



## Research Article

# Study of phytochemicals, antioxidant activity and antimicrobial properties of *Catharanthus roseus* (L.) G. Don

Dhruv Mishra, Manoj Kumar Chitara, Preeti Chaturvedi

### Abstract

Several anticancer medicines have been developed by using plant-derived compounds in the last few decades. *Catharanthus roseus* (L.) G. Don is a valuable therapeutic plant belonging to the Apocynaceae family. *C. roseus* leaves are exploited in the research of cancer treatment. Free radicals have a significant part in pathophysiology; natural substances that inhibit free radical accumulation can assist to prevent diseases and the ageing process. The antioxidant activity and total phenolic content (TPC) of *C. roseus* leaf extract were evaluated using DPPH free radical and the Folin-Ciocalteu method. Antioxidant activity increased with an increase in dose, roughly 80.21% inhibition at 500 µg/ml. The TPC is 2344.65 mM GAE g<sup>-1</sup> FW and a maximum inhibition zone of 12 mm was shown against *E. coli*. A qualitative study of leaf extract revealed the existence of flavonoids, alkaloids, phenols, and proteins.

**Keywords** antioxidant activity, antibacterial, *Catharanthus roseus*, DPPH free radical, total phenolic content

### Introduction

*Catharanthus roseus* (L.) G. Don (Family- Apocynaceae) is a popular endemic sub-shrub species of Madagascar but has now spread pan-tropically. Leaves of *C. roseus* generally known as Madagascar periwinkle have been utilized in folk medicine since ancient times. Vinblastine and vincristine, the two terpenoid compounds obtained from *C. roseus* leaves were revealed to be the first natural anticancer drugs. These phytochemicals have a wide range of applications in the treatment of leukemia in children, including lymphocytic leukemia, Wilkins' tumors, neuroblastoma, and choriocarcinoma. The vinblastine and vincristine biosynthesis route has been studied extensively on *C. roseus* leaves [1-2]. This plant's aqueous extracts are utilized for different purposes, i.e., bleeding control, diabetes, influenza, and arthritis. In addition, the plants' leaves were eaten to decrease hunger and weariness symptoms [3]. *C. roseus* shows a high concentration of volatile compounds and total phenolic content, which have antioxidant activity. It acts as an antioxidant against reactive oxygen species, which is an important part of the body's defense mechanism [4-5]. In the present investigation, screening of phytochemicals, estimation of total phenols, antioxidant and antibacterial activity were measured in the leaf extract of *C. roseus*.

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## Methodology

### Collection of plant material

Fresh *C. roseus* leaves were obtained G. B. Pant University of Agriculture & Technology, Pantnagar. The leaves were washed thoroughly under running tap water. An oven temperature of 40 °Celsius for 5 days was used to dry the leaves, then powdered with a home mixer blender and stored at room temperature till further use.

### Preparation of *C. roseus* leaf extract

To make methanol extract, 2 g fine powder of dried leaf sample was extracted in 250 ml of methanol using the Soxhlet apparatus. The liquid extract obtained from the Soxhlet apparatus was evaporated by using a rotary evaporator. The rest of the crude extract was collected and kept at 4 °C for further experiments.

### Screening of phytochemicals

Phytochemical screening in crude extracts of *C. roseus* was carried out using standard methods with minor modifications [6]. The findings were classified as either positive (+) or negative (-).

### Flavonoid's detection

**Sulphuric acid ( $H_2SO_4$ ) test** - A few drops of  $H_2SO_4$  were added to 1ml of methanol extract. The presence of flavonoids has been shown by orange color appearance.

Table 1 Phytochemical analysis of *C. roseus* leaf extracts

S. No	Phytochemicals	Method applied	Plant extract
1	Flavonoids	$H_2SO_4$ test	+
2	Phenols	Ferric chloride test	+
3	Alkaloids	Mayer's test	+
4	Carbohydrates	Benedict's test	+
5	Proteins	Xanthoproteic test	+
6	Saponins	Foam test	-

(+) indicates presence, (-) indicates the absence of the phytoconstituent in *C. roseus* extract

### Phenol's detection

**Ferric chloride ( $FeCl_3$ ) test** – One or two drops of  $FeCl_3$  solution were heated with 2ml crude extract, resulting in a blue-black coloration, indicating the presence of phenols.

### Alkaloid's detection

**Mayer's test:** 1 ml HCl was added to 1 ml extract. One or two drops of Mayer's reagent were added, and a yellow-colored precipitate developed, indicating the existence of alkaloids.

### Carbohydrate's detection

**Benedict's test** - 1 ml extract was added with one or two drops of Benedict's reagent and heated, yielding a reddish-brown precipitate that indicated the existence of carbohydrates.

### Protein detection

Xanthoproteic test - A few drops of conc. nitric acid ( $HNO_3$ ) was added to 1ml extract and subjected to heat, resulting in a yellow color that confirms the presence of proteins.

### Saponins detection

Foam test - 2 mL extract was combined with 2 mL dist. water and thoroughly shaken. Saponins were detected by the creation of a 1cm layer of foam.

### Estimation of total phenol

The total phenol content was estimated using 0.2 g fresh leaf samples blended with 4 ml of 80 percent methanol and cooked for 20 minutes at 80°C. To 1ml of this extract, 5 ml distilled water and 250 µl of Folin-Ciocalteu reagent (1 N) was added in a 5 mL vial. 1 mL saturated sodium carbonate (20%) was mixed immediately, and the mixture was incubated for 30 minutes at 25°C. A Genesys 10S UV-Vis Spectrophotometer was used to record the absorbance of the generated blue color at 725 nm [7]. The Gallic acid standard curve was used to determine phenolic content, which was noted as g GAE g<sup>-1</sup> fresh weight.

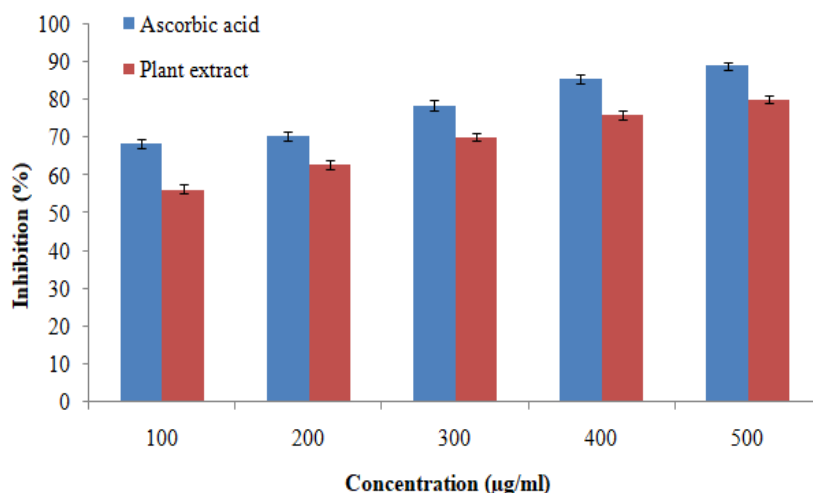


Figure 1. Antioxidant activity of different concentrations of *C. roseus* leaf extract by DPPH scavenging assay

### Measurement of antioxidant activity

The experiment was conducted using DPPH activity estimation [8]. At room temperature in the dark, 1 mL of 0.1mM DPPH solution in methanol was added to various volumes of *C. roseus* leaf extract (100, 200, 300, 400, 500 µg/ml), the solution mixture was incubated for 30 min. The Gallic acid standard curve was used to determine phenolic content, which was expressed as g GAE g<sup>-1</sup> fresh weight. For the control DPPH solution in methanol was utilized. Vitamin C was used as a standard, and methanol was utilized as a blank solution. The DPPH-free radical scavenging activity was measured by using the formula below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### Antibacterial activity

The antibacterial activity of *C. roseus* was investigated using disc diffusion assay against pathogenic bacteria *Escherichia coli* [9]. Stock solutions were prepared by growing bacteria in nutrient broth. Later, 100 µl of bacterium inoculums were distributed across sterile nutrient agar plates. 36 µl of stock solution were impregnated into paper filter discs (6 mm). To allow the solutions to diffuse, the plates were held at room temperature for 30 minutes. The inhibition zone around the disc was observed after a 24-hour incubation period at 37 °C.

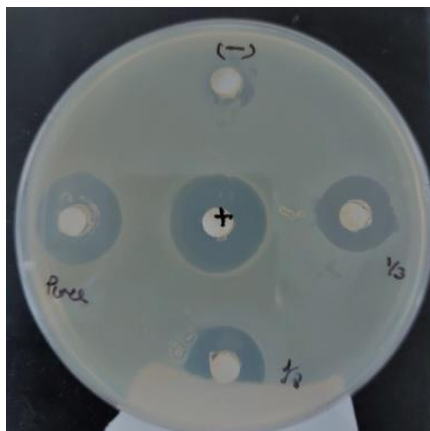
## Results and Discussion

### Preliminary screening of phytochemicals

Qualitative evaluation of phytochemicals displayed the existence of flavonoids, alkaloids, phenols, and proteins in the crude extract (Table 1).

### **Total Phenolic Content (TPC)**

Phenolic compounds provide hydrogen or electron to free radicals and hence function as antioxidants [10]. Total phenolic content (TPC) is measured in mM GAE g<sup>-1</sup> of fresh weight. The samples had a content of 2344.65 mM GAE g<sup>-1</sup> FW (2.34 mg GAE g<sup>-1</sup> FW).



**Figure 2. Antibacterial activity of *C. roseus* leaf extract**

### **Antioxidant activity**

Antioxidant activity of *C. roseus* leaf extract at various concentrations (100 to 500 µg/ml) was measured and the results were compared to the standard (Ascorbic acid). The antioxidant activity of *C. roseus* leaf extract was enhanced by the existence of flavonoids, alkaloids, and phenols. Figure 1 shows a dose-dependent increase in the antioxidant activity, with roughly 80.21% inhibition at 500 µg/ml, which is equivalent to the degree of inhibition found with normal ascorbic acid. Bhutkar and Bhise [11] discovered the antioxidant activity of the root extract of *C. alba* and *C. roseus*. Tiong et al., [12] found that Vindolicine, a compound of *C. roseus*, had the highest antioxidant potential in the DPPH assay.

### **Antibacterial activity**

The findings are depicted in Figure 2. The antibacterial activity of *C. roseus* against *E. coli* was assessed as the area of inhibition zone which is measured as the diameter of the inhibition zone generated around the disc. *C. roseus* showed a 12 mm zone of inhibition against *E. coli*. In another study, the effectiveness of the leaf extract of *C. roseus* was reported for antimicrobial activity against *Salmonella typhimurium* and *Staphylococcus aureus* [13].

### **Conclusion**

Medicinal plants are important in human health care. From time immemorial, the majority of the world's population depends on medicinal plants for their therapeutic uses. These are still vital therapeutic intervention in the treatment of many illnesses. *C. roseus* is a plant with a remarkable therapeutic value. The anticancer effects of this plant have been one of the major focus of researches.

Medications for the treatment of other illnesses are also required. According to the findings, *C. roseus* contains different secondary metabolites such as flavonoids, alkaloids, phenols and proteins in the crude extract as well as a high phenolic content, indicating that it has a high antioxidant potential. Furthermore, the findings revealed that secondary metabolites found in leaf extract have significant antibacterial activity. These medicinally useful metabolites found in leaves, which can allow a more effective and sustainable harvest without uprooting the plants and damaging their variety.



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