

Original Research Article

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Invitro Phytochemical Screening and Antibacterial Potentiality of *Catharanthus roseus* Leaves Extract Against Some Pathogenic Bacteria

Pooja Sahu¹, Manish Kumar¹ and Arvind K. Sharma^{2*}

¹Department of Science, P.K. University, Shivpuri (M.P.), India

²Advanced Environmental Testing & Research Lab (P) Ltd., Gwalior (M.P.), India

*Corresponding author

ABSTRACT

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Catharanthus roseus is one of the most important plant as described in ayurveda. This plant is known as sadabahar is grown in every home throughout India as ornamental plant. This plant comprises a no of significant bioactive compounds to cure various critical diseases as described in ayurveda. These incurable diseases have necessitated its continued exploration and consumption. The presence of bioactive compounds also has antimicrobial activity. Therefore, this study is carried out to evaluate the antimicrobial property and phytochemical components of *Catharanthus roseus* plant's leaf extract. Moreover about the study phytochemical screening of different extracts namely methanol extracts of *C.roseus* plant's leaves was done. Antimicrobial activity of Methanols extract against *Salmonella*, *Staphylococcus aureus* and *E.coli* was carried out. The 100 µg/µl methanolic extract showed good activity against *Salmonella* and *Staphylococcus aureus* comparing to *E.coli* organisms.

Introduction

Ayurveda is playing a significant role as natural remedies from ancient times in Indian subcontinent. Currently the demand for Traditional medicine system in India is growing. The commercial drug producers are now focusing on the medicinal potential of plants and natural products.

In India a vast diversity persists among plant grown in temperate as well as tropical area under the different climatic condition. A wide variety of plants having therapeutic importance can be found all over the world. Many weeds

in our environment are unknown to us, comprises highly effective medicinal characteristics that can cure a variety of significant health issues (Parihar *et al.*, 2021; Chaudhary *et al.*, 2021). From ancient times, India has long been known as a potential source of natural remedies among ancient cultures (Parihar *et al.*, 2021).

In present scenario, exhaustive usages of chemically synthesized antibiotics have badly affected the human immune system by generating drug resistance pathogenicity. This problem has led the extensive search of natural remedies to withstand such conditions. The growing demand for novel drugs that can overcome the

problem of drug resistance contribute to a revival of interest in plant based compounds for drug discovery. Nature endows medicinal plants for our health and survival (Prajapati *et al.*, 2002). In present time, the use of herbal has become even more important, while pharmaceuticals are making synthetic drugs based on natural constituents from medicinal plant. A great part of herbal compounds constitutes the main part in drug discovery to produce synthetic molecular analog. This specifies the importance of study of phytochemicals in diversity oriented synthesis (DOS) of natural product like pharma compounds.

Medicinal plants grow worldwide, which are intended for medicinal purpose. Among those *Catharanthus roseus* is a globally significant plant which found in tropical areas. Ayurveda states the use of plant as traditional medicine system in India that comprises the therapeutic importance of plants and natural products. Some plants of family Apocynaceae are medicinally important including *Catharanthus roseus* L.

Catharanthus roseus is a perennial tropical medicinal plant (Malar Retna *et al.*, 2013). Family Apocynaceae comprises eight species of *Catharanthus*, out of which, seven species are endemic to Madagascar and *C. coriaceus*, *C. lanceus*, *C. Inifolius*, *C. ovalis*, and *C. roseus* widely spread in India (Chandrashekhar *et al.*, 2021). Furthermore, about *C. roseus* plant, this produces more than 130 terpenoids indole alkaloids (TIAs). Some terpenoids indole alkaloids are very useful pharmaceutical and medicinal plants due to their strong and important pharmacological activities (Van der Heijden *et al.*, 2004; Almagro *et al.*, 2015).

Catharanthus roseus is a significant curative plant (Das *et al.*, 2017). This dicotyledonous angiospermic plant integrates two therapeutically important compounds terpene indole alkaloids: vinblastine and vincristine, which are the major constituents of the plant and fight against a variety of diseases. *Catharanthus* is a greek word that means "unadulterated blossom." It has many synonyms as Madagascar periwinkle, bright eyes, Cape periwinkle, graveyard plant, old maid, pink periwinkle, and rose periwinkle myrtle. In India this is frequently referred to as "Nayantara" or "Sadabahar" (Rai *et al.*, 2021; Dubey *et al.*, 2020).

Catharanthus roseus is a well-recognized herbal medicinal plant (Jamal *et al.*, 2024). *Catharanthus roseus* is widely distributed in South Africa and Australia and

grows well in the United States and Southern Europe. This is an ornamental plant, widely distributed throughout India. Different parts of *C. roseus* are used in folklore herbal medicines to treat many types of infectious diseases. Vinblastine and Vincristine, these two vinca alkaloids, anticancer chemicals, are abundant in *C. roseus* leaves (Arulvendhan *et al.*, 2024). Previous researches reveal that *Catharanthus roseus* contains more than 130 alkaloids. the presence of active components vincristine and vinblastine make this plant specific and are widely used in the treatment of various cancers, such as breast cancer, lung cancer, and melanoma (Leveque *et al.*, 2007; Ferreres *et al.*, 2008). *Catharanthus roseus* leaves contain 70 different bioactive compounds including ajmalicine, reserpine, and serpentine. Alkaloids are the main therapeutic compound of *C. roseus* and are significantly used in the remedy of severe complications like diabetes, high blood pressure, dysmenorrhea, asthma, constipation, and cancer (Pham *et al.*, 2020). Two major derivatives terpene indole alkaloids, namely vincristine and vinblastine are produced in the plants (Bhambhani *et al.*, 2021) along with large amounts of volatile and phenolic compounds such as cafe oil, quinic acid, and flavone glycosides. These compounds confer antioxidant activity against reactive oxygen species and play an important role in the plant's defence system (Prabha *et al.*, 2020). Plants compounds which impart antioxidant activity are mainly used for hypertension, diabetes, blood cancer, malaria, non-small-lung cancer, Hodgkin's lymphoma, and improving memory. These plant synthesized compounds also has antimicrobial, antioxidant, anti-diarrheal, and hypolipidemic activity, as well as wound-healing activity (Allamsetty *et al.*, 2020). The current work is focused on the phytochemicals and antimicrobial activity of methanolic extract of *Vinca rosea*.

Materials and Methods

This work was carried out at Advanced Environmental Testing & Research Lab (P) Ltd, Gwalior M.P. The plant was a perennial so, there was no difficulty in getting the material for this study.

Microbes used

A total of three microbes *Salmonella*, *Staphylococcus aureus* and *E. coli* were procured from MTCC, Chandigarh (Punjab). Bacterial cultures were maintained on the agar media as per given instruction of MTCC.

Collection of plant material

The fully mature *Catharanthus roseus* plants were collected from Botanical section of Jiwaji University (M.P.). Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant material using filter paper before they were used for extraction. Separated Plant leaves were shed dried. Dried leaves were grinded and extracted with methanol.

Extract preparation

Solvent extract

10 gm of grinded plant leaf material was dissolved in 100 ml organic solvent Methanol using Soxhlet apparatus. This methanolic extract was further dried using rotary evaporator.

Assessment of antimicrobial activity of plant extract against pathogenic microbes

The antimicrobial activities of plant extract of *Catharanthus roseus* were measured against the procured pathogenic microbes.

Agar well diffusion method (Perez *et al.*, 1993)

Muller Hinton Agar medium was prepared as per manufacturer's instructions. Sterilized media was poured into sterile Petri dishes each contains approximately 25 ml. This was allowed to solidify and spread the Bacterial culture and fungal spores on set petri plates. Five agar wells per plate were made in the set medium by using a sterile cork-borer of 9 mm diameter.

Thereafter, the wells were filled with the prepared extract solution at varying concentrations. This was done in duplicate and plates were incubated at suitable temperature for 24 hrs at 37°C for Antibacterial activity. The plates were observed for zone of inhibition (mm) around the wells.

Phytochemical screening

These methods performed with slight modifications were used to test for the presence of the active ingredients in the test samples (Harborne, 1978).

Test for steroids

1 ml of plant leaf extracts was taken in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tubes. The upper layer turns red and sulphuric acid layer show yellow colour with green fluorescence. This indicated the presence of steroids.

Test for saponins

Take 20 ml of distilled water in a test tube and mixed plant leaf extract and vigorously shake that test tube for 10 minutes. The formation of foam showed the presence of saponins.

Test for tannins

Take 5 ml of the extract and add few drops of 1% lead acetate. Formation of yellow precipitate indicated the presence of tannins.

Test for triterpenes

Take 10 mg of extract in a test tube and add 1 ml of chloroform later 1 ml of acetic anhydride. In the last add 2 ml of conc. H₂SO₄. Reddish violet colour indicated the presence of triterpenoids.

Test for alkaloids

Take 300 mg of leaf extract in a test tube and digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature and examined the alcoholic layer for the pink colour, indicating the presence of alkaloids.

Test for flavonoids

Take 1 ml of extract in a test tube and add few drops of dilute sodium hydroxide. An intense yellow colour was produced in the plant extract, which became colourless on the addition of a few drops of dil. acid indicating the presence of flavonoids.

Test for anthraquinones

Take 5 ml of the extract in a test tube and hydrolysed with dil. Sulphuric acid, later extracted with benzene and add 1ml of dil. ammonia to it. Rose pink rings suggested the positive responses for Anthraquinones.

Test for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete.

Results and Discussion

In the present study, Methanolic and aqueous extract from leaves of *Catharanthus roseus* were extracted and analysed for presence of phytochemicals namely steroids, saponins, tannins, Triterpenes, flavonoids, anthraquinones, alkaloids. Phytochemicals Tannins, triterpenes and Anthraquinones were found absent in methanolic extract of *Catharanthus roseus*.

However, tannins was found present in aqueous extract. Similarly methanolic extract, anthraquinone was found absent in aqueous extract also. Steroids, saponins, flavonoids, and alkaloids were found present in both methanolic and aqueous extract as shown in table 1.

Chinnavenkataraman Govindasamy and Rajendran Srinivasan, (2012) investigated phytochemicals in medicinal plant *Catharanthus roseus*. The phytochemical analysis of organic solvent methanolic extracts of plant leaf revealed the presence varying degree of major phytoconstituents Saponins, terpenoids, alkaloids and tannin (Chinnavenkataraman Govindasamy and Rajendran Srinivasan, 2012).

The present study was carried out to evaluate the phytochemical screening & antimicrobial activity of Methanolic and aqueous extracts of *Catharanthus roseus* leaves. Methanolic and aqueous extracts were prepared in 1mg/ml. Bacterial strains used in this study were *Staphylococcus aureus* (MTCC), *Salmonella* and *E.coli*.

In agar well diffusion assay performed against *Catharanthus roseus* methanolic extracts of leaves in different concentration 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. Highest activity 17.0 mm was measured against *Staphylococcus* for aqueous extract when applied in a 100 mg/ml.

Minimum Zone of inhibition was measured for aqueous extract against *E.coli* (10.0 mm). Highest Zone diameter has been measured against *Staphylococcus* (15.0 mm) followed by *salmonella* (13 mm) for a methanolic extract when used in a concentration of 75 mg/ml. On the other hand lowest inhibition zone is recorded in aqueous extract against the *E.coli* (9.0 mm). aqueous and

methanolic extract has not given any activity against *E.coli* when used in a concentration of 25 mg/ml and 50 mg/ml. Likewise *E.coli*, Both extracts methanolic and aqueous extracts not gave n used in a concentration of 25 mg/ml against *Staphylococcus* and *Salmonella*.

When a concentration of 50 mg/ml against all three bacteria Extract showed no activity against *E.coli*, aqueous extract showed activity against *staphylococcus* (10 mm). Both extract showed antimicrobial activity against *salmonella*. The Inhibition zones were 11 mm for methanolic extracts and 12 mm for aqueous extracts.

In another study, the antimicrobial activity was assessed for the leaf extract of *C. roseus* against *Salmonella typhimurium* and *Staphylococcus aureus* was similar to the present study (Patil *et al.*, 2010).

Goyal *et al.*, (2008) studied antibacterial activity of *C. roseus* against *E. coli* along with 5 other bacterial strains and found that ethanolic extracts were most effective than methanol and which showed maximum inhibition against *Klebsiella pneumoniae* followed by *E.coli* (Goyal *et al.*, 2008). This study also supports the findings of Goyal *et al.*, (2008) for antibacterial activity of plant leave extract against inhibition of *E.coli*.

Figures 1 showing the antibacterial activity of methanolic extract of Leaves against (A) *Salmonella* (B) *E.coli* (C) *Staphylococcus aureus*.

Ramya *et al.*, (2008) observations stated that antibacterial activity of ethanolic extracts of flower, leaves, stem and roots were found to show antibacterial activity. However No antimicrobial activity was observed for methanolic and aqueous extracts of different plant parts of *C. roseus* using disc diffusion assay.

Similarly, the present study methanolic and aqueous extract of leaves is not showed antibacterial activity for some concentration against *E.coli*, *Staphylococcus* and *Salmonella*.

For both plant varieties best antibacterial activity was found in methanolic leaf extract at 70 µl concentrations. Methanolic stem extract of *C. roseus var. "rosea"* gave better zones at 100 µl conc. as compared to 70 µl conc. of *C. roseus var. "alba"*. The present study also showed the similar results for three varying concentrations 50 mg/ml, 75 mg/ml and 100 mg/ml of methanolic leaves extracts (Ramya *et al.*, 2008).

Table.1 Qualitative analysis of phytochemical present in methanolic and aqueous extracts of *Catharanthus roseus*.

Phytochemicals	Leaves of <i>Catharanthus roseus</i>	
	Methanol	Aqueous
Steroids	++	+
Saponins	++	++
Tannins	-	+
Triterpenes	-	+
Flavonoids	++	++
Anthraquinines	-	-
Alkaloids	+++	+++

Table.2 Antimicrobial activities of *C. roseus* against different pathogenic microbes

Bacteria	Zone of inhibition (mm)					
	<i>E.coli</i> (MTCC 1687)		<i>S.aureus</i> (MTCC 96)		<i>Salmonella</i> (MTCC 98)	
	ME	Aq E	ME	Aq E	ME	Aq E
Concentration (mg/ml)						
25	0	0	0	0	0	0
50	0	0	0	10±0.2	11±1.5	12±0.3
75	10±1.25	9±1.65	15±1.1	12±1.25	12±1.5	13±1.15
100	12±1.3	10±0.65	17±1.9	13±1.5	13±1.35	14±0.85
Streptomycin Standard (50µl)	22±0.3	19±0.65	17±1.9	15±0.5	29±0.4	25±0.55

Abbreviations: Aq E-Aqueous Extract; ME-Methanolic Extract

Figure.1 Antibacterial activity of Methanolic extract of *Catharanthus roseus* leaves

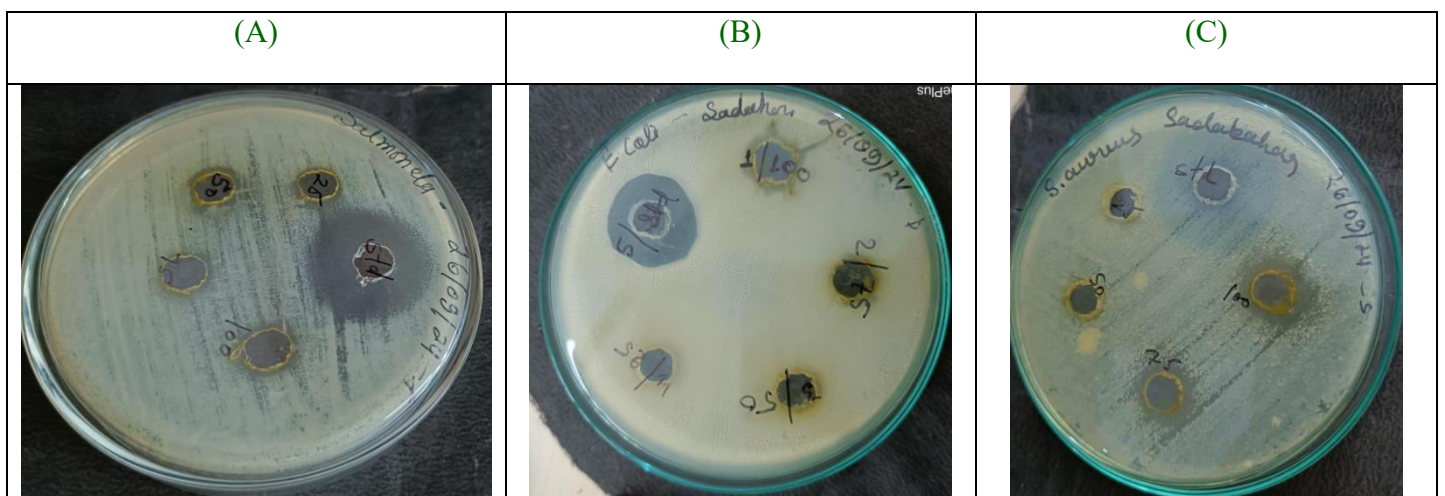
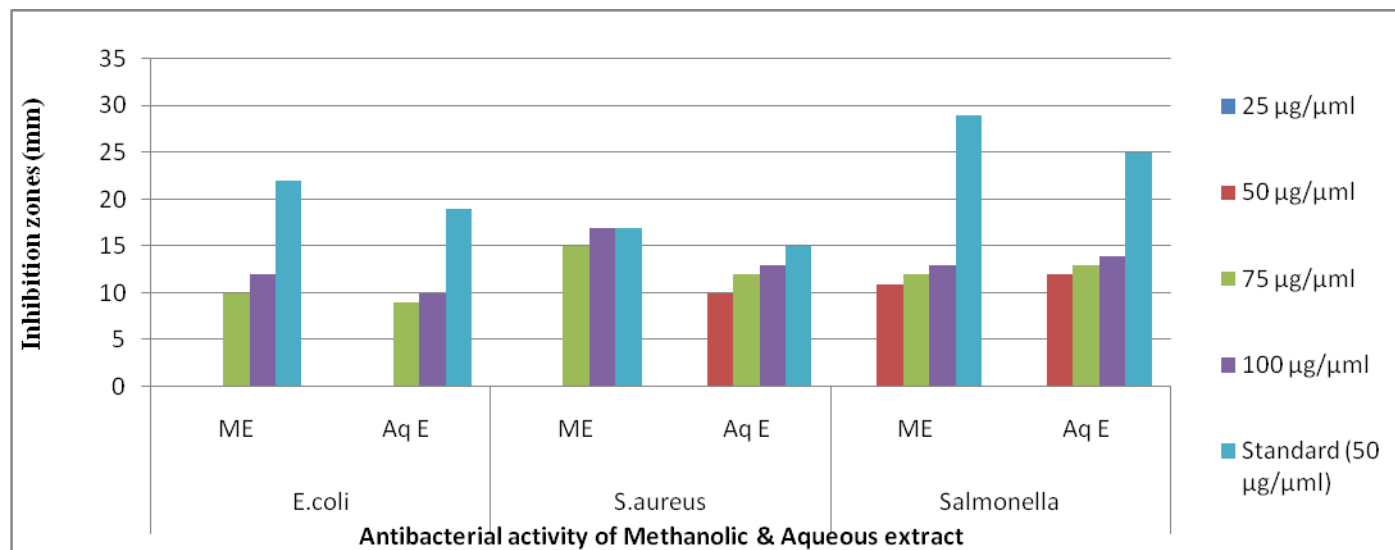


Figure.2 Graphical representation of Antibacterial activity of Methanolic & Aqueous extract



In the present study widely grown *catharanthus roseus* was collected from the Jiwaji University, Gwalior (M.P.). The plant leaf were segregated, shed dried and extracted with methanol and double distilled water for *in vitro* phytochemical and antibacterial studies. The qualitative analysis of phytochemicals revealed the presence of steroid, saponins, alkaloids and Flavonoids in methanolic extract and steroids, saponins, tannins, Triterpenes, flavonoids, and alkaloids in aqueous extract.

The Highest and lowest antibacterial activity of *Catharanthus* plant leaves extract were observed against *Staphylococcus aureus* and *E.coli* respectively in varying concentrations.

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Author Contributions

A Pooja Sahu: Conceived the original idea and designed the model and wrote the manuscript.; B Manish Kumar: Designed the model and the computational framework and analysed the data.; C Arvind Sharma: Review the Manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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