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Quantitative estimation of vinca alkaloid in *Catharanthus roseus* under stress conditions

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Abstract

Vinca alkaloids are anti-mitotic and anti-microtubule alkaloids derived from the periwinkle plant, Catharanthus roseus. Vinca alkaloids are utilized in the treatment of cancer. They are a type of cytotoxic medication that functions by preventing cancer cell division during the cell cycle. They work on tubulin to prevent it from forming microtubules, which are essential for cellular division. As a result, they prevent beta-tubulin polymerization in dividing cells. Vinca alkaloids are the second most frequently utilized family of cancer medications. For therapeutic purposes, vinca alkaloids are commonly used in combination with chemotherapies. Periwinkle plants produce vinca alkaloids naturally in their leaves, but the concentration of these alkaloids is quite low within the plant. The expense of extracting and isolating these alkaloids from vinca plants has been expensive throughout. Vinca alkaloids are secondary metabolites and their synthesis can be increased in response to stress and unfavorable environmental conditions. Synthetic vinca alkaloids have been produced to fight cancer and immunological suppression, however, it is still under research and is not extensively used. The purpose of this study is to examine if vinca alkaloids concentrations increase when plants are subjected to binary stress. We have extracted the phytochemical, vinca alkaloid from Catharanthus roseus by cultivating it under different controlled conditions to inspect the concentration of the phytochemical produced. Further, we have identified the stress circumstances that may result in the enhanced production of vinca alkaloid. Thereafter we have analyzed the extracts of vinca alkaloid via qualitative and quantitative examination.

Keywords: Vinca alkaloids, Anti-microtubule, Cytotoxic, *Catharanthus roseus*, Phytochemical, Chemotherapies.

Introduction

Plant-derived substances are known as phytochemicals. Plants produce phytochemicals (phyto, which means "plant") as a result of primary or secondary metabolism. They often have a biological impact on the plant host and aid in the development of defensive systems against competitors, pathogens, and predators[1]. Some phytochemicals have been exploited as poisons, while others have been utilized in traditional medicine[2]. Because of the evidence of their potential health benefits, phytochemicals are often classed as research compounds instead of vital nutrients[3]. Polyphenols and carotenoids, which include flavonoids, phenolic acids, and stilbenes/lignans, are two essential phytochemicals being studied. Flavonoids are divided into many types based on their chemical structure, including flavones, flavanols anthocyanins, flavanones, and isoflavones[4].

Over 25,000 phytochemicals have been discovered in total, and the bulk of these phytochemicals are located in the colorful portions of the plant[4]. The English Yew tree was known to be highly poisonous to animals who grazed on its leaves or children who ate its berries, paclitaxel was extracted from it in 1971 and has since become a vital cancer medicine. Plants produce alkaloids, which are nitrogen-rich compounds.

The pharmacological effects of several alkaloids are significant[5]. Among the alkaloids are cocaine, morphine, nicotine, caffeine, pilocarpine, strychnine, atropine, mescaline, ephedrine, methamphetamine, and tryptamine[3]. Alkaloids are often concentrated in certain sections of the plants such as leaves, bark, or roots. Alkaloids include neuroactive chemicals like caffeine and nicotine, and also life-saving drugs like emetine (used to treat oral intoxication), and antitumoral vincristine and vinblastine[6]. Alkaloids can operate as defensive molecules in plants owing to their toxicity, making them effective against diseases and predators[5,6].

A plant defensive system must be able to recognize threats and unfavorable environmental circumstances quickly, followed by effective and specific signal transduction for alkaloid production[1,6]. Toxic effects differ based on the quantity of exposure, the duration of exposure, and character traits such as sensitivity, location of the action, and developmental stage. Vinca alkaloids are isolated from *Catharanthus roseus*, a periwinkle plant, and other vinca species. Periwinkle leaves have a high concentration of vinca alkaloid. These alkaloids are anti-mitotic and anti-microtubule[7]. They hinder beta-tubulin polymerization in dividing cells[7]. Vinca alkaloids are used in cancer chemotherapy to treat the ailment[6,8].

The drugs work by inhibiting cancer cells from multiplying[8]. By blocking tubulin synthesis, they prevent tubulin from forming into microtubules, which are required for cell division[7,9]. Vincristine (VCR), Vinblastine (VBL), Vinorelbine (VRL), and Vindesine (VDS) are the four principal vinca alkaloids in therapeutic usage[4]. They've been employed as disinfectants and anti-cancer agents, as well as used to treat diabetes and high blood pressure. As a result, vinca alkaloids have cytotoxic characteristics, causing cell division to stop and cell death to occur[4]. Catharanthine, an indole nucleus, and vindoline, a dihydroindole nucleus, make up the chemical structure of vinca alkaloids[9]. Vinca alkaloids have been used to treat a variety of lymphomas and acute leukemias[8]. They can also be used to treat some solid tumors[8]. Intravenous administration of vinca alkaloids is common[8]. It prevents polymerization and, as a result, microtubule assembly, causing mitotic M-phase arrest and preventing cancer cell division[7]. Vinblastine and vincristine are two medicinal drugs that are widely used in cancer therapy. Although chemically nearly similar, the two alkaloids differ significantly in the types of tumors they target and their poisonous effects.

Catharanthus roseus is a member of the Plantae kingdom. It belongs to the Magnoliopsida class. This plant belongs to the Apocynaceae family and belongs to the Gentianales order. Catharanthus is the genus of this plant, and it belongs to the *C. roseus* species. Pink Periwinkle, Madagaskar Periwinkle, and Sadabahar are common names for this plant.

Zhu Wei et al. published a paper on Binary stress induction in a range of plants, which enhances the production of indole alkaloids in *Catharanthus roseus*. Artificial stress promotes alkaloids and other forms of phytochemical production in plants, according to their findings[10].

The properties of "Vinca alkaloids" were studied by Moudi, Maryam, and colleagues. The characteristics of vinca alkaloids were described in detail in this publication, including their anticancer and antitumor effects[5].

Prakash V and Timasheff SN investigated the mechanism of vinca alkaloid's interaction with tubulin: catharanthine and vindoline. Vinca alkaloids are active molecules that are used to treat cancer in convergence with other medications. Their research elucidates the method through which vinca alkaloids interact with cancer cells to kill them. The alkaloid molecule prevents the cell from producing microtubulin and, as a result, kills it[7]. Jai Mishra and Navneet Verma summarized the properties and advantages of *Catharanthus roseus*[1].

M. Naeem et al. published Current Research and Future Prospects of *Catharanthus roseus*. This study highlights all of the recent improvements made in the previous ten years. This also summarizes the value and future potential of *Catharanthus roseus* in the medicinal field[11].

Lee CT et al. used vinca alkaloids to investigate drug delivery methods and combination treatment, and their findings were published in Current themes in medicinal chemistry. Their findings showed that vinca alkaloid might be used in conjunction with other cancer treatments[2]. R. Biradar and D. Rachetti worked on secondary metabolite extraction and thin layer chromatography from various sections of Acacia farnesiana L. As a result of their research, several secondary metabolites have been discovered and isolated from various parts of the plant[12].

Methodology

Plants of *Catharanthus roseus* were grown for 45 days in a greenhouse following planting, with constant temperature (28^0C) and humidity (70%). Few of the plants were transported to the field for natural cultivation for 7 days as a control. The remaining plants were exposed to UV radiation for 1 hour, 2 hours, and 3 hours (Figure-1). Plants were then grown in the dark for 72 hours[10] (Figure-2). All of the plant's leaves were collected separately, sterilized, dried, and crushed into a coarse powder.

1.5g of the dried leaves samples were weighed on a weighing balance and packaged in little Whatman paper packets (Figure-). The packets were immersed in a 9:1 solvent mixture of ethanol and acetic acid (150ml) for 48 hours (Figure-4, 5). To keep the pH at 2, HCl was added drop wise[12].

The leaf extract solution was filtered and concentrated to one-quarter of its original volume in a water bath (Figure-6). The solution was treated with concentrated ammonium hydroxide until the precipitation was complete, then the solution was left undisturbed for 1 hour. The precipitate was recovered and rinsed twice with a solution of dilute ammonium hydroxide and then washed with distilled water. Precipitate containing alkaloids has been dried and weighed[12] (Figure-7).

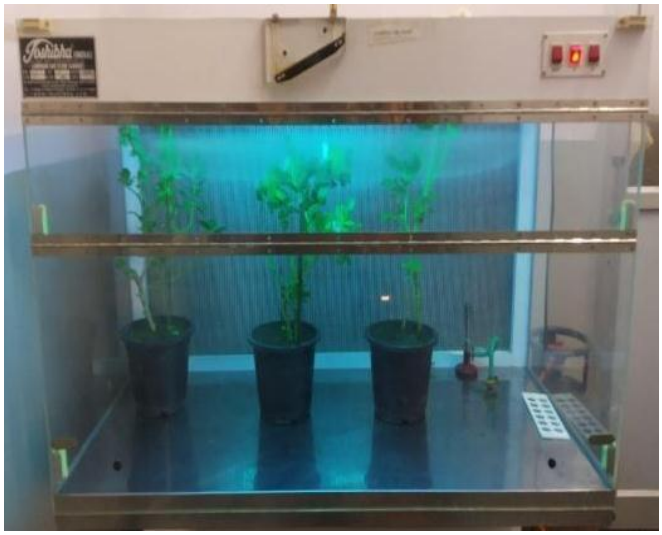

Figure-1: Plant's exposure to UV radiations.

Figure-2: Plants under observation.

Figure-3: Sample packed inside Whatman paper.

Figure-4: Packets immersed in a 9:1 solvent mixture.

Figure-5: Packets immersed in a 9:1 solvent mixture after 24 h.

Figure-6: Filtered Sample.

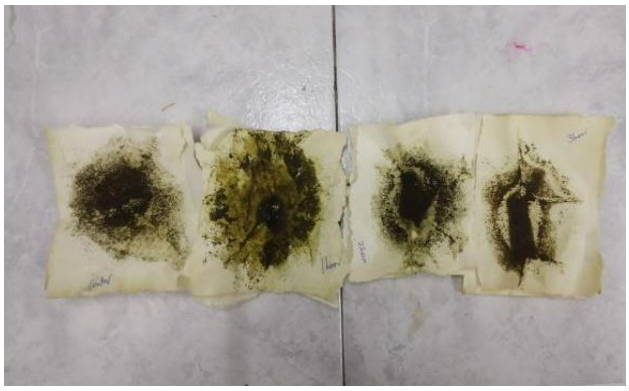

Figure-7: The precipitate obtained after extraction.

Qualitative Analysis for alkaloids: Wagner's test was used as a confirmatory test for alkaloids. Wagner's reagent was prepared by combining 2g of iodine and 1.27g of potassium iodide in 100ml of distilled water. 4-5 drops of reagent were added to 2ml extract in a test tube[13]. The presence of alkaloids was confirmed by the production of a brown precipitate (Figure-8).

Calculations

$$\text{Alkaloids (g)} = \frac{\text{Weight of alkaloids}}{\text{Weight of Sample}}$$ [12]

$$\text{Alkaloids \%} = \frac{\text{Weight of Alkaloids}}{\text{Weight of Sample}} x\ 100\%$$ [12]

Results and discussion

According to the previous studies, vinca alkaloids were traditionally utilized for numerous conditions such as cancer, cardiovascular disorders, high blood pressure, and so on[3]. The plant was identified and botanically authenticated. In this experiment, the plants were subjected to binary stress conditions. Following that, the plant's leaves were separated, shade dried, and grounded into a coarse powder, and the alkaloids were extracted using a soaking process with ethanol and acetic acid[12]. In a water bath, all of the extracts were filtered and concentrated. The solution was treated with concentrated ammonium hydroxide until the precipitation process was completed. Indole alkaloids were isolated into an aqueous acid

media and precipitated with an alkaline solution as water-insoluble complexes. The quantity of alkaloids in C. roseus leaves increased as the time of UV exposure increased from 1 hr to 2hr to 3hr, followed by a 72-hour dark incubation period (Figure-9). After 3 hours of UV irradiation, the alkaloid contents of *C. roseus* leaves increased the most, followed by 72 hours of dark incubation. As a result, we may deduce that because the stress circumstances at 3 hours of UV exposure were the most adverse, the concentration of alkaloids in the plants was the highest.

Conclusion

Although UV rays are an adverse environmental element for plant growth and development, they have been proven to trigger the synthesis of a number of bioactive secondary metabolites in medicinal plants, such as indole alkaloids[14]. Indole alkaloids offer resistance to microbial infection, herbivore feeding, and abiotic environmental stresses[6]. In this study, the concentrations of indole alkaloids in *Catharanthus roseus* leaves were increased by dual stress of UV irradiation and dark incubation. As a result, both UV irradiation and dark incubation are required stress conditions to increase alkaloid synthesis[10]. According to the results of this experiment, increasing the amount of UV exposure to the plant increases the contents of alkaloids extracted. Indole alkaloids were isolated from *C. roseus* and precipitated as water-insoluble complexes with an alkaline solution in an aqueous acidic media. The plants that were under exposure to UV light for the longest period of time produced the highest yield of alkaloids under the provided conditions. However, for higher yields of vinblastine and other dimeric alkaloids, parameters such as UV irradiation time, temperature, pH, and reagent concentrations added during the reaction can be optimized further[15]. With more advanced technology we can separate and purify different vinca alkaloids from the mixture of alkaloids obtained[15].

Figure-8: Formation of Brown Precipitate (Confirmatory Test for Alkaloids).

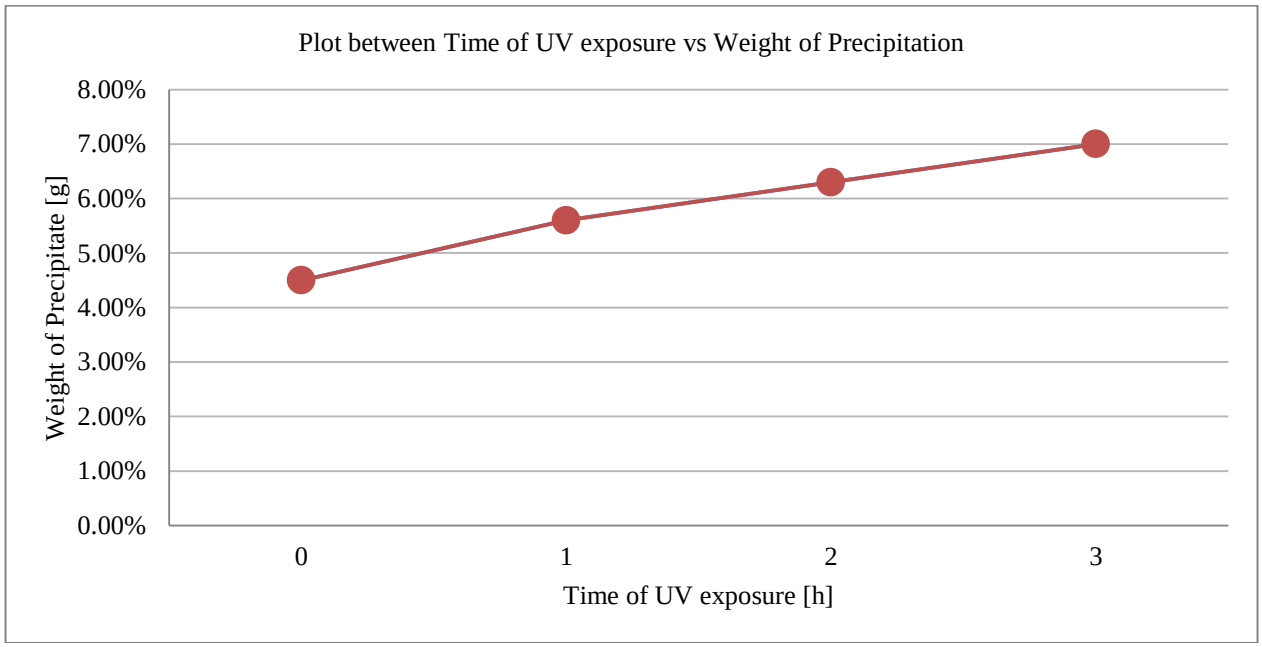

Figure-9: Graph plot between Time of UV exposure vs Weight of Precipitate.

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