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Anticancer Molecules from *Catharanthus roseus*

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ABSTRACT

Catharanthus roseus is an important medicinal plant found in various parts of the world and the bioactive compound has been extracted and used as anti-cancer agent to treat the cancer over decades. However, the extraction of bioactive compound also results in the generation of large quantities of pollution with wasted solvents. Toxic pollution occurs when synthetic chemicals are discharged or natural chemicals accumulate to toxic levels in the environment, causing reductions in wildlife numbers, degrading ecosystem functions and threatening human health. This review covers the extraction and phytochemical obtained leading to chemical compounds related to anti-cancer property of *C. roseus*. Additionally, recent advances of using biological cell cultures were also addressed. Thus, this work can be used for further investigation of *C. roseus* to be undertaken in future for its anti-cancer property further development and efficient production in drug industry.

Key words: *C. roseus*; Anticancer; Extraction; Phytochemical; Tissue culture

INTRODUCTION

Cancer is a disease that in which a group of abnormal cells grow uncontrollably beyond usual boundaries by disregarding the normal rules of cell division and causing a lump known as tumour. Tumour formed can then invade adjoining parts of the body and spread to other organs, this process is known as metastasis which is the major cause of death from cancer. According to the World Health Organization (WHO), cancer is the second leading cause of death globally; nearly 1 in 6 deaths is due to cancer. Cancer was responsible for 8.8 million deaths in 2015 and approximately 70% of deaths from cancer occur in low and middle income countries. In additional, according to Ministry of Health Malaysia, cancer is one of the fatal non-communicable diseases in Malaysia and contributed to 13.56% of all deaths in 2015 (Azizah *et al*., 2016). It was the third most common cause of death following diseases of circulatory system and respiratory system. *C. roseus* is a plant belonging to Apocynaceae family and the name Catharanthus comes from Greek word which means 'pure flower'. *C. roseus* is a perennial plant that commonly grows in tropical countries and Catharanthus is more known as Madagascar periwinkle.

Figure 1. The variety of *C. roseus* flowers.

C. roseus has long been cultivated for herbal medicine and also as an ornamental plant. In Indian and Chinese traditional medicine, the extracts have been used against numerous diseases such as malaria, diabetes and Hodgkin's disease. It has been reported that *C. roseus* contain more than 70 different types of chemical constituents such as indole type of alkaloids, ajmalicine, serpentine and reserpine (Kabesh *et al*., 2015). The Catharanthus alkaloids provide protection against microbial infection, abiotic environmental stresses such as UV irradiation and are wide importance in clinical medicine due to the anti-hypertensive and antispasmodic properties (Sain and Sharma, 2013; Nejat *et al*., 2015).

The Catharanthus alkaloids, vincristine and vinblastine which found abundantly in the leaves part of the plant inhibited the growth of tumour and hence contribute the anticancer property of *C. roseus* (Das and Sharangi, 2017). Vinblastine and vincristine were the first natural drugs used in cancer therapy and still among the most valuable agents used in cancer treatment (Costa *et al*., 2008). Vinblastine and vincristine are able to inhibit the cell mitotic and widely used medically to treat different kinds of cancers such as breast cancer, Hodgkin's lymphoma and leukemia. Vinblastine and vincristine bind to tubulin which is a structural protein that can be found in the cytoplasm, thereby inhibit the assembly of microtubule structures.

GEOGRAPHIC DISTRIBUTION OF *C. Roseus*

C. roseus is a tropical or subtropical plant which belongs to the family of Apocynaceae and known as Madagascar periwinkle. The origin of the *C. roseus* is in Madagascar. The plant is used for the medical and decorative in many countries such as Malaysia (Ong *et al.,* 2011), India, China, South Africa and Mexico (Patel *et al.,* 2012). It is incorporates a high salt tolerance which up to 2000 ppm and typically found close to water level where somewhat higher altitudes. *C. roseus* can grow 20-60cm high and wide and it also had variety color of flower such as pink, white or rosy-purple. The flowers are pollinated by butterfly and it self-fertilized. It has a basal tube which 2.5-3.0cm long with a corolla about 2.0-5.0cm diameter. It has five sepals which 2-6mm long, narrow and with hairs. Besides that, the leaves are oppositely arranged on the stem with leaf margin and have pinnate venation structure. The length of the blade is about 2-4 inches. The fruit pod found to be a pair of a follicle of about 2.0-4.5 cm long and 3 mm wide whereas the fruit characteristic invisible.

MEDICINAL USES OF *C. Roseus*

Since the ancient time, people had been used this plant as one of the medication alternative for various diseases. In most country, *C. roseus* was used for cancer treatment as it is one of the plants that produced phytochemical that fights cancer. In India, people had been used the leaf extract in order to cure bee sting or wasp sting. Other than that, in the decoction of the leaves was used for diabetes (Nammi *et al.,* 2003). The Philippines and African people use the leaf infusion for menorrhagia. The raw extracts of the leaves and roots were used to treat cancer activity and severe diarrhea. In Mauritius, the extract used for dyspepsia. The people in the Bahamas used the flower decoction to treat asthma, tuberculosis, and flatulence. In Malaysia, West Indie and Nigeria the plant was used to treat diabetes.

Following that, people in Madagascar used the leaf as evoke vomiting while in Hawaii the extraction was used to arrest bleeding (Sharangi, 2017).

EXTRACTION METHODS AND PHYSICO-CHEMICAL ANALYSIS OF ANTI-CANCER MOLECULES FROM *C. Roseus*

Chemical solvents that used for bioactive compound extraction might lead to environmental pollution. Long term exposure to the hazardous solvent waste from many industrial processes can lead to deleterious effect on respiratory, haematological and thyroid functioning (Babu and Reddy, 2014). The extraction conducted by using water as the solvents is an alternative method to extract the bioactive compound. However, in water extraction method, high temperature of water is required to extract the compound. The increase in temperature during extraction might deteriorate the quality of extract and degrade the desired bioactive compound. Moreover, water extraction method required a long time in order to complete the extraction process (Wojdylo *et al.,* 2013).

C. roseus produces low levels of two dimeric terpenoid indole alkaloids, vinblastine and vincristine, which are widely used in cancer chemotherapy (Costa *et al.,* 2008). The extraction and purification of several alkaloids found in *C. roseus* was first developed by Eli Lilly in the 1970s using organic solvents methods (Falcao *et al.,* 2017). These extraction methods using water with sulphuric acid includes four steps such as fractionation by partition with benzene, two chromatographic columns, crystallization in ethanol and sulphuric acid. Extraction of vindoline, catharanthin, and vinblastine from *C. roseus* leaves has been extensively carried out using ultrasound extraction with dilute or methanol as solvent, heating, boiling and refluxing with methanol. Additionally, several researches have been reported the used of supercritical fluid extraction (SFE) to extract phytochemical from *C. roseus* a (Verma *et al.,* 2008; Falcao *et al.,* 2017). Several phytochemical compounds obtained from *C. roseus* using advances technology to develop anti-cancer property (Table I).

CHEMICAL COMPOSITION, STRUCTURE AND MODE OF ACTION OF ANTICANCER MOLECULES OF *C. Roseus*

C. roseus produce several indole alkaloids, named as Vinka alkaloids, which is widely used as antimitotic drugs in the treatment of cancer (Almagro *et al.,* 2015). This includes natural products like vincristine and vinblastine, the first anticancer agents used clinically (Sottomayor et al., 2005) and their semisynthetic derivatives like vindesine, vinorelbine and vinflunine (Van der Heijden *et al.,* 2004; Moudi *et al.,* 2013). In comparison with vinblastine structure, the velbanamine moiety characterize vinflunine and vinorelbine which are both synthesized from the precursor alkaloids catharanthine and vindoline to increase their therapeutic action (Nirmala *et al.,* 2011; Ngan *et al.,* 2000).

Vinca alkaloids provoke apoptosis and cell growth repression by altering the microtubular dynamics. These Microtubules (MTs) are components of the cytoskeleton which are the major constituents of mitotic spindles involved in chromosome separation during meiosis and mitosis, they are also involved in maintaining cell structure, transport and many others cellular processes (Wang *et al*., 2012; van der Heijden *et al*., 2004). The basic units of the MTs are α-tubulin and β-tubulin heterodimers which are in a dynamic polymerization and depolymerization at their ends; this assembly and disassembly of the MT polymers, also called as "treadmilling" and "dynamic instability", is regulated by the binding of tubulin and guanosine 5-triphosphate (GTP), then, any destabilization or interruption of this dynamics can arrest the cell cycle and lead to programmed cell death or apoptosis (Jordan *et al*., 2002). Since, two groups of destabilizers are distinguished; the first group compounds are the MT-stabilizing agents that prevent the depolymerization and the second group compounds are MT-depolymerizing agents that inhibit MTs assembly (Perez *et al*., 2009; Islam and Iskander, 2004).

To arrest tumour cells cycle during mitosis, Vinca alkaloids and their derivatives act by binding at the surface between two tubulin heterodimers next to the exchangeable GTP-binding site and depolymerizing the MTs (Morris and Fornier, 2008; Bolanos-Garcia, 2009). The affinity of both natural Vinca alkaloids from *C. roseus* or their semi-synthetic analogues and derivatives for tubulin heterodimers appears to be the same but it is characterized by the decrease of the overall equilibrium constants as following order: vincristine > vinblastine > vinorelbine > vinflunine (Gigant *et al*., 2005; Okouneva *et al*., 2003). The complexes of these alkaloids with α, β-tubulin are stabilized by van der Waals forces and electrostatic interaction energy through common binding site.

Table I. Phytochemicals extracts and fractions obtained from *C. roseus*

No.	Bioactive Phytochemicals	Extraction/determination method	References
1	Vindoline, vinblastine, catharanthine, vincristine	Ultraviolet-c treated CMCs extraction	Moon *et al*., 2018
2	Vincristine, vinblastine, ajmalicine, catharanthine, serpentine, vindoline	Ultra HPLC-quadrupole time-of-flight (UPLC-Q-TOF) mass spectrometry method	Jeong *et al*., 2018
3	Vinblastine, vincristine	Modular transcriptional control of MIA (monoterpoid indole alkaloid) biosynthesis	Schweizer *et al*., 2018
4	Vincristine, vinblastine, vindesine, ajmalicine, ajmaline, reserpine, vindoline	Ultra HPLC – tandem mass spectrometry	Kumar *et al*., 2018
5	Vinblastine, vincristine	Yeast extract elicitation	Maqsood *et al*., 2017
6	Catharanthine, vinblastine, vincristine, vindoline	HPLC and qualification reverse – transcription polymerase chain reaction qRT-PCR, ultraviolet-C	Moon *et al*.,2017
7	Vincristine, vinblastine, Vindoline, catharanthine, yohimbine	HPLC with diode array detector (HPLC-DAD)	Liu *et al*., 2016
8	Catharanthine and vindoline	Centrifugal partition chromatography	Kotland *et al*., 2016
9	Cathacunine, dimeric indole alkaloids	Nuclear factor kappa-light chain-enhancer of activated B cells(NF_KB) and c-jun N terminal kinase (JNK), HPLC	Wang *et al*., 2016
10	Ajmalicine, Serpentine, Catharanthine, vindoline, vindolinine, vincristine, vinblastine, anhydrovinblastine	HPLC –DAD method simultaneous analysis of terpenoid indole alkaloids(TIAs)	Pan *et al*., 2016
11	Vincristine, vinblastine and catharanthine	Agilent HPLC, C18 column, HPLC with UV	Hanafy *et al*., 2016
12	Vindogentianine, Vindoline, vindolidine, vindolinine, vindolicine, serpentine and perivine	NMR, MS, UV and IR	Tiong *et al*., 2015

Also, this electrostatic interaction is improved in the case of vinflunine probably due to its two fluorine atoms that differentiate it from vinorelbine (Coderch *et al*., 2012; Miyamoto *et al*., 2003), then, tubulin heterodimers binding is ensured by the vindoline domain, while the cytotoxic effect is provided by the catharanthine domain (Sertel *et al*., 2011). Several authors have reported that Vinca alkaloids acts following dose-dependent model leading to stop the cell mitosis when the levels of Vinca alkaloids are low, then, the cells die after a long time of incubation. However, at high concentration, Vinca alkaloids stop the mitosis of the tumoral cells by the formation of paracristals (large tubulin polymers) and immediately provoke the cell death (Takanari *et al*., 1990; Attard *et al*., 2006). New mechanisms of action of these alkaloids have been recently reported by many authors, then, they can interplay with MTs associated protein, they can interact with calmodulin and they can inhibit the amino acid metabolism (Barbier *et al*., 2013; Schutz *et al*., 2011). However, differences of Vinca alkaloids activity were observed with this new mode of action. Then, the better efficacy of vinflunine in comparison with vinblastine against murine tumours and human tumour xenografts can be explained by its interaction with calmodulin in spite of its lower binds to tubulin than vinblastine or vincristine (Van der Heijden *et al*., 2004; Almagro *et al*., 2015).

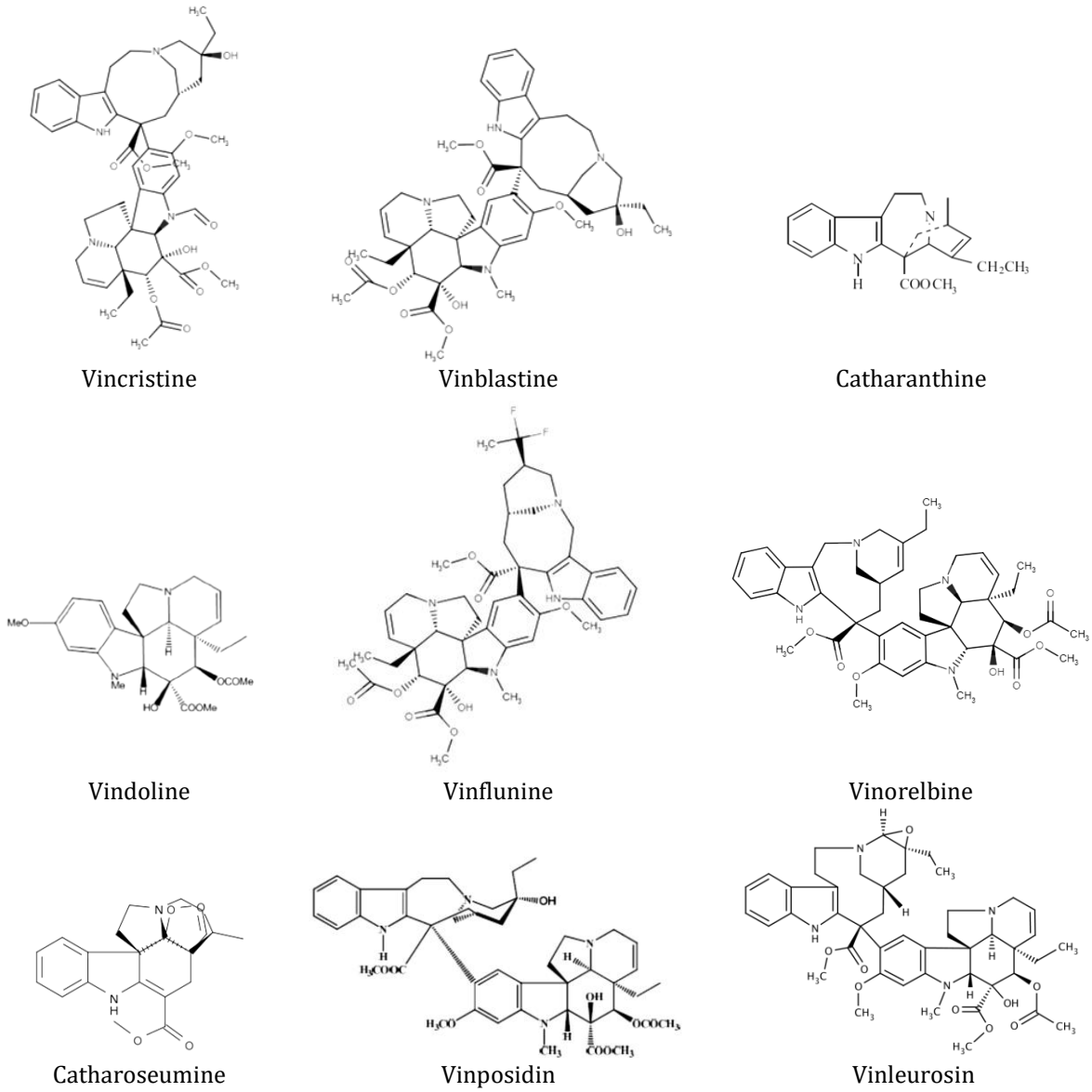

Figure 2. Chemical Structure of main bioactive alkaloids from *C. roseus* Vinca alkaloids.

Other indole alkaloids from *C. roseus* were reported as cell growth inhibitors. However, their mechanism of action is still not well-known. Catharoseumine, the monoterpenoid indole alkaloid isolated from *C. roseus* by Wang *et al*. (2012), possess a unique peroxy bridge moiety and show cytotoxic effect when tested in human tumour cell lines but only a moderate cytotoxic effect against HL-60 cell line. In 1974, Eli Lilly Company has patented a preparation process of vinposidin (Leurosidin) used as an antimitotic agent (Eli Lilly, 1974; Keglevich *et al*., 2012) (Table II).

PRODUCTION OF ANTICANCER COMPOUNDS OF *C. Roseus* IN SUBMERGED CULTURE SYSTEM

Beside the conventional approach of using grown plant in the field as initial materials of the production of anticancer compound of *C. roseus*, during the recent years many research have been focused on the cultivation of plant cells for more efficient production of anticancer compound. This method of cultivation offer many advantages such as: reducing the number of purification steps, highly efficient production system in shorter time, and well controlled production

Table III. Alkaloids from *C. roseus* used as antimitotic drugs.

Molecules	Cancer Type Targeted	*In vitro / in vivo testing*	Reference
Vincristine (Oncovin)	Leukaemia	HL60 human acute promyelocytic leukemia cells, K562 human chronic myelogenous leukemia cells and EA.hy926 human umbilical vein cells	Wang *et al*., 2016
	Lymphomas	B-cell lymphoma cell line	Qiu *et al*., 2018
Vinblastine (Velban)	Leukimia	Chronic Lymphocytic Leukaemia (CLL)	Bates *et al*., 2014
Vinorelbine, (Navelbine)	Solid tumours	Clinical trials	Bahleda et al., 2018
	Breast cancer	Clinical trials	Nazir *et al*., 2016
	Non-Small-Cell Lung cancer	Clinical trials	Nazir *et al*., 2016
Vinflunine	Non-Small-Cell Lung cancer	C	Krzakowski *et al*., 2010
	Urothelial carcinoma	Human patients	Schinzari *et al*., 2018
Catharanthine	Colorectal Carcinoma	Human Colorectal Carcinoma Cell Line (HCT 116).	Siddiqui *et al*, 2010
Cathachunine	Leukemia	B cells (NF-κB) and c-Jun *N*-terminal kinase (JNK) pathways	Wang *et al*., 2016

process under sterile conditions to cope with the cGMP requirements for the production of bioactive drugs. Therefore, in recent years production of high value medicinal product by *C. rosesus* is carried out in submerged cultivation systems either using shake flask or different types of bioreactors (Ducos *et al*., 2009; Mujib *et al*., 2014). Bioreactors of different scales have been proven to be suitable for the cultivation of hairy root lines of *C. roseus* for large scale production of ajmalicine, serpentine, and catharanthine (Verma *et al*., 2012). Other anticancer bioactives such as vincristine and vinbalastine were successfully produced in stirred tank bioreactor. The maximal production of these alkaloid is carried out under uncontrolled pH and aeration rate of 0.5 v/v/min. The alkaloid production was 13.47 and 7.94 folds higher compared to intact plant for vincristine and vinblastine, respectively (Taha *et al*., 2014). However, addition of chemical to induce stress on cells can increase also the alkaloid compound production. It has been reported that addition of chromium to culture medium in very low concentration (in range between 10-100 µM) reduced cell growth concomitant with a significant increase in vinblastine and vincristine production (Rai *et al*., 2014). Addition of sodium chloride to increase osmotic stress in culture can lead also to various stress signalling, enzyme activities and increase both of vinblastine and vincristine production as well (Fatima *et al*., 2015). Other study also reported that the addition of fungus elicitor of Aspergillus flavus lead to a significant stimulation of cell growth and increase in both vinblastine and vincristine production (Tonk *et al*., 2016). Recent research reported on the positive effect of yeast extract supplementation to culture medium on increasing the vinblastine and vincristine when added in concentration as low as 1.5g/L in suspension culture of *C. roseus* (Maqsood and Abdul, 2017).

CONCLUSION

During recent years, the demand for natural bioactive compounds have been increased as alternative or in combination with chemo- and radiotherapy in cancer treatment to reduce their side effects. Based on many years research, *C. roseus* is considered as one of the plant biofactory for the production of highly potent anticancer molecules. Of different compounds studied, vincristine and vinblastine are the widely used compounds in pharmaceutical industries. However, other molecules such as cathachunine, catharanthine, vinflunine, and vinorelbine have also high potential application as anticancer compound based on many in vivo and in vitro studies. The most attractive features of this plant is

the ability to cultivate in submerged culture using bioreactors. This make it easy to cultivate and production of the anticancer compounds in high concentration, in shorter time, and under fully sterile condition with full compliance with cGMP for drug manufacturing.

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