

Extraction and Characterization of Bioactive Alkaloids from Madagascar Periwinkle (*Catharanthus roseus*)

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Abstract: *Catharanthus roseus* (L.) G. often known as *Vinca rosea*, Madagascar periwinkle, Sadabahar. It is evergreen, periwinkle, sadabahar plant. It is evergreen, perineal, erect and long-lived flowering plant it belongs to the family Apocynaceae. The utilization of natural products as a medicinal herb in the form of extract or specific formulation to cure the diseases is an age-old concept. These remedial properties are used in a different medicinal system like Homeopathic, Ayurvedic, Unani and modern medicine etc. due to the efforts of great pioneer scientists. Today various studies have been conducted on the medicinal herbs for their traditional utilization, phytoconstituents, clinical applications and toxicity. Drugs like morphine, vinblastine, vincristine, quinine, aspirin, digitoxin are the example of the medicine derived from traditional herbs. Around twenty five percent prescriptions are directly or indirectly obtained from natural sources and isolation of bioactive alkaloids to investigate their therapeutic potential for various diseases.

Keywords: *Catharanthus roseus*, Alkaloids, HCL, Different reagents

I. INTRODUCTION

Catharanthus roseus (L.) often known as *Vinca rosea*, Madagascar periwinkle, sadabahar plants. It is an evergreen, periwinkle, sadabahar. It is an evergreen, perineal, erect, long lived flowering plant It belongs to Family Apocynaceae. It utilized as a decorative as well as therapeutic plant. The flower may vary in colour from pink to purple and leaves are arranged in opposite. It is an important medicinal plant. It arises from the Indian ocean Island of Madagascar. Grow tropical and sub-tropical region like India, Pakistan, Bangladesh, Australia and Malasia. Currently it is a common plant. *Catharanthus roseus* is an evergreen herbs or herbaceous plant growing to 1m. tall. The leaves are oval to oblong with glossy green with a pale midrib and short petiole. Leaves arrange in opposite pairs. The flower area white to dark pink. The fruits is a pair of follicles. It is cultivated ornamental plants in the tropical and sub-tropical region It grows to about 2 feet in length. It is highly branched and woody base. The leaves on the plant is petiolate, oblong, thick and leathery in texture, while the arrangements are apposite or alternate (Virmani et al 1978). The utilization of natural products as medicinal herbs in the form of extract or specific formulation as cure of various diseases as a age-old concept. These remedial properties are used in different type of medicinal system like Homeopathic, Ayurvedic, Unani, and modern medicine etc. Due to the efforts of great pioneer scientists' various studies have been conducted on the medicinal herbs for their traditional utilization, phytoconstituents, clinical applications and toxicity. Some drugs like morphine, vinblastine, Vincristine, Quinine, aspirin and digitoxin are the example of the medicine derived from traditional herbs. The therapeutic vegetation of south Asia is a well spring of various pharmacologically significant mixture that are generally consumed as home cures in restoring or treating different sorts of illness from normal cold to malignant growth (Patel,2011). The plant with multiple pharmacological applications is *Catharanthus roseus*. It is one of the commercially explored flowering plants due to its wide range of medicinal properties in *Catharanthus roseus*



it contains alkaloids, saponins, carbohydrates, flavonoids etc. Every part of these plants like roots, barks, leaves, etc contain the essential chemical constituents. These plants contain many chemical constituents which have the various significant pharmacological activities like Vincristine and Vinblastine are well known for the anticancer activity and it has other essential pharmacological activities such as antidiabetic, anthelmintic, antimicrobial, antioxidants and Alzheimer wound healing activity. More than one thirty alkaloids are present in the plants, which are used as pharmaceutical, agrochemical, flavour, fragrance, food additive and pesticides. The alkaloids like Actineo Plastidemic Vinblastine, Vincristine, Vindesine, Vindesine Tabersonine etc are mainly present in serial part. (Jai Narayan Mishra, Navneet Kumar Verma; 2017). The Cytotoxic drugs that work by inhibiting the ability of cancer cells to divide. The Vinca Alkaloids thus prevent microtubules polymerization. As opposed to the mechanism of action of taxanes (Harshal Ashok Pawar et.al.2022). Vincristine another alkaloid is used for leukaemia in children. Vinblastine is used particularly to treat Hodgkin's disease besides Lymphosarcoma, choriocarcinoma, neuroblastoma, carcinoma of breast, lung and other organ in acute and chronic leukaemia. Vincristine arrest mitosis in metaphase and is very effective for treating acute leukaemia in children's children and lymphocytes leukaemia (Hadagali Ashoka; 2017).

II. REVIEW OF LITERATURE:

Sangita Zongluju, Pankaj Kumar et al., (2024) Study the phytochemical screening and Extraction of *Catharanthus roseus* and he concluded presence of alkaloids and one of most widely available plants with life-savings quality in Vinca and it has anticancer and antitumor properties. Their research showed that the ethanol extract of Vinca contain the greatest concentration of alkaloids.

Harshal Ashok Pawar et al., (2022) work on Extraction, Isolation and analysis of Vinca alkaloids concluded that *Catharanthus roseus* is an important medicinal plant and several applications in pharmaceutical and industrial products. In the present, vinblastine and vincristine are two alkaloids for the treatment of childhood leukaemia and Hodgkin's lymphoma. Production rate of vinblastine and vincristine in *Catharanthus roseus* is very low it's extraction costly and too inefficient to be industrialized.

Jai Narayan Mishra, et al., (2017) worked on a brief study on *Catharanthus roseus* and concluded that *Catharanthus roseus* is one of the 21000 important medicinal plants found. It is used for the cure a number of diseases such as diabetes, sore mouth, mouth ulcer, and leukaemia It produces about 130 alkaloids such as reserpine, vinceine, raubasin and ajmalicine, Anti-leukaemia activity is showed by vinblastine and vincristine.

Rajesh Arora et al., (2022) he studied anticancer alkaloid of *Catharanthus roseus* and form *Catharanthus* alkaloids and their semisynthetic derivatives have been used in the clinic since a long time and continue to benefit innumerable cancer patients. The case of *Catharanthus* has been successful one.

Surya Nath Pandey et al., (2020) Phytochemicals and Pharmacological studies of *Catharanthus roses*. He concluded *Catharanthus roseus* contain many active chemical constituents such as Vincristine and Vinblastine are well known for their anti-cancer's activity. Every plant such as bark, root, leaves and flower contains secondary metabolites and responsible for various pharmaceutical activities.

Arooj Aftab, et al., (2020) Phytochemistry and phytochemicals potential of *Catharanthus roseus* they concluded the leaves of this plant incorporates diverse compound with medicinal properties, which includes alkaloids. *Catharanthus roseus* is used economically contain catharanthine and vindoline, which are substance that make anticancer dimers. Vinblastine and Vincristine extract from *Catharanthus roseus* leaves have additional proven anti-diabetes and anti-tumour results in laboratory.

Rupavath Chandrasekhar et al., (2021) he studied vinca alkaloids from Madagascar periwinkle and *Catharanthus roseus* (L.) G. Don: An overview reported that there are different medicinal compounds present in the Madagascar periwinkle. The two semisynthetic analogs, vinorelbine and vindesine obtained from alkaloids of periwinkle. It is considered that most efficient drugs introduced in the last decades from all vinca alkaloids, vincristine and Vinblastine exhibits less toxicity.



Parul Sharma, Nancy Singla et al., (2024) he studied a review on phytochemical constituents and pharmacological properties of *Catharanthus roseus* (L.) G. Don. He found roseus utilization for the treatment of cancer and diabetes has been widely accepted. Part from this the chemical compounds of *Catharanthus roseus* and its pharmaceutical uses have been intensively studied however, there is an increase in the number of researches of finding new chemical compounds from *Catharanthus roseus*, so their researches finding the new chemical compounds and also finding the new biological roles and their mechanism along with their toxicity. *Catharanthus. roseus* was conventionally used for the treatment of various disorders like ulcers, memory loss, malaria, stomach problems, menorrhagia and tooth problems but very few modern kinds of researches are available to support these uses.

III. MATERIAL AND METHODOLOGY:

Fresh, health leaves of *Catharanthus roseus* was collected from surrounding. An approximate 1 kg of fresh, matured leaves of the plant was thoroughly cleaned with tap water, after which they were rinsed with distilled water. The leaves were then allowed to air dry for a week at ambient room temperature (27 °C) for 7-10 days. The sample was then manually pounded into a fine powder using a sterile, clean mortar and pestle to enhance surface area, and the powder was weighed, assembled, and packaged into clean cellophane bags and labelled to avoid mix-ups. The sample was stored in a cool, dry location until needed, while fresh leaves were used promptly after being collected from the tree branches. The extraction was prepared using maceration method. Thus, 100g of the powdered plant material was weighted and extracted Leaf powder was soaked in ethanol for 30min. The mixture was filtered to obtained ethanolic extract. The filtrate was evaporated on water bath to remove solvent and diluted HCL was added in remaining residue Proper filtration was carried out using filter paper after which the filtrate was boiled for proper extraction. The extract was tested with several reagents like Mayers, Dragondraffs, Wagners to determine the presence of alkaloids.

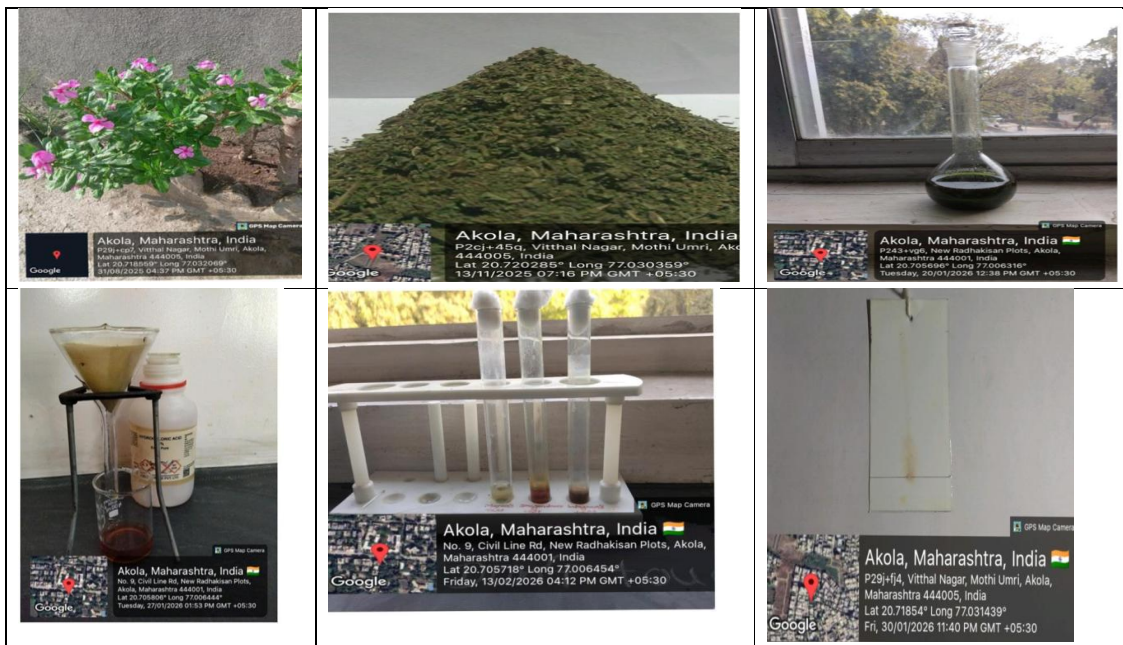
Pre coated Silica gel plate were used as stationary phase, Chloroform: Methanol (9:1) are used as mobile phase. Using a capillary tube, small spots of the sample and standard solutions were applied on the baseline of the TLC plate. The plate was placed in a TLC chamber pre-saturated with the mobile phase and allowed to develop until the solvent front travelled approximately 80% of the plate length after development, the plate was removed and dried. Alkaloid spots were visualized using. Dragendorff's reagent (orange/brown spots indicate alkaloids). Measure the distances using a ruler, and calculate the RF value of observed spots.

IV. OBSERVATION AND RESULT:

To determine the presence and Quantity of Alkaloids from *Catharanthus roseus* leaf. The powdered leaf sample was soaked in acidified solvent ethanol. The mixture gradually changed from light green to dark greenish-brown, indicating extraction of phytochemicals. After filtration, the filtrate appeared clear to dark greenish solution After evaporation of solvent, by putting ethanolic solution in water bath a brownish-yellow residue was obtained. The residue was slightly sticky and bitter in taste, characteristic of alkaloid extracts. After adding dil. HCL in residue reddish colour obtained indicating the present of alkaloids. The extract was subjected to qualitative tests for alkaloids, which produced characteristic precipitates confirming their presence.

Sr. No.	Test	Observation	Alkaloid ++/--
1	Mayer's Test	Creamy precipitation	++
2	Dragendorff's Test	Red Precipitation	++
3	Wagner's Test	Orange precipitation	++





Alkaloids were successfully extracted from the leaves of *Catharanthus roseus* using an acid–base extraction method. The qualitative chemical tests confirmed the presence of alkaloids. The extract showed characteristic precipitates with Dragendorff's, Mayer's, and Wagner's reagents. The final dried extract contained crude alkaloids, which may include important compounds such as Vincristine and Vinblastine, known for their pharmaceutical significance. Weight of dried leaf powder is 5 g and weight of crude alkaloid extract is about 0.01 and the Percentage yield is near about 1–2%. Thin Layer Chromatography (TLC) separates compounds based on different adsorption between a stationary phase and a mobile phase. Stationary phase is Silica gel TLC plate Mobile phase are Organic solvent mixture Detection: Alkaloid-specific spray reagents (e.g., Dragendorff's reagent) Alkaloids move different distances depending on their polarity, producing spots with different R_f values. Orange or yellow spots appear indicating alkaloids. Different spots correspond to different alkaloids present in the leaf extract's analysis confirms the presence of alkaloids in *Catharanthus roseus* leaves, indicated by coloured spots after spraying with Dragendorff's reagent.

$$R_F = \frac{\text{Distance Traveled by Compound}}{\text{Distance traveled By Solvent}}$$

$$\frac{5.4}{7.7} = 0.701$$

V. DISCUSSION

Alkaloids are nitrogen containing secondary metabolites widely recognized for their pharmacological properties, including anticancer, antimicrobial activities. *Catharanthus roseus* L.G. Don (commonly known as Madagascar periwinkle) is an important medicinal plant known for its rich alkaloid content, particularly Vinca alkaloids such as vincristine and vinblastine, which are used in chemotherapy. The extraction of alkaloids from plant material requires careful selection of solvents and procedure to maximize yield while preserving the bioactive compound. In this, ethanol was used as the primary extraction solvent due to its polar nature and ability to solubilize a wide range of alkaloids. Ethanol is safe and environment friendly as compared to other organic solvents like methanol or chloroform. The powered leaves of *Catharanthus roseus* were mixed with ethanol, allowing to diffuse from the plant matrix into the solvent. The solution was then filtered to remove insoluble plant residues, ensuring a clear extract for subsequent



processing. Filtration is a critical step to prevent clogging during solvent evaporation and avoid co-extraction of unwanted solids that may interfere with the alkaloid yield. The ethanol solution was subjected to evaporation using a water bath. This step serves to remove ethanol completely, concentrating the alkaloids in a crude residue form. Controlled heating in a water bath is essential because excessive heat can degrade alkaloids, reducing their bioactivity. Once the solvent was evaporated, the remaining residue was treated with hydrochloric acid (HCL). Acidification converts free base alkaloids into their respective water-soluble salt forms, facilitating further purification or analysis. This step is crucial in alkaloid extraction because it enhances the solubility of alkaloids, allowing for easier separation from non-alkaloid components and preventing precipitation. Overall, this extraction method demonstrates a practical approach to isolating bioactive alkaloids from *C. roseus* leaves. The combination of ethanol extraction, solvent evaporation, and acid treatment provides a foundation for subsequent qualitative and quantitative analyses, and it can be adapted for larger-scale pharmaceutical applications. TLC was employed for the qualitative analysis and preliminary identification of alkaloids. This method allows the separation of individual alkaloid compounds based on their differential migration on a silica gel or alumina plate under the influence of a suitable mobile phase. The development of distinct spots, when visualized under UV light or with appropriate spraying reagents, confirms the presence of alkaloid constituents. TLC is not only rapid and cost-effective but also provides valuable insight into the complexity and diversity of alkaloids present in *Catharanthus roseus* leaves. Overall, the described method effectively combines solvent extraction, concentration, acid treatment, and chromatographic analysis to isolate and identify alkaloid compounds. This approach demonstrates a systematic and reproducible procedure, which can be adapted for further pharmacological and phytochemical studies of *Catharanthus roseus* and other medicinal plants.

VI. CONCLUSION

This research has been successfully demonstrated that the extraction and preliminary analysis of alkaloids from *Catharanthus roseus* leaves highlighted the plant's potential as a valuable source of bioactive compounds. The study involved standard extraction technique, which proved effective in isolating the alkaloids fraction and confirming the presence of important phytochemicals such as vincristine and vinblastine, which are known for their therapeutic application in oncology. The finding emphasizes the influence of extraction parameters including solvent type, pH, and duration on yield and purity, providing critical insights for optimizing alkaloids recovery. Moreover, the qualitative and quantitative assessments of the extract underscore the significance of *Catharanthus roseus* as a medicinal resource and reinforce the importance of sustainable harvesting and processing practice. This work not only contributes to the growing body of knowledge on plant-derived alkaloids but also lays the foundation for further research on pharmacological efficacy, formulation development, and potential commercial exploitation. Overall, the study proves that the *Catharanthus roseus* leaves are a rich source of biologically active alkaloids and underscore the relevance of systematic extraction methodologies, encouraging continued exploration of plant-based therapeutic innovation.

REFERENCES

1. Arooj Aftab, Muhammad Akram, Umme Laila, Rida Zainab, Momina Iftikhar, Maghchiche Abdelhak, Gawel Solowski, Feth Ahmet Ozdemir, Ebrahim Alina – Ahandani, Adonis Sfera. (2023): Phytochemistry and phytochemicals Potential of *Catharanthus roseus*. 10: 6670-6676.
2. Ashishdeep Kaur. (2023): of Vinca alkaloids from *C. roses* and Mechanism of Alkaloids in Cancer. 20: 1-6.
3. Gautam Kumar, Ravi Kumar, Girendra Kumar Gautam, Harshit Rana. (2021): The Phytochemical and Pharmacological Properties of *Catharanthus roseus* (Vinca). 4: 429-434.
4. Handagali Ashoka, Prashanth Hegde, Mansa K. H Chandraprasad Madihalli, Pradeep S and Ashok Kumar. (2017): Isolation and Detection of Vinca alkaloids From *Catharanthus roseus*. 4: 675-683.
5. Harshal Ashok Pawar, Anjali Harshal Pawar, Sandip Ashok pawar, Prashant Ashok Pawar. (2022): Extraction, Isolation and analysis of Vinca alkaloids. 1: 1-10.
6. Jai Narayan Mishra, Nevneet Kumar Verma. (2017) A brief study of *Catharanthus roseus*. 2: 20-23.



7. Kashav, Khilnani. (2018): Phytochemicals Analysis of Catharanthus roseus L. (G.) Don. 5 :1-8. • Maryam Moudi, Rusea Go, Christina Yong Seok Yien, Mohd. Nazre. (2013): Vinca Alkaloids. 4: 1231-1235.
8. Parul Sharma, Nancy Singla. (2024): A review on phytochemical constituents and pharmacological properties of Catharanthus roseus (L.) G. Don. 10: 131-157.
9. Poonam Ingalwad, Vikram Veer, Dr. Ashok Bhosale. (2022): Overview on a Vinca Alkaloid & Its Medicinal, Therapeutic Properties Poonam Ingalwad, Vikram. 4: 846- 849.
10. Rajesh Arora, Poonam Malhotra, Ajay K Mathur, Archana Mathur, CM Govil, PS Ahija. (2009) : Anticancer Alkaloids of Catharanthus roseus 9: 293-309.
11. Rupavath Chandrasekhar, Jyothi Chaitanya Pagadale and N. Lakshmi Bhavani. (2020): Vinca Alkaloids from Madagascar periwinkle. (Catharanthus roseus (L.) G. Don). 5: 187192. 14
12. Sangita Zongluju, Pankaj Kumar, Aman Saini, Jugroop sing. (2024): Catharanthus roseus Extraction and Phytochemical Screening. 9: 1450-1458.
13. Surya Nath Pandey, Vijay Pratap and Naresh Kumar. (2020): Phytochemicals and Pharmacological Studies of Catharanthus roseus Linn. 9: 1407-1415.
14. Varunesh Chaturvedi1, Saloni Goyal1, Mohammad Muki, Monika Meghani3, Faheem Patwekar, Mohsina Patwekar, Shabana Kausar Khan, G.N. Sharma. (2022): Comprehensive Review on Catharanthus roseus L. (G.) Don: Clinical Pharmacology, Ethnopharmacology and Phytochemistry. 4: 17-36.
15. Virmani OP, Srivastava GN, Singh Pratap. (1978): Catharanthus roseus the tropical periwinkle. Indian Drugs. 8: 231-49.
16. Yash Srivastav, Aayush Singh Chauhan, Abdul Hameed. (2024): Catharanthus roseus (Vinca rosea), Taxonomy, Phytochemicals and Pharmacological (Anti-Cancer) properties: A comprehensive overview. 2: 236-254.

