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Catharanthusroseus: Extraction and Phytochemical Screening

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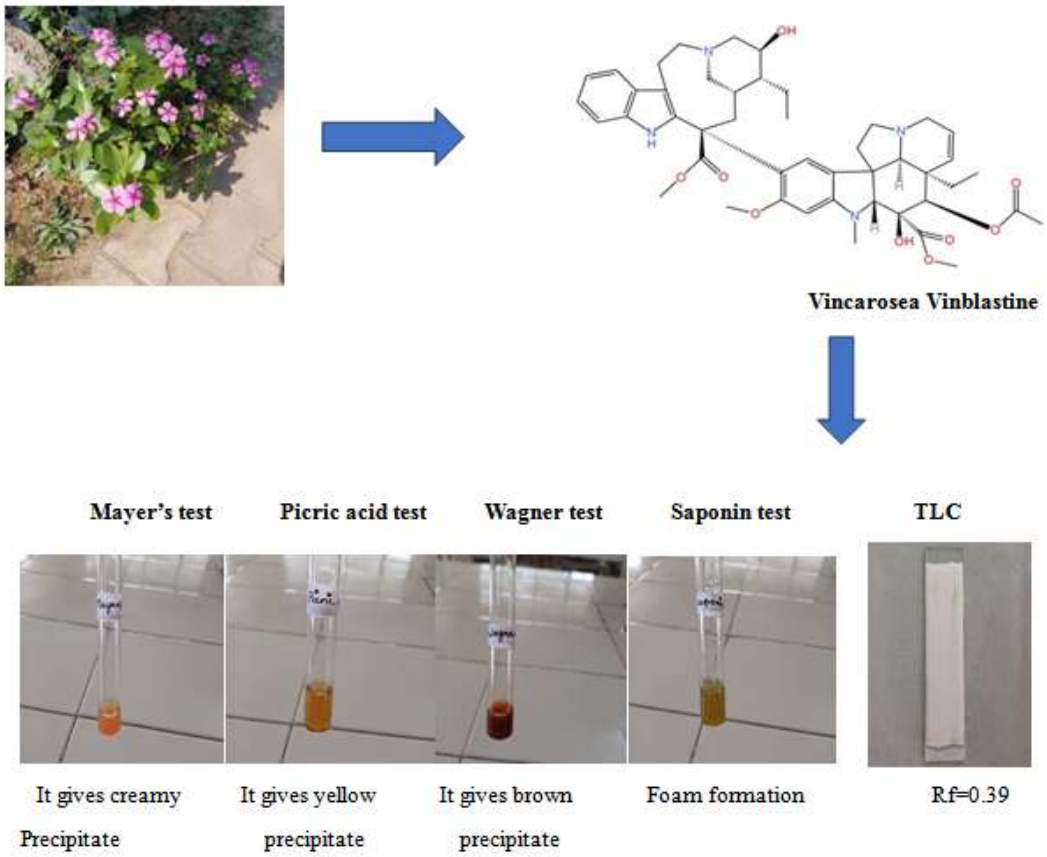
ABSTRACT:



KEYWORDS: Catharanthusroseus, Vinca Alkaloids, Extraction, Vinblastine, Vincristine, Phytochemical.

I. INTRODUCTION:

There are many naturally occurring plants that can be utilized medicinally are all around us. Among them is the plant Catharanthus roseus, which is widespread in tropical regions. A perennial plant native to Madagascar, Catharanthus roseus Linn is primarily found in tropical and southern Asia.(1, 2) Many popular names for Catharanthus roseus include vinca roseus, rose periwinkle myrtle, sparkling eyes, cape periwinkle, graveyard plant, old maid, pink periwinkle, and rose periwinkle. It has various colours, including pink, purple, and white, and is utilized as a decorative as well as a therapeutic plant. In Malaysia, the term that is used locally is KemuntingCina. The vinca alkaloids are the oldest class of plant alkaloids that have been used as cancer treatments.(3)More than 70 distinct indole alkaloids can be found in the stem of the vinca rosea plant, which yields a milky sap. Among these are two anti-neoplastic substances called vinblastin and vincristine that are derived from plants.(4)Vinblastine is used to treat paediatricleukaemia, and vincristin is used as a chemotherapeutic regimen for Hodgkin's lymphoma. Peripheral neuropathy, hair loss, hyponatremia, and constipation are the main

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adverse effects of vinca alkaloids, which inhibit the metaphase of cellular mitosis. Their primary functions are blood cancer, malaria, hypertension, diabetes, non-small lung cancer, Hodgkin's lymphoma, and improverecall. In addition, it possesses antimicrobial, antioxidant, anti-diarrhoeal, hypolipidemic, and wound-healing properties.(5)Peckolt (1910) reported on the use of a leaf infusion in Brazil for the treatment of chronic wounds, as a mouthwash for toothaches, and to control bleeding and scurvy. Related species have been utilized in Europe to suppress the flow of milk in a proprietary manner. It is a known effective oral hypoglycaemic agent in the Philippines and has been used to treat diabetic ulcers in the British West Indies. In a more recent study, Chopra et al. found that the total alkaloids have a notable and long-lasting hypotensive effect in addition to a limited antibacterial activity Ajmalicine, one of the alkaloids isolated from this plant, has been reported to have a transient depressor effect on arterial blood pressure, but the hypoglycaemic and antibacterial activities have not been verified. The name "Periwinkle," or Catharanthus roseus (Family Apocynaceae), is derived from the Greek word meaning "pure flower." It is also referred to as "Nayantara" or "Sadabahar." Roseus, on the other hand, means red, rose, or rosy.(6)

Scientific classification:(7)

Botanical Name: Vinca Rosea (Catharanthus roseus)
Family Name: Apocynaceae
Kingdom: Plantae
Division: Magnoliophyte(Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Order:Gentianales
Genus: Catharanthus

Species:c.roseus

Geographical distribution:

Catharanthus roseus is originated from the Indian Ocean Island of Madagascar. In the wild, it was thought to be an endangered species of plant. Nonetheless, it is now a common plant in many tropical and subtropical regions across the world, including the Southern United States.(8)The roots, leaves, stems, and flowers are all beneficial parts of the plant. It likes soil that has good soil drainage, is moist, and is sandy loam in nature.Cuttings and seeds can be used to propagate it, and in temperate regions, it can withstand cooler growing conditions.(9)

Chemical Constituents:(8, 10)

(a) Vinblastin-Vinblastin is a medication sold under various brands, including Velban. It is used in conjunction with various medications in certain areas. This department treats a wide range of cancer types, such as malignant melanoma, bladder cancer, brain cancer, seminoma, bladder cancer, and Hodgkin's malignant neoplastic disease. It is given by intravenous injection.

(b) Vincristine- In chemotherapy, vincristin is also known as leurocristine and is marketed under several brand names, including Oncovin. It ought to be considered when treating various cancer kinds. These comprise, among other things, neuroblastoma, Hodgkin's disease, acute lymphocytic leukaemia, acute myeloid leukaemia, and small cell lung cancer. IV administration is used to administer it. Vincristine Sulphate is also used as an immunosuppressant.

(c) Vindesine-Vindesine, an organic compound belonging to the opposing mitotic genus Vinca, is utilized in therapy. It should be used to treat a variety of cancer types, such as carcinoma, lymphoma, malignant neoplasm disease, multiple skin cancers, and carcinoma beneath malignant neoplasm diseases. The blood vessel is responsible for administering it.

(d) Tabersonine-Vincarosea is a medicinal plant that contains the indole alkaloid terpenetabersonine. The enzyme called tabersonine 16-hydroxylase (T16H) hydroxylates these at position sixteen, forming 16-hydroxytabersonine. Vinblastine biosynthesis requires the two precursors, of which this is the first intermediate that yields type vindoline.

(e) Vinpocetine-These are the synthetically derived chemical form of vincamine, an organic compound found in vinca. These were

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taken from the seeds of Voacanganafricona or the leaves of Vinca minor, also known as lesser periwinkle. Because of its potentially dangerous nootropic properties, vinpocetin supplements were not allowed for sale in Canada, Australia, or New Zealand.

The major constituents in different part ofCatharanthus roseus has been explained in the tableno.1

Table 1. Major constituents in different part of Catharanthus roseus

Part of plant	Constituents
Leaf	Vindoline, vinblastine, vincristine, alkaloids, carbohydrates, saponins, tannins, flavonoid, steroids, triterpenoids.
Steam	Carbohydrate, steroids, tannin, alkaloids.
Flower	Steroids, carbohydrates, tannins, saponins, alkaloids.
Root	Saponins, carbohydrates, alkaloids, triterpenoid, alkaloids, tannins, ajmalicine, steroids.
Whole part	Catharanthine, vindoline, vincristine, mono terpenoids, glycosides, phenolic, flavonoids, vinblastine.

Uses:

In Ayurvedic medicine, the leaves, flowers, and roots are used.The plant's extract is used in Chinese medicine to treat conditions like diabetes, malaria, leukaemia, and Hodgkins'sdisease. In conventional medicine, flower extracts are used as an infant eye wash, leaf juice is used to treat wasp stings, and gargles are used to soothe sore throats. Cough and diabetes can both be treated with periwinkle tea. The alkaloids with anti-tumour and anti-cancer properties are found in the leaves and stems. The leaves are used to manage hypertension and diabetes. Moreover, the alkaloids have calming and sedative effects. Because of its ability to detoxify and counteract poison, it is used to treat wasp stings and relieve depression and muscle pain. This herb treats sore throats, bleeding gums, mouth ulcers, and nosebleeds. When taken internally, it can help treat conditions like diabetes, diarrhoea, gastritis, cystitis, and enteritis.Vinca rosea is a plant that promotes brain health. The constituents of itscatives enhance the brain's blood flow and augment its oxygen consumption capacity. Additionally, it prevents abnormal blood coagulation and increases serotonin levels. In addition to maintaining blood thinning, the alkaloid vincamine improves memory. Therefore, it is beneficial in preventing dementia, particularly vascular dementia.If taken orally, periwinkle can be harmful.Women who are expecting should steer clear of the plant.

Extraction:

Extraction is the process of separating the parts of a plant that have medicinal properties using specific solvents and accepted practices.(11)Every extraction process aims to extract the soluble plant metabolites while leaving the insoluble cellular marc (residue) behind. Many plant metabolites, including alkaloids, glycosides, phenolics, terpenoids, and flavonoids, are present in complex mixtures in the initial crude extracts prepared using these techniques. In the form of tinctures and fluid extracts, some of the initially obtained extracts might be ready for use as medicinal agents, while others require additional processing. Commonly used extraction methods are discussed below: -

Maceration, infusion, decoction and percolation:

Maceration is a process, which is used to make wine and has been embraced extensively employed in studies on medicinal plants. During the maceration process, coarse or powdered plant materials were soaked in a solvent in a stoppered container and left to stand at room temperature for at least three days while being constantly stirred.(11)The procedure was designed to break down and soften the plant's cell wall, releasing the soluble phytochemicals. The mixture is filtered or pressed after three days. Convection and conduction are the methods used in this traditional method to transfer heat, and the type of compound extracted from the samples depends on the solvent selected. The principles of maceration are also applied to infusion and decoction, which involve soaking in either cold or boiling water. For decoction, the sample is boiled in a predetermined volume of water (e.g., 1:4 or 1:16) for a predetermined amount of time, but the maceration period for infusion is shorter.[14] In comparison to maceration and infusion, decoction typically yields a higher concentration of oil-soluble compounds and is only appropriate for extracting heat-stable compounds from hard plant materials (such as roots and bark). Another technique that uses a similar basic principle is percolation, which requires

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special equipment called a percolator. Samples that have been dried and powdered are placed inside a percolator, filled with boiling water, and macerated for two hours. To obtain a concentrated extract, the percolation process is typically carried out at a moderate rate (such as six drops per minute) until the extraction is finished.(12)

Soxhlet extraction or hot continues extraction: The method involves placing a finely ground sample in a porous bag or "thimble" made of cellulose or strong filter paper. The thimble chamber of the Soxhlet apparatus is then filled with the sample (Figure 1). Heating the extraction solvents in the bottom flask causes them to evaporate into the sample thimble, condense in the condenser, and then drip back. The process is repeated when the liquid content reaches the siphon arm (Figure 1) and empties into the bottom flask once more. When compared to maceration, this method uses less solvent.(12)

Fig 1. Soxhlet extraction

II. MATERIALS AND METHODS:

Plant material collection:

Fresh Catharanthus roseus (Leaves) parts were gathered from the various Gharuan areas within the Mohali district of Punjab, India. The fresh Catharanthus roseus plant material was thoroughly cleaned under running water to guarantee that it was free of any solid debris, fungi, organisms, or foreign organic matter. Following complete evaporation of the water molecules, the plant materials were shade dried until they were sufficiently dry for grinding. Following a period of drying, the plant materials were finely ground with a mortar and pestle to a powder and then placed into airtight containers labelled appropriately for future use.

Preparation of plant extracts:

Solvent extraction:

The Soxhlet extraction method was utilized to prepare the extract of crude plant. 250ml of solvent were used to extract 100 grams of powered material that had been packed into a thimble. Ethanol or methanol were the solvents used. The solvents in the extractor's siphon tube remain colourless after the extraction process has been going on for 24 hours. Subsequently, the extract collected in round-bottom flasks was placed on a heating mantle and heated to between30 and 40℃until the solvent had evaporated. Dried extract was kept in refrigerator at 4℃ for their future use in phytochemical analysis.(13)

Qualitative phytochemical analysis:

Standard procedures were followed to determine whether bioactive compounds were present in the extract.(12, 14, 15)

Test for Saponin:

A drop of Na2CO3 solution was added to 5ml of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponin.

Test for Alkaloids:

Mayer's Tests:

Mayer's reagent is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36g) and of potassium iodide (5.00g) in water (100ml). 3ml of extract was taken in a test tube. Few drops of Mayers reagent were

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added along sides of test tube. Gives creamy precipitate.

Picric acid Test: Picric acid test is carried out by using Hager's reagent. Hager's reagent is prepared by adding 1g of picric acid in 100ml of distilled water. 3ml of extract was taken in a test tube. Few drops of Hager's reagent were added along sides of test tube. It gives yellow precipitate.

Wagner Test: Alkaloid sample reacts with Wagner reagent (Iodine – Potassium – Iodine Solution) which gives brown colour precipitate which indicates the presence of alkaloids. Iodine 1.3g, potassium iodide 2.0g and water to make 100ml.

TLC Method:

In thin layer chromatography, the solid phase is applied to a thin glass plate using either silica gel or aluminium oxide. A solvent is selected for the mobile phase based on the characteristics of the mixture's constituent parts. TLC works on the basis of dispersing a compound between a liquid mobile phase (eluting solvent) that moves over the solid phase and a solid fixed phase (the thin layer) that is applied to a glass or plastic plate. Starting slightly above the bottom of the TLC plate, a small amount of a compound or mixture is applied. Next, the plate is developed in the developing chamber, which has a small solvent pool that is slightly lower than the level at which the sample was applied. By means of capillary action, the solvent is drawn up through the plate's particles. As the solvent passes over the mixture, each compound either stays in the solid phase or dissolves in the solvent and ascends the plate. The physical characteristics of each compound, which in turn depend on its molecular structure, particularly its functional groups, determine whether the compound advances up the plate or stays behind. The "Like Dissolves Like" solubility rule is adhered to. The longer a compound remains in the mobile phase, the more similar its physical properties are to those of the mobile phase. The compounds that are most soluble will be transported to the top of the TLC plate by the mobile phase. The substances that have a stronger affinity for the particles on the TLC plate and are less soluble in the mobile phase will remain behind.(16, 17)

Rf values: A quantity, represented as a decimal fraction, that serves as a defining characteristic for a single compound in TLC is called Rf. The compound's distance from the starting position is divided by the solvent's (the solvent front) distance from the starting position to determine the Rf.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Procedure:

Preparation of standard solution:

- Dissolve 1ml of vinca leaf extract in 2-3ml of distilled water.

Preparation of mobile phase:

- Mix Acetonitrile and Benzene in (30:70) and mix them.
- Transfer the mobile phase to TLC chamber and leave it for 30 min for saturation.

Preparation of spraying agent:

- Dissolve 1% solution of ceric ammonium sulphate in 85% phosphoric acid.
- Transfer the solution in the spraying bottle.

Preparation of stationary phase:

- Take the mortar pestle and silica gel in the mortar.
- Add sufficient amount of water to make a uniform slurry.
- Take the glass plate of 20× 5cm (dimension) and pour the slurry on the glass plate.
- Activate it at 105℃ for 30 minutes in hot air oven.

Spotting:

- Take the dry silica gel glass slide. Prepare an origin line leaving 2cm on the slide.
- Place a spot of sample on the glass slide with the help of capillary tube.
- After drying of slide put the slide in the saturated chamber and keep it in such a way that it just touches the surface solvents. Cover the chamber and allow the solvent to rise 3/4 of plate.
- Remove the plate and draw a solvent front on back side of the plate and air dry it.
- Now spray the detecting agent onto the slide with the help of spraying bottle.
- Measure the distance of developed spots from the origin.

Ash value:

The inorganic residues found in herbal medications, such as phosphates, carbonates, and silicates, are typically represented by the ash values. These are significant indicators that show the potency and purity of herbal medicine.(18)

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Total ash: The crucible was weighed after adding 1 g of the air-dried plant material (weight of sample). In an electrical muffle furnace, the sample was gradually lit and the temperature was raised to 500–600° until it turned white, signifying the absence of carbon. It was then reweighed (ash weight) after being cooled in a desiccator. % total ash was used to calculate the total ash content.(19)

% Total ash = Ash weight / Weight of sample × 100

III. RESULTS:

Table for Identification Test

S. No	Identification Test	Observation	Inference
1. (a)	**Test for Alkaloids** **Mayer's test:** Mayer's reagent is an Alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent freshly prepared by dissolving a mixture of chloride (1.36g) and of potassium iodide (5.00g) in water (100ml). 3ml of extract was taken in a test tube. Few drops of Mayer's reagent were added along sides of test tube.	 It gives creamy precipitate**.**	It shows the presence of Alkaloid.
(b)	**Picric acid test:** Picric acid test is carried out by using Hager's reagent. Hager's reagent is prepared by adding 1g of picric acid in 100ml distilled water. 3ml of extract was taken in a test tube. Few drops of Hanger's reagent were added along sides of test tube.	 It gives yellow precipitate.	Slight amount of Alkaloid is present.

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(c)	**Wagner test:** Alkaloid sample reacts with Wagner reagent (Iodide-Potassium-Iodide solution). Iodide 1.3gm, Potassium iodide 2.0gm and water to make 100ml.	 It gives you brown colour Precipitate.	Alkaloid is present.
2.	**<u>Test for saponin</u>** A drop of Na2CO3 solution was added to 5ml of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicates the presence of saponin.	 Foam formation	Slight amount of Alkaloid is present.

TLC Method:

1.	Sample preparation	Dissolve 1ml of vinca leaf extract in 1ml of ethanol
2.	Stationary phase	Silica gel G
3.	Mobile phase	Acetonitrile: Benzene (30:70)
4.	Detecting Agent	1% solution of Ceric ammonia sulphate in 85% Phosphoric acid
5.	Rf Value	0.39
6.	Colour spot	Pale Green

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Rf = Distance travelled by sample / Distance travelled by solvent

Distance travelled by sample -6.3
Distance travelled by solvent - 16
Rf value obtain is 0.39

Ash content:
% Total ash = Ash weight / Weight of sample × 100
= 0.2/1×100
= 20%
% Total ash value is 20%

IV. CONCLUSION:

One of the most widely available plants with life-saving qualities is vinca. The majority of diseases that are currently in vogue are cancer, and vinca has anticancer and antitumor properties. The goal of this study is to extract vinca (Catharanthus rosea) and conduct a preliminary phytochemical analysis. The phytochemical test for different constituents showed that saponin and alkaloids are present in the plant extracts. Our research showed that the ethanol extract of vinca contained the greatest concentration of alkaloids.

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