and synthesize the similarities, differences, and patterns between two or more cases that have a similar focus or objective in order to generate knowledge about causal questions—that is, how and why specific programs or policies succeed or fail—that is easier to generalize. Studies comparing the quality control of two different brands of paclitaxel have been conducted.

Keywords: Anti-cancer, paclitaxel, biotechnology, endophytic, tumor.

Introduction :

As part of the screening effort of the National Cancer Institute, more than thirty thousand plants were examined in 1958 for potential anticancer properties. Monroe E. Wall reported in 1963 that the crude extract from the bark of the Taxus brevifolia, or western yew tree, was discovered to exhibit cytotoxic effects against a variety of cancers. Monroe E. Wall and Mansukh C. Wani identified paclitaxel as the plant's active constituent in 1967, and in 1971, they revealed the compound's structure. The western yew, a 40-foot-tall plant that may have taken 200 years to reach that height, produced only half a gram of paclitaxel, which resulted in restricted supply of the drug. Consequently, 10-deacetylbaccatin, a similar molecule present in several nonthreatened yew species, Taxus, which can be harvested without destroying the entire tree, was transformed into paclitaxel by Robert A. Holton's research group using a four-step process. Its development on an industrial scale was highly complex, despite its strong anticancer efficacy. The medicine was extracted in extremely low yield from a slow-growing and low-growing tree, which presented challenges due to its low solubility in water. In order to address the issue of PTX's poor water solubility, ethanol and "Cremophor EL," a polyethoxylated castor oil, were added to the formulation. Long-term side effects were also disclosed by this remedy, in addition to its favorable clinical outcomes.

Hypersensitivity responses and other unwanted side effects are brought on by the increased concentrations of the surfactant Cremophor EL that are necessary for the administration of PTX. With positive clinical outcomes, PTX was authorized by the Food and Drug Administration (FDA) in 1992 for the treatment of breast and ovarian cancer. This medication is in high demand and is currently regarded as one of the most significant anticancer medications used in clinics to treat ovarian, breast, and lung cancer as well as AIDS-related Kaposi's sarcoma when combined with cisplatin.

Pharmacokinetic Properties

The powder form of Paclitaxel is white to off-white in color. It is strongly lipophilic, insoluble in water, and has a fusion point close to 216C. It is discovered that its plasma clearance is biphasic. The drug's distribution to the central compartment and subsequent elimination cause the first sharp decrease, whereas the drug's efflux from the peripheral compartment contributes to the later phase. 200–250 mg/m2 is the typical dose, which is administered as a 3- or 24-hour infusion. The drug's pharmacokinetics exhibit a significant standard deviation. The results indicated that the terminal half-life fell within the range of 1.3–8.6 hours (average 5 hours) and that the steady-state volume of distribution was 87.1 mL/min/m2.

The medication has a significant hepatic metabolism mediated by the enzymes CYP3A and CYP2C, which results in less than 10% of the drug being eliminated in the urine in its unaltered form. Over 90% of the PTX attaches to plasma proteins quickly and widely, and the majority of the medication is eliminated in the feces. After six hours of infusion, the medication was essentially removed from the rats' brains and testes, with the largest concentration occurring in the lung, liver, kidney, and spleen.

Mechanism of action

PTX functions differently from most cytotoxic drugs in its mode of action. This medication does not directly interact with nuclear components (DNA and RNA), in contrast to several other medications used in chemotherapy. Paclitaxel primarily acts on microtubules; however, unlike vinca alkaloids or derivatives of colchicine, which cause the microtubules to depolymerize, paclitaxel acts during the mitotic phase of cell division, encouraging the

polymerization of tubulin proteins and their assembly. This leads to the stabilization of microtubules and the blocking of the cell cycle, which ultimately results in cell death.

Within the cytoskeleton of eukaryotic cells, microtubules are a key component and perform a variety of vital biological tasks. Cell signaling, the creation of mitotic spindles during cell division, the development and maintenance of cell shape, and the movement of vesicles, mitochondria, and other elements in the intracellular environment all depend on them. The microtubules consist structurally of thirteen tube-shaped structures arranged lengthwise. Tubulin alpha and beta heterodimers make up each linear protofilament in multiples. Alpha tubulin subunits terminate at the negative end of the microtubules, while beta tubulin terminates at the positive (C) end. The ability of microtubules to randomly shorten and lengthen due to the addition or loss of a/b-tubulin heterodimers from microtubule ends is one of their defining characteristics. This process is known as "dynamic instability." Guanosine triphosphate (GTP)/guanosine diphosphate (GDP) exchange mediates dynamic instability; hence, tubulin's a-subunit binds irreversibly to achieve the proper configuration for microtubule polymerization, while tubulin's b-subunit establishes a reversible binding with GTP or GDP, resulting in either polymerization or depolymerization of the microtubule.

Every microtubule has two ends with radically differing dynamic properties. There is greater dynamism at the positive end than the negative. The mitotic spindle's microtubules are continuously polymerizing at their positive ends and depolymerizing at their negative ends as a result of these variations. Many endogenous cellular proteins are involved in this regulation of the microtubules that the cells impact, which is essential for the numerous cellular functions to take place. The mitotic phase is characterized by increased microtubule dynamics, and it is during this phase that chromosomal fixation, segregation, and separation require proper kinetochore production and stress.

The various isotypes of a and b tubulin and their interactions with microtubule-associated proteins (MAPs), which are proteins that connect with microtubules, are two of the elements that determine the dynamic capacity of microtubules. There are MAPs that have the ability to stabilize microtubules (MAP4 and Tau) as well as MAPs that destabilize them (stathmin family proteins). In the lumen of polymerized microtubules, taxanes and other Microtubule-Stabilizing Agents (MSA) can bind to tubulin's b-subunit, suppressing and stabilizing microtubular dynamics. As a result, cell aggregation during the G2/M phase of the cell cycle interrupts mitosis, preventing cell division and the subsequent growth of malignant cells.

In addition to altering the course of the cell cycle, MSA's action on microtubules also modifies signaling pathways, leading to apoptosis. Even with these incredibly obvious morphological alterations, the exact process causing cell death remains unknown. The duration of cell exposure and medication concentrations affect cell death. Furthermore, it has been discovered that PTX has the ability to kill cells even in the absence of mitosis. It has the ability to attach to the Bcl-2 protein, which controls apoptosis, phosphorylates cells, and triggers a convoluted process that results in cell death. But PTX has a lower propensity to bind to this protein than it does to tubulin.

Drug delivery system

For PTX loading, a variety of delivery methods can be used, including liposomes, hydrogels, dendrimers, polymeric nanoparticles, micelles, inorganic nanoparticles, carbon nanotubes, and cyclodextrin nanoparticles. Because they have various advantages over normal therapy, nanocarriers have garnered growing attention in recent years, particularly for cancer therapies. The following are the main benefits of using nanocarriers for PTX delivery: The solubility of the drug can be enhanced by nanoparticles due to their small size, which facilitates drug delivery to the tumor through the permeability and retention (EPR) effect. Additionally, due to steric hindrance caused by PEGylation, nanoparticles can evade the recognition of the reticuloendothelial system (RES), reducing side effects and ultimately improving the pharmacokinetic profiles of the drug from nanocarriers. In order to enhance the solubility of PTX and the physicochemical stability of liposome compared to the existing Taxol formulation, Yang and associates created a PEGylated liposomal of paclitaxel. The findings demonstrated that, in comparison to Taxol, PEGylated liposomes prolonged the biological half-life of PTX, reduced absorption in the liver, spleen, and lung, and enhanced absorption in tumor tissues following injection. Jin synthesized PTX-encapsulated PLGA nanoparticles and assessed their cytotoxicity against two hypoxic human tumor cell lines, carcinoma services (HeLa) and breast cancer (MCF-7). After retaining its bioactivity to block cells in the G2/M phase and exhibiting a cytotoxic effect on both cell lines, the authors concluded that PTX-loaded PLGA nanoparticles could be a viable drug delivery system to eradicate hypoxic tumor cells. Furthermore, the cytotoxicity of the formulation was found to be more significant than that of free PTX. In addition, active ligands like monoclonal antibodies, transferrin, folate, peptides, or aptamers can be functionalized on the surface of nanocarrier systems for targeting purposes. This will enhance the absorption of the drug into the tumor and lessen its side effects. Eloy and colleagues created functionalized liposomes with antibodies (Trastuzumab) that contained PTX and rapamycin (RAP) for this reason. The study's findings showed that, in comparison to the control groups, immune-liposomes were able to better limit tumor growth because of their increased cell absorption. Consequently, more clinical research on immunoliposomes targeted at breast cancer may be possible.

The study examined the potential of a third-generation (G3) dendrimer-based carrier based on polyamidoamine (PAMAM) to improve PTX permeability and get beyond cellular barriers. Lauryl chains were used to modify the surface of dendrimers, and PTX was coupled with them using a glutaric anhydride linker. The authors proposed that G3 and surface-modified G3 PAMAM dendrimers could function as viable nanocarriers to improve the permeability of P-gp efflux transporter substrates, such as paclitaxel, which are weakly water-soluble medications. In order to overcome difficult biological barriers, the development of delivery systems incorporating PTX may find tremendous interest in the dendrimer-based prodrug.

Emami created a receptor-targeted micelle that was loaded with paclitaxel and based on tocopherol succinate-chitosan-polyethylene glycolic acid (TS-CS-PEG-FA). They found that, as a result of folate receptor-mediated endocytosis, the folate on the micelle surface greatly enhanced the cytotoxic action of PTX. Additionally, the tissue distribution tests showed that the PTX/TS-CS-PEG-FA micelles showed longer blood circulation residence times and higher drug accumulation in tumor tissue.

In order to test the effectiveness of paclitaxel in treating prostate cancer, Sahoo and associates conjugated transferrin into the nanoparticle. The scientists showed that the usage of transferrin increased the absorption of the nanoparticles by cells and that the encapsulation of PTX in the nanoparticles inhibited tumor growth, indicating that the transferrin conjugation is mediated by the transferrin receptors.

A novel commercial formulation was created by American BioScience, Inc., located in Santa Monica, California, in which paclitaxel is attached to 130 nm-sized albumin nanoparticles. Paclitaxel is reversibly bound by the inherent qualities of albumin in this formulation, which helps it pass through endothelial cells and concentrate in tumor regions. The use of albumin as a vehicle to remove solvent-related toxicities and, as a result, the requirement for steroid and antihistamine premedication are two benefits of Abraxane. In addition, this formulation reduces toxicity and, thus, the length of the infusion.

Methodology

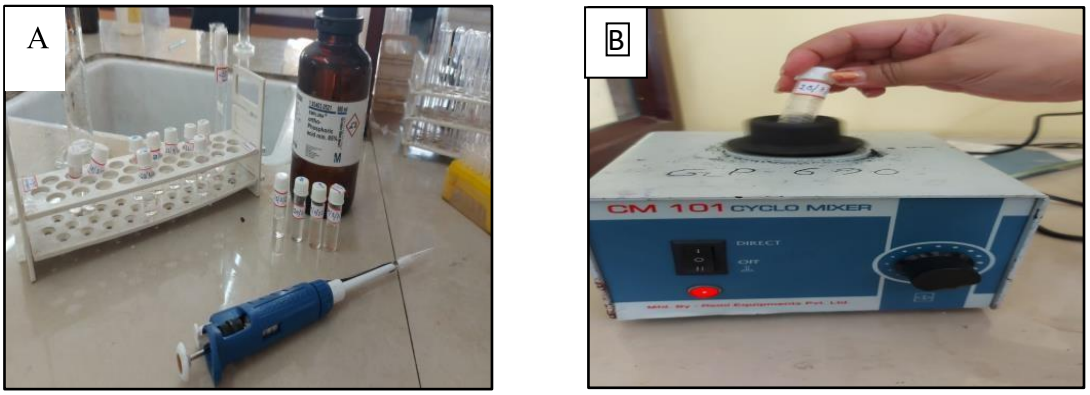
Preparation of Buffer System: To prepare buffer ethanol and orthophosphoric acid is required. 2ml of ethanol 50 microlitre H3PO4 was added, then put in acyclo-rotator for proper mixing and a sonicator for the buffer preparation.

Preparation of stock solution:

1. To the above-mentioned buffer solution 1mg of paclitaxel was added to make theconcentration of 1mg/2ml.
2. From the above-mentioned sample solution serial dilution for concentrations 5µl/2ml,10µl/2ml, 15µ/2ml, 20µl/2ml, 25µl/2ml was performed.
3. Checking OD for the different concentrations under UV Spectrophotometer: the ODobserved for the above-mentioned concentrations are respectively 1.67,1.86,1.94, 1.94,
   2.1 at 230nanometer.

For both Intaxel and Paclitero samples were prepared in the same above-mentioned way as the API.

Figure 1: A & B show the prepared samples of paclitaxel and proper mixing of all theingredients in a cyclo-mixer respectively.



Sterility testing

Prior to being released and administered to patients, sterile items must undergo microbiology testing to ensure they are free of live bacteria.
Disk-diffusion testing was used to verify sterility.

Disk diffusion technique (DDM): The plant extract to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism, which is why it is categorized as an agar diffusion method (ADM). The reservoir is often an agar surface put above a filter paper disk.

1. If any agar medium is available, prepare it for bacterial growth.

2. Preparing the reservoir (filter paper disk).
3. Preparing Agar media

To produce a 100ml solution, you'll need medium materials including peptone, yeast extract, sodium chloride, and agar.

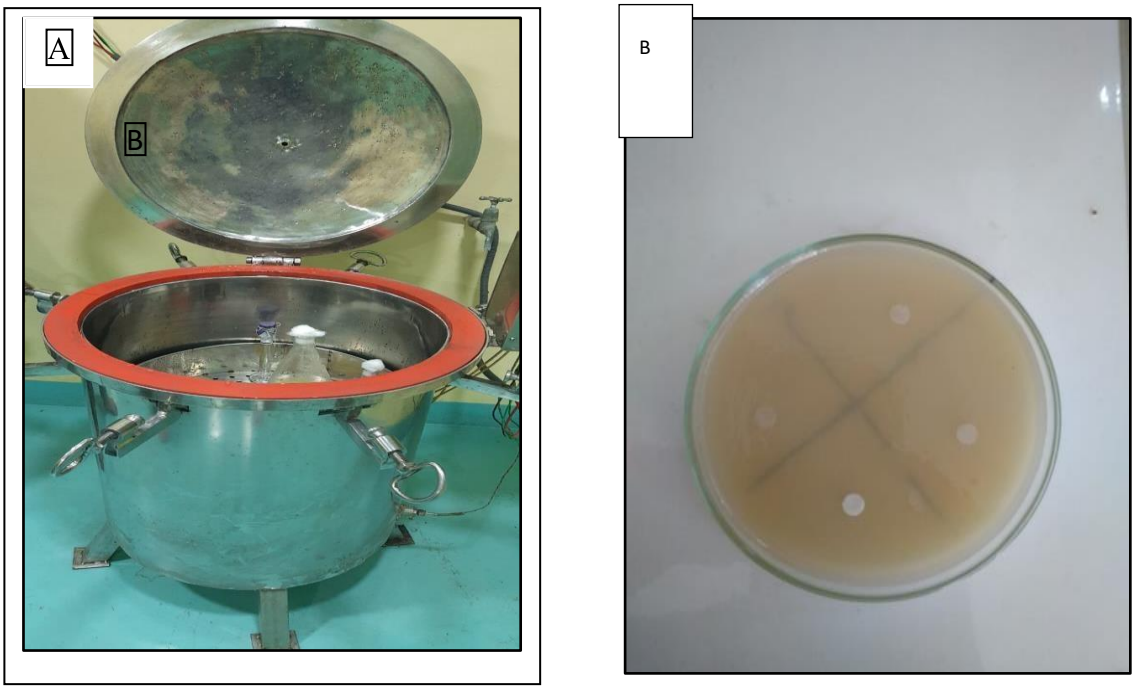
1. Using a measuring balance, all the materials were measured according to specifications and combined with sterile water in a conical flask.
2. Next, using sterile water, the volume was adjusted to 100 ml.
3. To eliminate any remaining microorganisms, the solution was autoclaved.
4. Calmed down.
5. To produce agar plates, the agar solution was put into previously sanitized plates.

Sterile conditions were maintained during the entire aforesaid process.

Setting Up a Reserve

1. The goods were put onto filter paper discs, which were then positioned over the previously prepared agar plates.
2. After that, these plates were placed in the incubator for a whole day to see if any microbiological growth had occurred.

Figure 2: A & B showing the autoclave method to sterile the products preparedand preparation of plates respectively

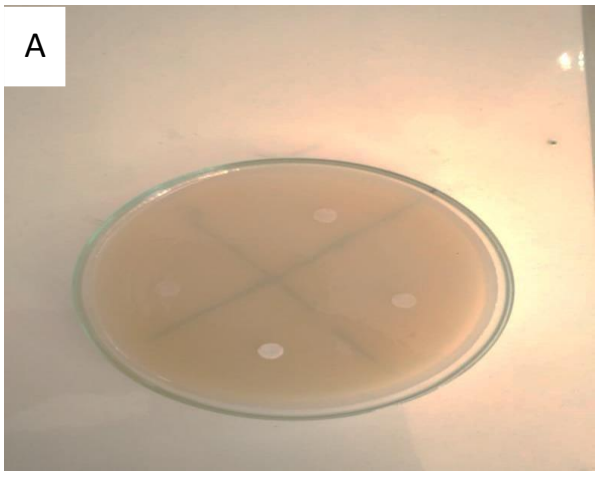


Results and discussion

Study on sterility testing

The purpose of sterility testing is to find live germs that can lead to an infection or other health problems.
Upon examining the plates a day later, there was no indication of any microbial activity, indicating the sterility of the goods.

Figure 3: showing no microbial growth



The research demonstrating that paclitaxel is present in branded goods

Utilizing UV-Vis spectroscopy, the amount of paclitaxel was determined. UV-Vis spectroscopy is an analytical method that counts the number of distinct UV or visible light wavelengths that a sample absorbs or transmits when compared to a reference or blank sample. Each and every molecule has a certain wavelength. The wavelength of paclitaxel is 230 nanometers; for 20µg/2ml and 25µg/2ml at 231 nanometers, the OD was given as 1.94 and 2.1, respectively. In contrast, for the branded drugs Intaxel and Paclitero, the OD was given as 2.03,2.3,2.03,2.2, for the same concentrations of 20µg/2ml and 25µg/2ml at 231 nanometers.

Graphs Obtained

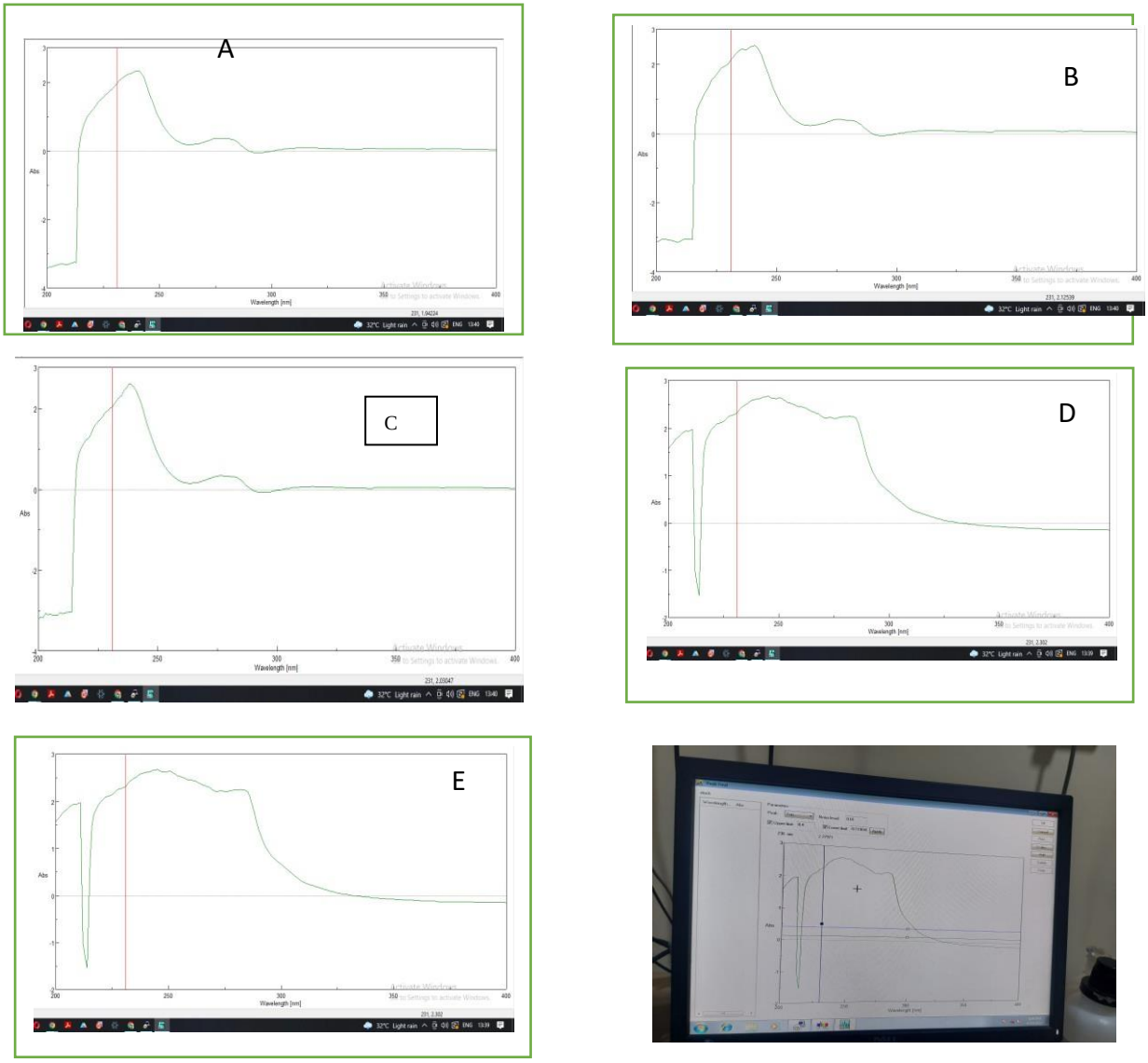


Figure 4: Showing the presence of paclitaxel

- Figures A & B show the OD given by API samples at 20µg, and 25µg concentrations was 1.94, 2.15 respectively.
- Figures C & D show the OD given by INTAXEL samples at 20µg, and 25µg concentrations was 2, 2.3 respectively.
- Figures E & F show the OD given by PACLITERO samples at 20µg, and 25µg concentrations was 2, 2.2 respectively.

Standard Curve Obtained

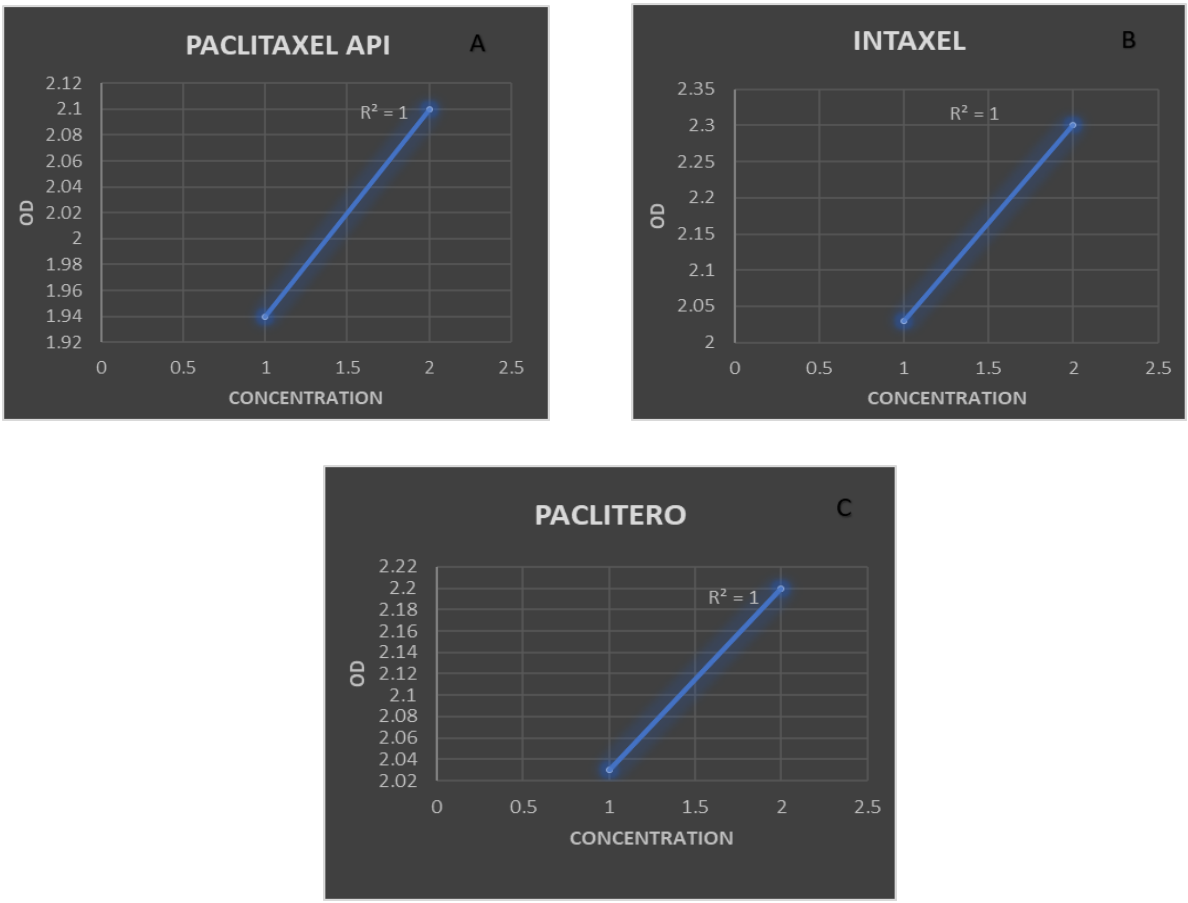


Figure 5: Showing the curves obtained for samples of Paclitaxel, Intaxel and Paclitero

Conclusion :

A pharmaceutical product's quality is crucial to guaranteeing patients' safety. Various quality control measures for pharmaceutical goods can ensure their optimal therapeutic action, as well as their purity and bioavailability. Thus, the purpose of the current investigation was to guarantee the caliber of the two distinct paclitaxel products. The two tests mentioned above demonstrated the lack of microorganisms and the presence of the active medicinal ingredient, proving the safety of both goods.

REFERENCE :

1. Rao S, Krauss NE, Heerding JM, et al. 3-(p-Azidobenzamido) taxol photolabels the N-terminal 31 amino acids of beta-tubulin. J Biol Chem 1994; 269:3132-4.
2. O'Shaughnessy, J. A.; Cowan, K. H. Current Status of Paclitaxel in the Treatment of Breast Cancer. Breast Cancer Res. Treat. 1995, 33 (1), 27–37. PMID: 7749130.
3. Kingston, D. G. I. The shape of things to come: Structural and synthetic studies of taxol and related compounds. Phytochemistry 2007, 68 (14), 1844–1854DOI: 10.1016/j.phytochem.2006.11.009.
4. Weiss, R. B.; Donehower, R. C.; Wiernik, P. H.; Ohnuma, T.; Gralla, R. J.; Trump, D. L.; Baker, J. R.; Van Echo, D.; Von Hoff, D. D.; Leyland-Jones, B.
5. Rowinsky, E. K.; Donehower, R. C. Paclitaxel (Taxol). N. Engl. J. Med. 1995, 332 (15), 1004–1014 DOI: 10.1056/NEJM199504133321507.
6. Parise RA, Ramanathan RK, Zamboni WC, Egorin MJ (2003) J Chromatogr B 783:231–236
7. Sparreboom A, de Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J (1998) J Chromatogr B 705:159–164
8. Merler E, Villa L, Lucchini R (1996) Med Lav 87(3):207–221
9. Skov T, Maarup B, Olsen J, Rørth M, Winthereik H, Lynge E (1992) Br J Ind Med 49:855–864
10. Grever MR (1992) Semin Oncol 19(6):622– 638
11. Mortier KA, Renard V, Verstraete AG, Van Gussem A, Van Belle S, Lambert WE (2005) Anal Chem 77(14):4677–83
12. Hempel G, Lehmkuhl D, Krumpelmann S, Blaschke G, Boss J (1996) J Chromatogr A 745:173–181

13. Huang Q, Roessner CA, Croteau R, Scott AI. Engineering Escherichia coli for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. Bioorg Med Chem Lett. 2001;9(9):2237–42.
14. Vassileva V, Allen CJ, Piquette-Miller M. Effects of sustained and intermittent paclitaxel therapy on tumor repopulation in ovarian cancer. Mol Cancer Ther. 2008;7:630–7.
15. Tan JL, Chan ST, Lo CY, Deane JA, McDonald CA, Bernard CC, et al. Amnion cell- mediated immune modulation following bleomycin challenge: controlling the regulatory T cell response. Stem Cell Res Ther. 2015;6(1):8
16. Fukuda K, Funakoshi T, Sakurai T, Nakamura Y, Mori M, Tanese K, et al. Peptide-pulsed dendritic cell vaccine in combination with carboplatin and paclitaxel chemotherapy forstage IV melanoma. Melanoma Res. 2017;27:326–34
17. Jiang JL, Yao M, Liu CD. Efficient synthesis of α-quaternary α-hydroxy-β-amino esters via silyl glyoxylate-mediated three component reactions. Org Lett. 2014;45:318–21
18. Liu WC, Gong T, Zhu P. ChemInform abstract: advances in exploring alternative Taxol sources. Cheminform. 2016;47: 48800–9.
19. Zhao C, Song G, Fu C, Dong Y, Xu H, Zhang H, Yu LJ. A systematic approach to expound the variations in taxane production under different dissolved oxygen conditions in Taxus chinensis cells. Plant Cell Rep. 2016;35:541–59
20. Chen G, Sheng L, Du X. Efficacy and safety of liposome-paclitaxel and carboplatin based concurrent chemoradiotherapy for locally advanced lung squamous cell carcinoma. Cancer Chemoth Pharm. 2018;82(3):505–10.