

EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE STEM OF THE *CATHRANTHUS ROSEUS*

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ABSTRACT

An ancient Indian medicinal system called Ayurveda places a strong emphasis on plants' ability to cure. A well-known herb in Ayurveda is *Catharanthus roseus*. It is well documented to have effects on oxidants, bacteria, diabetes, cancer, and mutagenic disorders. The native home of this evergreen shrub was Madagascar. The color of the blooms ranges from pink to purple, and the leaves are arranged in opposing orientations. More than 130 distinct alkaloids are produced by it, such as vinblastine, reserpine, vincine, raubasine, and vincristine. Vincristine and vinblastin are used as treatments for a number of illnesses, including lymphoblastic leukemia, Hodgkin's disease, and breast cancer. Because of its endangered status, this species needs to be preserved by techniques like micropropagation. Its potent therapeutic properties require further investigation. Stem extracts from *Catharanthus roseus* plants were prepared using methanol as a solvent, and their antibacterial effectiveness against pathogenic microorganisms was evaluated. Maximum antibacterial efficacy against all pathogenic pathogens was shown by *Catharanthus roseus* stem extract. A study evaluating plant extracts revealed that *B. subtilis* was more susceptible to the stem extract of *Catharanthus roseus*, but *E. coli* was less susceptible. Of all the extracts tested, methanol had the strongest antibacterial properties. This research uses the agar well diffusion technique to determine the antibacterial activity of stem extract from *Catharanthus roseus*. As the study's findings show, *catharanthus roseus* is an effective weapon against pathogenic bacteria that cause infections and illnesses. Further research should be done to determine the plant's therapeutic potential when combined with other herbal drugs.

Keywords: *Catharanthus roseus*, Antimicrobial activity, Methanol extract

INTRODUCTION

Plants are mostly employed in the treatment of cancer-related diseases. Alkaloids, flavonoids, saponins, steroids, cyanogenic glycosides, terpenoids, and other secondary metabolites are produced by plants as a means of defense against natural diseases, insect pests, and environmental stresses. The solvent and extraction procedures employed should have an impact on the compounds' above activity [1,2]. Most likely, the variety of bioactive chemicals found in herbal plants utilized in traditional Many infectious illnesses may be prevented or treated with the use of medication as an alternative therapeutic tool. Comparable to pharmaceutical antibiotics, medicinal herbs are thought to be safer and more clinically effective [3, 4]. Elevation to one meter is possible for the evergreen subherb species *Catharanthus roseus*. Entire leaf: 2.5–9.5 cm long, 1-3 cm wide, green, hairless, glossy, oval to oblong. They are grouped in opposite pairs and have a light midrib and a short, around 11.8 cm long petiole. A 2.5–5 cm diameter corolla with a hue ranging from white to dark pink and a dark crimson center is adorned with five petal-like lobes in the flowers. The basal tube is around 2.5–3 cm long. The fruit consists of two follicles that are 3 mm wide and 2-4 cm long. Notable within the apocynaceae family, *Catharanthus roseus* is a herb used to treat lung, breast, uterine, non-Hodgkin's lymphoma, and melanomas, among other cancers. Together with chemotherapeutic drugs, it comprises around 70 distinct kinds of alkaloids [12].

Names like *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea* are often used to describe it. Native to the Indian subcontinent and southern Asia, *Catharanthus roseus* grows wild across these regions [5].

Two common names for *Catharanthus roseus* are Pink (Rosea) and White (Alba), which are named for their bloom colors[6]. Many ailments, such as menorrhagia, rheumatism, dyspepsia, dysmenorrhea, diabetes, hypertension, cancer, menstrual problems, skin issues, and bleeding diarrhea, have long been treated medicinally using leaves of the *catharanthus roseus* plant. Sedative and antiviral properties are also present in it. That plant has more than seventy distinct chemical compounds in its leaves, such as reserpine, ajmalicine, serpentine, and indole type alkaloids. Two common names for *Catharanthus roseus* are Pink (Rosea) and White (Alba), which are named for their bloom colors[6]. *Catharanthus roseus* leaves have long been used as medicine to treat a variety of conditions, including menorrhagia, rheumatism, dyspepsia, dysmenorrhea, diabetes, hypertension, cancer, menstrual disorders, skin conditions, and bleeding diarrhea. It also possesses sedative and antiviral qualities. More than 70 different chemical compounds, including reserpine, ajmalicine, serpentine, and indole type alkaloids, are found in the leaves of that plant. It promotes healing of wounds. It was attempted through experimentation to demonstrate *Catharanthus roseus*'s antibacterial efficacy against isolates from clinical wounds. Many antibiotics are made from this to treat wound infections, but they also have unfavorable side effects[9]. Compared to dietary plants, medicinal plants are likely to include a greater diversity of naturally occurring antioxidants, specifically phenolics, flavonoids, and tannins. There are notable concentrations of volatile and phenolic chemicals in *Catharanthus roseus*, such as flavonal glycosides and caffeoylquinic acids, Its antioxidant qualities are well established.

Reactive oxygen species (ROS) are harmful substances that are created during regular cell aerobic respiration. It serves as an antioxidant and is a crucial part of the body's defensive mechanism[10]. Pathological diseases such as Parkinson's disease, ischemia, asthma, arthritis, inflammation, neurodegeneration, mongolism, aging, and maybe dementia can be brought on by an accumulation of free radicals[11]. Parts of the *Catharanthus roseus* flower, including the petals and seeds, have antioxidant properties. Consequently, because of their redox properties, phenolic compounds may behave as singlet oxygen quenchers, hydrogen donors, metal chelators, and reducing agents. It is used extensively in the culinary, cosmetics, and pharmaceutical sectors. In addition to their antioxidant properties, these substances have anti-inflammatory, antibacterial, anti-thrombotic, cardioprotective, and vasodilatory properties[12]. Many environmental and geographic factors have a role in this. Antioxidants that are naturally occurring can be used to identify potentially safe, affordable, and efficient antioxidants. Because of this, they are referred to as free radical scavengers in unison. The primary purpose of these plant antioxidants is to stop lipid peroxidation in the food industry [13].

Plant-based compounds are becoming more and more popular for drug discovery, partly due to the growing desire for new medications that can solve the issue of drug resistance. Many medicinal plants are provided by nature to aid in human survival and wellbeing. In [14] In order to create synthetic molecular analogs, a large portion of herbal composites are utilized

as the primary particle in drug discovery. This demonstrates the importance of studying DOS of pharmacompounds that resemble natural products. In [15]

Through breeding techniques, more than 100 variants have been generated during the last 20 years. Along with enhanced floral characteristics, they have also increased herbage and alkaloid output, and made the plant more disease-tolerant.[16] with a deep crimson core, a corolla measuring two to five centimeters in diameter and five petal-like lobes on a basal tube that is roughly 2.5 to 3 cm long.[17] The fruit consists of two 2-4 cm long and 3 mm wide follicles covered with many black seeds.[18]



Figure 1- Madagascar periwinkle-rosea.

Classification

CLASSIFICATION	
Domain	Eukarya:eukaryotes
Kingdom	Plantae:plants
Sub-Kingdom	Tracheobionta:vascular plants
Superdivision	Spermatophyta:seed plants
Division	Magnoliophyta:Flowering plants
Class	Magnoliopsida:dicotyledons
Subclass	Asteridae
Superorder	Gentianane
Order	Gentianales
Family	Apocynaceae:dogbane
Subfamily	Rauvolfioideae
Tribe	Vinceae
Specific epithet	Roseus(Linnaeus)G.don

MATERIALS AND METHODS

AUTHENTIZATION AND GATHERING OF PLANT:

When the plant was in bloom and fruit, Mature *Catharanthus roseus* stems were gathered from the city of Coimbatore and the National Botanical Research Institute (NBRI) of CSIR performed the authentication.

EXTRACT PREPARATION:

The plant stem powder was immersed in 10g/100ml of methanol for a whole night in a conical flask.

METHANOL EXTRACTION :

In a conical flask with cotton wool inserted, After combining 10 g of air-dried powder with 100 ml of methanol, the mixture was agitated for an entire day at 190–220 rpm. Following a complete 24-hour period, the solvent was removed to provide a final volume equal to one-fourth of the starting volume (12), and the sealed bottles were stored at 4 °C. The supernatant was then collected.

Qualitative Examination of Physical

Examine for Alkaloids: One milliliter of extract should be mixed with one milliliter of Mayers reagent and one or two drops of iodine solution. If a yellow-colored precipitate forms, It signifies that alkalioids are present.

Examine for Terpenoids: Boil for two minutes after adding one milliliter of concentrated H_2SO_4 to one milliliter of crude extract. When terpenoids are present, the color becomes grayish.

Measure Tannin and Phenol Content: One milliliter of $FeCl_3$ was added to milliliters of crude extract. Tannins are indicated by colors that are either blue-green or black.

Examine for reducing Sugar: To one milliliter of extract, add one milliliter of Fehling's solutions A and B, in that order. When redness starts to appear, sugar is present.

Examine for Saponins: Saponins may be detected by thoroughly swirling 1 milliliter of extract with 2 milliliters of distilled water until a 1-centimeter-thick layer of foam appears.

Examine for Flavonoids: Two drops of strong hydrochloric acid were added dropwise to one milliliter of extract, along with a few bits of magnesium ribbon. When the pink scarlet color appeared, it was confirmed that flavonoids were present.

Examine for Protein : One milliliter of extract received a little addition of mercuric chloride. To show that proteins are present, a yellow tint appears.

Examine for Steroids: One milliliter extract mixed sideways with one milliliter of concentrated H₂SO₄ and one milliliter of chloroform. Steroids are shown by a red color at the bottom of the chloroform layer.

Examine for Quinines: One milliliter of extract and one milliliter of 1% NaOH were combined, and everything was well combined. When quinines look red or blue-green in color, they are present.

TLC Study:

To extract a single chemical from a mixture, TLC is frequently utilized. By examining the intensity, the chemical and R_f value of the isolated spots may be determined. Both qualitative and quantitative analysis now heavily relies on TLC as a tool.

- Chromatographic Condition
- Preparation of sample: The previously described procedure was followed to extract the sample and obtain the alkaloid fraction, which was then employed for the TLC investigation.
- Stationary phase: Plates pre-coated with silica gel GF254 (Merck).
- Plate activation: The plate was heated to 1100 °C for a duration of 60 minutes.
- Chloroform in a mobile phase with 5% MeOH / NaOH (11:1)
- Spray reagent: Dragendroff's reagent
- Sample application: A TLC plate had spots of the sample extract on it in accordance with standard procedure, which was then positioned vertically in the saturated chamber. It is OK for the solvent to run. All of the resolved spots' R_f values were recorded.
- Visualization: Three were conducted after spraying Dragendroff's reagent: three thereafter, one under long UV (366 nm), one under short UV (254 nm), and one under.

Phytochemical Screening by GC-MS Analysis

The sample was diluted in methanol and then injected into a column of a Shimadzu® GC-MS QP2010 model, with a splitless injection mode, 30 m by 0.25 mm by 0.25 μm. The following operational parameters were used to the GC-MS set analysis: Oven temperature ramping up from 45 °C to 280 °C in two minutes, then 140 °C at a rate of 5 °C per minute, which was held at that temperature isothermally for 10 minutes. A volume of 2 μL of the sample was injected, and 1 mL/min of helium was used as the carrier gas. At 70 eV, the sample's constituent parts were ionized. The GC happened in the span of 9.10 and 52.0 minutes. Subsequently, a NIST14.L library (2020) search was performed in order to compare the compound structures with those that were discovered in the NIST database. Retention times

and mass spectra were then used to identify substances that were previously known to exist in the NIST library (C:\Database\NIST14.L).

Antibacterial assay

In this study, the bacterial strains *B. subtilis*, *S. aureus*, and *P. aeruginosa* were used. It was prepared using sterile Muller-Hinton Agar (MHA). The agar plates were thoroughly coated with diluted bacterial cultures, which were equally distributed using a sterile cotton brush. It took fifteen minutes for the plates to incubate at room temperature. One to five 5 mm diameter wells were made for medication loading using a well puncher. The plant extracts (methanol) were prepared and then dissolved in dimethyl sulfoxide (DMSO). After rough labeling each well, 10µl of plant extracts were added using a micro-pipette. The plates were kept in an incubator at 37°C for an entire day. To quantify the antibacterial activity, the examined microorganisms were tested, and the diameter of the zones of inhibition was used.

Antifungal assay

The study made use of the fungal strains listed below. *Parapsilosis* and *Candida albicans*. The usual protocol was followed when implementing the well diffusion approach. In a sterile tube, 2 mg of plant extract were dissolved in 1 ml of 2% DMSO to form a stock solution. Dextrose agar with Sabouraud was made. Using a sterile cotton swab, the fungal spores were distributed evenly across the agar plate surface. At room temperature, the plates were incubated for 15 minutes. A well puncher was used to create one to five 5 mm diameter wells for drug loading. After processing, DMSO was used to dissolve the plant extracts (methanol). Using a micro-pipette, 10µl of plant extracts were added to each well after they were roughly labeled. After incubating the plates for seven days at 27±2°C, Both the fungal growth (mm) and For every plate, measurements and records were made about the diameter of the zones of inhibition against the fungus.

RESULT

Preliminary phytochemical analysis of *Catharanthus roseus*:

The findings of a phytochemical examination of *C. roseus* in methanol extracts are displayed below.

Table1 Preliminary Phytochemical screening of (*catharanthus roseus*) stem

S.NO	TEST	Stem
1	Alkaloids	Positive
2	Terpenoids	Positive
3	Phenols and Tannins	Positive
4	Flavanoids	Negative
5	Reducing sugar	Negative
6	Saponins	Positive

TLC Study:

The various R_f values seen under the various visualizations are shown in Table 6.2. When Dragendroff's reagent was sprayed, the fewest number of spots (7) were seen, and the greatest number of spots (9) were seen in long UV (366 nm). All three visualizations show that the R_f values of 0.13 and 0.23 correspond to similar chemical separation at these values. This indicates that there is a separation of chemicals in the stem that may be more easily seen at wavelengths of 366 nm. This method will benefit the pharmaceutical Ayurvedic sector as a fundamental tool for identifying the stem of *Catharanthus roseus* and will make it easy to determine whether a medicine is genuine.

Table 2 R_f values of the Saptaparna (*C. roseus*) stem at various display settings

S.No	Visuaalization	No of spots	R _f Values
1.	Shortest uv (254mm)	8	0.12,0.13,0.23, 0.27,0.43,0.61, 0.78,0.90
2.	Long uv (366mm)	9	0.13,0.23,0.31,0.46,0.56,0.64,0.74,0.79,0.86
3.	Following the use of Dragendroff's Reagent.	7	0.13, 0.17, 0.23,0.35,0.43,0.46,0.52

PHYTOCHEMICAL SCREENING BY GCMS ANALYSIS

Numerous chemicals starting with the methanol's GC fractions extracts of *Catharanthus roseus* are identified as a consequence of the GC-MS analysis. Mass spectrometry coupled with GC was used to identify them. For the purpose of profiling secondary metabolites in plant species, GC-MS is an essential technical instrument. The methanol extract of *Catharanthus roseus*' stem bark had the mass spectra of every phytochemical that was found. The presence of key phytochemicals was discovered by GC-MS analysis of methanol stem bark extract from *Catharanthus roseus*. The biological activity of the available phytochemicals was shown.

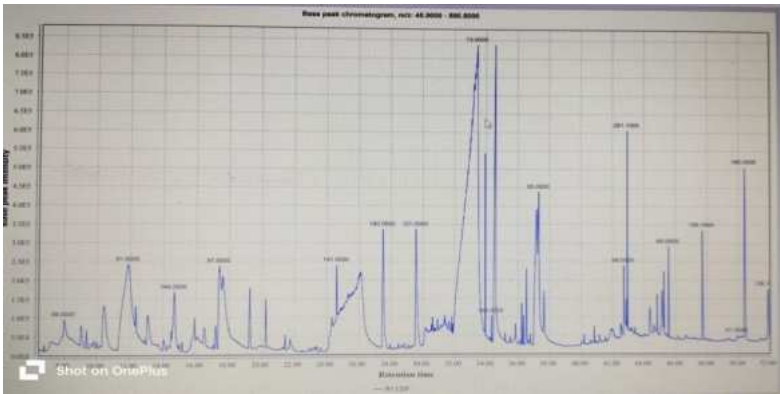


Figure-1

Table 3 Antibacterial activity of methanol extract

ORGANISM	DESCRIPTION	ZONE OF INHIBITION
B.subtilisas	Methanol	8mm
S. aureus	Methanol	9mm
P. aeruginosa	Methanol	9.66mm
E.coli	Methanol	27mm

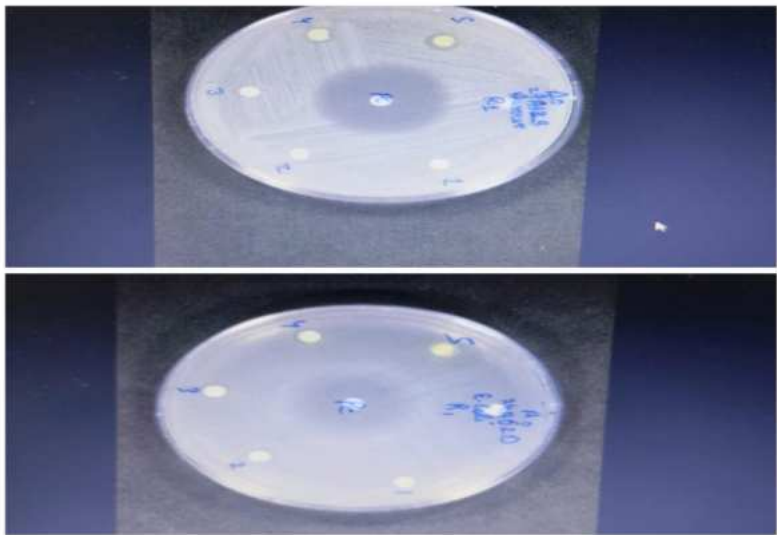
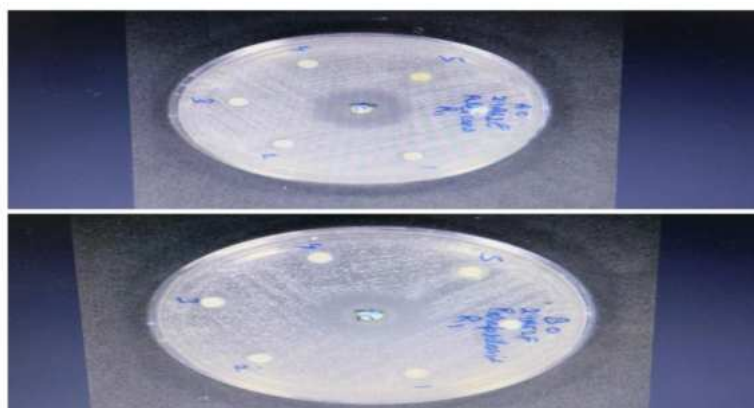


Figure-2

Table 4 Antifungal activity of Methanol extracts

ORGANISM	DESCRIPTION	ZONE OF INHIBITION
C.albicans	Methanol	7.66mm
C.parapsilosis	Methanol	8mm

**Figure-3**

CONCLUSION

The present research investigated the phytochemical and antibacterial properties of methanol extracts derived from the *Catharanthus roseus* plant. The most effective extract for each trial was found to be methanol extract. Following GC-MS analysis, a silica gel column was used to further purify the methanol extract. The chemical was found in the plant extract, according to a TLC analysis. *Bacillus subtilis* and *Pseudomonas aeruginosa* were among the pathogens used in the antibacterial studies that demonstrated antimicrobial action. Chemicals with antibacterial qualities were found in the extracts according to a GC-MS study.

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