Journal of Advances in Science and Technology
Vol. 20, Issue No. 2, September-2023, ISSN 2230-9659

Isolation of Vinca Alkaloids from C.Roseus and Mechanism of Alkaloids in Cancer

Ashishdeep Kaur*

Research Scholar

Abstract - Malagasy periwinkle, or Catharanthus roseus (CULTIVAR- ALBA), is a strong medicinal plant that has been studied for its potentially therapeutic alkaloids. This research investigates the mechanisms of action of C. roseus (CULTIVAR- ALBA) vinca alkaloids and total alkaloids, with a particular emphasis on their anticancer properties. The wider consequences of anticancer therapies, such as possible side effects and influence on general quality of life, are also investigated in this research. This study explores methods for reducing the severity of these side effects, which may ultimately enhance adherence and the quality of care patients receive. Vinca alkaloids were successfully isolated and characterised from C. roseus (CULTIVAR- ALBA) in this work.

Keywords - vinca alkaloids, C.roseus, mechanism, alkaloids, cancer

----------X----------

INTRODUCTION

Plants include organic compounds like vinca alkaloids, which are composed of carbon, hydrogen, nitrogen, and oxygen. It's important to remember that not everything that sounds alkaline in name really is. Because of their effects on the body, certain alkaloids may be utilised therapeutically even if many of them are hazardous.The vinca alkaloids [1] were among of the first plant alkaloids to be utilised in the treatment of cancer.The periwinkle plant of Madagascar may contain vinca alkaloids, according to [2]. The pink periwinkle, or Catharanthus roseus G. Don, may provide an all-natural or partially synthetic nitrogenous basis.[3] For illustration purposes, consider Figure 1. Canadian scientists Robert Noble and Charles Beer found vinca alkaloids in the 1950s. Researchers are keeping a close check on the hypoglycemic and cytotoxic effects of this plant due to its possible medicinal uses.As well as treating diabetes and high blood pressure, these medicines have also been used as disinfectants [4]. Vinca alkaloids, however, are crucial due to their ability to combat cancer. Only vincristine (VCR), vinblastine (VBL), vinorelbine (VRL), and vindesine (VDS) — four of the most important vinca alkaloids used in medicine — have received FDA clearance for usage in the United States. Recently, vinflunine, a synthetic vinca alkaloid, was given the green light for medical use.[5,6]

Mechanism of action

Vince alakaloid cytotoxicity leads to metaphase arrest by inhibiting microtubule activity in the mitotic spindle. This happens predominantly via interactions with tubulin.In addition to affecting microtubules, they may engage in a plethora of other metabolic functions. [7]. Vinca alkaloids must be administered to cells in clinically insignificant amounts to produce several effects that do not need microtubule disruption. Microtubules are engaged in numerous processes outside of the mitotic cell cycle, making vinca alkaloids and other antimicrotubule drugs harmful to both non-malignant and malignant cells during the non-mitotic cell cycle.[3]

Unlike taxanes, colchicine, podophyllotoxin, and guanosine-5'-triphosphate, the binding of vinca alkaloids to tubulin is not competitive.[8] Fast binding may happen in either direction. Based on these results, we may conclude that there are two binding sites for vinca alkaloids for every mole of tubulin dimer.There are about 16–17 high-affinity binding sites at each microtubule end [9]. However, at low drug concentrations, the most noteworthy effect is the creation of a "kinetic cap" that limits growth and shortening of microtubules at the assembly end. This occurs when vinca alkaloids adhere to specific sites on the microtubule, preventing assembly.[10] At drug doses below those that lower microtubule mass, vinca alkaloids induce metaphase arrest by altering microtubule dynamics, especially at the poles of the mitotic spindle.Anti-cancer effects of vinca alkaloids and other microtubule-disrupting drugs have been shown in vitro [11]. At doses between 0.1 and 1.0 pmol/L, VBL inhibited endothelial proliferation, chemotaxis, and spreading on fibronectin[12], but it had no effect on normal fibroblasts or lymphoid tumours. Low dosages of VBL dramatically improved antitumor response when combined with antibodies against vascular endothelial growth factor, even in tumours resistant to the drug's direct cytotoxic effects.[13] By stabilising microtubules, vinca alkaloids prevent cell division and may even cause apoptosis. By adhering to tubulin and preventing polymerization, VCR and

similar chemicals increase microtubule instability.[14]

Medicinal uses

Vinca alkaloids are a typical component in chemotherapy combinations. Drug resistance does not develop in response to alkylating agents because of their distinct mode of action.Treatment plans that include VBL exist for a variety of diseases, such as testicular cancer, Hodgkin lymphoma, and non-Hodgkin lymphoma [3, 4].[15] This medication is also effective against breast cancer and germ cell tumours. Possible negative effects to VBL include toxicity to white blood cells, stomach discomfort, diarrhoea, trouble breathing, chest pain, tumour pain, wheezing, and a high fever. Antidiuretic hormone secretion is connected with this illness very rarely.[3]

VBL is synonymous with VRL. Patients with breast cancer may have potent anticancer effects, and such effects may extend to osteosarcoma tumour cells. Furthermore, lipid bilayer membrane stability is decreased by VRL. VRL therapy for patients with advanced lung cancer is now available in the United States.[16] Potential side effects of VRL include lowered immunity to infection, bleeding, bruising, anaemia, constipation, diarrhoea, nausea, tingling and numbness in the limbs, extreme fatigue (peripheral neuropathy), and inflammation at the injection site. Hair loss and allergic reactions are two side effects that patients seldom experience.Appropriate indications for VCR include the treatment of acute leukaemia, rhabdomyosarcoma, neuroblastoma, Wilm's tumour, Hodgkin's disease, and various lymphomas [3]. Non-malignant hematologic disorders such as refractory autoimmune thrombocytopenia, hemolytic uremic syndrome, and thrombotic thrombopenic purpura have all responded well to treatment with VCR. Peripheral neuropathy, reduced bone marrow function, constipation, toxicity to the nervous system, nausea, and vomiting are the most prevalent adverse reactions to VCR.[3,15]

In this respect, VDS is analogous to VBL. Several types of cancer, such as acute lymphocytic leukaemia, blast crisis chronic myeloid leukaemia, and metastatic renal, breast, esophageal, and colorectal carcinomas, have been shown to respond well to VDS treatment.[17] Recently, utilising superacidic chemistry, a synthetic vinca alkaloid named vinflunine was developed.[6] First of its type, vinflunine is a fluorinated vinca alkaloid. This chemical was first developed as a treatment for urothelial transitional cell carcinoma (TCCU), but it is now being researched in the hopes that it may be used to treat other types of cancer as well. Clinical trials using it have examined a wide variety of solid cancer types. The most promising clinical results have been shown in the treatment of urothelial transitional cell carcinoma, non-small cell lung cancer, and breast cancer. Patients with TCCU have also been studied for their response to vinflunine in the treatment of advanced breast cancer.[5]

Toxicity

Vinca alkaloids share a number of structural characteristics yet exhibit widely varying degrees of toxicity. Although all vinca alkaloids are peripherally neurotoxic, VCR seems to be particularly potent. Peripheral, symmetric, fluctuating sensory-motor and autonomic polyneuropathy is the hallmark of neurotoxicity.[7] Axonal degeneration and decreased axonal transport are the principal pathogenic outcomes, which may be brought on by drug-induced disruption of microtubule function. Cognitive impairment, mood problems, hallucinations, restlessness, sleeplessness, seizures, coma, incorrect antidiuretic hormone production, and visual impairments are uncommon side effects of VCR usage because of its limited brain absorption. An announcement of laryngeal paralysis has also been made. Stopping therapy or reducing the amount or frequency of medication administration is the only established treatment for vinca alkaloid neurotoxicity. Thiamine, vitamin B12, folic acid, pyridoxine, and neuroactive medicines are only some of the many additional potential antidotes that have been tried without success. Any vinca alkaloid has the potential to produce neurotoxicity, albeit VBL and VRL are far less likely to do so than VCR. The primary dose-limiting effect for VB, DV, and VRL is neutropenia. The numbers of those affected by anaemia and thrombocytopenia have gone down. VCR is associated with extreme drug exposure and hepatic failure, two of the uncommon diseases associated with significant myelosuppression.In addition to their effects on the autonomic nervous system, vinca alkaloids have been associated with gastrointestinal toxicity [3, 4].[18] Large doses of VCR and other vinca alkaloids may cause gastrointestinal distress such gas, bloating, constipation, ileus, and abdominal discomfort by interfering with the gut's autonomic nerve system. Mucositis is more prevalent with VBL than it is with VRL. Other adverse responses include nausea, vomiting, and diarrhoea. Vinca alkaloids have been hypothesised to be potent vesicants and a potential source of severe tissue injury.[3] In addition to being linked to Raynaud's phenomenon and pulmonary and hepatic poisoning, vinca alkaloids have also been linked to abrupt cardiac ischemia, chest pains without evidence of ischemia, inexplicable fever, and acute pulmonary consequences. Women who are or might become pregnant or who are breastfeeding should not use these medications [19]. This drug shouldn't be used by patients who are also receiving injections. VCR usage has been linked to an increased risk of disease and a lowered resistance to illness. Patients receiving chemotherapy should inform their doctor of any other health conditions they may have, such as chicken pox, herpes zoster infection, gout, kidney stones, infections, liver illness, nerve or muscle damage, and so on.[20] Drug accumulation and cytotoxicity may be predicted to some extent by both drug concentration

and treatment time, although the former seems to be more essential.[21]

MATERIAL AND METHODS

Plant Material

The *Catharanthus roseus L.* specimens used in this research were obtained in June 2006 and were kindly donated by Professor Dr. K.H. El Batanouny of the Faculty of Science at Jiwaji University, India. There is a herbarium catalogued and kept at the phytochemistry lab.

Experimental

These Merck 60 F_{254} Silica gel TLC plates, measuring 20 x 20 cm, were put to good use. A 25% ammonia, ethyl acetate, benzene, and ethyl alcohol solution, stored in a glass trough (20 cm 20 cm). We utilised a CAMAG automated TLC sampler III to place labels on each band individually, with these specifications: 8 mm from the bottom edge, 6 mm band length, 12 mm track distance, 15 mm from the left edge. After spraying the sample with Dragendorff's solution and then 10% HCl, the alkaloidal bands were visible using a CAMAG TLC Scanner and CATS assessment software.

- A study of the optical spectra at 254, 289, 366, 400, and 500 nm.
- Scanning speeds of up to 20ml/s.
- Multi-level calibration is possible using peak-area-based linear regression.
- For the VLC separation, we used Silica gel (Merck) and alumina (Neutral Aluminium Oxide 60 GF254, type E, Merck).
- Elution in chloroform:methanol 95:5 (v/v) at 6 ml/min in a centrifuge-accelerated radial chromatograph (chromatotrone) using silica gel 2 mm thick (type 7749, Merck, Darmstadt, Germany).
- Waters High Performance Liquid Chromatography (600E pump), 486 variable-wavelength detector, Nova pack C18 (Waters, 3.9 150 mm), and Millenium 32 chromatography for data fusion.
- We prepared our standard solutions by dissolving 0.5 mg of vincristine (Vc) and 1 mg of vinblastine (Vb) in 1 mL of methylene chloride.

C. roseus total alkaloid extraction

Total alkaloids were produced by a number of different processes. Here is a rundown of the top five tried-and-true techniques:

Method I

In an hour, two percent tartaric acid was used to rehydrate five kilogrammes of *C. roseus* powder, and then nine litres of benzene were added. When the thirty minutes were over, we drained the liquid and reacted it with benzene. The extraction process was carried out three times. By distilling the benzene extract in a vacuum at 50 degrees Celsius, the volume was reduced to around 150 millilitres. Tartaric acid was employed to extract the last of the benzene in a vacuum at 50 degrees Celsius (2% in 300 ml of benzene extract). An acidic solution might be obtained and purified. Six separate 50 ml extractions of methylene chloride were performed on the filtrate. A vindoline-rich fraction (A) was obtained by washing the combined methylene chloride extract with water until free of acidity, drying it over anhydrous sodium sulphate, filtering it, and then concentrating it in vacuo at 50 °C until dry.

After removing the vindoline-rich fraction (A), we neutralised the residual acidic aqueous solution to a pH of 5.9 using a 25% NH4OH solution before extracting the alkaloids in four 500 ml volumes of methylene chloride. An aqueous citric acid (pH 2.5) solution (0.1 M, 750 ml) was used to extract the alkaloids. The alkaloids were extracted with methylene chloride (500 ml3), and the resultant clear acidic solution was neutralised with NH4OH solution (25%).

In order to get the vinblastine rich fraction (B), the mixed methylene chloride extract was vacuum dried at 50 °C. The extract was dried over anhydrous sodium sulphate to achieve this. A vincristine-rich fraction (C) with a pH of 5.9 was obtained by methylene chloride extraction of the freed alkaloids. The pH of the remaining aqueous layer was brought up from 4.4 to 5.9 with the help of an ammonia solution (25%).

Method II

Method I may be modified to extract 5 kilogrammes of dried, powdered *C. roseus* plant material in 10 litres of methanol (80%). The mixed methanolic extract was then evaporated in a vacuum at 50 degrees Celsius to remove the water. After dissolving the residue in a tartaric acid solution (2% in water), we used the same methodology described in method I to extract the vinblastine-rich fraction (D). The alkaloids were extracted in the same manner (described above for the pH 4.4 fraction in procedure I) to generate the vincristine-rich fraction (E). The pH of the remaining aqueous layer was brought down to 5.9 with the help of ammonia solution (25%).

Method III

An hour was spent infusing five kilogrammes of *C. roseus* plant powder in 95% methanol. After filtering, 500 ml of the methanolic extracts were concentrated in vacuo at 50 °C, 200 ml of water was added, 1 N sulfuric acid was added to reduce the pH to 2, and lastly, three extractions were performed using 500 ml of ethyl acetate. We threw away the EtOAc concentrate. Three extractions with 500 ml of methylene chloride were performed after the initial aqueous solution was brought to a pH of 6.4 using a 25% NH4OH solution. The alkaloidal fraction (F)

was prepared by first adjusting the pH of the combined methylene chloride extract with water, then drying it on anhydrous sodium sulphate, filtering it, and finally evaporating it at a lower pressure.

Method IV

For two days, 3.5 kilogrammes of air-dried plant powder was soaked in 30 litres of 80% methanol. A sticky residue was obtained after filtering the mixed methanolic extract and then evaporating it at 50 °C. A 5% HCl (750 ml) flush was used to eliminate any remaining gum. Chloroform was added after being refrigerated for 24 hours. After neutralising the very acidic solution with ammonia solution (25%, 200 ml), chloroform (5 1.5 L) was used to extract the solution. A crude alkaloidal combination (8.8 g) was obtained by drying the combined chloroformic extract over anhydrous sodium sulphate and then evaporating it at 50 °C under vacuum. In order to extract the alkaloids, eight grammes were mixed with fifty millilitres of chloroform, and then five hundred millilitres of phosphate buffer (pH 2). A total of 4.4 g of pure total alkaloidal combination was obtained when the chloroform layer was removed, dried over anhydrous sodium sulphate, and vacuum evaporated at 50 °C.

To facilitate dissolution, an additional 40 ml of chloroform and 60 ml of petroleum ether (both at a boil of 40-60 °C) were added to the alkaloidal combination. It was discovered that certain alkaloids precipitated. The alkaloid-rich precipitate was separated and discarded. At 50 °C, vacuum concentration of the filtrate left behind a sticky residue of 0.9 g. Phosphate buffer (pH 2) was used to recover the residue after it was dissolved in ethyl acetate (50 ml). After the aqueous layer was extracted using 3,200 ml of chloroform, the vindoline and catharanthine-rich fraction (G, 0.305 g) was obtained. After making the buffer layer alkaline (pH 10) using a 25% ammonia solution, the vinblastine rich fraction (H, 0.1225 g) was extracted with chloroform (3 200 ml).

Method V: Adsorption on charcoal column

One kilogramme of powdered *Catharanthus roseus* plant was mixed with three litres of 95% methanol and left to percolate overnight. After being filtered and vacuum concentrated at 50 degrees Celsius, the whole methanolic extract was diluted with 500 millilitres of water and acidified to a pH of 2 using 1N sulphuric acid. The aqueous acidic extract of the resinous substance was cooled for a whole night before filtering. A glass column 4 centimetres in diameter and 100 centimetres in height was used to filter the filtrate through 500 grammes of charcoal heated to 120 degrees Celsius for two hours. Mayer's reagent was used to check for the presence of alkaloids in eluates collected at different times to make sure the alkaloidal mixture was absorbed properly. The column was washed with 2 litres of distilled water before being eluted in a gradient of methanol concentrations: 500 millilitres of 30% methanol, 500 millilitres of 50% methanol, 500 millilitres of 70% methanol, and 500 millilitres of 100% methanol.

Without adding any additional solvents, the eluted fractions were concentrated by centrifugation at 50 degrees Celsius in a vacuum. The pH of the water was decreased to 6.4 using a 25% NH4OH solution, and the solution was extracted with methylene chloride (500 ml x 5). Following alkali-free washing of the combined methylene chloride extract with distilled water, drying over anhydrous sodium sulphate, and vacuum evaporation to dryness, the vincristine and vinblastine extremely rich fraction eluted by 70% methanol (I, 1.033 g) was identified.

Thin layer chromatography (TLC) was used to independently examine each of the produced fractions. Based on the data, we know that all four components are rich in pure alkaloid.

RESULTS AND DISCUSSION

Three litres of 95% methanol were percolated with one kilogramme of powdered *Catharanthus roseus* plant for a whole night. The entire methanolic extract was filtered, vacuum concentrated at 50 degrees Celsius, diluted with 500 millilitres of water, and acidified with 1N sulphuric acid to a pH of 2. After the resinous substance's aqueous acidic extract had been chilled for a whole night, it was filtered. The filtrate was filtered through 500 grammes of charcoal heated to 120 degrees Celsius for two hours in a glass column that was 4 centimetres in diameter and 100 centimetres in height. To ensure that the alkaloidal combination was absorbed adequately, the presence of alkaloids in eluates collected at various periods was determined using Mayer's reagent. Two litres of distilled water were used to clean the column before elution with a series of methanol solutions ranging from 30% to 70%. The final elution volume was 500 millilitres.

The eluted fractions were concentrated by centrifugation at 50 degrees Celsius in a vacuum without the addition of any additional solvents. We used a 25% NH4OH solution to lower the pH of the water to 6.4, and then we extracted the solution using methylene chloride (500 ml x 5). The vincristine and vinblastine extraordinarily rich fraction eluted by 70% methanol (I, 1.033 g) was isolated after the mixed methylene chloride extract was washed with alkali-free distilled water, dried over anhydrous sodium sulphate and vacuum evaporated to dryness.

Each of the separated fractions was analysed separately using thin layer chromatography (TLC). The statistics show that all four parts contain high concentrations of pure alkaloid.

Table 1: *C. roseus* of alkaloidal fractions: Quantitative determination (VB & VC rich fractions)

Method		I		II		III	IV		V	
Frations		Vindoline rich fraction (A)	Vinblastine rich fraction (B)	Vincristin rich fraction (C)	vinblastine rich fraction (D)	Vincristin rich fraction (E)	vinblastine rich fraction (F)	Vindoline and catharanthine	rich fraction(G)	Vinblastine rich fraction (H)	Vinblastine & Vincristin rich	fraction (I)
*% of alkaloidal	fractions	0.748	0.00274	0.0075	0.002	0.0015	0.1153	0.0087		0.0035	0.1033	
*HPLC % of Vb & Vc rich fractions	Vb	-	0.00399	0.00014	0.00253	0.0009	0.1417	-		0.0041	0.095	
	Vc	-	0.00012	0.00772	0.0007	0.00184	0.00017	-		0.0004	0.011	

*Relative to plant dry weight, Mean of duplicate analysis.

Vinblastine (Rf 0.54) was found in fractions B, D, F, and H from the five methods used to synthesise total alkaloids, while vincristine (Rf 0.21) was found in fractions C and E (Table 1). Fraction I mainly consisted of vinblastine and vincristine.

Under these conditions, HPLC analyses were performed on all fractions produced by the five methods of synthesising total alkaloids, and the findings are shown in Figure 1. Vinblastine and vincristine, the two actual alkaloids of relevance, have 3.77-minute differences in retention times.

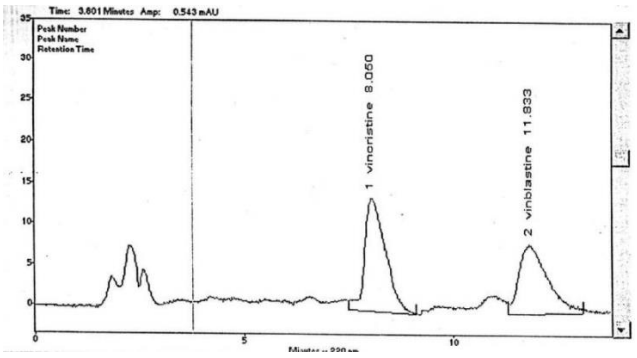

Figure 1: Vb and Vc as Standard

The fraction eluted from the charcoal column with 70% methanol (K) contained the desired alkaloids vinblastine (Vb) and vincristine (Vc), along with only six other alkaloids; this fraction was then subjected to VLC (5g on mixed bed column of equi portions of silica gel g for TLC and The desired alkaloids were found to be present in a ch. Co-chromatography with TLC, HPTLC, and HPLC was then used to further purify the 750 mg of target alkaloids present in the collected fractions. The mixture of chloroform and methanol was 95:5, by volume, and the flow rate was 6 ml/min.

CONCLUSION

It was shown that employing a charcoal column to isolate extremely pure versions of the total alkaloids, especially Vb and Vc, was very repeatable, even on a big industrial scale. The HPTLC findings were positive, falling within the expected range for the HPLC values.

REFERENCES

1. Satoshi Y, Toshihiro U, Satoshi K, Ayato S, Takeshi K, et al. (2012) Stereocontrolled Total Synthesis of (+)-Vinblastine J Am Chem Soc 124: 2137– 2139. Available: http://pubs.acs.org/doi/abs/10.1021/ja0177049. doi:10.1021/ja0177049
2. Sreekanth D, Syed A, Sarkar S, Sarkar D, Santhakumari B, et al. (2019) Production, purification and characterization of taxol and 10DAB III from a new endophytic fungus Gliocladium sp. isolated from the Indian yew tree, Taxus baccata. J Microbiol Biotechnol 19: 1342–1347. Available: http://www.ncbi.nlm.nih.gov/pubmed/19996685. doi:10.4014/jmb.0904.04041
3. Rangarajulu SK, Hyung JK, Byung-Ki H (2010) Taxol promising fungal endophytes, Pestalotiopsis species isolated from Taxus cuspidate. J Biosci Bioeng 110: 541–546. Epub 2010 Jul 15. Available: http://www.ncbi.nlm.nih.gov/pubmed/20634132. doi:10.1016/j.jbiosc.2010.06.007
4. Deng BW, Liu KH, Chen WQ, Ding XW, Xie XC (2009) Fusarium solani, tax3, a new endophytic taxol-producing fungus from Taxus chinensis. World J Microbiol Biotechnol 25: 139–143. DOI 10.1007/s11274-008-9876-2 Available: http://link.springer.com/content/pdf/10.1007/s11274-008-9876-2.pdf.
5. Kusari S, Zu¨hlke S, Spiteller M (2009) An endophytic fungus from Camptotheca accuminata that produces camptothecin and analogues. J Nat Prod 72: 2–7. Available: http://www.ncbi.nlm.nih.gov/pubmed/19119919. doi:10.1021/ np800455b
6. Azra AA, Babak DH, Hassan E, Ahmad M (2018) High in vitro production of anti-canceric indole alkaloids from periwinkle (Catharanthus roseus) tissue culture. Afr J Biotechnol 7:2834–2839. Available: http://www.academicjournals.org/AJB. Available: www.ajol.info/index.php/ajb/article/download/59172/47477.
7. Kharwar RN, Verma VC, Strobel G, Ezra D (2008) The endophytic fungal complex of Catharanthus roseus (L.) G Don Curr Sci 95: 228–233. Available: http://www.currentscience.ac.in/Downloads/article_id_095_02_0228_0233_0.pdf.
8. Eyberger AL, Dondapati R, Porter JR (2016) Endophyte fungal isolates from Podophyllum peltatum produce podophyllotoxin Amy. J Nat Prod 69: 1121–1124. Available: http://www.ncbi.nlm.nih.gov/pubmed/16933860. doi:10.1021/ np060174f
9. Zhou H, Tai Y, Sun C, Pan Y (2015) Rapid identification of vinca alkaloids by direct-

injection electrospray ionization tandem mass spectrometry and confirmation by HPLC-MA. Phytochem Anal 16: 328–333. Available: http://www.ncbi.nlm.nih.gov/pubmed/16223089. doi:10.1002/pca.852

10. Guttman A, Khandurina J, Budworth P, Xu W, Hou Y, et al. (2004) Analysis of combinatorial natural products by HPLC and CEZLGGC: Europe 17:39–41. Available: http://www.chromatographyonline.com/lcgc/data/articlestandard//lcgceurope/072004/84873/article.pdf.
11. Tikhomiroff C, Jolicoeur M (2012) Quantification of indole alkaloids and irridoid precursor in Catharanthus roseus hairy roots by HPLC. J Chromatogr A 955: 87–93.
12. Tung CY, Yang DB, Gou M (2012) A preliminary study on the condition of the culture and isolate of endophytic fungus producing Vincristine. J Chuxiong Normal Univ 6:39–41.
13. Favretto D, Piovan A, Filippini R, Caniato R (2001) Monitoring the production yield of vincristine and vinblastine in Catharanthus roseus from somatic embryogenesis. Semi quantitative determination by flow injection spray ionization mass spectrometry. Rapid Commun Mass Spectrom 15: 364–369. Available: http://www.ncbi.nlm.nih.gov/pubmed/11241768.
14. Zhang LB, Gou LH, Zeng SV (2010) Preliminary study on the isolation of endophytic fungus of Catharanthus roseus and its fermentation to produce product of therapeutic value. Chinese Trad Herb Drugs 11: 805–807.
15. Guo B, Kunming LH (2021) A middle vinblastine fungi isolated. J Yunnan Univ 20: 214–215
16. Aniruddha D, Srivastava PS (2021) Variation in vinblastine production by Catharanthus roseus, during in vivo and in vitro differentiation. Phytochem 46: 135–137. Available: http://dx.doi.org/10.1016/S0031-9422(97)00165-9. Available: http://www.sciencedirect.com/science/article/pii/S0031942297001659.
17. Chu I, Bodnar JA, White EL, Bowman RN (2020) Quantification of vinblastine and vincristine in Catharanthus roseus plant by capillary zone electrophoresis. J Chromatogr A 755: 281–288. b Goldsmith Seeds, Inc., P.O. Box 1349, Gilroy, CA 95021, USA. Available: http://dx.doi.org/10.1016/S0021-9673(96)00614-0. Available: http://www.sciencedirect.com/science/article/pii/S0021967396006140.
18. Strobel GA, Yang XS, Sears J, Robert K, Sidhu RS, et al. (2020) Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology 142: 435–440. Available: http://www.ncbi.nlm.nih.gov/pubmed/8932715. doi:10.1099/13500872-142-2-435
19. Volkov SK (2020) Methods of analysis of some antitumor- active alkaloids from Catharanthus roseus. Pharm Chem J 30: 391–399. Available: http://link.springer.com/article/10.1007%2FBF02219327.
20. Paulo RH, Heijden R, Verpoorte R (2019) Cell and tissue cultures of Catharanthusroseus: A literature survey. Plant Cell Tiss Organ Cult 42: 1–25. Available: http://link.springer.com/article/10.1007%2FBF00037677.
21. Nelson PE, Dignani MC, Anaissie EJ (2019) Taxonomy, biology, and clinical aspects of Fusarium species. Clin Microbiol 7: 479–504. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC358338/.

Corresponding Author

Ashishdeep Kaur*

Research Scholar