*THINK INDIA JOURNAL*

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

# *Catharensus Roseus* Based Alkaloids: Applications and Production

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**Abstract**

Alkaloids are sole or key components for many natural herbs based drugs. Alkaloids play important role in alleviating ill effect of many human disease. *Catharanthus roseus* is an herbaceous plant having ornamental and medicinal value. It has been part of traditional herbal medicine. Crude extract obtained from different plant parts containing alkaloids as well as other phytochemicals has been documented for various therapeutic purposes. Purified alkaloids and their derivatives have now being well characterized as anticancer, anti-diabetic and antioxidant. Under natural conditions the production of alkaloids in wild species is low. This is responsible for low availability and high price of resulting drugs. Routine breeding, tissue culture, metabolic engineering and mutagenesis approaches can be employed to improve yield of alkaloids. There are some concerns regarding safety of alkaloids based medicines that can be solved using in-depth toxicity evaluation. Further, chemical modification of existing alkaloids to more safe medicines is recommended.

## 1. Introduction

Plants contain large number of diverse primary and secondary metabolites. Primary metabolites have always been considered most important as they play a major role in plant

growth, development and reproduction. With advances in healthcare sector and techniques available to test the potential of plant secondary metabolites for therapeutic purpose, secondary metabolites became equally valuable to mankind. Plant secondary metabolites possess diverse structural and chemical diversity. The chemistry of these diverse natural products plays an important role in plant metabolism, physiology and appearance. The secondary metabolites also protect plants from predation and various natural adversities. Most of the plant metabolites have been used to treat one or another disease. There are wide ranges of plant secondary metabolites. More than 100,000 secondary metabolites have already been reported from various plant species. Secondary metabolites biosynthesis and its accumulation therafter occurs in a variety of cell types, epidermal cell, mesophyll cells, pericycle, phloem and companion cells. Identification of cells producing secondary metabolites is useful for their industrial scale production as the plant cells cultured *in vitro* are capable to synthesize and hyper accumulate secondary metabolites [1]. Among these there may be more than 12000 alkaloids [2-6]. Among various plant secondary metabolites, alkaloids are one class that has been part of human diet and various medicinal herb based formulation for long time. Not all the alkaloids are beneficial; some may have harmful effects [7, 8]. Pyrrolizidine classes of alkaloids are widely distributed in plants commonly used for human diet. This group contains toxic as well as pharmacologically important alkaloids [9]. Likewise several alkaloids of quinolizidine class are known to be very toxic. In some of the cases, the toxicity of alkaloids has been associated with their bitter taste [10]. We are still not even sure about the role of alkaloids that are consumed by us in a day-to-day life. Sophisticated techniques are available to characterize various secondary metabolites including alkaloids, flavonoids, steroids, carbohydrates saponins, tannins, proteins, terpenoids, glycosides, oils, fats and waxes [11].

Alkaloids are pharmacologically active compounds produced by bacteria, fungi, plants and animals. Alkaloid extraction from various parts of medicinal plants has been a regular practice from ancient times. These compounds hold a great promise even today and are responsible for preventing and treating wide range of diseases like blood pressure, asthma, malaria, cancer menstrual problems and inflammation. These compounds have also been used for relieving pain, constipation and stress [8]. The potential of alkaloids may be much more than currently

 

explored. Hence, in this review we are going to explore the alkaloids from medicinal herb *Vinca rosea* (*Catharanthus roseus*).

### 2.1 Alkaloids from *Vinca rosea*

*Catharanthus roseus* (L.) G. Don was named by Scottish botanist George Don. Later in 1759 Carl von Linne named first species of this genus, *Vinca rosea*. There was long debate over its nomenclature and hence known by more than one names. *Catharanthus roseus* (L.) G. Don (synonyms, *Vinca rosea L.*) is a tropical perennial plant with 7 species native to Madagascar and 1 species is native to South Asia. Due to native availability of most of the species in Madagascar it is known as Madagascar periwinkle. The stem sap appears milky and has more than 70 different indole alkaloids of medicinal importance. Two of the common anti-cancer drugs which are derived from this plant are vincristine and vinblastine as named after *Vinca* [12, 13]. First study reporting the antitumor activity of the vinca alkaloids was reported from extracts of *Vinca rosea*. Authors isolated and tested more than 30 alkaloids and found that only four of these namely, vinleurosine, vinrosidine, vinblastine, vincristine with reasonable antitumor activity. Two of these, vindoline and catharanthine were documented to belong to new type of large complex dimeric alkaloids. This class of alkaloids contain both indole and dihydroindole groups [14].

For last four decades vinca type of alkaloids has been used for therapeutic purpose. Plant based alkaloids has also been explored for their curative effect on various neurodegenerative diseases. Specifically isoquinoline, indole, pyrroloindole, oxindole, piperidine, pyridine, aporphine, vinca, β-carboline, methylxanthene, lycopodium, erythrine and their modified products has been explored for neuroprotective action against diseases like Epilepsy, Parkinson's disease, Alzheimer's disease, stroke and Huntington disease [15].

Vinca derived compounds are successively being used due to their anti cancer potential. A number of semi-synthetic derivates have since been identified and tested. Some of these, vinflunine, vindesine and vinorelbine are identified as potent anti-cancerous drugs [16-18]. These alkaloids have also been used in combination with other chemotherapeutic agents [19]. Plant alkaloids have mainly been explored for therapeutic activity however, they also affect plant growth and development [20].

 

ISSN: 0971-1260

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### 2.2 Alkaloids: *Basic properties, structure and classification*

Alkaloids are heterocyclic nitrogen containing compounds derived from some of the primary metabolites and their derivatives. The term "alkaloid" was first used by W. Meißner in 1819 due to their alkali like appearance. The name came one and half decade after first alkaloid morphine was isolated by F. Sertürner [21].

### 2.3 Medicinal importance of Alkaloids from *C. roseus*

Alkaloids were initially considered as waste to plants. However, they play several regulatory roles as involved in seed formation and defense against insects. But still their exact role in plant growth and survival may be unexplored. Although, usefulness of alkaloids are well known to humans from ancient times. Currently, therapeutic potential of alkaloids have been variously reported and documented.

#### 2.3.1 Anticancer effect

*C. roseus* is a source of many anticancer alkaloids known as Vinca alakloids. Vinblastine, vincristine, vindesine, and vinorelbine are commonly found alkaloids of *C. rosea*. Vinca alkaloids are cell cycle dependent anticancer agents. These alkaloids interfere with the formation and growth of microtubules and thus prevent mitosis [22]. The vinca alkaloids are broad-spectrum mitotic inhibitors used to treat various form of malignancies [23]. Polychemotherapeutic approach is followed to treat neuroblastoma and Ewing sarcoma. In this approach, Vinca alkaloids are generally administered with cell cycle-arresting drugs. So, there is less assessment of individual abilities of Vinca alkaloids. Ehrhardt group found better anti-tumour response of Vinca alkaloids when administered individually without being used in addition to cell cycle arresting drugs. Interestingly, including caffeine in polytherapy restored the antitumour activity of Vinca alkaloids and led to knocking down of the p53 gene or

maintainenance of the active cell cycle [24]. Individual Vinca alkaloids are also being used separately as anticancer agents.

Vinblastine has been potentially used as anticancer agent, although it is also associated with some side effects like oxidative stress, disruption of intestinal tract and constipation [25, 26]. Vinblastine resistance has also been reported in some cancer cell lines. C20′-urea derivatives of vinblastine have been reported for anticancer activities against vinblastine resistant cancer cell lines [27, 28]. Vinblastine has also been reported to induce neurotoxicity that can be reduced by using caffeine [29]. Vinorelbine is a new semi-synthetic vinca alkaloid that is also known as Navelbine. It chemically differs from vinblastine by substitutions on the catharantine moiety. It has anticancer activity against non-small lung and breast cancer [30].

Vindesine, a deacetyl amide sulfate derivative of Vinblastine has been documented to be effective against Ridgeway osteogenic sarcoma and Gardner lymphosarcoma in rodent models. Parental alkaloid, vinblastine was ineffective against both kind of cancer [31]. It has been used for treatment of malignant melanoma, and head and neck cancer in combination with other chemotherapeutic agent cisplatin. Surgery is the only potential therapeutic strategy for curing non-small cell lung, head and neck cancer. However, recently vindesine has also been used for neoadjuvant chemotherapy to reduce cancerous tissue so that it can be completely removed with surgery thereafter. However, vindesine has poor anticancer activity against breast cancer [32, 33].

Vincristine is part of a front-line therapy for the treatment of acute lymphoblastic leukaemia [34]. In 1959, it was isolated from *C. roseus* and named leurocristine. While it was first used for humans in 1962 as a single-agent therapy in 13 patients from the National Cancer Institute by Karon group.

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

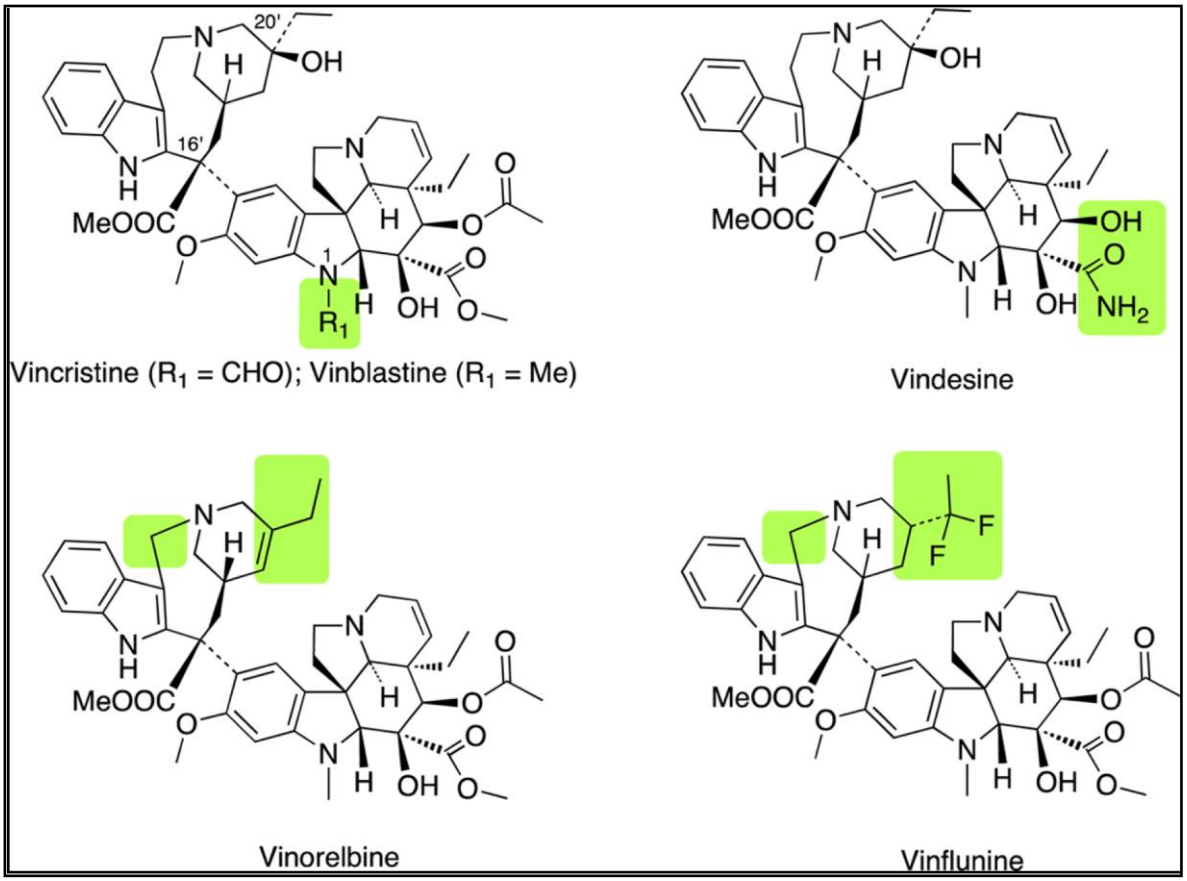


**Fig. 1 Common Vinca alkaloids of therapeutic use (Copyright ACS,** Silvestri, 2013) [25]**.**

Out of these 13 patients, 12 have acute lymphoblastic leukemia. More than 50% of the patients achieved complete diminution of the leukemia. New formulation for better drug delivery has been used by encapsulating vincristine in sphingomyelin-cholesterol liposome [35, 36]. It is commonly used in pediatric oncology due to being comparatively safe and effective in children. However, peripheral neuropathy is a common side effect in pediatric patients that limits the use of vincristine [37]. It is also used to treat multiple myeloma and lymphoma along with doxorubicin and dexamethasone [38, 39]. Liposome encapsulated vincristine has been found to have lower neurotoxicity and better anticancer activity as compared to free vincristine in mice models having non-Hodgkin's lymphomas [40].

Vinflunine is a fluorinated derivative of vinorelbine. It is also an anticancerous agent. It is used as second-line chemotherapy for advanced transitional cell carcinoma. It is also under

clinical trial phase for its use as chemotherapeutic agent for breast cancer and metastatic bladder cancer cure [41-43].

### 2.3.2 Analgesic and antipyretic activities

Analgesic compounds relieve pain selectively by altering sensory perception without blocking the conduction of nerve impulses. These compounds also do not affect consciousness. Alkaloids are known for their pain relieving effect from ancient times. Morphine is a very effective pain reliever but has addictive unwanted effect. However, its methyl ether derivative, Codeine is a non-addictive analgesic. Likewise, curare obtained from *Chondrodendron tomentosum* is commonly used as muscle relaxant in surgery [44]. Cocaine obtained from *Erythroxylon coca* is a local anesthetic drug [45]. *C. roseus* plant also posses compounds with analgesic properties. However, exact characterization of these compounds in *C. roseus* is still not done [46].

### 2.3.3 Anti-diabetic and antioxidant activity

Alkaloids from *C. roseus* have been documented for lowering blood glucose level thus possessing anti-diabetic activity. Vindogentianine, indole alkaloid has been reported to have anti diabetic activity against type 2 diabetes in cell lines and rat models [47-50]. *C. roseus* phytochemicals has been documented to have very good antioxidant activity. The alkaloid component of *C. roseus* extracts has also been found to possess antioxidant potential [49, 51, 52].

### 2.3.4 Anti-ulcer, Anti-malarial, anti-parasitic and anti-neurodegenerative property activity

Vincamine and Vindoline have been reported for their antiulcer potential on experimental rat models. Vincamine is also known for cerebro-vasodilatory and neuroprotective activity [53-55]. Malaria is caused due to infection by parasitic protozoan *Plasmodium falciparum*.

 

Monoterpenic indole alkaloid, catharoseumine isolated from *C. roseus* has been reported to inhibit growth of *P. falciparum* [56]. Likewise, vinblastine and vincristine has been documented for antiparasitic activity against *Trypanosoma cruzi*. *T. cruzi* is known to cause trypanosomiasis in humans [57].

Neurodegeneration is progressive loss of function and structure of neurons that may lead to one and/or another disease like Alzheimer's disease, Parkinson's disease, and myasthenia gravis. Alkaloids from different plant sources have been documented to cure one or another form of neurodegeneration [58]. Root extract of *C. roseus* containing alkaloids mainly containing serpentine has been reported to have acetylcholinesterase inhibiting activity [59].

So overall alkaloids are very useful to mankind and may be a potential therapeutic agent for many existing health problems. As per World Health Organization majority of the developing countries population uses traditional medicine for health care. Further most of the traditional medicine uses plant extracts as key ingredients. As *C. roseus* is mainly known for anticancer potential. Among more than 87 anticancer drugs approved over the past ten years, more than 60% are natural or derived from natural compounds. The natural production of these pharmaceutically active compounds including alkaloids is generally low. This makes resource availability very difficult to achieve for industrial scale production thus the market prices for drugs is very high [60]. Likewise, the production of these alkaloids is limited in source plant, *C. roseaus* [61]. So, different biotechnological approaches are applied to maximize production of overall as well as specific Vinca alakloids in *C. roseus* as discussed below and shown in Fig 1.

### 2.4 Improving alkaloid content of *C. roseus*

#### 2.4.1 Breeding approaches to improve alkaloid production

Hybridization of *C. roseus* and *C. trichophylhs* has shown overall increment in alkaloid production as compared to individual plants [62]. Breeding approach to enrich medicinal value of *C roseus* has earlier been reviewed in detail [63].

 

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

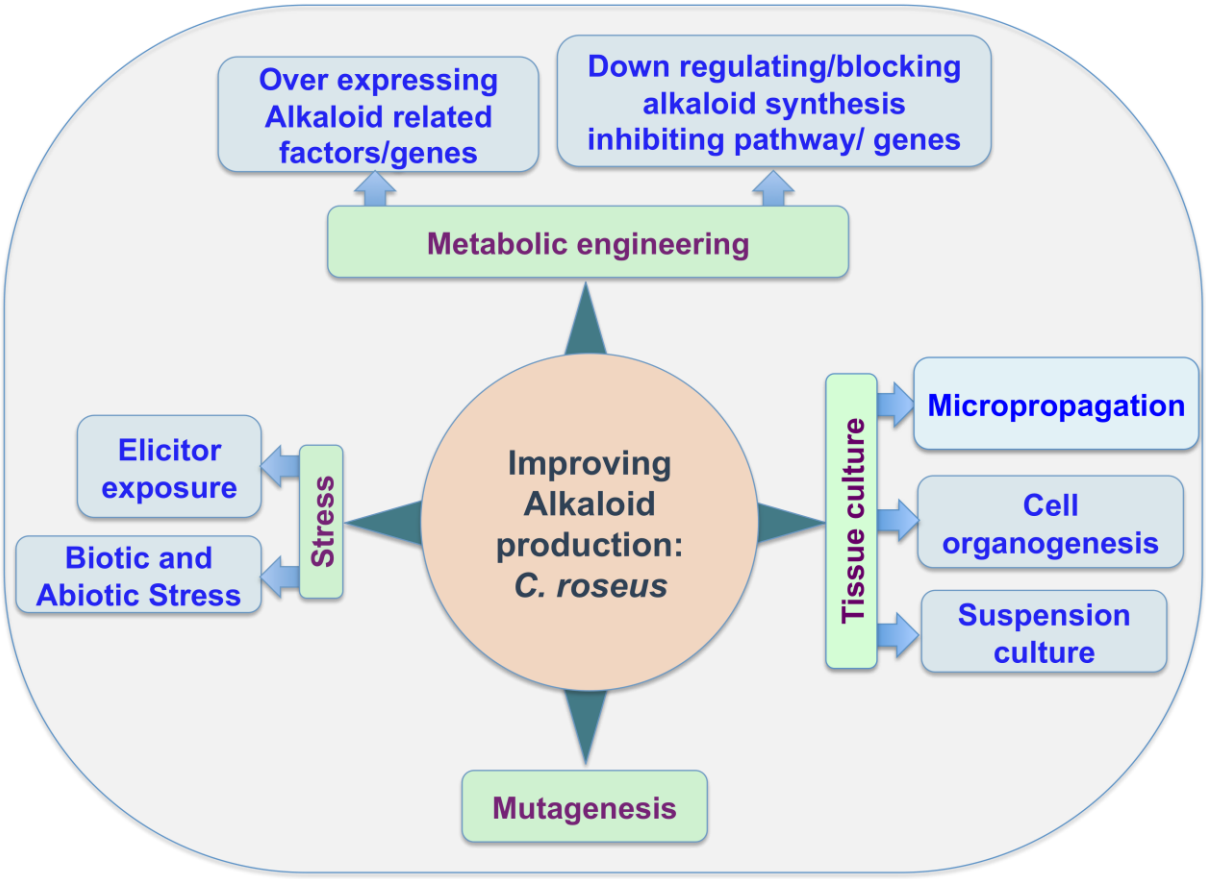


**Fig. 2. Commonly used strategies for increasing alkaloid production in *C. roseus*.**

### 2.4.2 Plant tissue culture based approaches to produce alkaloid from *C. roseus*

#### 2.4.2.1 Micropropagation and root culture

*In vitro* propagation and micropropagation can be used for the production of high quality plant based medicines in higher amount. Different phytohormone has been reported to affect and promote micropropagation of *C. roseus* [64-67]. Hypocotyls regenerated calluses have been use to obtain multiple somatic embryos that can be used as strategy for industrial scale production of *C. roseus* alkaloids [68]. Woody plant tissue culture media has also been successfully used for regeneration of shoots from *in vitro* grown plant leaves and internodes [69].

Hairy root culture has been used to increase the growth and alkaloid production of *C. roseus* [70]. Ajmalicine and catharanthine production has been documented using different hairy root cell lines [71, 72]. UV-B light exposure has been reported for ochnericine, serpentine, and ajmalicine synthesis using hairy root disease [73, 74]. Low strength MS medium and dark conditions were used for root culture using a novel temporary immersion system. Dark conditions were more useful for root culture growth [75].

#### 2.4.2.2 Cell culture

Cell suspension culture systems act as a reliable tool for industrial scale production of alkaloids [76]. Tissue, hypocotyls derived *Catharanthus roseus* callus has been reported for alkaloid production. The alkaloid production capabilities of the original tissue and callus propagated tissue subcultured callus tissue were same [77].

Jasmonate exposure has been reported to induce the accumulation of alkaloids in *C. roseus* cell cultures [78]. Small-scale bench top modified airlift bioreactor can be used for increased production of alkaloids. The bioreactor was effective in enhancing production of alkaloids in presence of external biological inducer, chitin and chemical inducer, mannitol, methyl jasmonate [79]. Interestingly, supplementation of 3% sucrose carbon source 1-5% glucose, lactose and maltose in cell suspension cultures significantly increased the alkaloid production. The yield enhancement was better in cell suspension culture as compare to solid media grown plants [80].

### 2.4.3 Molecular and genetic engineering approaches to improve alkaloid production

#### 2.4.3.1 Genetic Transformations and metabolic engineering

Genetic Transformations is very useful technique for the production of several biologically active natural compounds such as alkaloids [81, 82]. *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* have been used as a vehicle for the transfer of alkaloid producing genes into the plant genome resulting in the transfer and integration of genes of the plasmids from the bacteria into the plant genomic DNA [83, 84]. Ri plasmid mediated transfer of foreign genes into shooty teratomas of *C. roseus* epicotyls and stem nodal explants exhibited a

 

ten times more production of vincristine as compared to untransformed control cultures [85]. rol ABC gene containing hairy root transgenic *C. roseus* line expressed higher levels of terpenoid indole alkaloids [86, 87]. Overexpression of 1-deoxy-D-xylulose synthase (DXS) and geraniol-10-hydroxylase (G10H) in hairy root culture has been reported to induce ajmalicine, lochnericine and tabersonine production [88]. Feedback resistant anthranilate synthase expressing transgenic hairy root culture increased the release of catharanthine, ajmalicine, lochnericine and tabersonine in the culture [89-92]. Strictosidine synthase and strictosidine -D-glucosidase gene expressing transgenic hairy root on methyl jasmonate exposure were reported for secologanin expression [93]. Ri T-DNA expressing hairy root culture has been reported for the hyperaccumulation of ajmalicine and serpentine [78]. Terpenoid pathway has also been indirectly involved in alkaloid production. Loganin exposure to *C. roseus* root culture has been documented for enhanced alkaloid production. Transformed *C. roseus* root culture interfering terpenoid pathways were reported to reduce alkaloid production [94]. Vincristine and Vincristine production was enhanced in *Agrobacterium tumefaciens C58* transformed *C. roseus* epicotyl and stem node [85]. Axillary bud based micropropagation of *C. roseus* was potent for increased production of alkaloids. Randomly amplified polymorphic DNA marker analysis also revealed true type of somaclones [95]. Indole and terpene secoiridoid pathways play as critical role in synthesis of monoterpene indole alkaloids. Intermediates of these pathways combine to form vinblastine and vincristine. Low availability of alkaloids acts as feedback molecule to control alkaloid production. Transgenic expressing more terpenes has been documented to enhance alkaloids production [96]. Soamtic embryo mediated micropropagation and higher production of vincristine and vinblastine has been reported in *C. roseus* [97, 98]. Improved alkaloid content in *A. rhizogenes* transformed roots culture of *C. roseus* has been reported [99]. Over expression of CrWRKY1 transcription factor in *C. roseus* hairy root culture cells can enhance the production of serpentine [100]. Vinblastine is produced in low quantity naturally in *C. roseus*. Production of precondylocarpine acetate synthase and dihydroprecondylocarpine synthase enzyme in *C. roseus* can be used ultimately for production of higher concentration of vinblastine [101].

 

### 2.4.3.2 Mutation, stress and elicitor mediated enhancement in alkaloid production

Mutation has also been used as a strategy to obtain high yield of selected alkaloids [82]. In another study use of ethyl methyl sulfonate as mutagen yielded *C. roseus* mutagenic plants having high levels of ajmalicine. The mutagenic plants have lower level of another alkaloids namely, catharanthine and vindoline [102]. Mutation based strategies to enhance specific and overall alkaloid has been reviewed earlier [103].

Exposure of nitrogen, phosphate, tryptophan, and phenylalanine through nutrient medium in suspension culture can induce overall alkaloids production [104]. Plant growth promoting rhizobacteria also promoted synthesis of alkaloids in *C. roseus* [105]. Interestingly, stress condition has also been reported for increased production of alkaloids. Chromium stress has been reported to enhance vincristine and vinblastine content [106]. Likewise, rare earth metal salts namely, cerium, yttrium and neodymium were documented to increase ajmalicine and catharanthine alkaloid production in suspension cultures [107]. Vanadyl sulphate exposure has also reported similar increase [108]. Ultra violet B light exposure has been found to increase production of vinblastine and vincristine in multiple shoot and cell suspension cultures. Root culture exposed to UV-B light also enhanced the lochnericine, serpentine, and ajmalicine production [73]. Appropriate level of wounding stress has induced increase in alkaloid production. Likewise, cytokinin, ethylene gas and methyl jasmonate vapor can also induce hyper accumulation of alkaloids [109-112]. Seawater salt stressed *C. roseus* showed enhanced formation of vindoline, catharanthine, vinblastine and vincristine [113]. Water stress combined with *Psudomonas fluorescens* infection or ketoconazole exposure to *C. roseus* cell suspension has been documented to enhance accumulation of ajmalicine [114, 115].

Elicitor treatment also induced increase in alkaloid production. Extract obtained from *Pythium aphanidermatum* culture as well as yeast can induce hyperproduction of alkaloids in suspension culture [116,117]. Betaine, n-propyl gallate, succinic acid, malic acid and tetramethyl ammonium bromide treatment has also been documented for increase in ajmalicine yield [118]. *Aspergillum niger* based elicitor has also been used for increase the alkaloid production [119]. Oxidative burst and lipid peroxidation were main factor behind increase in indole alkaloid production. Exposure of well known reactive oxygen species,

 

hydrogen peroxide was tested to have same effect over alkaloid production that proves role of oxidative stress in enhanced alkaloid production [120]. Combined treatment of *Aspergillum niger* mycelium and tetramethyl ammonium bromide enhanced ajmalicine production in *C. roseus* suspension cultures [121]. Protein prenylation exposure can induce more formation of monoterpenoid indole class of alkaloids via induction of jasmonate signalling pathway [122]. Phytoplasma infection can induce more accumulation of catharanthine, vindoline, vincristine and vinblastine in *C. roseus* flowers as compared to leaves [123, 124].

## Conclusion

Alkaloids are plant secondary metabolites with immense therapeutic potential. *C. roseus* has been a source for alkaloids containing analgesic, anticancer, antioxidant, antiageing, and antimicrobial properties. Apart from good medicinal value, alkaloids are also important component of most of the human diets. Alkaloids may also be very useful for plant applications. The challenges regarding low production of alkaloids in *C. roseus* can be overcome by breeding, tissue culture and metabolic engineering approach. Further *C. roseus* plants may be improved in context to biotic and abiotic stress tolerance using tissue culture and genetic engineering approach. There exist some uncertainties regarding toxicity behaviour of alkaloids that can be eliminated by following rigorous and appropriate toxicity testing approach.

## Acknowledgement

VK is thankful to The Chancellor, Pro-Chancellor and Vice-Chancellor Lovely Professional University for providing research facilities and encouragement. PG is thankful to Chancellor and Vice Chancellor DAV University for continuous encouragement and support. PG is also thankful to DST for financial assistance in the form of Startup Project YSS/2015/000719.

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