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Mini-review

Constituent Phytochemicals from *Catharanthus roseus*

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ABSTRACT
Catharanthus roseus belonging to family Apocynaceae is a plant with high medicinal importance and wide occurrence all over the world. It shows a number of therapeutic activities like antimicrobial, antidiabetic, anticancer, antiallergic, anti-inflammatory, cardioprotective activity etc. The present study is aimed to throw a light on the analysis and distribution of phytochemical constituents of *C. roseus*, transporters involved and influence of various factors on their abundance. A thorough analysis of the past and recent studies done in the field was made. *C. roseus* whole plant as well as individual parts contain diverse types of phytochemicals like alkaloids, phenols, saponins, tannins, terpenoids, steroids, flavonoids etc. The medicinal properties of *C. roseus* are attributed to the bioactivity of the above compounds. As the amount of phytochemicals extracted from the plant is less than the need, other different strategies like in-vitro cell culture technique, hairy root cultures, compost application, use of endophytes and arbuscular mycorrhizal fungi etc. should be employed to enhance the production of phytochemicals.

Keywords: Phytochemicals, *Catharanthus roseus*, Alkaloids, Phenols

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INTRODUCTION

Catharanthus roseus is an ornamental plant and belongs to family Apocynaceae. It is widely distributed in tropical, subtropical and also temperate regions of the world. It is a hard plant, can grow well in arid and nutritionally deficient places. Its ornamental value and hardiness are the reasons for its wide occurrence. The plant possesses chromosome number 2n=16. Due to its small genome size and self-compatibility it is preferred for transcriptomic, proteomic and metabolomic studies on secondary metabolism (Murata *et al.*, 2008; Rischer *et al.*, 2006). The plant is diploid but polyploids have also been produced artificially.

From ancient times whole plants, their parts or decoctions prepared from them are used to treat various ailments. Medicinal properties of plants are attributed to their phytochemical constituents. Phytochemicals are naturally occurring, non-nutritive and biologically active compounds found in plants. Phytochemicals can be categorized into primary and secondary metabolites. Primary metabolites are produced during growth phase of organisms and are involved directly in growth, development and reproduction of living organisms. Secondary metabolites are derivatives of primary metabolites. They do not play direct role in the above life processes but play a relational function. They are produced at certain developmental stages. Primary metabolites include purines, pyrimidines, amino acids, proteins and carbohydrates. Secondary metabolites include alkaloids, flavonoids, terpenes, saponins, steroids, phenols, lignans, glucosides etc.

C. roseus is one of the best studied medicinal plants and is a rich source of many important phytochemicals. Phytochemical analysis of *C. roseus* has revealed the presence of tannins, flavonoids, alkaloids, steroids, phenolic compounds, carbohydrates, terpenoids, quinones and saponins (Kabesh *et al.*, 2015; Philosia and Dhivya, 2017; Shoba *et al.*, 2018). It has antidiabetic, antimicrobial, antihelminthic, cardioprotective, anti-inflammatory, antiallergic and many other curative properties. Many studies have been conducted from time to time to isolate, purify, characterize, identify, quantify and to check therapeutic potential of various phytochemicals from *C. roseus*. These studies are essential for full elucidation of medicinal potential of *C. roseus*. Keeping all these facts in mind the present study has been carried out. The present work is prepared by a comprehensive study of the past and recent studies related to phytochemicals profiling, their transport and factors influencing their content in *C. roseus.*

Distribution and description of *C. roseus*: *Catharanthus* genus includes eight species. Out of them seven species are indigenous to Madagascar and the eighth species *C. pusillus* occurs in Sri Lanka and India. The name *Catharanthus* in Greek means "pure flower". The plant *C. roseus* is indigenous to Madagascar but due to its great ornamental value it is widely naturalized around the world. It can be found in diverse types of habitats whether it is a waste place, rocky area, sandy soil, along the coast etc. Its high salt tolerance (upto 2000 ppm) is the reason for its wide occurrence (Plaizier, 1981). It is a herbaceous, erect and bushy plant. Plants can be up to 1m in height. It can be annual or perennial. Leaves are thinly coriaceous, hairless and are oppositely arranged. Their shape ranges from oval to oblong. Flowers are pentamerous, actinomorphic and can have purple, pink or white color. Petals are velvety. Fruit is composed of two follicles. Seeds are black, oblong and have lateral hilum. It is also known as Periwinkle, Old Maid, Ceyenne Jasmine, Sadabahar, Sadaphool, Chichiriea, Nichinchi, Kembang Saritijha, Nithyakalyani and by many other regional names in various countries.

Taxonomic Position
Kingdom: Plantae
Sub-Kingdom: Tracheobionta
Class: Magnoliopsida
Sub-class: Asteridae
Order: Gentianales
Family: Apocynaceae
Genus: *Catharanthus*
Species: *roseus*

Phytochemical studies: Medicinal plants contain bioactive compounds which provide them the capability to have physiological influence in the body of living organisms. Phytochemical evaluation of extracts of *C. roseus* has revealed the presence of various primary and secondary metabolites like terpenoids, alkaloids, tannins, flavonoids and cardiac glycosides (Paikara *et al.*, 2017). In the extracts prepared from *C. roseus* plants the presence of proteins, amino acids, reducing sugars, phenols, ortho-dihydroxyphenols, lipids and soluble sugars have also been reported (Komathi and Vanmathiselvi, 2014). *C. roseus* produces more than 130 terpenoid indole alkaloids. Terpenoid indole alkaloids are nitrogen containing cyclic compounds. Some of the terpenoid indole alkaloids have high medicinal value (Heijden *et al.*, 2004). Alkaloids found in *C. roseus* like vindolinine, leurosine, catharanthine and vindoline reduce blood glucose and provide relief against diabetes (Chattopadhyay, 1999). Dimeric alkaloids for e.g. vincristine, anhydrovinblastine and vinblastine have antitumor activities. Vindesine, vincristine, vinorelbine and vinblastine are the four major vinca alkaloids used in cancer chemotherapies (Roepke *et al.*, 2010). Antimicrobial activity of the alkaloids has also been reported (Grellier *et al.* 1999). Table 1 gives a glimpse of phytochemicals analysed in different parts of *C. roseus*.

Plate 1
Images of *Catharanthus-roseus* and some of its varieties (Source: Sawant, 2019).

Table 1.

Phytochemicals analyzed in different parts of *C. roseus*

Phytochemicals analysed	*C. roseus* plant material	Analytical Method	Reference
Catharanthine, vindoline, ajmalicine and serpentine	Hairy root cultures	HPLC and GC-MS	Bhadra *et al.*, 1993
Serpentine, tabersonine, ajmalicine, tryptamine and catharanthine	Hairy root cultures	HPLC	Tikhomiroff and Jolicoeur, 2002
Vanillyl alcohol, glucovanillic acid and vanillic acid	Cell suspension culture	RP-HPLC	Yuana *et al.*, 2002
Petunidin 3-O-(6-Op-coumaroyl), Petunidin 3-*O*-glucosides, Hirsutidin 3-O-(6-Op-coumaroyl) and Hirsutidin 3-Oglucosides	Flowers and cell suspension culture	ESI-MS/MS	Filippini *et al.*, 2003
Tryptamine, tryptophan and lochnericine	Hairy root cultures	HPLC	Hughes *et al.*, 2004
Gallic acid, Vanillic acid, Hydroxytyrosol and ferulic acid	Whole plant	RP-HPLC	Proestos *et al.*, 2005
Linolenic acid, linoleic acid, capric acid, myristic acid, stearic acid, lauric acid, palmitic acid and oleic acid	Leaves	AIMIL-Nucan gas chromatograph	Mishra *et al.*, 2006
Catharanthine and vindoline	Leaves	UPLC-MS	Roepke *et al.*, 2010
Vindoline	Leaves	Automatic column chromatography	Chandrasekaran *et al.*, 2014
Vinblastine, vindoline, catharanthine, vinleurosine and vincristine	Roots, stem and leaves	HPLC-ESI-Ms/MS	Lin *et al.*, 2014
Terpenoids, alkaloids, saponins, tannins, phenols, proteins and quinines	Leaves	TLC and GC-MS	Kabesh *et al.*, 2015
Stearic acid, phytol, linolenic acid ethyl ester and hexadecanoic acid	Leaves of pink flowered plants	GC-FID and GC-MS	Lawal *et al.*, 2015
Phytol, limonene and linolenic acid ethyl ester	Flowers of pink flowered plants		
Geraniol, dodecyl alcohol, citral and limonene	Leaves of white flowered plants		
Dotriacontane and limonene	Flowers of white flowered plants		
Alkaloids, steroids, proteins, amino acids, phenols, terpenoids, polysterols, fats, saponins, glycosides, flavonoids, carbohydrates and tannins	Leaves	GC-MS	Mohan *et al.*, 2015
Reducing sugars, phenols, proteins, amino acids, tannins, total alkaloids, flavonoids and vincristine	Whole plant	Different colorimetric tests, TLC and LCMS/HNMR	Nayaka and Babu, 2015
Vindoline	Leaves	HPLC	Pandey *et al.*, 2016
Alkaloids, glycosides, phenols and carbohydrates	Leaves	Different colorimetric tests	Begum and Padmalatha 2017):
3,4-anhydrovinblastine, vinblastine and vincristine	Leaves and flowers	Tissue Spray-Mass Spectrometry	Junior and Furlan, 2017
Tridecanoic acid; octadecyne; N-hexadecanoic acid; squalene; hexatriacontane; undecanoic acid; 2h-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-Tetramethyl-2(4,8,12-Trimethyltridecyl) acetate; DI-N-Decylsulfone; 1,2-Bis (Trimethylsilyl)Benzene; Hexatriacontane and 4-Heptanol, 2-Methyl	Leaves	GC-MS	Nathiya *et al.*, 2017
Flavonoids, terpenoids, tannins, cardiac glycosides and alkaloids	Leaves	Different colorimetric tests	Paikara *et al.*, 2017
Phenols, tannins, proteins, alkaloids, quinines, terpenoids and flavonoids	Leaves	Different colorimetric tests	Philosia and Dhivya, 2017
Saponins, phenols, alkaloids and proteins	Leaves	Different colorimetric tests	Josphine *et al.*, 2018
Phenols, tannins, alkaloids (vinblastine, vindoline and vincristine), terpenoids, glycosides, quinines, phytosterol, saponins, steroids and flavonoids	Leaves	TLC	Kale *et al.*, 2018
Saponins, cardiac glycosides, tannins, flavonoids and terpenoids	Whole plant	Different colorimetric tests	Mir *et al.*, 2018
Ajmalicine	Roots	HPLC	Monnerat *et al.*, 2018
Catharanthine, vincristine, vindoline and vinblastine	Leaves	HPLC	Saadedin, 2018
Phenols, tannins, steroids, alkaloids, flavonoids, proteins, terpenoids, carbohydrates and saponins	Leaves	Different colorimetric tests	Shoba *et al.*, 2018
Hentriacontane; Methyl 7,11,14 Eicosatrienoate; N-Hexadecanoic acid; Dodecanedioic acid, Bis (Trimethylsilyl) ester; Alpha Linolenic acid, trimethylsilyl ester; Sulfurous acid, octadecyl 2-Propyl ester; Sulphurous acid, butyl tridecyl ester and Methyl-19-methyl-Eicosanate	Leaves	GC-MS	

Alkaloids profiling has been done in twelve cultivars of *C. roseus* grown in Iraq (Saadedin, 2018). The twelve cultivars were Titan Icy Red, Pink Cooler, Pacifica xp Apricot, Pacifica xp Cherry Red Halo, Iraqi cultivar, Lavender Hue Cooler, Mediterranean xp Rose Halo, Titan Icy Pink, Pacifica xp Burgundy Halo, Pacifica xp White, Jams n Jellies Blackberry and Pacifica xp Polka Dot. The alkaloids: vincristine, catharanthine, vinblastine and vindoline were quantified in leaves of the plants. Highest amount of vincristine was found in Titan Icy Pink (0.0310 %), catharanthine in Pacifica xp White (0.0283), vinblastine in Lavender Hue Cooler (0.0342 %) and vindoline in Iraqi cultivar (0.0653%). Highest percentage of vindoline in Iraqi cultivar was attributed to the growth habit of the cultivars (Iraqi cultivar takes more time in reaching the flowering stage) (Saadedin, 2018). It was in agreement with the other findings (Naaranlahti *et al.*, 1991;

Pan, 2014). Good acceptable amount of vincristine upto 148 µg/g is also noted in *C. roseus* plants (Ayyash *et al.,* 2016). A sensitive method of quantitative determination of five alkaloids (vinleurosine, vinblastine, catharanthine, vindoline and vincristine) simultaneously in *C. roseus* by HPLC-ESI-MS/MS was developed. The mobile phase used was methanol-15 nmolL-1 ammonium acetate consisting of 0.02% formic acid (65:35 v/v). The five alkaloids had recovery rate ranging from 79.9% to 91.5%. The five analytes were stable for 2 h at room temperature, for 12 h at 4oC and for two weeks at -20oC. Trace alkaloids and their precursors can be determined quantitatively in *C. roseus* by using this method (Lin *et al.*, 2014).

Root extracts of *C. roseus* are a rich source of saponins and phenolics (kaemferol, apigenin and gallic acid) (Pham *et al.,* 2019). Ajmalicine is an antihypertensive drug. It is used to treat high blood pressure. It was observed in the adventitious roots of *C. roseus* (Phuc *et al.,* 2017). Although cortical cells and protoderm of *C. roseus* roots appears to be the site of biosynthesis of complex alkaloids such as catharanthine and tabersonine (Laflamme *et al.*, 2001) the pathway seems to be compartmented in multiple cell types in aerial parts like leaves, flowers and stem of the plants (St-Pierre *et al.,* 1999). Some studies indicate that young developing leaves are the sites of production of precursors like vindoline and catharanthine. Older leaves play role in production and accumulation of dimers. Vindoline is found within leaf cells whereas catharanthine accumulates in leaf wax exudates. Catharanthine accumulation in the wax layer of the plant leaves has a biological significance. It was shown that incubation of zoospores of fungus *Phytopthora nicotianiae* with catharanthine (10 µg/ml of culture medium) inhibited the zoospores growth and formation of hyphae. Insecticidal role of catharanthine was also observed. *Bombyx mori* larvae died after being fed on mulberry diet mixed with various amounts of *C. roseus* leaves and with diet consisting of catharanthine-enriched leaf surface extracts (Roepke *et al.,* 2010). For detailed study epidermal cell contents were extracted from *C. roseus* leaves using carborundum abrasion technique (Murata *et al.,* 2008). The extract was used for mRNA isolation. Epidermome or complement of proteins which express in epidermis was sampled. cDNA library was derived and randomly sequenced. It established 3655 unique expressed sequence tags which consisted of 1142 clusters and 2513 singletons. In the set of expressed sequence tags all known monoterpenoid indole alkaloid pathway genes were found. Identification of several novel monoterpenoid indole alkaloid pathway genes was done. Triterpene biosynthesis pathways were identified and oleanane-type triterpenes were found to be localized to cuticular wax layer. The biosynthesis pathways for very long chain fatty acids and flavonoids, were also localized in the cell type. All the results revealed that *C. roseus* leaf epidermis is specialized for the synthesis of multiple classes of metabolites (Murata *et al.,* 2008).

Essential oils obtained from leaves and flowers of *C. roseus* pink and white flowered plants were characterized by means of gas chromatography mass spectrometry and gas chromatography flame ionization detector (GC-FID). Leaf oil of *C. roseus* plants with pink flowers was found to consist of phytol (7.3%), ethyl ester (43.9%), hexadecanoic acid (6.8%) and stearic acid (10.6%) and flower oil showed the presence of linolenic acid ethyl ester (14 %), phytol (29.6%) and limonene (34.1%). Essential oil obtained from the leaves of white flowered plants contained geranoil (7.3%), dodecyl alcohol (9.8%), limonene (23.2%) and citral (7.0%) and the oil from their flowers consisted of dotriacontane (16.1%) and limonene (37.2%) (Lawal *et al.*, 2015).

Quantitative screening of ethanol extracts of *C. roseus* by GC-MS has indicated the presence of two major compounds: tridecanoic acid (20.02%) and octadecyne (43.66%) and ten minor compounds i.e. N-hexadecanoic acid (2.34%), squalene (4.34 %), hexatriacontane (1.92%), undecanoic acid (2.24%), 2h-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-Tetramethyl-2(4,8,12-Trimethyltridecyl) Acetate (6.54%), DI-N-Decylsulfone (2.09%), 1,2-Bis (Trimethylsilyl) Benzene (2.963%), Hexatriacontane (3.76%) and 4-Heptanol, 2-Methyl (4.68%). Many of the above compounds like N-Hexadecanoic acid, 4-Heptanol, 2-Methyl, squalene etc. are bioactive compounds (Nathiya *et al.,* 2017).

Lipid profile of *C. roseus* leaf changes at different stages of development. Earliest appreciable amounts of fatty acids and free sterols can be observed during early maturation stage. 68-fold enhancement occurs in sterols/phospholipids ratios in abscised leaf relative to that at full maturity. Senescent leaf showed far lower unsaturated/saturated fatty acid ratio in comparison to the fully expanded leaf. The changes in lipid profile results in concomitant changes in the ultrastructure and functions of membranes. It in turn influences indole alkaloids accumulation capability of the plant tissues (Mishra *et al.*, 2006).

Phytocystatins are thiol proteinase inhibitors. These compounds provide protection against abiotic stress, insect attack, cancer etc (Franco and Melo, 2000; Ryan, 1990; Schelp and Pongpaew, 1988). Two phytocystatins i.e. *Catharanthus roseus* Cystain I (CRCI) and *Catharanthus roseus* Cystain II (CRCII), were isolated and purified from *C. roseus*. Ammonium sulphate fractionation and gel filtration chromatography methods were employed. The percent yield of CRCI was 18.18% and of CRCII was 16.35%. Both of the phytocystatins showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Sharma *et al.*, 2011).

A comparative analysis of efficiency of three solvents (DMSO, water and acetone) in extraction of various phytochemicals from *C. roseus* has revealed water as the most efficient solvent. Phytochemicals like tannins, saponins, flavonoids, cardiac glycosides and terpenoids were present in the water extract. Antioxidant activity of the water extract was also high as compared to the other two extracts (Mir *et al.*, 2018). Whereas, phytochemical profiling of *C. roseus* using GC-MS has revealed that ethanol extract was richer in bioactive compounds as compared to the aqueous extract and exhibited antidiabetic activity in diabetes affected rats (Mohan *et al.*, 2015).

Dimeric alkaloids like vincristine and vinblastine are derived by coupling of vindoline and catharanthine. Although chemists are making remarkable efforts in synthesizing dimeric alkaloids, even then the dimeric alkaloids and their monomeric precursors (vindoline and catharanthine) are still extracted and purified from *C. roseus* plants (Almagro *et al.*, 2015). The extraction of the alkaloids from the plants is an expensive process due to the low amount of these compounds in the plants. The need to produce these compounds in high amount has stimulated the research to obtain bioactive compounds from in vitro *C. roseus* cell cultures. Genetic engineering can also revolutionize the production (Heijden *et al.*, 2004). The synthesis of vinca alkaloids is a complex multistep pathway and is regulated by environment, organ, development and cell specific controls (Facchini and De Luca, 2008). These controls over monoterpenoid indole alkaloids are coupled to secretary mechanisms. It keeps vindoline and catharanthine separated from each other in plants. This spatial separation of the two monoterpenoid indole alkaloids is a reason for low content of dimeric anticancer drugs in plants (Roepke *et al.*, 2010).

Production of valuable compounds from hairy root cultures has been investigated for last several years. Transformation with *Agrobacterium rhizogene*s, a soil bacterium, helps in the production of hairy roots. Here the production of secondary metabolites is higher than the cell cultures (Ahn *et al.*, 2011). A wide variety of alkaloid compounds like ajmalicine, catharanthine, serpentine, vindoline, tubersonine, vinblastine, vincristine, secologanine etc. have been reported in hairy root cultures of *C. roseus* (Bhadra *et al.*, 1993; Parr *et al.*, 1988; Tikhomiroff and Jolicoeur, 2002). Other compounds like flavonoid glucosides and phenolic compounds are also detected in extracts prepared from *C. roseus* hairy roots cultures (Chung *et al.*, 2007; Chung *et al.*, 2009). So, hairy root cultures are efficient means of secondary metabolites production.

Many beneficial activities of endophytes are reported in host plants like improving nutrient supply, promoting plant growth and protecting plant from abiotic and biotic stresses (Knoth *et al.*, 2014; Rodriguez and Redman, 2008). Endophytes are the microbes that reside inside the host plant without causing any harm. Two fungal endophytes were isolated from leaves of *C. roseus* (Pandey *et al.*, 2016). These were *Choanephora infundibulifera* CATDLF-6 and *Curvularia* sp. CATDLF5. The fungal endophytes had no effect on the primary metabolism of the plants but increased the amount of vindoline by modulating regulatory and structural genes associated with the biosynthesis of terpenoid indole alkaloid. Expression of transcriptional activators like BPF1, MYC2 and ORCA3, associated with terpenoid indole alkaloids biosynthesis was enhanced, whereas the expression of repressors like GBF2, ZCT1, ZCT2, ZCT3 was downregulated in plants inoculated with the fungi as compared to the control plants (Pandey *et al.*, 2016).

C. roseus plants inoculated with mixed inoculums of arbuscular mycorrhizal fungi (*Gigaspora margarita* + *Rhizophagus intraradices*) and supplied with nitrogen fertilizers have shown enhancement in total ajmalicine yield in the plants (69.63 mg/kg) (Monnerat *et al.*, 2018).

Feather compost application which consists of 90% keratin and is rich in amino acids and nitrogen, boosted the contents of primary (reducing sugars, proteins and amino acids) and secondary metabolites (alkaloids, flavonoids, tannins and phenols) in *C. roseus* plants (Nayaka and Babu, 2015).

The biosynthesis pathways of the compounds are complex and different types of cells participate in it (Bernonville *et al.*, 2015; Facchini and De Luca, 2008). A precise transport system is also required by the biosynthesis pathways. ABC transporters might play a role in monoterpenoid indole alkaloids secretion by hairy roots of *C. roseus* (Monribot-Villanueva *et al.*, 2015). Inhibitors of different transporting systems like glibenclamide, potassium cyanide, quinidine and sodium orthovanadate were used for the analysis performed on hairy root cultures of the plants. ATP plays a central role in the functioning of ABC transporters. When *C. roseus* hairy roots were treated with potassium cyanide, the accumulation of ATP was inhibited. It occurred both in the absence and presence of acetyl salicylic acid. It suggested that potassium cyanide had decreased the level of ATP in the cells. Treatment with inhibitors glibenclamide and quinidine led to a substantial decrease in the secretion of serpentine in the hairy roots when compared with the control. The results indicated the role of ABC transporters in MTAs secretion (Monribot-Villanueva *et al.*, 2015).

Agroclimatic conditions vary from place to place and so the phytoconstituents in plants. *C. roseus* plants collected from three different locations in India i.e. Sivakasi, Ooty and Theni showed different contents of secondary metabolites like phenolic compounds, saponins, terpenoids, steroids, tannins and alkaloids. The different agroclimatic conditions of the three regions like soil type, amount of precipitation, temperature etc. were responsible for the above differences (Santhi *et al.*, 2017).

Conclusion

Diverse types of phytochemicals in *C. roseus* like phenols, alkaloids, terpenoids, quinones, flavonoids, saponins, tannins, steroids etc. make it a pharmacologically important plant. But due to the low content of the phytochemicals in the plant, their extraction becomes expensive. So other strategies like in vitro cell culture technique, hairy root cultures, compost application, use of endophytes and arbuscular mycorrhizal fungi etc. should be employed to increase their production. More research needs to be done in genetic engineering and other areas to increase the yield of phytochemicals from *C. roseus* so that humanity can benefit from *C. roseus* in a big way.

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