



CODEN [USA]: IAJ PBB

ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: <http://www.iajps.com>

Research Article

EVALUATION OF ANTI-MICROBIAL PROPERTY OF VINCA ROSEUS PLANT EXTRACT

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Abstract:

Ayurveda, the traditional system of Indian medicine, emphasizes the therapeutic potential of plants. *Catharanthus roseus* (L.) commonly known as periwinkle is a significant medicinal plant recognized for its wide range of pharmacological activities, including anti-tumour, anti-diabetic, anti-microbial, antioxidant, and anti-mutagenic properties. Native to Madagascar, this evergreen plant contains over 130 alkaloids, such as ajmalicine, vincristine, vinblastine, reserpine, and Rauba sine, which are clinically important for treating various cancers including Hodgkin's disease, breast cancer, skin cancer, and lymphoblastic leukaemia. Owing to its endangered status, conservation through micro propagation techniques is essential.

In the present study, leaf extracts of *Catharanthus roseus* were prepared using acetone, ethanol, and chloroform to evaluate their antimicrobial potential against selected pathogenic microorganisms through the agar well diffusion method. Among the tested solvents, ethanolic extracts showed the highest antibacterial activity. *Streptococcus* species exhibited maximum susceptibility, followed by *Escherichia coli*. The findings indicate that *Catharanthus roseus* possesses promising antimicrobial activity and can be utilized as a potential source for developing novel herbal formulations. Further studies are recommended to explore its synergistic effects when combined with other medicinal plants.

Key Words: *Catharanthus roseus*, Diffusion method, Antimicrobial potential

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Please cite this article in press Umesh Mitte et al., Evaluation of anti-microbial property of
Vinca roseus plant extract, Indo Am. J. P. Sci, 2025; 12(11).

INTRODUCTION:

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease.^[1] The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues.^[2] Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties.^[3] Medicinal herbs as potential source of therapeutics aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health care.^[4] The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, phenols, glyco-alkaloids, flavonoids, sesquiterpenes lactones, and terpenoids.^[5] Various plant species have served as a source of medicine for people all over the world.^[6] Herbal plants information is a special type of information dealing with medicinal herbs.^[7] Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects.^[8] Over the last decades, herbal supplements have gained popularity among patients and consumers.^[9] Knowledge about herbal medicine can be contributed from experts in several cultures.^[10] The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments.^[11] Ayurveda is considered by many scholars to be the oldest healing science Ayurvedic knowledge originated in India more than 5,000 years ago and is often called the "Mother of All Healing."^[12] There has been an increase demand for the pharmaceutical products of Ayurveda in all over the world because of fact that the allopathic drugs have a side effect. Ayurvedic medicinal plant products are most convenient and have greater acceptance amongst the users due to their easy availability easy biodegradability, easy to handling, economic cost, mankind and environment friendly nature both and minimum side effect.

[13] Two of the largest users of medicinal plants are China and India. Recently even developed countries, are using medicinal systems that involve the use of herbal drugs and remedies.^[14] India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. India is one of the 12 mega

biodiversity centres having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000-18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms. The World Health Organization (WHO) has recently defined herbal drugs as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today.^[15] The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacogenetic and pharmacological studies to ascertain their therapeutic properties.^[16] The scientific study of traditional medicine is thus of great importance.^[17]

PLANT: VINCA ROSEA**Vinca rosea SCIENTIFIC****TERMS:^[18]**

Botanical name: - *Catharanthus Roseus* Linnaeus
 Domain: - Eukarya: Eukaryotes. Kingdom: - Plantae: plants.
 Subkingdom: - Tracheobionta: vascular plants.
 Subdivision: - Spermatophyte: seed plants.
 Division: - Magnoliophyte: flowering plants. Class: - Magnoliopsida: dicotyledon.
 Subclass: - Asteridea. Suborder: - Gentiananae.
 Order: - Gentianales.
 Family: - Apocynaceae. Subfamily: - Rauvolfioideae. Tribe: - Vinceae.
 Genus: - *Catharanthus* G. Don. Species: - *Roseus*.

VERNACULAR NAMES:

English: Cayenne jasmine, old maid, Periwinkle.
 Hindi: Sada-bahar, Sadabahar.
 Kannada: Batla hoo, bili kaasi kanigalu, ganeshana
 hoo, kempu kaasi kanigalu.
 Malayalam: Banappuvu, nityakalya, savanari.
 Marathi: Sadaphool, sadaphul, sadaphuli.
 Sanskrit: nityakalyani, rasna, sadampuspa, sadapushpi.

Tamil: cutkattu malli, cutukattu malli, cutukattuppu.

Telugu: billaganneru.

Gujarati: Barmasi.

Bengali: noyon tara.

Vinca rosea also known as *Catharanthus roseus*, bright eye care periwinkle, graveyard plant, Madagascar periwinkle, old maid, pink periwinkle, rose periwinkle, is a species of flowering plant in the family Apocynaceae. *Vinca rosea* plant has a popular reputation in indigenous medicine in various parts of the world. The plant *Vinca rosea* is grown commercially for its medicinal uses in Australia, Africa and India. The rainfall of about 100 cm is most suitable for *Vinca rosea*. The plant formulation of *Vinca rosea* is mainly used for several diseases associated with cancer treatment. *Vinca Rosea* has been cultivated in mostly China as a flowering evergreen ornamental plant. Mainly flowering tops and leaves of plant of *Vinca rosea*

are used for the extraction of oil. *Vinca rosea* for Hypertension, diabetes, blood cancer, improve memory. It also has some Pharmacological properties. ^[19]

The present study is designed to evaluate the antimicrobial activity of *Vinca Roseus* (Madagascar periwinkle) plant extract against selected bacterial strains, and to determine its potential as a natural source of antimicrobial agents.

METHODOLOGY:

COLLECTION OF PLANT SAMPLE:

Healthy *Catharanthus roseus* Leaves were collected from Kalaburagi district, Karnataka, India. The plant material like leaf were washed thorough with tap water and then with Sterile distilled water for the removal of dust and sand particles. The leaf was shade Dried and powdered by Mechanical blender. ⁽²⁰⁾

Selection of the Plant



Shade drying of the Plant



EXTRACTION PROCEDURE STEPS:

1. **Apparatus Setup:** Assemble the Soxhlet extraction apparatus by connecting a distillation flask with solvent, a Soxhlet extractor, and a reflux condenser.
2. **Sample Preparation:** Place the powdered solid sample, enclosed in a porous thimble, inside the extraction chamber of the apparatus.
3. **Solvent Loading:** Add the chosen solvent (e.g., ethanol, n-hexane) to the distillation flask, ensuring there are anti-bumping granules to promote smooth boiling.
4. **Heating:** Place the distillation flask on a heating mantle to gently heat the solvent.
5. **Cooling:** Attach a water source to the condenser, ensuring cold water flows through the condenser's lower arm and out the upper arm to facilitate efficient cooling and condensation.
6. **Extraction Cycle:**
 - The solvent heats and vaporizes from the flask.
 - The vapour rises and enters the condenser, where it cools and condenses into liquid.
 - The condensed solvent drips onto the

sample in the thimble, dissolving the soluble compounds.

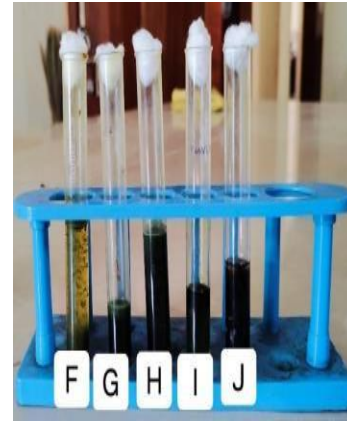
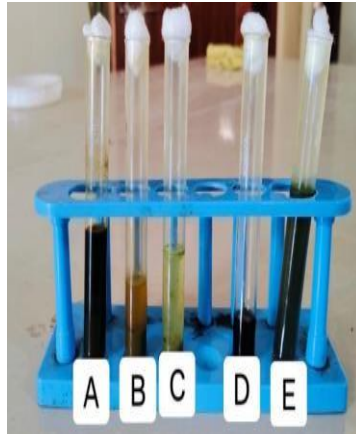
- As the solvent level in the extractor rises, it reaches the siphon tube, which then automatically siphons the solvent, now rich with extracted compounds, back into the distillation flask.
7. **Completion:** The cycle continues until the extraction is considered complete.
 8. **Product Isolation:** After the process, the solvent is evaporated from the distillation flask, leaving the pure extract behind. ⁽²¹⁾

Principle of Soxhlet Procedure: The principle of Soxhlet extraction is continuous, uninterrupted extraction of compounds from solid material using a small amount of solvent that is repeatedly recycled through the sample. Heat is used to vaporize the solvent, which then condenses and drips onto the solid sample, dissolving soluble components. As the solvent fills the extraction chamber, a siphon action causes the solvent and dissolved compounds to flow back into the flask, and this cycle repeats until the extraction is complete and the maximum yield of the desired compound is obtained. ⁽²²⁾



PHYTOCHEMICAL TESTS ⁽²³⁾

Phytoconstituents	Test Name & Procedure	Expected positive results for C. Roseus	Significance
Alkaloids (Primary)	(A) Wagner's Test: Extract+ Wagner's reagent (iodine & potassium iodide)	Formation of reddish-brown precipitate	Strongly positive confirms the Presence of Alkaloids like Vincristine, Vinblastine, Ajmalicine, Vindoline etc...
	(B) Dragendorff's test: -Extract+ Dragendorff's reagent Potassium Bismuth Iodide)	Formation of an orange or orange-red precipitate.	Strongly Positive. A standard test for alkaloid detection.
	(C) Mayer's Test: Extract + Mayer's reagent (Potassium Mercuric Iodide)	Formation of a creamy white or yellowish precipitate.	Strongly Positive.
Terpenoids	(D) Salkowski Test: Extract+ Chloroform+ concentrated Sulphuric acid.	Formation of a reddish-brown coloration at the interface.	Positive. TIAs are derived from the terpenoids pathway.
Flavonoids	(E) Alkaline Reagent Test: Extract + Sodium hydroxide (NaOH).	Formation of intense yellow colour which becomes colourless upon addition of dilute acid.	Positive. Indicates presence of flavones.
Saponins	(F) Foam Test: Extract + water; shake vigorously.	Formation of a stable, persistent foam (≥ 1 cm) for 15 minutes.	Positive (usually in aqueous extract).
Tannins & Phenolics	(G) Ferric Chloride Test: Extract + few drops of 5% FeCl ₃ .	Formation of a blue-black, green, or blue-green coloration.	Positive
Cardiac Glycosides	(H) Keller-Killian Test: Extract+ Glacial acetic acid + FeCl ₃ + concentrated H ₂ SO ₄ .	Formation of a brown ring at the interface and a green colour in the acetic acid layer.	Maybe Positive.
Anthraquinones	(I) Borntrager's Test: Extract + Ether + 10% Ammonia solution.	Appearance of a pink, red, or violet colour in the ammoniacal layer.	Typically, Negative. Not a major constituent.
Steroids	(J) Liebermann- Burchard Test: Extract+ Chloroform+ Acetic anhydride + conc. H ₂ SO ₄ .	Formation of a greenish or blue-green colour.	Positive. Steroids are precursors for alkaloid biosynthesis.



C



STERILIZATION PROCESS

- Sterilization process was carried out by autoclave.
- Autoclave sterilization uses high pressure steam to destroy microorganisms.
- Process Involves Loading of materials into autoclave
- Close the lid and remove air
- Set conditions for temperature 121°C and pressure 15 psi for 15-20mins
- Steam penetrates and microorganisms killed until the holding time completed
- After the complete microorganisms killed release the pressure and exhaust the steam
- Drying and cooling of sterilized materials
- Remove all the safe sterile materials/items. ⁽²⁴⁾



PREPARATION OF CULTURE MEDIA

- ▶ 2.60g of Nutrient agar powder was mixed with 100ml of distilled water to prepare Nutrient agar media.
- ▶ The nutrient agar media was sterilized at 121°C under 15 lbs pressures for 30 minutes.
- ▶ The petri plates were prepared by pouring 20 ml of Nutrient agar medium.
- ▶ The plates kept at room temperature for solidification and stored at 4°C until use. ⁽²⁵⁾

**ANTIMICROBIAL ACTIVITY****Introduction: -**

Antimicrobial activity by Agar well diffusion is a widely used technique for antimicrobial activity. It is the process of inhibiting the growth of microorganisms and decreasing their shelf life and prevent from the formation of microbial colonies. This process can be done by various methods like: agar well diffusion, disc diffusion method, etc. ⁽²⁶⁾

Chemicals: -

1. MHA agar
2. MHA broth
3. Ethanol

Instruments: -

1. Laminar air flow
2. Autoclave
3. Incubator
4. Weighing balance



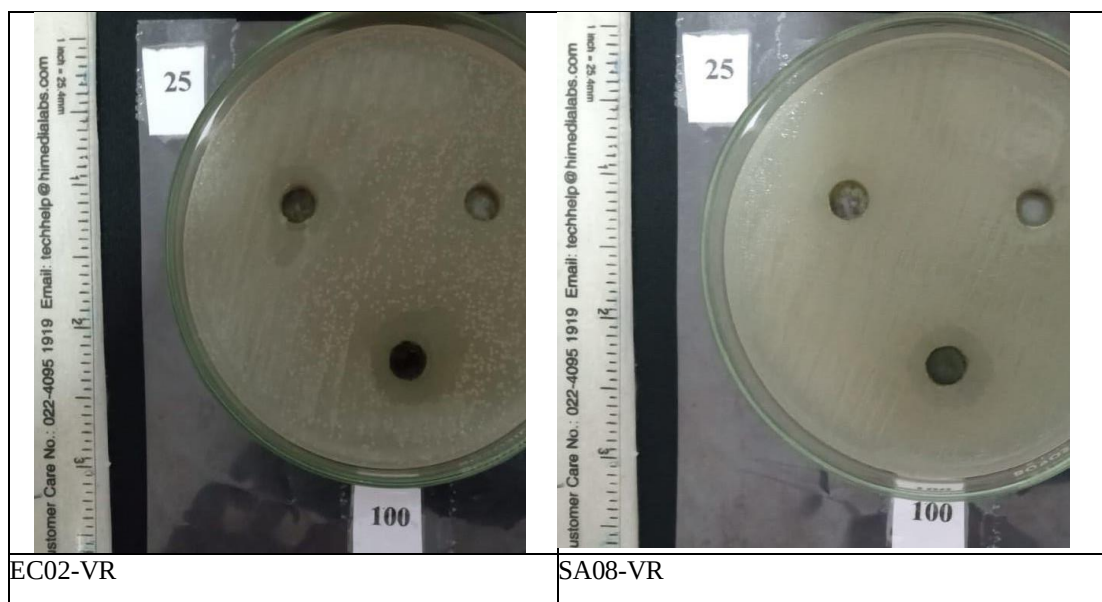
Methodology:

- The Antibacterial activity of Vinca rosea extract was performed by Kirby-Baur well diffusion method (Bauer et.al., 1966).
- 24 hours freshly grown (0.5 McFarland turbidity Standard) cultures of *S. aureus* (Gram positive), *E. Coli* (Gram negative) in nutrient broth medium were inoculated by lawn culture technique on sterile solidified Muller Hinton Agar (HIMEDIA-M173) plates with the help of sterile cotton swabs.
- The stock solutions of concentration 1 mg/ml were prepared in ethanol solvents.
- Three wells were bored on the MHA media plates with the help of sterile cork borer (8mm). Each well loaded with solution following concentration, 10µl, 25µl, 100µl respectively.
- Then allowed to diffuse at room temperature for 2-3 hours. The plates were incubated in the upright position at 37 °C for 24 hours. Then the zone of inhibition was observed.
- The control plates were kept in which DMSO 100 µl was added as negative control and Antibiotic disc as a positive control were used. For *E. Coli*, *S. aureus*, -Amikacin 30 mcg is used. ⁽²⁷⁾

RESULTS:

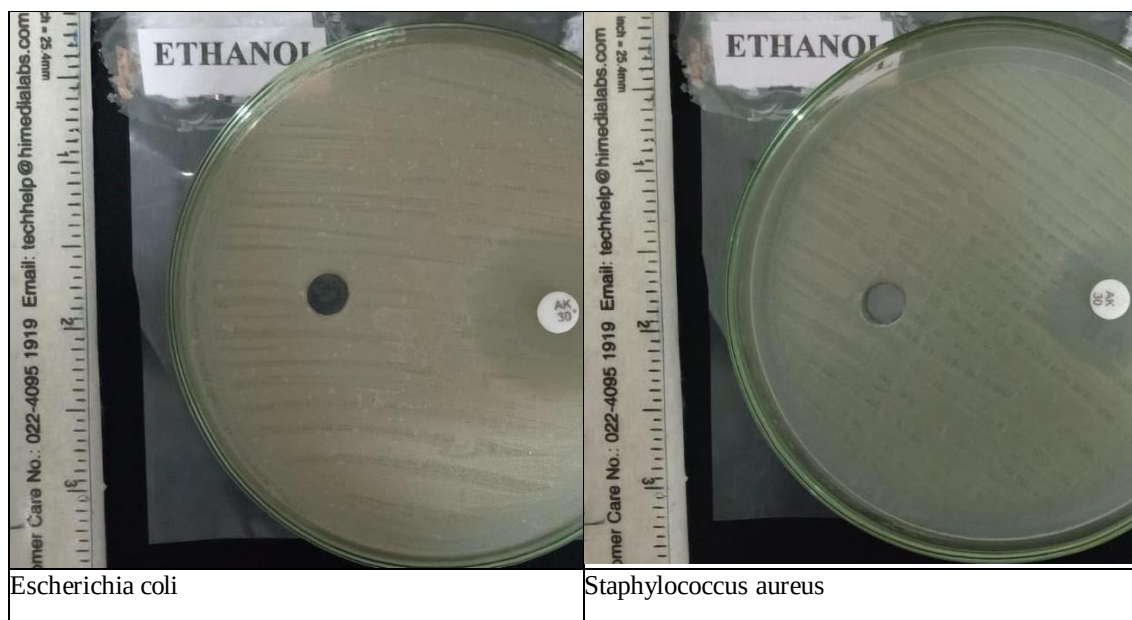
Antibacterial activity			
Zone of Inhibition in mm			
Organisms	10 µl	25 µl	100 µl
EC02-VR	NI	13	17
SA08-VR	NI	13	15

Note :- NI- No Inhibition, Strain EC02- Escherichia coli, Strain SA08- Staphylococcus aureus, VR- Vinca rosea



Control	AB (Amikacin 30 mcg)	Ethanol
Escherichia coli	25	NI
Staphylococcus aureus	18	NI

Note- NI-No Inhibition, AB-Antibiotic



DISCUSSION:

- The antibacterial activity was tested using the agar well diffusion method against *E. coli* and *S. aureus*.
- At concentrations of 10 μ l, no inhibition was observed for either bacteria, indicating that lower concentrations of *Vinca rosea* extract are ineffective.
- Both organisms exhibited zones of inhibition at 25 μ l and 100 μ l: *E. coli* had 13 mm and 17 mm, *S. aureus* 13 mm and 15 mm, showing a dose-dependent response.
- Compared to the positive control (Amikacin 30 mcg), which produced 25 mm (*E. coli*) and 18 mm (*S. aureus*) zones, *Vinca rosea*'s activity remains lower, suggesting its limitations as a standalone antibacterial agent.
- The negative controls (DMSO, ethanol) showed no inhibition, confirming that the activity is due to the plant extract.

CONCLUSION:

The study concluded that *Vinca rosea* extract exhibits moderate antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, with higher concentrations leading to greater zones of inhibition, though these effects are less pronounced than standard antibiotics.

- *Vinca rosea* extract demonstrates measurable antibacterial effects at higher concentrations on both *E. coli* and *S. aureus*, validating its traditional use for microbial infections.
- However, since its efficacy does not match

standard antibiotics, further refinement and concentration adjustments or synergistic studies may be needed for practical applications.

- The extract exhibited a clear dose-response, suggesting that its active compounds could be studied further for potential therapeutic use, but its limited potency means it is currently best considered as a supplementary agent rather than a replacement for conventional antibiotics.

SUMMARY:

Ayurveda, the traditional system of Indian medicine, emphasizes the therapeutic potential of plants. *Catharanthus roseus* (L.)—commonly known as periwinkle—is a significant medicinal plant recognized for its wide range of pharmacological activities, including anti-tumour, anti-diabetic, anti-microbial, antioxidant, and anti-mutagenic properties. Native to Madagascar, this evergreen plant contains over 130 alkaloids, such as ajmalicine, vincristine, vinblastine, reserpine, and Rauba sine, which are clinically important for treating various cancers including Hodgkin's disease, breast cancer, skin cancer, and lymphoblastic leukaemia. Owing to its endangered status, conservation through micro propagation techniques is essential.

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exhibited maximum susceptibility, followed by *Escherichia coli*. The findings indicate that *Catharanthus roseus* possesses promising antimicrobial activity and can be utilized as a potential source for developing novel herbal formulations. Further studies are recommended to explore its synergistic effects when combined with other medicinal plants.

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