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Review Article

CATHARANTHUS ALKALOIDS AND THEIR ENHANCED PRODUCTION USING ELICITORS: A REVIEW

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Abstract:

Catharanthus roseus poses a wide range of medicinally important alkaloids such as Vincristine and Vinblastine that are used in the treatment of cancer. The other alkaloids such as Ajmalicine and Serpentine are used as sedative compounds. Industrial production of these alkaloids, extremely exploits *Catharanthus roseus*, however, tissue culture is an alternative approach but low yield is a major problem. Elicitor treatment is an effective strategy used in improvement of production of alkaloids. Both biogenic, abiogenic and their combinations showed some promising results. Concentration of elicitors and application time is crucial phenomenon in production of different alkaloids.

Key words: Ajmalicine, Alkaloids, Elicitors, Vinblastine.

Introduction

Organism such as animals and plants are known to naturally produce organic molecules as a mean of chemical protection [1, 2]. The chemical connections of these molecules play a crucial role in the survival of such organisms. The use of plants as traditional medicines has proven to be one of their most important functions. Extracts from plants have been used as active constituents of poisons, teas etc. Fossil records date human utilization of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago [3]. The common use of natural remedies in India led to the development of the Ayurvedic system of medicine, which has been recognized since 1000 BC. The search for new plant-derived chemicals should thus be a priority in current and future efforts towards sustainable conservation and rational utilization of biodiversity. Biotechnological approaches, specifically plant tissue culture play a vital role in search for alternatives to produce desirable medicinal compounds from plants.

Catharanthus roseus in India is popularly known as Sadabahar, posing a wide range of indole alkaloids with some pharmaceutical potential. Vincristine and Vinblastine are the most widely used alkaloid in cancer treatment [(4)] where as Serpentine as hypertensive compound and ajmalicine are used as vasodilating agents as cited in http://www.genome.jp/kegg/drug/ besides containing valuable alkaloids *Catharanthus roseus* has capacity to survive in harsh conditions such as high temperature and high salinity [(5)].

Due to its rich properties, especially alkaloids, *Catharanthus roseus* has been widely exploited, plant tissue culture paved the way as an alternative but the major bottle neck is low yield of alkaloids, to overcome the problem scientists used various strategies to enhance the production. Elicitor treatment (both biogenic and abiogenic) till date has been found an effective mode of increasing the production of alkaloids [(6)]. Elicitation technique triggers the compounds responsible for secondary metabolite production hence detailed knowledge of metabolic pathway is requisite. In this review we examine the various alkaloids of *Catharanthus roseus* their metabolic pathway and various elicitors enhancing alkaloid production.

Catharanthus Alkaloids

Catharanthus roseus (Madagascar) is an evergreen sub or herbaceous plant of about 1 m in length. Almost each shoot bears flowers that are white or dark pink having dark red centre. This periwinkle is native to Madagascar. It has escaped cultivation and naturalized in most of the tropical world where it often becomes a rampant weed. It is established in several areas in the southern U.S. *Catharanthus roseus* is grown commercially for its medicinal uses in Australia, Africa, India and southern Europe [(7)].

More than 200 monoterpenoid indole alkaloids are produced by *Catharanthus roseus* (Madagascar periwinkle), some of them with pharmaceutical uses. The aerial parts of the plant contain maximum amount of alkaloids. These contain ajmalicine (vasodilating) (Figure 1), serpentine (hypertensive). Vincristine (Figure 2), vinblastine (anti-cancer) (Figure 3) vinorelbine, vinflunine: an active compound in the management of Metastatic Breast Cancer

(MBC) [8]. It has also been found that vinflunine inhibits stable tubule only polypeptide (STOP) for calmodulin (CAM) better than vinblastine [9], gomaline, 11-methoxy tabersonine, tetrahydroalstonine, catharanthine, mitraphylline [10]. Recent studies had shown the anti-diabetic effect of *Catharanthus roseus* extract [11].

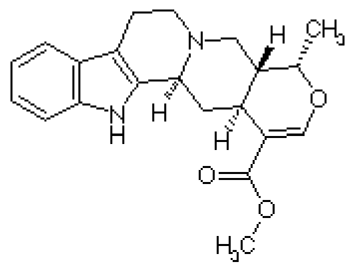


Figure 1: Molecular structure of ajmalicine.

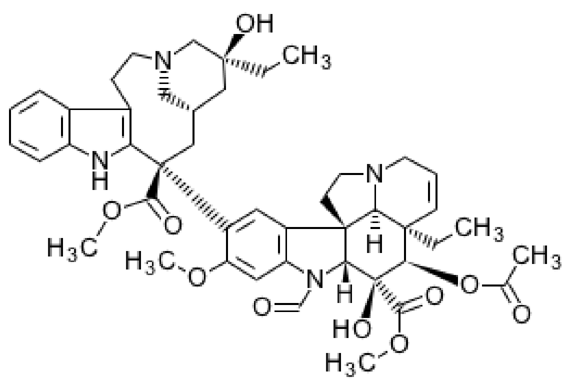


Figure 2: Molecular structure of vincristine.

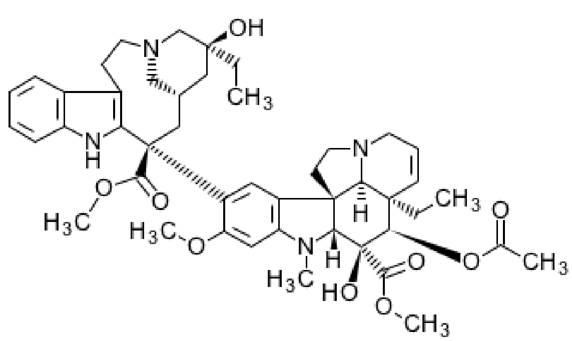


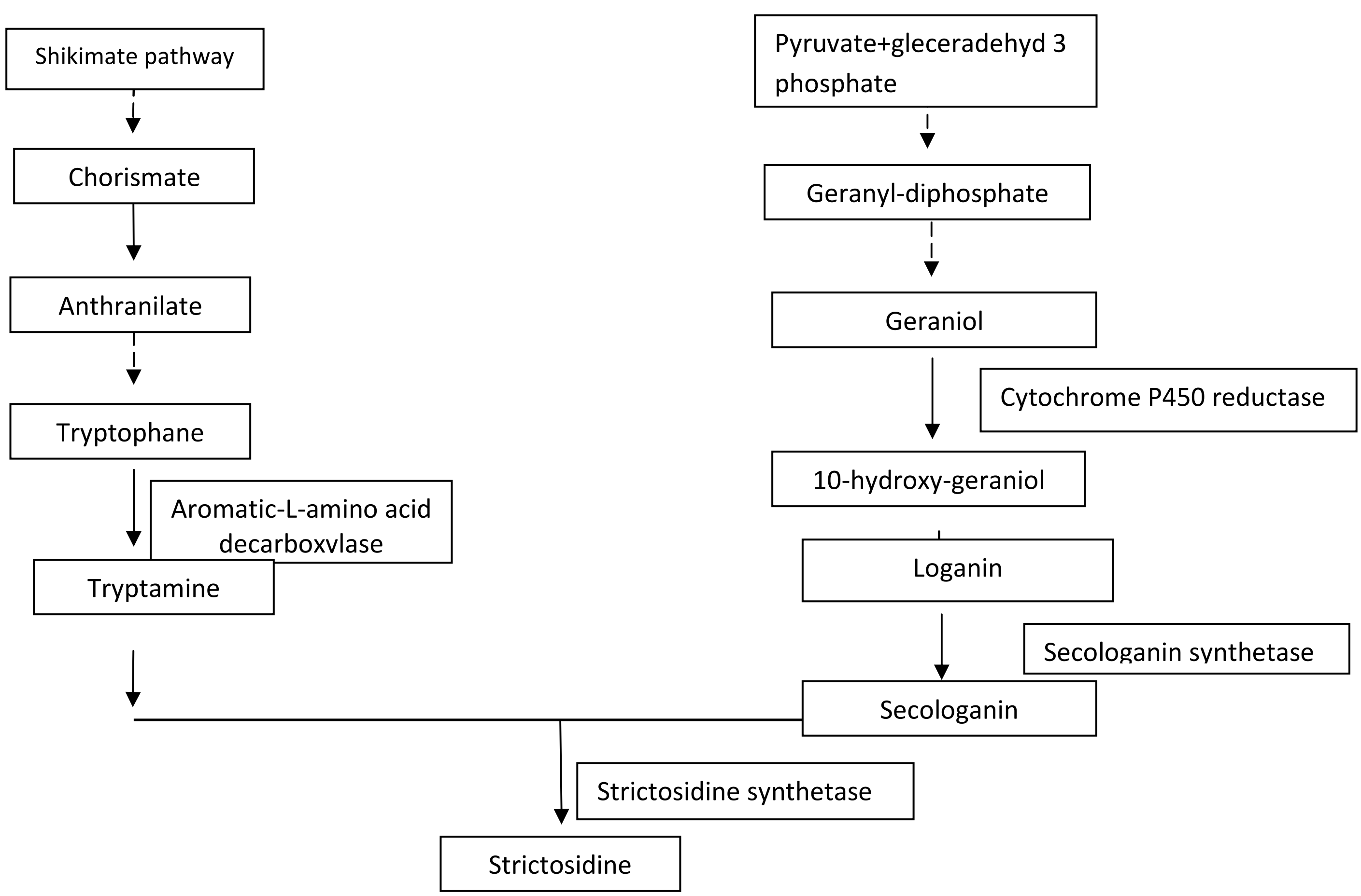
Figure 3: Molecular structure of vinblastine.

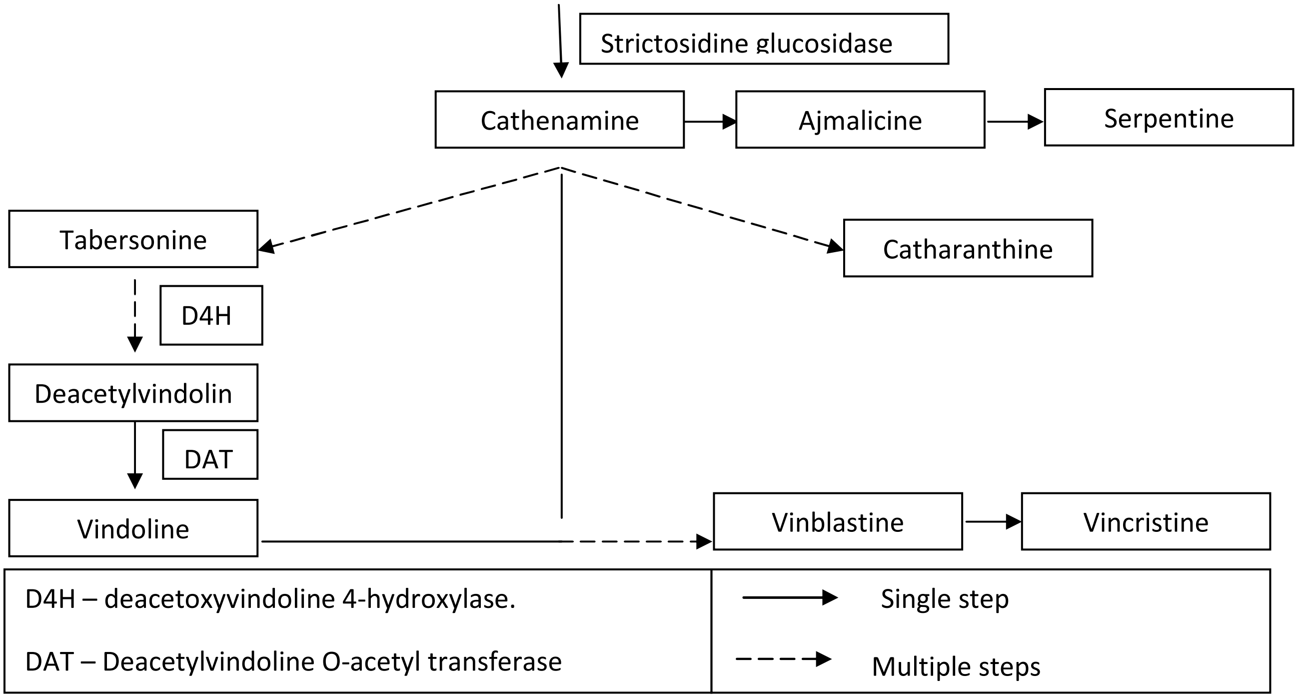
Biosynthesis of *Catharanthus roseus* Alkaloids

There are thousands of different plant products that have nitrogen in their structures. Perhaps the most diverse of these types of compounds (found in 20 to 30% of vascular plants) are the alkaloids that, like most other nitrogen-containing compounds, are synthesized from amino acids. Alkaloids are especially interesting because they are toxic to both herbivores and humans; yet they have some very important medicinal properties for humans. The nitrogen atom, which in these substances is almost always part of a heterocyclic ring origin, is found innately in the structure of the amino acids from which they came or are the result of the circularization of the given amino acid [12].

The various Catharanthus alkaloids belong to the first class of terpenoid indole alkaloids, that is, they consist of two moieties derived from two separate metabolic pathways – The Mevalonate pathway which gives the non tryptophan moiety and the tryptophan moiety is obtained from tryptophan (Figure 4) [13]. The complex structure of these alkaloids usually contains two nitrogen atoms, one is indole nitrogen (in the tryptophan – derived moiety) and the second is generally two carbons removed from mevalonic acid and it is a C_{10}-geranoil (monoterpenoid) contribution in the case of these alkaloids [14].

Figure 4: Biosynthesis of Catharanthus alkaloids.





A key intermediate in the biogenesis of the monoterpene indole alkaloids is 3alpha (S)- strictosidine, formed by the enzymatic condensation of tryptamine and secologanin [15]. The enzyme responsible for this important reaction, strictosidine synthase, has been isolated and characterized from cell cultures of a number of species including *Catharanthus roseus* [16]. Strictosidine then leads to formation of cathenamine (a coryanthe- type of alkaloid); enzyme involved is cathenamine synthase. Cathenamine further gives rise to ajmalicine (enzyme- ajmalicine synthase) and serpentine. Both ajmalicine and serpentine are also coryanthe- type alkaloids [17]. Cathenamine through a series of reactions also leads to formation of catharanthine (iboga- type) and vindoline (aspidosperma-type) [18]. Catharanthine and vindoline are monomeric indole alkaloids and occur free in the plant. 3, 4-Anhydrovinblastine is a key intermediate from the coupling of catharanthine and vindoline and the enzymes involved are peroxidases. It is further converted to vinblastine and vincristine [19].

Mode of Action

Vinblastine and vincristine are antimitotic. They bind to tubilin and prevent the formation of microtubules which help in the formation of mitotic spindle. Thus, these compounds block mitosis and cause an accumulation of cells in the metaphase (metaphase arrest). This contributes to their major phamacological action [20]. Vincristine exhibited a higher overall affinity for porcine brain tubulin than vinblastine [21]. The effect of vinca alkaloids has been studied on the microtubule network and the recovery of its disruption of HeLa cells, at concentrations of 4615 µg/l (vincristine) and 9230 µg/l (vincristine) tubulin paracrystals appears at concentration of 4615 µg/l and 9230 µg/l vincristine [22]. Serpentine is used as sedative agent it is tetrahydroderivative of ajmalicine. The level of serpentine in *Catharanthus roseus* is higher in contrast to ajmalicine; bioconversion of ajmalicine to serpentine takes place in presence of peroxidase enzyme [23].

Effects of various elicitors

The use of elicitors is one of the effective strategies employed to increase the production of important alkaloids in cell and organ culture [24-30]. There are two groups of elicitors: biogenic and abiogenic. Biogenic elicitors are divided into exogenous (isolated from pathogens or their culture media) and endogenous (isolated from tissues of a plant itself) groups. Among abiogenic elicitors are heavy metal ions, inhibitors of some metabolic steps, some antibiotics and fungicides, and UV radiation. Various reports have been found in increasing the production of alkaloid using both biogenic and abiogenic elicitors [31-33].

Bhagwath studied the effect of both biogenic and abiogenic elicitors; among biogenic they used autoclaved cell wall filtrates of the fungi *Protomyces gravidus* and among abiogenic elicitors they used vanadyl sulfate, both types of elicitors reported increment in the alkaloid production [34]. Yeast extract (YE) are found to activate genes responsible for terpenoids indole alkaloids in *Catharanthus roseus* cells [35]. Low concentration of *Pythium* elicitor stimulated alkaloid production from *Catharanthus roseus* cell suspension culture but second treatment of same elicitors did not stimulate alkaloid accumulation and also affect the cell growth [36]. *Pseudomonas fluorescens* another biogenic elicitor caused a significant enhancement in the production of alkaloids like Ajmalicine, Catharanthine, Tabersonine, Serpentine and Vindoline when compared to untreated control plant [37]. Karthikeyan studied the combined effect of *Pseudomonas fluorescens* and *Azospirillum brasilense* on biomass yield and ajmalicine production in *Catharanthus roseus* and found considerable enhancement of biomass yield and alkaloid contents, Ajmalicine content can also be increases when *Catharanthus roseus* plant is exposed to a combination of phosphorus and arbuscular mycorrhizal (AMF) [38]. Similarly cell wall fragments of *Aspergillus niger (A.niger*), *Fusarium moniliforme (F. moniliforme*), and *Trichoderma viride (T.viride*) increased alkaloid concentration in suspension when used at high concentration and for longer period of time, suggesting that both concentration and exposure plays crucial role in enhancement of alkaloids [39].

Among abiogenic elicitors, UV-B radiations increases production of Catharanthine and Vindoline 3-fold and 12-fold respectively in *Catharanthus roseus* suspension culture compared to control [40]. Methyl jasmonate, a plant secondary metabolite, at a concentration of 10µM and 100µM when introduced to cell suspension on day 6 of cell growth increased ajmalicine and serpentine production respectively, re-elicitation showed negative effect on both growth and alkaloid synthesis [41]. Jaleel reported that increasing concentration of NaCl increases total alkaloid content [42]. Magdi studied the effect of phytoharmones 2, 4-dichlorophenoxyacetic acid, salicylic acid, methyl jasmonate and abscisic acid on growth and alkaloid accumulation by Catharanthus roseus suspension culture supplied with alkaloid precursor's tryptamine and Loganin. Methyl jasmonate was found to enhance the ajmalicine accumulation, whereas 2, 4-D suppressed it, however, salicylic acid and abscisic acid did not show any affect on ajmalicine production [43]. Similar investigation by three growth retardants i.e. Chlormequate chloride, AMO-1618 and Tetcyclacis were conducted on the production of serpentine in *Catharanthus roseus* cell suspension culture, AMO-1618 at high concentration increases serpentine accumulation two fold in comparison to cultures without growth retardants (control) [44]. Indole-3-acetic acid (IAA) and benzyladenine (BA) were studied on production of ajmalicine by multiple shoot cultures of *Catharanthus roseus*, high concentration of IAA i.e. 11.42 µM and a low concentration of BA i.e. 2.22 µM were taken in MS media that accumulated high levels of ajmalicine [45]. Combined elicitor treatment has also shown some prominent results, Jian enhanced the production of ajmalicine by combining both biogenic and abiogenic elicitors i.e. *Aspergillum niger* and tetra methyl ammonium bromide [46]. Polyamines such as putrescine and tryptophan enhance the growth as well as total alkaloid content in *Catharanthus roseus* [47].

High dissolved oxygen cultures gives higher production of ajmalicine in comparison to low dissolved oxygen cultures [48]. The accumulation of ajmalicine decreases in oxygen concentration less than adequate (a condition called hypoxia), reoxygenation did not re-establish hypoxia however addition of Loganin in the culture media restore the alkaloid production [49]. Several physical stress such temperature, salinity, drought has also been studied, and optimal temperature for the production of ajmalicine was found to be 27.5 ^{0}C [50]. High salinity and drought conditions favours the production of vincristine, the increased level of vincristine was due to increased level of arginine since high salinity and drought resulted in increased amount of amino acids serine, methionine and arginine [5]. These amino acids are precursors of various polyamines (putrescine) that are found to enhance nitric oxide biosynthesis [51].

Conclusion

Catharanthus roseus cell culture has been studied for many years as a potential way to produce therapeutically important indole alkaloids, such as vinblastine, catharanthine and ajmalicine [5, 27]. But few successful industrial applications are introduced because of many biological and technological limitations. One of the major limitations is the low yield of those compounds in cell cultures. Selection of high-yield cell lines faces some difficulties in storage of cells and somaclonal variance during a long time of subculture. Other strategies, such as employment of biotic and abiotic elicitors, therefore are of great interest [52]. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants to ensure their survival persistence and competitiveness. Elicitors are abiotic and biotic, or on their basis of 'origin' like exogenous elicitors and endogenous elicitors. Abiotic elicitors are the substances of nonbiological origin, i.e. inorganic salts and physical factors acting as elicitors like Cu^{2+} and Cd^{2+} ions, Ca^{2+} and high pH, where as biotic elicitors are substances of biological origin including polysaccharides derived from plant cell walls (pectin or cellulose) and microorganisms (chitin or glucans and glycol proteins or G-proteins or intercellular proteins). Exogenous elicitors are substances originated outside the cell like polysaccharides, polyamines and fatty acids, where as endogenous elicitors are substances originated inside the cell [53].

The use of plant cell culture for the production of chemicals and pharmaceuticals has made great strides building on advances in plant sciences. Better molecular understanding of elicitation and signal transduction in plants are emerging. The use of methyl jasmonate is one strategy that appears to be broadly useful and can result in rapid improvements in productivity of alkaloids in a short period of time. The interrelationship of methyl jasmonate signaling with other signal compound is beginning to emerge and will supplement the widely demonstrated strategy of using multiple productivity enhancement techniques to achieve synergistic increase in production of alkaloids.

Further, the effective application of genetic tools and an emerging picture of the structure and regulation of pathways for secondary metabolism will provide the basis to move from a brute-force empirical approach for the optimization of production conditions to a semi rational one; one can then predict a great reduction in the time required to move from the establishment of a culture to the point when conditions are optimal for the production of commercially acceptable levels of product.

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