Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 1695-1701
Received: 17-07-2017
Accepted: 18-08-2017

Suddhasuchi Das
Research Scholar, Department of Spices and Plantation Crops, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Amit B Sharangi
Professor and Head, Department of Spices and Plantation Crops, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Correspondence
Amit B Sharangi
Professor and Head, Department of Spices and Plantation Crops, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Madagascar periwinkle (*Catharanthus roseus* L.): Diverse medicinal and therapeutic benefits to humankind

Suddhasuchi Das and Amit B Sharangi

Abstract
The genus *Catharanthus* consists of eight species of which seven are native to Madagascar and one, *C. pusillus*, to India. *Catharanthus roseus*, Madagascar periwinkle is one of the few pharmacological plants that have a long history of therapeutic voyage from Mesopotamian folklore of 2600 BCE till today playing a considerable role as herbal and traditional medicine of various diseases. As previously published reviews on Madagascar periwinkle mostly concentrate on the pharmaceutical and chemical compounds of the herb, lack of information about the other features of the species is extremely tangible. This organised review provided insights into agro technological, biological, ecological, and medicinal aspects (particularly anticancer compounds) of Madagascar periwinkle along with possible pathways and mode of action. The diverse medicinal and therapeutic potentials of Catharanthus roseus have been revealed that this magical herb may be utilized to its full potential and future research orientation may be directed towards it.

Keywords: *Catharanthus roseus*, alkaloids, secondary metabolites, medicinal values, therapeutics

1. Introduction
Catharanthus roseus L. (Madagascar periwinkle) have several synonyms viz., *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*. Other English names occasionally used for the plant include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle and Old Maid. It is an important medicinal plant of Apocynaceae containing abundant useful alkaloids, used in diabetes, blood pressure, asthma, constipation, cancer and menstrual problems. Vinblastine and vincristine are two powerful anticancer natural products, belonging to the group of terpenoid-indole alkaloids isolated from the pan tropical plant *Catharanthus roseus*, in which they are present only as minor constituents of the complex mixture of about 130 alkaloids produced by this plant. During the last 40 years vinblastine and vincristine have been used for the treatment and cure of thousands of patients, both because of their unique mode of action and their effectiveness. The plant has a long history of use in Ayurvedic medicine, traditional Chinese medicine and other healing systems. Western medical science began researching *Catharanthus roseus* and its extracts during the 20th century, finding several compounds useful in cancer treatment. All parts of the plant have been used in regional herbal medicine, including the dried root, leaves, flowers and stalks. Alkaloids used in modern medicine are extracted from the whole dried plant.

2. Morphology
Catharanthus roseus is an evergreen sub herb or herbaceous plant. A native to Madagascar, this herbaceous plant grows to 80 cm to 1 m high and blooms continuously year-round with pink, purple, or white flowers (Hogan, 2003) [1]. The leaves are oval to oblong, 2.5- 9.0 cm. long and 1- 3.5 cm. broad glossy green hairless with a pale midrib and a short petiole about 1-1.8 cm. long and they are arranged in the opposite pairs. There are two common cultivars of *C. roseus* named on the basis of their flower colour, one producing pink flower "Rosea" (4) and the other, white flowers "Alba". The flowers are white to dark pink with a dark red centre, with a basal tube about 2.5- 3 cm. long and a corolla about 2-5 cm. diameter with five petal like lobes. The fruit is a pair of follicles about 2-4 cm. long and 3 mm broad.
The plant grows widely to about 1m tall at subtropical areas. Its leaves are found to be of oval to oblong, 2.5 – 9.0 cm long and 1.0- 3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole of about 1.0- 1.8 cm long and they are arranged in the opposite pairs (Gajalakshmi *et al*., 2013) [2].

3. Geographical distribution

Catharanthus roseus is native to the Indian Ocean Island of Madagascar. In many tropical and subtropical regions worldwide it has been introduced as a popular ornamental plant. It commercially cultivated in Spain, United States, China, Africa, Australia, India, and Southern Europe for its medicinal uses. The drugs derived from this plant find major markets in USA, Hungary, West Germany, Italy, The Netherlands, and UK (Anon., 2011; Lata, 2007) [3, 4]. It is now common in many tropical and subtropical regions worldwide, including the Southern United states.

4. Cytology, reproduction and genetic diversity

The chromosome number of all species of the genus *Catharanthus* is $2n$=16 (Mendioro *et al*, 2005) [5]. Due to colchicines treatment doubling of chromosome number, tetraploidy, has been induced that resulted in an increase in TIAs, larger stomata, branches, and leaves, although there was reduced pollen fertility and poor seed set compared with diploid plants (Tang *et al*., 2011) [6].

Catharanthus roseus is a unique species because of its self-compatibility, unlike most of the other species in the family. However, intraflower self-pollination does not normally occur in periwinkle because of the physical separation between the stigma and anthers, a phenomenon known as reverse herkogamy, when the stigma is recessed below the level of anthers (Kulkarni *et al*., 2001, Sreevalli *et al*., 2005) [7, 8]. Although many aspects of alkaloid biosynthesis have been investigated, the genetic variation between accessions in relation to alkaloid content and the effects of breeding for flower color or growth habit on the levels of vinblastine and vincristine are still poorly understood.

Terpenoid indole alkaloids (TIAs) are accumulating by dissimilar cultivars in various parts of the plant. However Pan *et al*., (2015) [9], while working on gene transcript profiles of the TIA biosynthetic pathway of *Catharanthus roseus* in response to ethylene and copper opined that the change in TIA accumulation does not correlate with expression of the associated genes. So necessary efforts are to be initiated to identify accessions from the vast resources of naturally occurring *Catharanthus roseus* germplasm for their chemotherapeutic potential, finding out the type of plant tissues accumulating these active compounds, and foster genetic development towards higher yield of alkaloids (Van der Heijden *et al.*, 2004, Z´arate and Verpoorte 2007) [10, 11].

5. Traditional and folkloric uses

There are many traditional and folkloric uses of periwinkle which are time-tested and confirmed with peoples' belief. The paste prepared from the leaves is an excellent wound healer and also relieve the wasp sting pain. It can stop bleeding, thereby quickening the healing process. Many also say that periwinkle is useful in bringing relief from depression, headaches and fatigue.

Table 1: Uses of periwinkle in different country

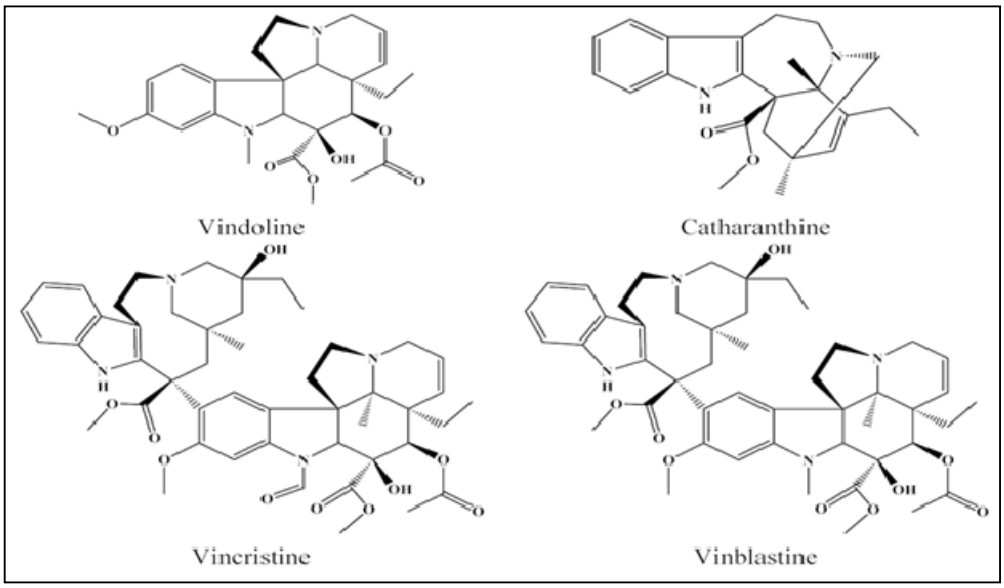
Sl No	Country	Use
1	India	The juice of leaves is used as application to bee sting/ wasp sting
2	Philippines	Decoction of leaves is used in diabetes and decoction of young leaves is used in stomach cramps, root decoction is used for intestinal parasitism. Infusion of leaves is used for treating menorrhagia. Crude leaf extracts and root has anti cancer activity. Roots used for dysentery
3	Madagascar	The bitter and astringent leaves are used as vomitive, roots used as purgative, vermifugl, depurative, hemostatic and toothache remedies
4	Mauritius	The juice of leaves is used for indigestion and dyspepsia
5	West Indies and Nigeria	The plant is used in diabetes
6	Cuba and Jamaica	Flower extract is used for eye wash in infants
7	Bahamas	Decoction of flower is used in asthma, tuberculosis and flatulence
8	Malaysia	The plant is used in diabetes, hypertension, insomnia and cancer
9	Hawai	Extract of boiled plant is used to arrest bleeding
10	America	Gargle of plant is used to ease soarthroats, chest ailments and laryngitis
11	Africa	Leaves are used for menorrhagia and rheumatism

6. Chemistry

C. roseus possess carbohydrate, flavinoid, saponin and alkaloids. Alkaloids are the most potentially active chemical constituents of Catharanthus roseus. More than 400 alkaloids are present in the plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance, ingredients, food additives and pesticides. The alkaloids like actineo plastidemeric, Vinblastin, Vincrestine, Vindesine, Vindeline Tabersonine etc. are mainly present in aerial parts whereas ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine etc are present in roots and basal stem. Rosindin is an anthocyanin pigment found in the flower of C. roseus.

6.1 Alkaloids and Other Secondary Metabolites

Some major products found in the plant are anhydrovinblastine, vindoline, catharanthine, ajmalicine and serpentine. Kisakurec and Hesse identified different alkaloid subclasses: vincosan, corynanthean, vallesiachotaman, strychnan, aspidospermatan, plumeran, ibogan, eburnan and bisindole alkaloids. Catharanthus is particular rich in bisindole alkaloids (about 40 compounds), most of those containing a plumeran (vindoline) and an ibogan (catharanthine) moiety. Some of the C. roseus alkaloids are marketed as pharmaceuticals: - Ajmalicine (Hydroserpan R, Lamuran R) was introduced in 1957 for the treatment of hypertensia. Another important source for this alkaloid is *Rauvolfia serpentina* (L.) Bentham. - Vinblastine (vincaleukoblastine, VelbeR) was introduced in 1960 and is used in the treatment of Hodgkin's disease, non-Hodgkin lymphomas, testiscarcinomas, and sometimes against breast cancer and chorio-carcinomas. - Vincristine (leurocristine, Oncovin R) is an oxidized form of vinblastine and was introduced in 1963. Although these compounds are available from the leaves, the plant itself, while alive, does not contain them in significant quantities. The plants contain precursor alkaloids vindoline and catharanthine (in the waxy coating of the leaves) Mixing of these precursors is essential for synthesis of specific chemicals towards fighting cancer.



7. Phytochemical components and medicinal uses

Study of periwinkle has increased because of its ability to produce secondary metabolites such as terpenoid indole alkaloids that may be used to treat cardiac diseases and certain tumors in mammals (Favali *et al.,* 2004) [12]. Periwinkle is also a natural host of many phytoplasmas. Phytoplasmas are cell wall-less organisms that infect over 300 plant species, including many woody species. They thrive in the phloem of infected plants and can cause symptoms such as curled and yellow leaves, witches-broom appearance, and phyllody, resulting in reduced crop yield and plant death (Lee *et al.,* 2000) [13]. Many factors, including genotype (Choi *et al.,* 2004; Obukosia *et al.,* 2005; Xu *et al.,* 2007) [14, 15, 16], medium (Trigiano and Gray, 2000) [17], explants type (Gambino *et al.,* 2007) [18], plant growth regulator (PGR) (Trigiano and Gray, 2000) [17], and culture condition (Choi *et al.,* 2001) [19], can affect plant regeneration.

Catharanthus roseus was the highly exploited and studied medicinal plants as it was found to produce more than 100 monoterpenoid indole alkaloids (MIAs) that includes the two major commercially important cytotoxic dimeric alkaloids that are used in the cancer chemotherapy. (Magnotta *et al.,* 2006) [20]. *C. roseus* was also found to be a good source of the non-enzymatic and enzymatic antioxidants. (Jaleel *et al*., 2006, 2007) [21, 22]. From the Traditional period itself, the plant has been used to cure diabetes and high blood pressure as it was believed to promote the insulin production or to increase the body's usage of the sugars from the food in case of diabetes. (Singh *et al.,* 2001) [23]. Alkaloids are the bitter-tasting plant compounds that contains mostly of nitrogen many of them was found to possess pain relieving or the anticancer properties. Especially two major alkaloids in *C. roseus* such as vinblastine and vincristine was developed into the prescriptions for the anticancer drugs. (Duflos *et al.,* 2002) [24]. These injectable drugs and its derivatives such as vinflunine acts in several pathways and was found to interfere with the division of the cancer cells. Recently, it was also found that certain chemicals in *C. roseus* involves in the prevention of the growth of the new blood vessels that is supporting the tumour growth. (Zhang *et al.,* 2000) [25]. Vincristine was found to be produced by *Fusarium oxysporum* which is an endophyte of this host, (Tung *et al.,* 2002) [26] while another group has isolated the vinblastine from an endophytic fungus. *Catharanthus roseus* L. is found to be an important source of the indole alkaloids that are present in all plant parts. The plant has been used for the treatment of diabetes, fever, malaria, throat infections, chest complaints, regulation of menstrual cycles and as a euphoriant. (The physiologically important antineoplastic alkaloids such as vincristine and vinblastine are present in the leaves and the antihypertensive alkaloids are found in the roots such as ajmalicine, serpentine, and reserpine. (Mishra *et al.,* 2001) [27]. Other pharmacological uses of *C. roseus* include wound healing, analgesic, vasodilatory and hypoglycaemic (Nayak *et al.,* 2006) [28]. The *Catharanthus* alkaloids was found to comprise a group of about 130 terpenoid indole alkaloids (TIAs) (Van der Heijden *et al.,* 2004) [29]. Wide differences has been noted in the compositions of the alkaloids that are isolated from the underground and aerial tissues of the plant. (Mishra *et al.,* 2000; Shukla *et al.,* 2005) [30, 31]. Low "dimeric" alkaloid contents in the plants has encouraged the intense research for the alternative production methods by involving the usage of cell cultures (Verpoorte *et al.,* 2002) [32] and metabolic engineering (De Luca *et al.,* 2000) [33]. Total synthesis was found to be difficult due to the structural complexity of the molecules and also the complicated reaction steps that involves the stereochemical constraints.

8. Therapeutic properties

8.1 Anti cancer property

The anticancer alkaloids Vinblastine and Vincristine are derived from stem and leaf of *Catharanthus roseus*. (Banskota *et al.,* 2002) [34]. Different percentage of the methanolic crude extracts of *Catharanthus* was found to show the significant anticancer activity against numerous cell types in the *in vitro* condition (Ueda *et al.,* 2002) [35] and especially greatest activity was found against the multidrug resistant tumor types (Wang *et al.,* 2004) [36]. They are also used for treatment of leukemias, lymphomas, and testicular cancer. (Retna and Ethalsa, 2013) [37].

Widowati *et al*., (2013) [38] has demonstrated that methanol extracts of aerial and roots of *C. roseus* reduced the proliferation of the human ductal breast epithelial tumour cell lines (T47D) with a mean IC50 of 2.8% by apoptosis. Also, aqueous extract of leaves induced cell death of human leukemic T-cells (Jurkat) in a time – and dose – dependent manner with mean IC50 value of 2.38 µg/ml by DNA

fragmentation (Ahmad *et al*., 2010) [39]. Preliminary cytotoxicity study has demonstrated further a dose-independnet activity of methanol extracts of *C. roseus* against HCT-116 colorectal carcinoma cell lines at 200 µg/ml (Siddiqui *et al*., 2010) [40].

8.2 Anti diabetic property

The aqueous extract was found to lower the blood glucose of about 20% in diabetic rats when compared to that of the dichloromethane and methanol extracts which lowered the blood glucose level to 49-58%. The hypoglycemic effects has appeared due to the result of the increased glucose utilization in the liver.

All four alkaloids viz., vindoline,vindolidine, vindolicine and vindolinine were isolated and identified from the dichloromethane extract of Catharanthus leaves induced relatively high glucose uptake in pancreatic β-TC6 or myoblast C2C12 cells, with vindolicine showing the highest activity. In addition, compounds vindolidine, vindolicine and vindolinine demonstrated good protein tyrosine phosphatase-1B (PTP-1B) inhibition activity, implying their therapeutic potential against type 2 diabetes (Tiong *et al*., 2013) [41].

8.3 Anti microbial property

The antimicrobial activity of the leaf extracts of the plant was checked against the microorganisms like *Pseudomonas aeruginosa* NCIM 2036, *Salmonella typhimurium* NCIM 2501, *Staphylococcus aureus* NCIM 5021 and was found that the extract coud be used as the prophylactic agent in the treatment of many of the diseases. (Prajakta and Ghosh, 2010) [42].

8.4 Anti oxidant property

The antioxidant potential of the ethanolic extracts of the roots of the two varieties of *Catharanthus roseus* L. namely 'rosea'(pink flowers) and 'alba'(white flowers) was obtained by using different systems of assay such as Hydroxyl radical-scavenging activity, superoxide radical-scavenging activity, DPPH radical- scavenging activity and nitric oxide radical inhibition method. The results obtained proved that the ethanolic extracts of the roots of Periwinkle varieties extracts has exhibited the satisfactory scavenging effect in all the radical scavenging assays in a concentration dependent manner but *Catharanthus rosea* was found to possess more antioxidant activity than that of *Catharanthus alba* (Alba Bhutkar and Bhise, 2011) [43]. Vindolicine, present in the plant showed the highest antioxidant potential in ORAC and DPPH assays and it also alleviated H2O2-induced oxidative damage in β-TC6 cells at 12.5 µg/mL and 25.0 µg/mL (Tiong *et al*., 2013) [41].

8.5 Antihelminthic property

Helminthes infections are the chronic illness, affecting human beings and cattle. *Catharanthus roseus* was found to be used from the traditional period as an anti helminthic agent. The anti helminthic property of *C. roseus* has been evaluated by using *Pherithema postuma* as an experimental model and with Piperazine citrate as the standard reference. The ethanolic extract of the concentration of 250 mg/ml was found to show the significant anti helminthic activity. Helminthes infections are the chronic illnesses affecting human beings and cattle. *Catharanthus roseus* was found to be used from the traditional period as an anthelminthic agent. The anthelminthic property of *Catharanthus roseus* has been evaluated by using *Pherithema posthuma* as an experimental model and with Piperazine citrate as the standard reference. The ethanolic extract of the concentration of 250 mg/ml was found to show the significant anthelminthic activity with death time of 46.33 min whereas the standard drug at 50 mg/ml was found to show the death time of 40.67 min This investigation supported the ethnomedical claims of *Catharanthus roseus* as an anthelminthic plant. (Rawat and Gupta *al.,* 2011) [44].

8.6 Anti ulcer property- Vincamine and Vindoline alkaloids of the plant showed anti ulcer property. The anti ulcer property of the *C. roseus* plant leave is reported in ulcer induced test animals (Babulova *et al*., 2003) [45]. Vincamine and Vindoline alkaloids of the plant show anti ulcer property. Vincamine is known for cerebro-vasodilatory and neuroprotective activity (Sain *et al.,* 2013) [46].

8.7 Hypotensive property

Alkaloids that are isolated from C. roseus are found to be hypotensive, sedative and possess tranquilising and anti cancerous properties. The leaves have been known to contain 150 useful alkaloids among other pharmacologically active compounds. Significant antihyperglycemic and hypotensive activity of the leaf extracts (hydroalcoholic or dichloromethane-methanol) have been reported in laboratory animals (Pillay *et al.,* 1959) [47].

The leaves extract of Catharanthus roseus was investigated for hypotensive effects in adrenaline-induced hypertensive rats (AIHR) and compared with those of Atenolol in a crossover design. Catharanthus roseus leaves extract treated animals have shown the hypotensive effects. Hypotensive effects were also shown by Atenolol. (Ara *et al.,* 2009) [48]. The pharmacologically Active components responsible for hypotensive activities were isolated from plant using bioassay guided purification approach and the structure of the compounds was proposed by spectroscopic methods. The Catharanthus roseus leaves extract made significant changes in each cardiovascular parameter after investigation.

8.8 Hypolipidimic effect

Patel *et al*., 2011 [49] observed that significant anti atherosclerotic activity as suggested by reduction in the serum levels of total cholesterol, triglycerides, LDL-c, VLDLc and histology of aorta, liver and kidney with the leaf juice of Catharanthus roseus (Linn.) G. Donn. could have resulted from the antioxidant effect of flavonoid, and probably, vinpocetine like compound present in leaf juice of Catharanthus roseus (Linn.) G. Donn.

8.9 Anti diarrheal property

The anti diarrheal activity of the plant ethanolic leaf extracts as tested in the wistar rats with castor oil as a experimental diarrhea inducing agent in addition to the pretreatment of the extract. The anti diarrheal effect of ethanolic extracts *C. roseus* showed the dose dependant inhibition of the castor oil induced diarrhea**. (**Rajput *et al*., 2011) [50].

8.10 Biopesticidal property

Biological activity of solvent extracts of *Catharanthus roseus* were evaluated against larvae of gram pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae). Ethyl acetate fractions of leaf extract of *C. roseus* was found to be a potent bio-pesticide (Ramya *et al*., 2008). Insecticidal properties of Catharanthus roseus have also been reported by Deshmukhe *et al*., (2010) [51].

The pupicidal action of plants was evaluated by treating the pre-pupal stage of Spodoptera litura with the leaf extract of Catharanthus roseus by topical application method. 73.33, 49.33, 33.33, 28.00 and 17.33 percent mortality was observed when pre-pupae were treated with 2.0, 1.5, 1.0, 0.5 and 0.1% of leaf extract of C. roseus respectively (Sandey and Sudha Summarwar, 2016) [52].

8.11 Phytoremediation property

Phytoremediation used to remove pollutants from environment components. Pandey *et al*., (2007) [53] observed that the impact of cadmium and lead on *Catharanthus roseus*. They concluded that during germination the toxic effects of cadmium and lead with respect to C. roseus are the maximum and the plant gradually becomes more resistant to these heavy metals as it attains maturity. The phytoremediation potential of *Catharanthus roseus* with respect to chromium has been analyzed by Ahmad and Mishra (2014) [54]. C. roseus was shown to absorb up to about 38% of the amount of Cr present in primary and secondary sludge amended soil through roots and accumulate it to about 22% in leaves, thereby, proved useful in the reclamation and remediation of chromium contaminated soil and land. *Catharanthus roseus* has been used for lead and nickel phytoremediation by Subhashini and Swamy (2013) [55]. *Catharanthus roseus* is used in plant pathology as experiment host for phytoplasmas. This is because it is easy to infect with a large majority of phytoplasmas and also often has very distinctive symptoms such as phyllody and significantly reduced leaf size.

9. Possible pathways and mode of action

Vinca alkaloids block cells in mitosis because they are cell cycle specific agents. The vinca alkaloids bind specifically to b-tubulin and block its ability to polymerize with a-tubulin into microtubules. In the absence of an intact mitotic spindle, duplicated chromosomes cannot align along the division plate and cell division is arrested in metaphase. Cells blocked in mitosis undergo changes characteristic of apoptosis (Fig 2). They are also used for treatment of leukemias, lymphomas, and testicular cancer (Retna *et al*., 2013) [57].

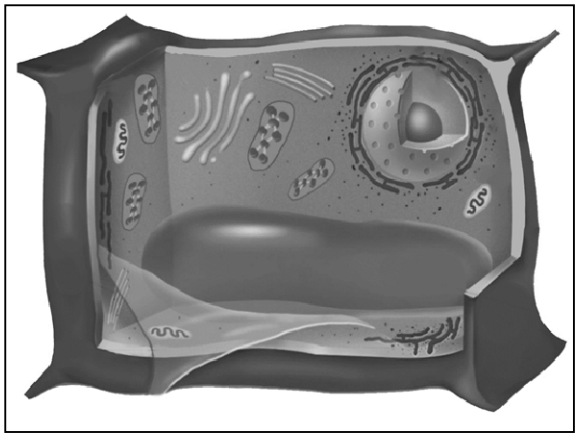


Fig 2: Possible pathways and mode of action of vincristine and vinblastine to act as anticancer

10. Conclusion

Medicinal plant is the most exclusive source of life saving drugs for majority of the world's population. They continue to be an important therapeutic aid for alleviating the ailments of human kinds. The search for defence mechanism, longevity and remedies to relieve pain and discomfort drove early man to explore these immediate natural surroundings. It led to the use of plants, animal products and minerals etc., and the development of a variety of therapeutic agents. Today, there is a renewal interest in traditional medicine and an increasing demand for more drugs from plant sources because green medicine is safe and more dependable then costly synthetic drug, many of which have adverse side effects. *Catharanthus roseus* was investigated from the ancient time for their phytochemical components and their therapeutic effect. The plant contains enormous phytochemical constituents of various medicinal applications. The plant also possess various property such as anti cancerous, anti diabetic, anti helmintic, anti diarrheal, anti microbial etc. Hence there are ample scope of research work to be done on the above plant to reveal the unknown mysteries which would help the need of the present pharmaceutical world.

11. References

1. Hogan S. Flora: A gardener's encyclopedia. Timber Press, Inc., Portland, OR, 2003.
2. Gajalakshmi S, Vijayalakshmi S, Devi RV. Pharmacological activities of *Catharanthus roseus:* A perspective review. International Journal of Pharmaceutical Science. 2013; 4(2):431-439.
3. Anonymous. Plant Resources of Tropical Africa. African Ornamentals. Proposals and Examples, PROTA Foundation, Wageningen, The Netherlands, 2011.
4. Łata B. Cultivation, mineral nutrition and seed production of Catharanthus roseus (L.) G. Don in the temperate climate zone. Phytochemistry Reviews. 2007; 6(2-3):403-411.
5. Mendioro MS, Diaz MQD, Alcantara MTB, Hilario OJ, Mateo P, Maghirang RDM. Cytological studies of selected medicinal plants: *Euphorbia pulcherrina* Willd. Ex Klotz, *Moringa oleifera* Lam., *Catharanthus roseus* (L.) Don. and *Chrysanthemum indicum* Linn, The Philippine Journal of Science. 2005; 134:31-37.
6. Tang, KX, Xing SH, Guo XB. Induction and flow cytometry identification of tetraploids from seed-derived explants through colchicine treatments in *Catharanthus roseus* (L.) G.Don, Journal of Biomedicine and Biotechnology, 2011, 793198.
7. Kulkarni RN, Sreevalli Y, Baskaran K, Kumar S. The mechanism and inheritance of intraflower self-pollination in self-pollinating strains of periwinkle. Plant Breeding. 2001; 120:247-250.
8. Sreevalli Y, Baskaran K, Kulkarni RN, Kumar S. Further evidence for the absence of automatic and intra-flower selfpollination in periwinkle. Current Science. 2000; 79 (12):1648-1649.
9. Pan YJ, Liu J, Guo XR, Zu YG, Tang ZH. Gene transcript profiles of the TIA biosynthetic pathway in response to ethylene and copper reveal their interactive role in modulating TIA biosynthesis in *Catharanthus roseus*. Protoplasma. 2015; 252(3):813-824.
10. Van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: Pharmacognosy and biotechnology. Current Medicinal Chemistry. 2004; 11:607-628.
11. Z´arate R, Verpoorte R. Strategies for the genetic modification of the medicinal plant *Catharanthus roseus* (L.) G. Don. Phytochemical Reviews. 2007; 6(2-3):475-491.
12. Favali M, Muestti R, Benvenuti S, Bianchi A, Pressacco L. Catharanthus roseus L. plants and explants infected

with phytoplasmas: Alkaloid production and structural observations. Protoplasma. 2004; 223:45-51.
13. Lee, IM, Davis RE, Gunderson Rindal DE. Phytoplasma: Phytopathogenic mollicutes. Annual Review of Microbiology. 2000; 54:221-255.
14. Choi P, Kim Y, Choi K, Chung H, Choi D, Liu J. Plant regeneration from hairy-root cultures transformed by infection with Agrobacterium rhizogenes in Catharanthus roseus. Plant Cell Reports. 2004; 22:828-831.
15. Obukosia S, Kimani E, Waithaka K, Mutitu E, Kimani P. Effects of growth regulators and genotypes on pyrethrum *in vitro In Vitro* Cell. Dev. Biol. Plant. 2005; 41:162-166.
16. Xu L, Liu G, Bao M. Adventitious shoot regeneration from *in vitro* leaves of Formosan sweetgum (Liquidambar formosana L.). Hort Science. 2007; 42:721-723.
17. Trigiano R, Gray D. Plant tissue culture concepts and laboratory exercises. CRC Press, Boca Raton, FL, 2000.
18. Gambino G, Ruffa P, Vallania R, Gribaudo I. Somatic embryogenesis from whole flowers, anthers, and ovaries of grapevine (Vitis spp.). Plant Cell Tissue Organ Culture. 2007; 90:79-83.
19. Choi J, Kim H, Lee J, Bae Y, Chung Y, Shin J, Hyung N. Efficient and simple plant regeneration via organogenesis from leaf segment cultures of persimmon (*Diospyros kaki* Thunb.). *In Vitro* Cell Developement Biological Plant. 2001; 37:274-279.
20. Magnotta M, Murata J, Chen J, De Luca V. Identification of a low vindoline accumulating cultivar of *Catharanthus roseus* (L.) GDon. by alkaloid and enzymatic profiling. Phytochemistry. 2006; 67:1758-1764.
21. Jaleel CA, Gopi R, Alagulakshmanan GM, Panneerselvam R. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Science. 2006; 171:271-276.
22. Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R *et al*. Pseudomonas fluorescens enhances biomass yield and ajmalicine production in Catharanthus roseus under water deficit stress. Colloids and Surf acesB: Biointerfaces. 2007; 60(1):7-11.
23. Singh SN, Vats P, Suri S, Shyam R, Kumria MM, Ranganathan S *et al*. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. Journal of Ethnopharmacology. 2001; 76:269-277.
24. Duflos A, Kruczynski A, Barret J M Novel aspects of natural and modified vinca alkaloids. Current Medicinal Chemistry - Anti-Cancer Agents. 2002; 2:55-70.
25. Zhang LB, Gou LH, Zeng SV. Preliminary study on the isolation of endophytic fungus of *Catharanthus roseus* and its fermentation to produceproduct of therapeutic value. Chinese Traditional Herbal Drugs. 2000; 11:805-807.
26. Tung CY, Yang DB, Gou M. A preliminary study on the condition of the culture and isolate of endophytic fungus producing vincristine. Journal of Chuxiong Normal University. 2002; 6:39-41.
27. Mishra P, Sharma S. Pattern of diversity for morphological and alkaloid yield related trades among the periwinkle *Catharathus roseus* accessions collected from in and around Indian Subcontinent. Genetic Resource and Crop Evolution. 2001; 48:273- 286.
28. Nayak BS, Lexley MPP. *Catharanthus roseus* flower has wound healing activity in Sprague Dawley rats. BMC Complimentary Alternative Medicine. 2006; 6:41.
29. Van der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The Catharanthus alkaloids: pharmacognosy and biotechnology. Current Medicinal Chemistry. 2004; 11(5):607-628.
30. Mishra P, Kumar S. Emergence of periwinkle Catharanthus roseus as a model system for molecular biology of alkaloids: phytochemistry, pharmacology, plant biology and *in vivo and in vitro* cultivation. Journal of Medicinal and Aromatic Plant Science. 2000; 22:306-337.
31. Shukla AK. Molecular studies on biosynthesis of shoot alkaloids in *Catharanthus roseus* (L.) G. Don. PhD thesis, Department of Biochemistry, University of Lucknow, India, 2005.
32. Verpoorte R, Contin A, Memelink J. Biotechnology for the production of plant secondary metabolites. Phytochemical Review. 2002; 1:13-25.
33. De Luca V, Pierre B. The cell and developmental biology of alkaloid biosynthesis. Trends in Plant Science. 2000; 5:168-173.
34. Banskota AH. Antiproliferative activity of Vietnamese medicinal plants. Biological Pharmaceutical Bulletin. 2002; 25(6):753- 60.
35. Ueda JY, TezukaY, Banskota AH. Antiproliferative activity of Vietnamese medicinal plants. Biological Pharmaceutical Bulletin. 2002; 25(6):753- 60.
36. Wang S, Zheng Z, Weng Y. Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. Life Science. 2004; 74(20):2467-78.
37. Retna AM, Ethalsha P. A review of the taxonomy, ethnobotany, chemistry and pharmacology of *Catharanthus roseus* (Apocyanaceae), International Journal of Engineering Research & Technology. 2013; 2(10):3899-3912.
38. Widowati W, Mozef T, Risdian C, Yellinaty Y. Anticnacer and free radical scavenging potency of *Catharanthus roseus*, *Dentrophthoe petandra*, *Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines. Oxid Antioxid Medicinal Science. 2013; 2(2):137-140.
39. Ahmad R, Misra N. Evaluation of Phytoremediation Potential of Catharanthus roseus with Respect to Chromium Contamination. American Journal of Plant Science. 2014; 5:2378-2388.
40. Siddiqui MJ, Ismail AFA, Aisha AMS, Abdul M. Cytotoxic activity of *Catharanthus roseus* (Apocynaceae) crude extracts and pure compound against human carcinoma cell line. International Journal of Phytopharmacology. 2010; 6:43-47.
41. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong *et al*. Antidiabetic and Antioxidant Properties of Alkaloids from *Catharanthus roseus* (L.) G. Don. Molecules. 2013; 18:9770-9784.
42. Prajakta JP, Ghosh JS Antimicrobial Activity of Catharanthus roseus A Detailed Study. British Journal of Pharmacology *and* Toxicology. 2010; 1(1):40-44.
43. Alba Bhutkar MA, Bhise SB. Comparative Studies on Antioxidant Properties of Catharanthus Rosea and Catharanthus. International Journal of Pharmaceutical Techniques. 2011; 3(3):1551-1556.
44. Rawat Sand Gupta. A Development and study of wound healing activity of an Ayurvedic formulation. Asian Journal of Research in Pharmaceutical Sciences. 2011; 1(1):26 -28.

45. Babulova A, Machova J, Nosalova V. Protective action of vinpocetine against experimentally induced gastric damage in rats. Arzneimittel forschung. 2003; 43:981-985.
46. Sain M, Sharma V. *Catharanthus roseus* (An anti-cancerous drug yielding plant) - A Review of Potential Therapeutic Properties. International Journal of Pure and Applied Bioscience. 2013; 1:139-142.
47. Pillay PP, Nair CPM, Santi Kumari TN. Lochnera rosea as a potential source of hypotensive and other remedies. Bulletin of Research Institute of the University of Kerala, Ser A6. 1959; 1:51-54.
48. Ara N, Rashid M, Amran MS. Comparison of hypotensive and hypolipidemic effects of Catharanthus roseus leaves extract with atenolol on adrenaline induced hypertensive rats. Pakistan Journal of Pharmaceutical Science. 2009; 22(3):267-71.
49. Patel Y. Evaluation of hypolipidemic activity of leaf juice of Catharanthus roseus (Linn.).Acta Poloniae Pharmaceutica - Drug Research. 2011; 68(6):927-935.
50. Rajput M, Nair V. Akansha Chauhan.Evaluation of Antidiarrheal Activity of Aerial Parts of Vinca major in Experimental Animals. Middle-East Journal of Scientific Research. 2011; 7(5):784-788.
51. Deshmukhe PV, Hooli AA, Holihosur SN. Bioinsecticidal potential of *Vinca rosea* against the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Recent Research Science and Technology. 2010; 2(1):01-05.
52. Sandey J, Summarwar S. Pupicidal activity of plant extracts of Catharanthus roseus against 6th instar of Spodoptera litura. International Journal of Fauna and Biological Studies. 2016; 3(4):9-10.
53. Pandey S, Gupta K, Mukherjee AK. Impact of cadmium and lead on Catharanthus roseus - A phytoremediation study. Journal of Environmental Biology. 2007; 28(3):655-662.
54. Ahmad NH, Rahim RA, Mat I. *Catharanthus roseus* aqueous extract is cytotoxic to Jurkat leukemic T-cells but induces the proliferation of normal peripheral blood mononuclear cells. Tropical Life Science Research. 2010; 21(2):101-113.
55. Subhashini V, Swamy AVVS. Phytoremediation of Pb and Ni Contaminated Soils Using *Catharanthus roseus* (L.). Universal Journal of Enviromental Research and Technology. 2013; 3(4):465-472.