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EXTRACTION AND DETECTION OF VINEBLASTINE SULPHATE FROM VINCA ROSEUS (CATHARANTHUS ROSEUS) PLANT LEAVES BY SOXHLET EXTRATION METHOD

Amruta Mokashi[1], Sachin Chivade[2*], Kartik Baradkar[3], Rohit Dhage[4], Adarsh Chougule[5], Atharva Gadekar[6]

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Abstract

Vinblastine as well as vincristine, two dimeric alkaloids are widely used as anti-cancer medications, are found in the Catharanthus roseus plant. Its cost can be reduced globally by employing an effective method for extracting it from plant matter. Soxhlet Extraction, Solid-liquid Extraction, Supercritical Fluid Extraction (SFE), and Microwave Assisted Extraction are some techniques for extracting chemicals (MAE). The proposed study evaluates the Soxhlet Extraction method which is used to extract the VBL from C. roseus. Extraction was carried out using Soxhlet extraction, in which 10g of vinca roseus plant sample is used with 300mL of ethanol (B.P 78.37 °C) as a solvent in a reflux for 6h at constant temperature 800C [2]. Simple distillation is used to obtain raw Vinblastine from a solution of ethanol at temperature 700C. Cooled extracted samples after dilution Methanol samples were analyzed using an Agilent HPLC 1200 series. The analysis was carried out on an Agilent Zorbax Eclipse XDB-C18 column with a gradient system and a flow rate of 0.2 mL/min. The gradient began with a combination of 70% ammonium acetate (20 mM) and 30% acetonitrile, and the analysis was conducted using a UV detector with a wavelength of 254 nm. [1]. Calibration curve was prepared for quantifying purpose of the actual VBL drug.

Keywords: *C. Roseus, VBL (Vinblastine), HPLC, Soxhlet Extraction, SFE, MAE, UAE.*

[1,2,3,4,5,6]Department of Chemical Engineering, Vishwakarma Institute of Technology, Bibwewadi, Pune.

[2*]sachin.chivade20@vit.edu

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1. Introduction

Catharanthus roseus, often known as Vinca rosea, is a perennial evergreen herb and a member of the *Apocyanaceae* family. [1]. Periwinkle or Madagascar periwinkle is the popular name for this plant. It is native to India and Madagascar, but it is now commonly grown as a decorative plant across the world. Vindoline, catharanthine, and 3′, 4′-anhydrovinblastine are the most important alkaloids identified in this plant. [2]. Catharanthus roseus has natural compounds that can be utilised to create medications, including vinblastine and vincristine. These molecules are also known as alkaloids, and they are generated naturally in plants. Humans have been fascinated by this plant since the 1950s, when they discovered the compounds in its leaves. Three of these compounds are now commercially available: ajmalicine, vinblastine (VBL), and vincristine. [1]. Out of them ajmalicine is an anti-hypertensive and vinblastine (VBL), and vincristine are antineoplastic.

Vinblastine is a chemotherapeutic drug that is used to treat a variety of cancers. Robert Noble and Charles Thomas Beer of the University of Western Ontario were the first to extract it from the Madagascar periwinkle plant. They discovered that the plant extract may have anticancer qualities while researching the plant's potential to cure diabetes. The extract reduced the quantity of white blood cells in rabbits, which might be effective in treating cancers of these cells, such as lymphoma. To treat cancer, the medicine, marketed under the trade name Velban, is often given in conjunction with other treatments and supplied through vein injection.

Studies for novel applications of established medications have grown over the first decade of the twenty-first century. A promising study utilizing VBL was reported in this decade, in which 51 pediatric patients with low-grade gliomas received VBL instead of carboplatin. Seven patients in this pilot research had a tumor size decrease of more than 25% after receiving weekly doses of the medication at a rate of 6 mg/m2. In addition to this investigation, a multicenter phase II pilot research was conducted from 2002 to 2006 in which nine patients with low grade gliomas aged between 1.4 and 18.2 years received 52 weeks of VBL therapy[1]. At the conclusion of the course of therapy, it was confirmed once more that the medicine had low toxicity, that the patients' quality of life had improved, and that 35% of them had less tumor growth.

Eli Lilly, a pharmaceutical company, developed the first method for extracting and purifying VBL, one of the numerous alkaloids discovered in C. roseus, in the 1970s. The extraction procedure consists of four steps: extraction with sulfuric acid and water, separation with benzene, passage through two chromatographic columns, and crystallisation in ethanol and sulfuric acid. Although there are alternative ways for extracting alkaloids from C. roseus, they are time-consuming and need many steps as well as organic solvents.

There are several methods for extracting VBL alkaloids, including Soxhlet extraction, which is the topic of this work. Soxhlet extraction is a technique that requires utilising a particular equipment to extract essential components from solid materials. The machine is made up of three parts: a condenser, a circular container, and the Soxhlet extractor. This approach is used when the target component is not soluble in water. The machine extracts the vital component using a specific liquid, similar to sucking juice from a fruit with a straw. The study investigates the efficacy of Soxhlet extraction in the extraction of VBL alkaloids.

2. Literature Survey

In the overall endeavor to ensure that high-quality herbal products are available to customers globally, Novel sample preparation procedures that provide considerable benefits over traditional approaches for extracting and analyzing medicinal plants are predicted to be critical. It is also expected that analytical methods for identifying plant components would be developedfound in botanical and herbal remedies depends critically on sample preparation. The operating principles of various extraction techniques, performance-influencing elements, research advancements, and the strengths and weaknesses of various extraction procedures.

The primary indole alkaloids and iridoid precursors discovered in Catharanthus roseus hairy roots have been examined using two direct HPLC analytical techniques. Both fluorescence detection and photodiode array were

used. A reversed-phase C column was used to achieve the separation. Catharanthine, 18 serpentines, tabersonines, vindolines, vinblastines, and vincristine could all be separated using the first approach in under 20 minutes. The chemicals were identified by comparing their retention times and UV spectra to verified standards.

Apocynaceae is the family of plants that includes Catharanthus roseus. 70 out of 120 alkaloids it generates have pharmacological activity. Vinblastine is a drug that is made by C. roseus and is used to treat Hodgkin's disease, testicular tumours, breast and choriocarcinomas, Kaposi sarcoma, and Letterer-Siwe disorder. Acute lymphocytic leukaemia, lymphosarcoma, lympho-granulomatosis, and solid baby cancers are all treated with vincristine. Vinblastine and vincristine's production rates in C. roseus are extremely low, their extraction is expensive, and they are too inefficient to be used industrially. Because intermediaries and precursors are essential, the semisynthesis also encounters numerous challenges. Vincristine and vinblastine are significant terpenoid indole alkaloids in medicine. They are, however, only present in trace levels in plants (approximately 0.0005% of dry weight), in vitro tissue, and in cell cultures.

3. Traditional Methods for Extraction of VBL

SFE (Super Critical Fluid Extraction)

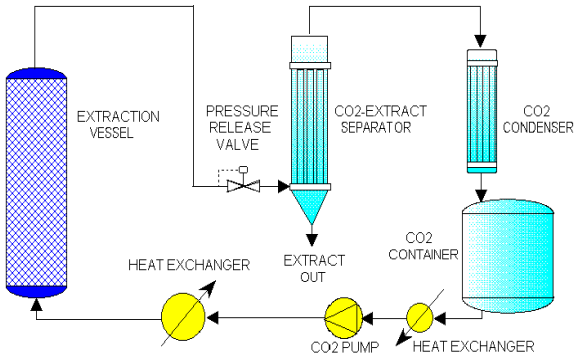

Figure 1: Supercritical Fluid Extraction Process Flow

Due to its numerous benefits over conventional or traditional extraction procedures, supercritical fluid extraction (SFE) has emerged as one of the significantly used green extraction technique today. The significant expansion of SFE has been largely attributed to factors including enhanced selectivity, higher extraction yields, better fractionation capabilities, and reduced environmental consequences. There are several benefits, including (i) quicker and more effective extractions, (ii) no residual solvent in the finished product, which lowers operating costs by reducing post-processing and clean-up steps[2], and (iii) the ability to separate sensitive and relatively non-volatile compounds under mild thermal conditions without decomposition.

MAE (Microwave Assisted Extraction)

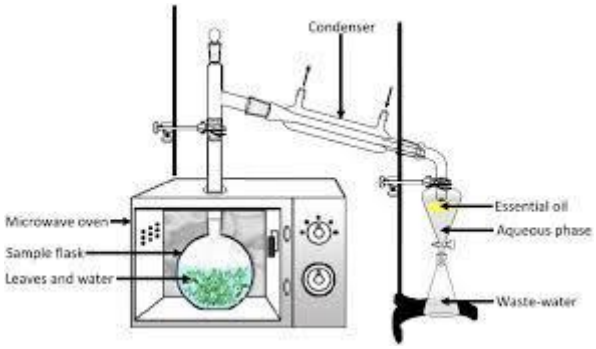

Figure 2: Microwave Assisted Extraction Process Flow

Microwave-assisted extraction (MAE) is a common method for extracting bioactive components from medicinal plants. MAE has the particular benefit of rapidly heating the sample and solvent mixture, making it

suited for the fast extraction of analytes, even those that are thermally labile. MAE efficiency is controlled by various parameters, including the solvent's characteristics, the kind of sample material, and the individual components being extracted, such as their dielectric constants. Several MAE parameters, such as the polarity and volume of the extracting solvent, sample size, extraction temperature and duration, and microwave power, should be tuned when designing procedures for extracting plant-based medicines.

UAE (Ultrasound-Assisted Extraction)

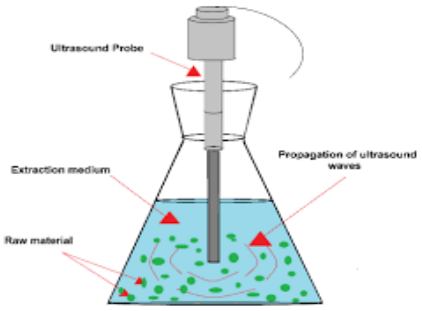

Figure 3: Ultrasound-Assisted Extraction Process Flow

The method of transmitting sound waves through a liquid solution containing solid particles is known as UAE, or Ultrasound-Assisted Extraction. As the waves collide with the material, they generate a force that might be perpendicular or parallel to the surface. As acoustic energy is turned into mechanical energy, shock waves with pressures comparable to several thousand atmospheres are produced. [3]. UAE is a technique for extracting substances from cells by breaking them open by sound waves. This allows the solvent to go inside and extract what is required. UAE is a fantastic option since it is inexpensive and does not require costly equipment. An ultrasonic probe system or an ultrasonic bath can be used to perform UAE. [4]. This approach may be used to obtain valuable substances such as isoflavones from soybeans and phenolic compounds from wheat bran and coconut shells. To achieve the greatest results, though, you must alter the process for each substance you're extracting as well as the liquid you're employing. It's similar to other methods of extracting substances from plants.

Traditional Extraction Methods and Experimental Process for VBL Extraction

Solid–Liquid Extraction

During three hours, a 100 mg plant sample was extracted in an ultrasonic bath containing a solution of 10 mL methanol and 0.5 M sulfuric acid. The extract was filtered, and the residual residue was treated for an additional hour with 10 mL of the same solvent. The combined liquid was mixed with an internal standard, filtered, and alkalinized with 2 mL of 25% ammonia. The resultant mixture was extracted twice with 10 mL of methyl tert-butyl ether, and the ether fraction was dried. The dry residue was diluted in 1 mL of methanol, filtered, and a 5 L sample was tested using HPLC.

Hot Water Extraction at 50, 70 and 90°C

Plant samples weighing 100 mg were extracted for three hours in a water bath at three different temperatures (50, 70, and 90°C) with constant shaking in the experiment. The extracts were then filtered through a Büchner funnel with Whatman no. 40 filter paper, and the residue was re-extracted for one hour with an additional 10 mL of water. The supernatant that resulted was mixed, filtered, and alkalized with 2 mL of 25% ammonia. After that, the mixture was extracted twice with methyl tert-butyl ether (2 * 10 mL), and the ether component was separated and dried to dryness. After dilution in 1 mL of methanol, the dry residue was filtered through a 0.45 m membrane, and a 5 L aliquot was injected into the HPLC for analysis.

Table 1: Dry Weight Content (µg/g) and RSD (%) of Major Alkaloids in Different Extraction Methods

Method of extraction	Catharanthine	Vindoline	3′,4′-anhydrovinblastine

	µg/g (RSD %)	µg/g (RSD %)	µg/g (RSD %)
hot water extraction at 90	5.9 (11.5)	8.2 (11.9)	4.5 (17.5)
hot water extraction at 70	95.4 (6.3)	153.4 (10.8)	12.9 (13.0)
hot water extraction at 50	101.2 (6.4)	159.1 (7.5)	12.4 (6.3)
solid–liquid extraction	132.8 (15.9)	287.6 (13.3)	156.8 (14.5)
Soxhlet extraction using dichloromethane	177.9 (11.6)	353.8 (11.4)	29.4 (14.0)
Soxhlet extraction using methanol	-	329.5 (4.1)	-
supercritical fluid extraction	198.8 (6.5)	208.2 (7.1)	77.8 (7.2)

4. Methodology

Soxhlet Extraction Method

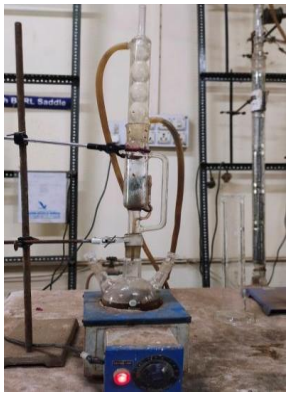

Figure 4: Soxhlet Extraction Setup

For extracting the Vinblastine from C.roseus, soxhlet extraction was performed by extracting 10g of the plant sample with 300mL of ethanol (boiling point 78.37 ℃) as a solvent in a reflux for 6h at constant temperature 80^0C[2]. Modern extraction methods include soxhlet extraction involve repeatedly running the same solvent through the extractor. Although it is a form of continuous extraction, we can refer to it as a succession of short macerations. The desired component must be soluble in the solvent at a high temperature in order to use a Soxhlet extractor. The soxhlet extraction process has an extraction cycle that comes after the solvent has evaporated. And theoretically, this cycle can be repeated as often as necessary to produce the target product with the highest yield. Compared to the conventional method, the Soxhlet extractor greatly improves the efficiency of the extraction process.

Simple Distillation

Figure 5: Simple Distillation Setup

Simple distillation is used to obtain raw Vinblastine from a solution of ethanol at temperature 70^0C. And after distillation 40 ml of raw extracted sample is obtained, which is then filtered for removing some plant particles to get a pure raw sample. One of the main separation processes used to separate light chemicals from solutions or

mixtures is distillation. Distillation consumes a lot of energy, especially when mixes are closely boiled. Efficiency and economy are necessary for the operation to be used effectively.

HPLC

After distillation is done then the main and final process is HPLC. The technique is used to check the presence of vinca alkaloids (vinblastine) in the raw extract obtained.HPLC is an effective technique for analyzing materials for a wide range of organic compounds, with advantages such as versatility, sensitivity, and applicability to complex mixtures. The experiment used an Agilent Zorbax Eclipse XDB-C18 column with a gradient system and a flow rate of 0.2 mL/min. The mobile phase was a 70:30 mixture of ammonium acetate (20 mM) and acetonitrile. The real VBL medication was measured using a calibration curve and a UV detector with a wavelength of 254 nm.

5. Experimental Work/ Raw Material

C. roseus leaves were acquired from natural sources. Dried it for 24hr under sunlight and was finely ground. For extracting the Vinblastine from C.roseus, Soxhlet extraction was performed by extracting 10g of the plant sample in Soxhlet with 300mL of ethanol (boiling point 78.37 ℃) as a solvent in a reflux for 6h at constant temperature 80^0C[2]. And was covered with polythene packs to keep away from dissolvable dissipation. As we know it is a form of continuous extraction. So, due to continuous extraction we found that the sample got extracted four times in the overall process (i.e. 6hr). Each sample has a duration of 80 min. For the extraction process we take 300ml of solvent out of which 80 ml is evaporated during extraction. Total solution we get is 260ml, which contains 40 ml of extracted raw sample and 220 ml of ethanol as a solvent, and this separation of solvent is carried out by simple distillation process at temperature 70^0C. The extracted sample was filtered using filter paper, because it gives problems during HPLC if the sample has not been filtered. After distillation is done then the main and final process is HPLC. The technique is used to check the presence of vinca alkaloids (vinblastine) in the extracted raw sample.

Figure 6: Extraction of Vinblastine (VBL) Using Soxhlet Extraction Method(at const. temp 800C)

Figure 7: Simple Distillation to Separate the Sample From Solvent (at temp 700C)

Figure 8: Raw Material Before Extraction

Figure 9: Raw Material After Extraction

6. Vinblastine Sulphate (VBL)

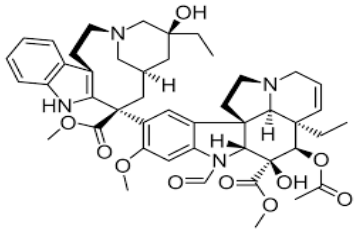

Figure 10: VBL (Vinblastine Sulphate) Structure

Figure 11: Market Sample of VBL Injection

Uses of Vinblastine (VBL)

Vinblastine is a chemotherapeutic agent that is commonly used in the treatment of cancers such as Hodgkin's lymphoma and non-lymphoma. Hodgkin's White blood cells are the source of these cancers. Vinblastine can also be used to treat testicular cancer and Langerhans cell histiocytosis, a disorder in which a certain kind of white blood cell overgrows in certaine area of the body. It's also used to treat breast cancer that hasn't responded to conventional treatments, as well as gestational trophoblastic tumors, which form in the uterus during pregnancy. Vinblastine, a vinca alkaloid, inhibits the proliferation of cancer cells in the body.

The Side Effects Can This Medication Cause

- Difficulty passing stools

- Feeling sick
- Regurgitation
- Decreased food intake
- Stomach discomfort
- Frequent bowel movements
- Cranial pain
- Loss of balance
- Rheumatic pain
- Thinning hair

7. Results and Discussion

As the experiment was carried out for dried leaves of C.roseus using Soxhlet extraction method. The raw sample is obtained from the extraction process which is further separated using simple distillation. Basically, this sample contained many alkaloids such as Catharanthine, 18 serpentines, tabersonines, vindolines, vinblastine, and vincristine. Out of which vinblastine is more efficient alkaloids as a drug for various cancer treatments. So, the proposed experiment is carried out to check the availability of these alkaloids in raw samples. There are various traditional methods for extraction of vinblastine such as SFE (supercritical fluid extraction), MAE (microwave assisted extraction), UAE (Ultrasound-Assisted Extraction). But these methods are very costly, so to overcome this problem the Soxhlet extraction method is carried out. Which is cost effective as compared to other methods.

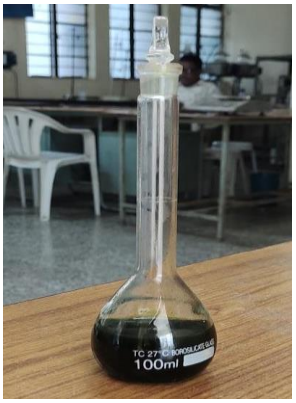

Figure 12: Raw Sample (Contain Many Alkaloids) After Extraction

For the extraction process we take 300ml of solvent out of which 80 ml is evaporated during extraction. Total solution we get is 260ml, which contains 40 ml of extracted raw sample and 220 ml of ethanol as a solvent, and this separation of solvent is carried out by simple distillation process at temperature 70^0C. The extracted sample was filtered using filter paper, because it gives problems during HPLC if the sample has not been filtered.

8. Conclusion

Extracted raw sample is obtained after the distillation, which contains high range of alkaloids, so to check the presence of VBL alkaloid in the sample, the HPLC is carried out.

According to literature, HPLC has been found to be one of the powerful analytical techniques used to separate, identify, and quantify components of complex mixtures. Then, further, to overcome the difficulty in analyzing VBL peak in HPLC result of obtained sample, market ready standard VBL injection is used for comparison of both sample's (actual VBL and extracted raw sample) HPLC results. For HPLC process of standard VBL, it was diluted with methanol, which resulted in complex type mixture.

Comparing HPLC results of both the samples resulted that VBL is detected at nearly same peak in both the samples. So, presence of VBL is extracted sample was confirmed. The table 2. represents the comparison of results of both the samples time (min) vs chromatogram (mV).

Table 2: HPLC comparison of standard VBL injection and extracted raw sample.

	HPLC of standard VBL injection	HPLC of extracted raw sample.
Time (min)	14min	10 min
Chromatogram(mV)	147mV	140mV

9. Acknowledgment

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