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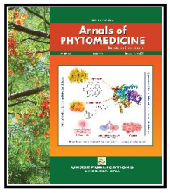

Review Article : Open Access

An updated review on biotechnological approaches for biosynthesis of anticancerous alkaloids, vincristine and vinblastine from *Catharanthus roseus* (L.) G. Don

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Abstract

Cancer is one of the most prevalent causes of death in present era, accounting for over 9.7 million deaths each year. It occurs as a result of genetic, environmental, and lifestyle factors. Current treatment methods like chemotherapy, radiotherapy, and surgery often come with serious side effects for patients and incur high costs. Herbal medicines and their products are considered a better alternative to overcome these barriers. Leaves of *Catharanthus roseus* (L.) G. Don (Apocynaceae), is an exclusive natural source of the anticancerous alkaloids, vinblastine and vincristine. These alkaloids are widely used to treat different malignancies, including leukemia, lymphomas, neuroblastoma, Wilms tumour, Ewing's sarcoma, multiple myeloma, rhabdomyosarcoma, brain tumours, breast cancer, testicular cancer, lung cancer, bladder cancer, and choriocarcinoma. However, the plant produces only trace amount of these compounds, making the situation hard to meet the surging demand. Scientists have been studying the biosynthesis of these alkaloids in plants to understand their importance and its potential for commercial use. Various biotechnological approaches aid in increasing the production of these alkaloids either through tissue culture techniques or through genetic engineering approaches, which manipulate the genes for the overexpression of these alkaloids. This review discusses the different methods of extracting these pharmaceutical compounds, their market value, the biosynthetic pathways, and various biotechnological approaches, such as *in vitro* culture techniques, metabolic engineering, and genetic modifications, to increase their production, including advancements in omics technologies.

1. Introduction

Catharanthus roseus (L.) G. Don, widely known as Madagascar periwinkle, is a member of the Apocynaceae family. The plant is indigenous to Madagascar Island and is medicinally important crop cultivated throughout India. There are nine different species under the genus *Catharanthus*, such as *C. lanceus* (Bojer ex A.DC.) Pich., *C. trichophyllus* (Bak) Pich., *C. longifolius* Pich., *C. pusillus* (Murr.), *C. scitulus* Pich., *C. coriaceus* Markgr., *C. ovalis* Markgr., *C. roseus* with the most recently identified species being *C. makayensis* (Allorge *et al*., 2015). *C. roseus* is the sole species reported to produce vinblastine and vincristine; other species may generate precursor alkaloids like vindoline and catharanthine but show no confirmed presence of these dimeric anticancer compounds (Verma *et al.*,2007). It is a persistent, evergreen herb or small herb which have a diploid genome and chromosome number 2n = 16. All parts of this plant (Figure 1) are rich sources of alkaloids in different proportions. According to the Global Bioresource Information facility, there are approximately 23,645 observations of *C. roseus* worldwide (Figure 2), with hotspots in India (5,447), followed by the USA (2,419), Italy (1,430), Mexico (1,231), Australia (972), and the Philippines (860) (Figure 2) (GBIF, 2025).

In India, it is widely distributed across India in the Northwestern and Northeastern Himalayas, the Western and the Eastern Ghats,

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the west and east coasts, the central Deccan Plateau, and the Indo-Gangetic Plain including states such as Assam, Bihar, Uttar Pradesh, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, and Tamil Nadu (Das *et al*., 2020). Periwinkle synthesizes two essential alkaloid compounds, like vinblastine and vincristine and their need remains higher in the therapeutic industries due to their valuable anticancerous properties. These compounds are mainly obtained from leaves of *C. roseus* and used to cure numerous types of carcinoma, including neuroblastoma, Hodgkin lymphoma, breast carcinoma, lung carcinoma, skin carcinoma, and blood carcinoma (Omino 1996; Jaleel *et al*., 2009; Kalidass *et al*., 2010; Aslam *et al*., 2010; Le Roux and Guéritte, 2016; Shala and Deng, 2018; Sharma *et al*., 2020; Singh and Singh, 2021; Arif *et al.,* 2022; Vasanthkumar *et al.,* 2024). In addition to vinblastine and vincristine, *C. roseus* has a varied range of bioactive compounds in different plant parts. The petals include tricin (Flavones) vingramine, apparicine, catharanthine, vindoline, leurosine, lochnerine, coronaridine and mitraphylline, while the stem possess catharanthine, vindoline, leurosine and lochnerine. The roots are specifically rich in compounds which includes serpentine, ajmalicine, and reserpine, that possess therapeutic effects and are used to relief of hypertension, diabetes, anxiety disorders, and cardiovascular diseases (Shanks *et al.,*1998; Ferreres *et al.,* 2008; Zhou *et al.,* 2009; Azharhusain *et al.,*2022; Chaturvedi *et al.,* 2022).

Figure 1: Morphological features of *C. roseus.* A: Completely matured *C. roseus* plant in its flowering stage; B, C and D: Flowers in the bloom of "Rosea" (pink), "Ocillata"(white with a rose-purple spot in the centre) and "Alba" (white) cultivars; E: Oval to elongated shape leaves; F: Stem, anthocyanin pigmentation in 'Rosea' cultivar; G: A close-up view of fruits composed of a pair of long, narrow seed pods; H: Dehisced matured fruit busted and seeds.

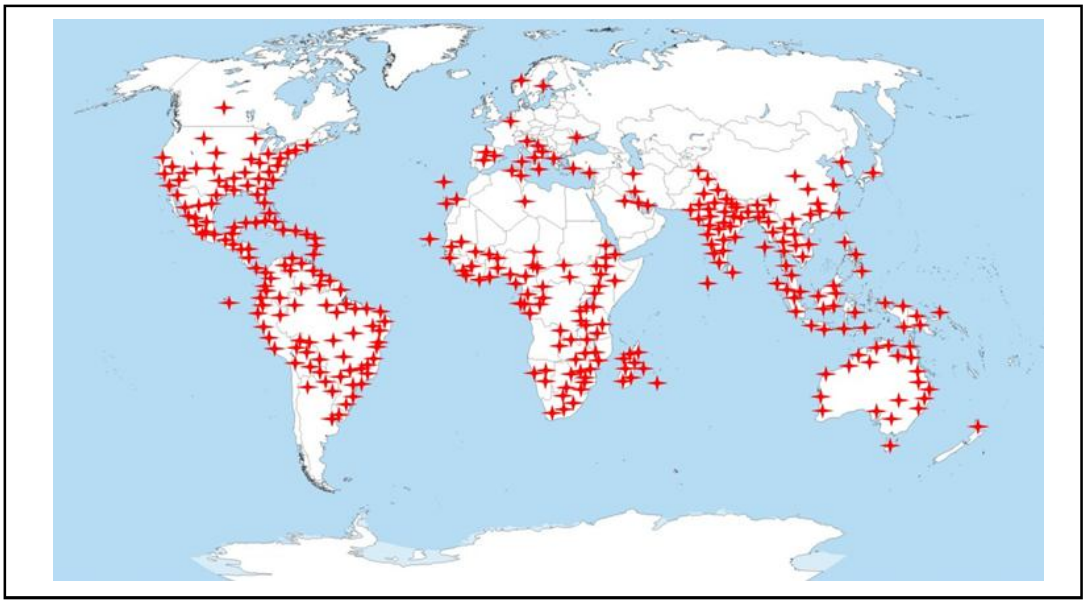


Figure 2: Global distribution and hotspots of *C. roseus* (Source: The Global Biodiversity Information Facility).

Researchers from Canadian state, Robert Noble and Charles Beer discovered compounds in *C. roseus* during 1950s. The total alkaloid content in leaves was 1%, while in roots it was 2-3%, nearly 9% in root fibre, 0.48% in the stem, 0.40% in the fruits, 0.18% in the seeds, and 1.14% in the pericarp (Karthikeyan *et al*., 2008). The chemical formula as well as molecular structures of vincristine and vinblastine are shown below (Figure 3). These alkaloids bind to tubulin, preventing microtubule polymerization then it blocks spindle formation, stop the cell cycle, and induce apoptosis, inhibiting tumour cells growth (Figure 4) (Jordan *et al*., 1992; Toso *et al*., 1993; Leveque and Jehl, 2007; Arif *et al.,* 2022; Vasanthkumar *et al.,* 2024). Besides *C. roseus*, various other plants also produce alkaloids with anticancer properties. These include camptothecin from *Camptotheca acuminata*, berberine from Berberis species, sanguinarine from *Sanguinaria canadensis*, evodiamine (*Evodia rutaecarpa*), noscapine (*Papaver somniferum*), matrine (*Sophora flavescens*), and homoharringtonine (Cephalotaxus species) (Potmesil and Pinedo, 1994; Lopes *et al.,* 2013). The yields of these two alkaloids are minimal, ranging from 0.0002 to 0.0005 % dry weight. Vincristine, for instance, is more expensive than vinblastine because of its lower natural abundance, complicated extraction procedure, and high need for cancer therapy (Jacobs *et al.,* 2002; Caputi *et al.,* 2018). To overcome this, vinblastine, which is found in higher amounts, is now used to produce vincristine in the pharmaceutical industry (Noble, 1990; Sharma, 2021). Furthermore, these compounds production and potency can be improved through current biotechnological developments.

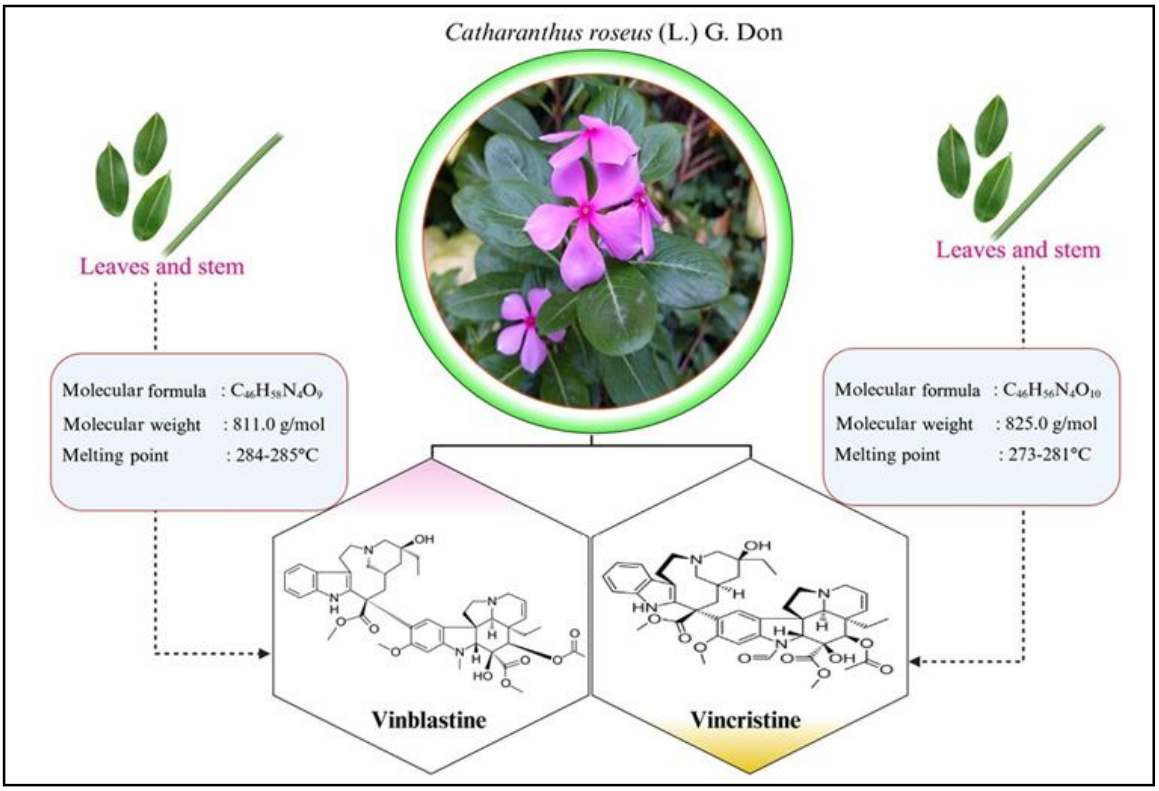
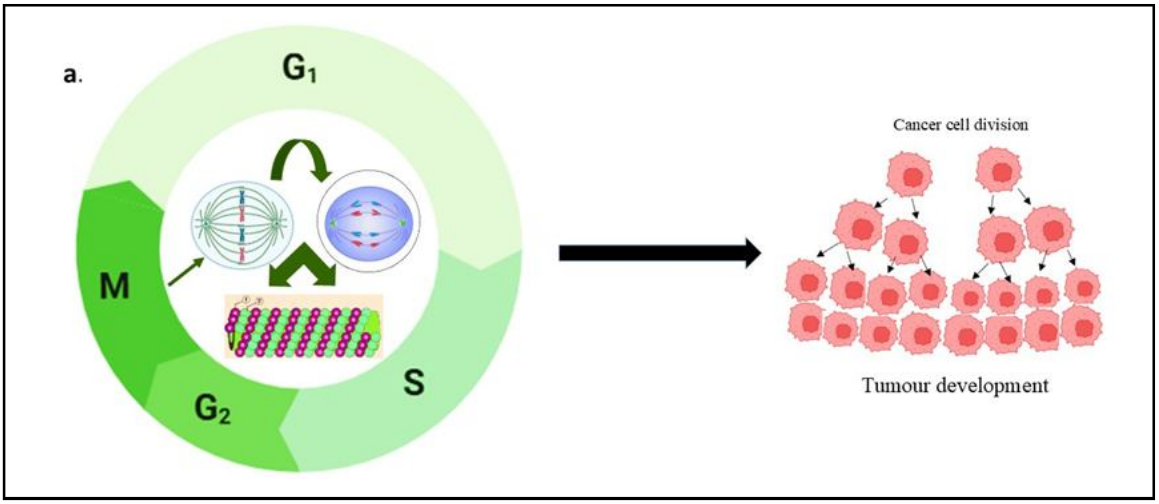


Figure 3: Chemical structure of vincristine and vinblastine.



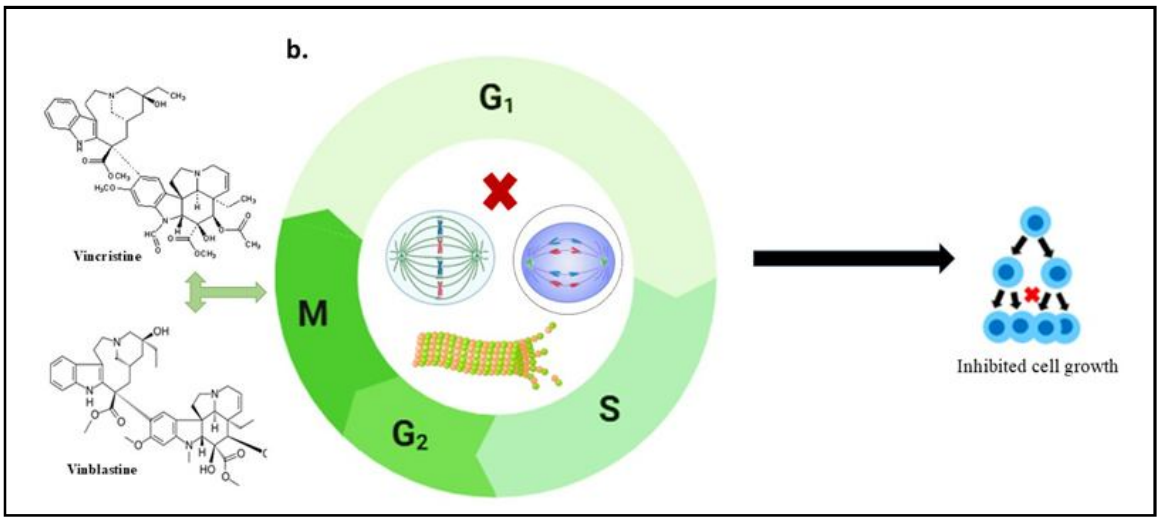


Figure 4: Mechanism of action of vincristine and vinblastine.

The cell cycle contains four phases: G_1: Gap 1, S: Synthesis, G_2: Gap 2, and M: Mitosis. a. During normal mitosis, microtubule polymerization helps chromosomes line up properly as the cell moves from metaphase to anaphase, supporting cell division as well as tumour growth; b. Inhibition of mitosis by Vinca alkaloids, Vincristine and vinblastine bind to tubulin, inhibiting microtubule polymerization. This blocks spindle formation, arrests the cell cycle, and induces apoptosis, preventing tumour cell growth (Dhyani *et al*., 2022).

2. Discovery of vincristine and vinblastine

In mid-1950s, two investigation teams, one at the University of Western Ontario in Canada and the other at the Eli Lilly Company in Indianapolis, USA, explored the traditional application of periwinkle as a potential treatment for diabetes. Although, they could not confirm its hypoglycemic effects in rabbits, they discovered that periwinkle leaf extracts exhibited remarkable anticancer properties, including a reduction in white blood cell count in leukemia-affected rats and an increase in their survival rate (Noble *et al*., 1958; Svoboda, 1975). The US Food and Drug Administration first isolated and approved these compounds in the 1960s under the brand names Oncovin (vincristine) and Velban (vinblastine) (Svoboda *et al*., 1975; Aslam *et al.,* 2010).

3. Market value of vincristine and vinblastine

It is estimated that retail prices for vinblastine and vincristine are 2 and 15 million dollars (USD) per kilogram, respectively. The source of these compounds is inadequate due to the exposure of the plant globally. The yearly market worth of vincristine and vinblastine ranges from USD 1,000,000-3,500,000/kg. The expenses of the drugs, Velban and Velsar, prepared from vinblastine sulphate, are USD 2/mg, which were applied in the cure of various cancers such as testicular cancer, Hodgkin's disease, and Kaposi's sarcoma. Likewise, vincristine sulphate (Oncovin and Vincasar PFS) is priced at $15/mg or $15 million/kg, used to cure childhood leukaemia (Alam and Sharaf-Eldin, 2016). The global market for *Vinca* alkaloid compounds, which includes vincristine and vinblastine, was priced at approximately USD 115.82 million in 2023, USD 126.35 million in 2024, and is estimated to produce at compound annual growth rate (CAGR) of 9.18%, reaching a predictable USD 214.21 million by 2030. Moreover, market survey reports from 2024 show an increasing demand for catharanthine in the nutraceutical sector, in addition to its use in producing anti-cancer drugs (Research and Markets, 2024).

4. Biosynthetic pathway of vinblastine and vincristine

Vinblastine and vincristine are synthesized through a sequence of enzyme-catalyzed reactions (Figure 5) in multiple locations including cytosol (16-hydroxytabersonine 16-O-methyltransferase, deacetoxy-vindoline 4-hydroxylase, and deacetylvindoline 4-O-acetyltrans-ferase), endoplasmic reticulum (tabersonine 16-hydroxylase), chloroplasts thylakoid membrane (N-methyltransferase), and vacuole (peroxidase) (Costa *et al*., 2008; Guirimand *et al*., 2011; Liscombe and Õ Connor, 2011). The dimeric indole alkaloids, vinblastine and vincristine originate from combination of two monomeric compounds, vindoline as well as catharanthine. This coupling process is catalysed by a key type of vacuolar III peroxidases (CrPrx1) (El-Sayed and Verpoorte, 2007; Costa *et al*., 2008). These monomeric compounds produce anhydrovinblastine, a reduction product obtained as a result of peroxidase activity. All these dimeric alkaloids have the common precursor α-32, 42-anhydrovinblastine and it is catalyzed by CrPrx1 (Sottomayor *et al*., 1998), which subsequently converts into vincristine and vinblastine through various steps (Zhu *et al*., 2015).

5. Extraction procedure of anticancer alkaloids from *C. roseus*

Various extraction and analysis methods for isolating vinblastine and vincristine have been reported. Supercritical fluid extraction is a common method to extract these alkaloids (Choi *et al.,* 2002; Falcão *et al*., 2017; Mandal *et al*., 2022). A supercritical fluid extraction method which uses chemicals such as carbon dioxide (CO_2), methanol with diethylamine, and methanol with triethylamine (10%, v/v) at various concentrations was used in a supercritical fluid extractor (SFX 3560 model) at a pressure range of 10.2-34.0 MPa, at 80°C, and for a static duration of 50 min. After extraction, the analyte was collected, solvent was removed, and the leftover compounds were dissolved again in 1 ml of methanol for further analysis (Choi *et al*., 2002). Supercritical fluid extraction with CO_2 and 2% ethanol resulted in 92.41% higher alkaloid concentration at 40°C at an optimal pressure

of 300 bar (Falcão *et al*., 2017). Vinblastine was extracted at a specific temperature of 35°C to 60°C, with pressure set between 100 and 300 bar using 2% ethanol as a co-solvent. An alternate method for extracting vinblastine at high pressure involves using a combination of ethanol and carbon dioxide (Mandal *et al*., 2022). There are multiple benefits of supercritical fluid extraction, which include non-toxicity, non-mutagenicity, low viscosity, high diffusivity, and low energy consumption. However, it has some drawbacks like the requirement of high pressure, high expense, and inadequate solubility of CO_2 (Khastan and Al-Athary, 2016).

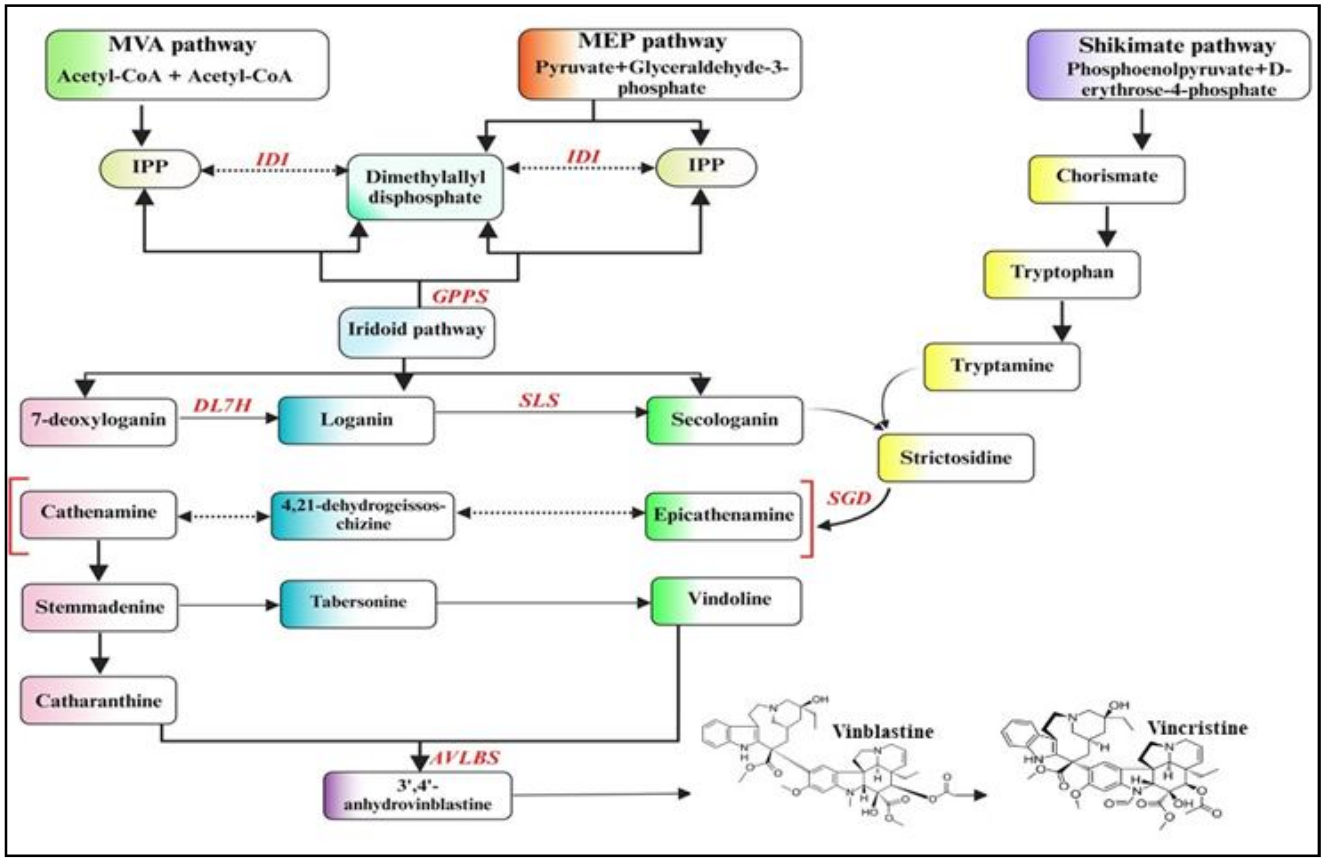


Figure 5: Terpenoid indole alkaloid synthesis pathway of *C. roseus*. Enzyme abbreviation stands IPP: isomerase, IDI: isopentenyl diphosphate isomerases, GPPS: geranyl diphosphate synthase, DL7H: deoxyloganic acid hydroxylase, SLS: secologanin synthase, SGD: strictosidine b-glucosidase. Broken arrows indicate various steps or uncharacterized enzymatic conversions (Adopted from El-Domyati *et al.* (2017).

Ultrasound method of extraction of vinblastine was reported by Verma *et al*. (2007). Vinblastine was extracted by treating 1g dried leaves with 100 ml of 0.1 M HCl solution in an ultrasonic bath for 30 min and then centrifuged for 10 min at 2000 rpm. Then, obtained pellet was re-extracted for 30 min using 100 ml of HCl. Following filtration, petroleum ether was used to eliminate the chlorophyll from the extract. Following the separation of the acidic and alkaline fractions, 10% embonic acid was gradually added to bring the pH down to 5.0 (Verma *et al.,* 2007). 500 mg of air-dried, powdered leaves were soaked in 5 ml of various solvents for an h at room temperature, they were placed in an ultrasonic bath at 50°C for 30 min and centrifuged for 20 min at 8,000 rpm. A 0.45 µm nylon filter was employed to filter the supernatant (Mihkel *et al.,* 2020). Another common method to extract vinblastine and vincristine is microwave-assisted extraction (Javid *et al*., 2016). 1g of powdered *C. roseus* leaves, 10 ml of solvents (ethanol and water were used separately), and 0.2 ml of HCl were combined for 30 to 90 sec at 500, 700, and 900 W microwaves. Using an ultrasonic device, 0.1 g of dried powdered leaf material was combined with 5 ml of methanol for 1 h at 30°C. The residue of the compound was then centrifuged for 15 min at 10,000 rpm at ambient temperature to obtain vincristine and vinblastine (Paul *et al.,* 2022). Dried leaves of 10g were soaked in water of 200 ml for 24 h at 40°C, these two alkaloids were separated using a shaker water bath. The extract was then dried after the mixture was centrifuged for 30 min at 3600 rpm (Abdul-Rahim *et al.,* 2018).

Alkaloids were also taken out from some other natural (endophytic fungi) sources (Abdusamatov *et al.,* 2024) like *Fusarium oxysporum* (Kumar *et al*., 2013; Ashraf *et al*., 2021) and *Chaetomium globosum* Cr95 (Zafari *et al*., 2019). Vincristine was extracted from the endophytic fungus *Nigrospora zimmermanii* (Birat *et al*., 2022). Incorporation of endophytic fungi, particularly *Fusarium solani* RN1 and *Chaetomium funicola* RN3, greatly boosted the production of alkaloids, including vinblastine (Linh *et al*., 2021). As elicitors, fungal extracts from *Piriformospora* and *Trichoderma* can influence the synthesis of secondary metabolites in *C. roseus* cell suspensions (Ramezani *et al*., 2018).

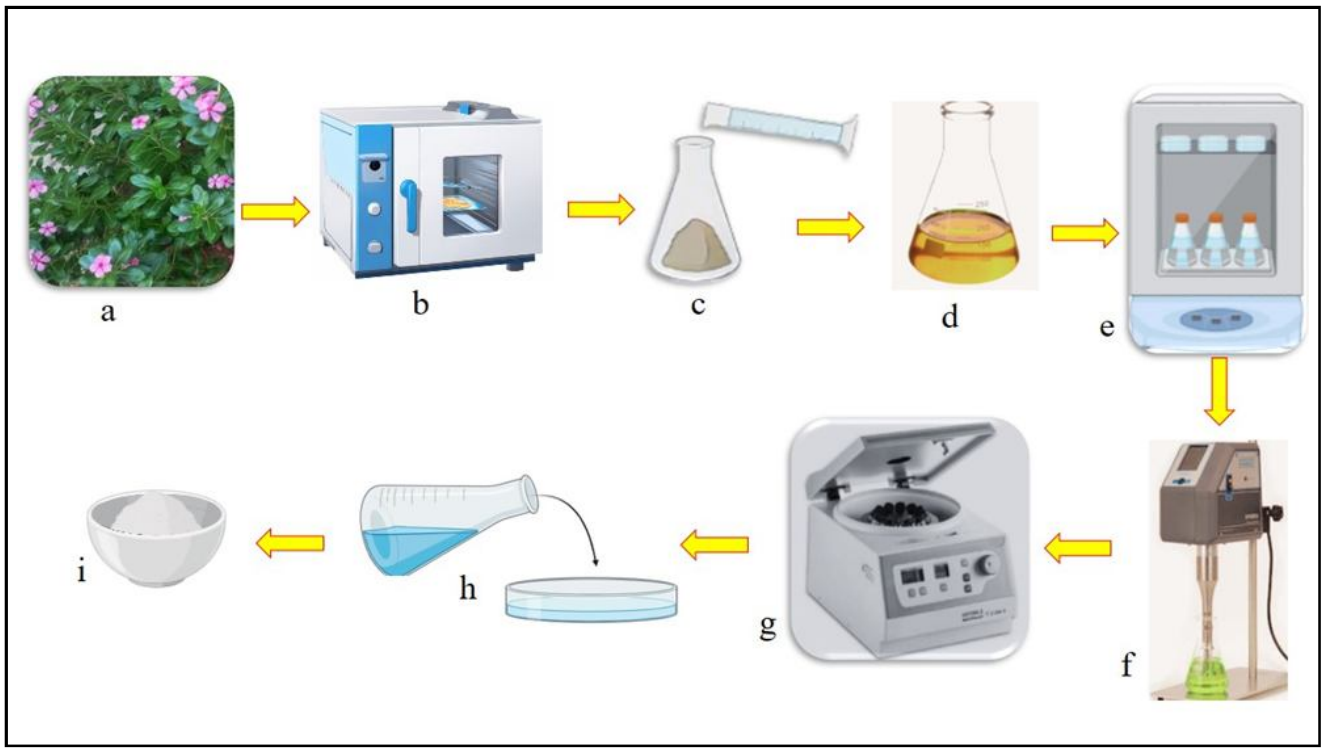


Figure 6: Schematic representation of the extraction process for vinblastine and vincristine. a. *C. roseus* plant; b. Hot air oven used for drying; c. Dried material mixed with solvent; d. Mixture prepared; e. Place in shaker for 15-30 mins; f. Ultrasonication; g. Centrifugation; h. Supernatant collected and allowed to dry in the refrigerator; i. Dried and powdered plant material.

6. Biotechnological approaches for alkaloid production

6.1 *In vitro* methods

In vitro production offers a controlled and sustainable approach to increasing the synthesis of beneficial secondary metabolites (Rao and Ravishankar, 2002). 2, 4-dichlorophenoxyacetic acid (2,4-D) encouraged the development of callus as well as the synthesis of vinblastine and vincristine compounds. When 0.5 mg/l kinetin and 1.0 mg/l 2, 4-D were applied, the utmost concentration of vincristine (0.7088 µg/g dry weight) was achieved. On the other hand, Solimani *et al.* (2013) found that 1.0 mg/l of 2, 4-D without kinetin produced the maximum vinblastine yield (0.7088 µg/g dry weight). Different auxin and cytokinin concentrations on callus development significantly increased the amount of alkaloid content. Using leaves as explants resulted in the maximum synthesis of total alkaloids in callus biomass at a concentration of 0.50 mg/l 2, 4-D, 1.0 mg/l BAP, and 6% sucrose (Verma *et al.,* 2012). *C. roseus* callus, roots, as well as petioles, were used to produce indole alkaloids at varying concentrations of kinetin and NAA (0.1, 5, 10, and 20 mg/l). The excessive synthesis of vindoline, catharanthine, and vincristine in MS medium was confined at 0.1 mg/l NAA and 0.1 mg/l kinetin (Ataei-Azimi *et al.,* 2008).

C. roseus leaf suspension and callus cultures can boost the production of alkaloids, such as vinblastine. The highest growth was obtained with 1.5 mg/l of 2,4-D and 1.5 mg/l of kinetin, which resulted 67-fold rise in callus growth (Linh *et al.,* 2021). Vinblastine and vincristine synthesis was enhanced in callus cultures of *C. roseus* cultivated on MS media with different growth hormones, such as Kin/IAA. Vinblastine levels were 3.39 times higher than those in the wild plant (Mekky *et al.,* 2018). *C. roseus* cultured *in vitro* on MS media with various PGRs such as auxins, cytokinins, gibberellic acid, abscisic acid, MeJA, salicylic acid, ethephon, chlormequat chloride, and triazoles had varying effects on alkaloid production. Among all these PGRs, salicylic acid, ethephon, chlormequat chloride, and triazoles significantly increased the synthesis of the anticancer alkaloids (Karur *et al*., 2017). *Trichoderma harzianum* and *sodium nitroprusside* applied in *C. roseus* cell suspension cultures taken from leaves, cultured in 8 µM 2, 4-D and 2 µM BAP significantly improved the synthesis of secondary metabolites (Farzaei and Sayyari, 2024).

Zygotic embryos and endosperm tissues of *C. roseus* cultured on MS media added with 2.5 µM BAP and 1.25 µM 2,4-D showed that endosperm-derived callus and cell cultures showed sustainable vincristine production (Patil *et al*., 2024). The effect of varying NaCl concentrations (0, 25, 50, and 75 mM) on callus growth and alkaloid production was studied and it was observed that high yields of total indole alkaloids, vincristine, and vinblastine were obtained from callus cultured with 50 mM NaCl (Abdulhussein Ihsan and Al-Hujamy, 2012). The maximum vinblastine quantity, reaching 0.81 mg/g dry weight was attained by adding specific elicitors and inhibitors such as 100 mg/l loganin, 100 mg/l tryptophan, 0.5 µmol/l benzotriazole, 20 µg/l hydrogen peroxide, 5 mg/l acetyl CoA, and 30 mg/l cerium chloride to the *C. roseus* cell culture medium (Guo *et al*., 2013). NaCl acts as an elicitor and increases the synthesis of alkaloids from *C. roseus* grown *in vitro* (Fatima *et al*., 2015). Low salt levels (25 mM) amended in MS medium increased vinblastine content in regenerated leaves (14.17 mg/g dry weight) and vincristine

accumulation (5.12 mg/g dry weight) in tissue culture. These studies revealed that salinity stress effectively stimulates alkaloid synthesis in *in vitro* grown embryogenic tissues.

Potassium in the form of KNO_3 significantly increased the vinblastine and vincristine levels compared to K_2SO_4. Suspension culture showed two to three times greater alkaloid amount and yield when compared to callus cultures grown on MS or B5 agar medium (Mishra *et al.,* 2018). Vinblastine and vincristine synthesis in *C. roseus* were increased by *Aspergillus flavus* elicitors (Tonk *et al.,* 2016). The callus obtained from the hypocotyls of seeds that germinated *in vitro* exhibited the highest growth and alkaloid yield of 0.15%, with vinblastine yielding 7.88% and vincristine yielding 15.50%. Leaf-induced callus tissues with addition of 1 mg/l 2,4-D and BAP achieved the maximum callus of 3.276 g. Abiotic factors, including drought and sucrose, significantly enhanced vinblastine and vincristine production, with sucrose at 40 g/l increasing the callus fresh and dry weights (Khashan and Athary, 2016).

Chitosan is found to impact the vincristine and vinblastine yields in cell suspension cultures after 14 days of initiation. The highest quantities of vinblastine and vincristine with values of 4.15 and 5.48 µg/mg dry weight were exhibited at stationary phase with 100 mg/l chitosan (Pliankong *et al.,* 2018). CaCl, acts as an elicitor in embryogenic cell suspension cultures, increasing vinblastine synthesis by 1% to 17.99% (Siddiquia *et al.,* 2023).

Drought stress led to a significant increase in compound concentrations in *C. roseus*, particularly during the first two weeks, compared to well-watered controls (Yahyazadeh *et al*., 2021). *C. roseus* cell suspension cultures under salinity (150 mM NaCl) and water stress (12% polyethylene glycol) conditions significantly enhanced the total alkaloid production and also yield (Mishra *et al*., 2019).

Hairy root culture of *C. roseus* treated by a precursor (1 µM phenylalanine) yielded the maximum vincristine and vinblastine in the 7th week, with a dry weight biomass of 0.306 g, using *Agrobacterium rhizogenes* strains (Vu *et al*., 2022). Use of *A. rhizogenes* enhanced production of vincristine and catharanthine compared to normal root cultures (Hanafy *et al*., 2016). Changing the carbon source from sucrose to sorbitol in *in vitro* shoot cultures from *C. roseus* significantly improved the levels of catharanthine, vinblastine, and vincristine (Khataee *et al.,* 2020). Treating *C. roseus* tissues and plantlets with 1.5 g/l yeast extract as an elicitor resulted in a 22.74% rise in vinblastine as well as a 48.49% rise in vincristine content (Maqsood and Abdul, 2017).

Salicylic acid and other elicitors can increase the activity of important genes that produce vinblastine and vincristine, including chorismate mutase geraniol-10-hydroxylase, tryptophan decarboxylase, strictosidine synthase, secologanin synthase, desacetoxyvindoline-4-hydroxylase, and deacetylvindoline-4-O-acetyltransferase (Soltani *et al.,* 2020). Combining methyl jasmonate and UV-B light increased the vinblastine levels peaked on the third day after MU60 treatment, whereas vincristine levels increased 6-fold after single exposure day (Rady *et al*., 2021). Likewise, UV-B light stress produced more synthesis of vincristine and vinblastine in *C. roseus* hairy roots (Lalaleo *et al*., 2016).

6.2 Genetic engineering approaches by overexpression of key pathway genes

Overexpression of the genes enhances the synthesis of targeted metabolites by increasing the expression of specific genes involved in the related biosynthetic pathways. Emerging approaches, such as nanotechnology-assisted genetic modifications, are being explored to enhance plant growth and metabolite accumulation (Husain, 2023). Overexpressing the CrTPT2 catharanthine transporter gene significantly increased the levels of catharanthine, by five times as that of control hairy roots (Wang *et al.,* 2019). Vinblastine production was increased by enhancing the metabolic flow of terpenoid indole alkaloid pathway. Gene expression levels were raised by agrobacterium transformation, CrTDC and CrSTR were enhanced by 38-fold and 65-fold respectively. The transgenic plants exhibited a 5-fold rise in vinblastine, a 2-fold rise in alkaloid production, and a 9-fold rise in vindoline and catharanthine levels (Sharma *et al.,* 2018). Overexpressing transcription factors like ORCA4 and ORCA5 in *C. roseus* hairy roots show a significant rise in ajmalicine, catharanthine, and tabersonine levels (Paul *et al.,* 2017, 2020). Overexpression of ORCA3 transcription factor, an essential precursor for the production of vinblastine and vincristine, regulates key genes of the terpenoid indole alkaloid biosynthetic pathway in *C. roseus* hairy roots, which contributed increased 2.49 in catharanthine and 4.2 times, increased in vindoline production (Tang *et al*., 2011). Treatment with 0.1 µg/ml 3 kDa chitooligosaccharides significantly upregulated key genes in terpenoid indole alkaloid pathway (SLS, STR, DAT, PRX1, and ORCA3) leading to enhanced accumulation of vindoline and catharanthine (Tang *et al*., 2022). CrERF5, an AP2/ERF transcription factor, adjusts the monoterpene indole alkaloid biosynthesis pathway in *C. roseus*. CrERF5 activates the TDC promoter when it detects ethylene and jasmonic acid signals in the nucleus.

Overexpressing CrERF5 in petals of *C. roseus*, improved the expression of important biosynthetic genes and enhanced the concentration of monoindole alkaloids (ajmalicine, vindoline, catharanthine) and bisindole alkaloids (anhydrovinblastine, vinblastine) (Pan *et al.,* 2019). Overexpression of CrMYC1, a key transcription factor, significantly enhanced the production of alkaloids in *C. roseus* with a 3-fold rise in catharanthine and a 2.5-fold rise in vinblastine (Sazegari *et al*., 2018). Likewise, 2,4-D, tryptophan, and IAA treatments enhanced the expression of key biosynthetic genes in *C. roseus,* in which 2,4-D increased tdc, str, and per 1 gene; tryptophan upregulated tdc, str, t16h, d4h, and dat; and IAA boosted d4h and dat. Thus, overexpression of these genes in *C. roseus* leaves may increase the production of vinblastine and vincristine (El-Domyati *et al*., 2014).

Transgenic *C. roseus* cv. Dhawal was developed by overexpressing the apoplastic peroxidase gene (CrPrx) using *Agrobacterium rhizogenes* infection. Fifteen independent hairy root clones (NV-C1 to NV-C15) were developed, with the NV-C7 clone showing overexpression of CrPrx. The NV-C7 root tissues showed 2 to 2.54-fold higher mRNA levels of strictosidine synthase and tryptophan decarboxylase compared to the controls (Mathur *et al*., 2022). Hub genes (ALP1, hbdA and Helicase ATP-binding domain) are promising genetic engineering targets for increasing terpenoid indole alkaloid production (Tabatabaeipour *et al.,* 2024). A new and improved Virus-Induced Gene Silencing (VIGS) technique for *C. roseus*, has been developed, and this method combines the target gene and a visual

marker gene into the same plasmid, allowing successful identification of silenced tissues directly in the plant (Yamamoto *et al*., 2021). Thus, genetic and metabolic engineering can improve the production of terpenoid indole compounds in *C. roseus,* and in the near future, scientists can explore advanced gene-editing techniques to identify new genes that are helpful in the production of these useful medicinal alkaloids.

7. Omics technologies

Omics technologies state to high-throughput methods like genomics, transcriptomics, proteomics, and metabolomics, which are referred to study the genes, RNA, proteins and metabolites. Multi-omics technologies were used to study monoterpene indole compound biosynthesis in *C. roseus* by identifying gene clusters on chromosomes, a secologanin transporter, and cell-type-specific roles in the pathway. Combining single-cell RNA sequencing with metabolomics revealed an involved reductase in the production of bis-indole alkaloid anhydrovinblastine (Li *et al*., 2023). UV-B radiation increased ATP export in the leaves through a protein called mitochondrial ATP synthase, and UV-B activated the methylerythritol phosphate pathway, which helps to produce monoterpene compounds (Zhong *et al*., 2021). Single-cell RNA sequencing and metabolomics together showed different metabolite patterns in monoterpene indole alkaloid biosynthesis across various plant organs (Dangwal *et al*., 2024). Metabolomics with advanced UPLC-ESI-QTOF-MS analysis studied the impact of LED light on *C. roseus* alkaloid synthesis (Nagy *et al.,* 2023). High blue LED light significantly increased the levels of vinblastine, vincristine, and 32, 42-anhydrovinblastine by up to 15-fold compared to normal conditions.

Transcriptome Illumina HiSeq sequencing was used to study the genes that are active in *C. roseus* cambial meristematic cells and dedifferentiated cells. Differentially expressed 9,564 genes were found between the two cell suspension types, the hormones and the MAPK signalling pathways were found to regulate the alkaloid production. The cambial meristematic cells are much more useful for the biosynthesis of alkaloids like vinblastine, vindoline, and ajmalicine than dedifferentiated cells (Zhou and Chen, 2022). Omics technologies, including transcriptomics, homology-based identification, and guilt-by-association analysis were used to study the monoterpene indole alkaloid biosynthesis pathway, which resulted to the discovery of Vm16OMT as an essential gene in this pathway (Stander *et al*., 2020).

8. Conclusion

Cancer is one of the most fatal health issues in the world, and medicinal plants like *C. roseus* are essential to fight the disease using life-saving treatments that use their alkaloid content in leaves. The plant *C. roseus* contains many natural compounds, especially the alkaloids vincristine and vinblastine, which effectively treat different types of leukaemia and tumours. The extraction methods discussed in the review have made these important drugs more accessible and easier to produce. In addition, new laboratory methods, such as bioreactors and cell cultures, can help to produce vincristine and vinblastine sustainably on a large scale. Another promising approach is the utilization of genetically modified microorganisms, such as yeast and bacteria, to synthesize these alkaloids. Using advanced biotechnological tools and innovative strategies will ensure that these important drugs are more affordable and accessible for cancer treatment. Research focuses more on the catalytic enzymes that produce vincristine and vinblastine. Since several enzymes remain unknown and require further comprehensive multi-dimensional research. Biotechnological tools other than tissue culture techniques can be explored to widen the scope of biopharming. Genes/ QTLs governing morphological traits like increased biomass and the genes involved in alkaloid production can be identified using different approaches of gene mapping and mutation mapping. Advanced genome engineering approaches like genome editing, and base editing can manipulate the genes to make plants produce more alkaloids through their natural pathways. New omics technologies are helping researchers understand the complex steps involved in producing these compounds and these technologies can identify missing enzymes, uncover gene networks, and improve production efficiency.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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