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Assessment of the Antioxidant potential and Antibacterial activity of *Catharanthus roseus*plant extracts on Periodontal Pathogens : An In vitro study

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Abstract

The present study aims to evaluatethe antioxidant potential and antibacterial activity of crude extracts from root and leaf parts of *Catharanthus roseus* against periodontalpathogens of clinical importance.Crude extract from root and leaf part of *C.roseus* involved active principles in appropriate solvent followed by evaluation of antibacterial activity by agar well diffusion assay against selective bacterial species. To determine minimum inhibitory concentrationfor the crude extracts of *C.roseus*.Among the extracts that were significantly active, extract obtained using chloroform and methanol exhibited maximum activity against bacterial species tested.Gram (-) bacteria were more sensitive when compared to Gram (+) bacteria. The study also focuses the determination of antioxidant activity and flavonoids, alkaloids terpenoids, and total phenolic content (TPC) of *C. roseus*crude extractusing DPPH assay and IC_{50} (Inhibitory concentration) using broth dilution assay. Phytochemical screening revealed that leaf and root extracts of *C. roseus* is a potential source for anti-leukemic alkaloids and phenolics. This study implicates that leaf and root extracts of *C. roseus* could potentially be exploited as therapeutic agents for various bacterial infections.

Keywords: Antibacterial activity, Antioxidant, bacterial strains, DPPH assay,*C.roseus*

1.Introduction

Catharanthus roseus which is an important medicinal plant of family Apocynaceaeused topreparation of traditional medicine which aretreated for many pathogenicdiseases.Several studies have shown that Catharanthusroseus has a high potential for many varieties of medicinal properties, such as antibacterial, antifungal, antioxidant, anticancer and antiviral activates [1]. *C. roseus* possess known antimicrobial properties continue to receive attention due to development of herbal drugs due to simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action [2].

In recent years, many drugs have been isolated from natural source as the herbal medicinecould prove useful in minimizing the adverse effects of various chemotherapeutic agentswhich are having prolonging longevity and rare chances of harmful side effects [3,4].In many countries, cultivated herbal plants mainly for their alkaloids, which are having antimicrobial activities.These natural products canprovide unique elements of molecular diversity andbiological functionality, which is finding new herbal chemicals for the development of new drugs[5].Among many herbal plants *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 because *C. roseus* shows a high concentration of volatile bio compounds and total phenolic content. It acts as an antioxidant against reactive oxygen species, which is an important part of the body's defense mechanism [6,7]. Two terpenoid compounds such as vinblastine and vincristine, obtained from *C. roseus* leaves were used to synthesize first natural anticancer drugs. These phytocompounds have a wide range of applications in the treatment of leukemia, Wilkins' tumors, neuroblastoma, and choriocarcinoma [8,9]. *C. roseus* plant's aqueous extracts are utilized for different purposes, i.e., bleeding control, diabetes, influenza, and arthritis. In the present study, screening of phytochemicals, estimation of flavonoids, alkaloids terpenoids, and phenols, antioxidant and antibacterial activity were measured in the leaf and root extract of *C. roseus*.Hence, the present study was undertaken to screening of phytochemicalsand evaluate the in vitro antimicrobial and antioxidant efficacy leaf and root extractsof *C. roseus*against standard gram positive and gram negative strains.

2.0. Experimental

2.1. Collection of Plant materials and Chemicals

The root part of *Catharanthus roseus* was collected from Palar river basin of Vellore district, Tamilnadu, India, in the month of March 2022. All chemicals and solvents were of analytical grade purchased from NICE chemicals Pvt.Ltd, Mumbai.The leaves was washed thoroughly under running tap water and dried in an oven at 40 °C in for 5 days. Then dried leaves was powdered with a home mixer blender and stored at room temperature till further use. The root part was washed and air dried over a period of one month. The dried samples were milled into fine powder by pounding manually with a clean sterile mortar, stored at room temperature till further use. Percent yield of crude extract was calculated using following formula:

$$\text{Percent yield} = \frac{\text{weight of crude extract obtained(g)}}{\text{total weight of plant powder (g)}} \times 100$$

2.2. Preparation of *C. roseus* leaf extract

2 g fine powder of dried leaf sample was extracted in 250 ml of methanol using the Soxhlet apparatus. The methanol 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.

2.2. Preparation of *C. roseus* root extract

100 g fine powder dried root samples was extracted in 1000 mL of hexane, chloroform, ethyl acetate, methanol and aqueous sequentially using Soxhlet apparatus. The process was run for 24hr after which the sample was concentrated using reduced pressure distillation under vacuum pump and freeze dried to powdered form. The dried crude extract was collected and kept at 4 °C for further experiments.

2.3. Screening of phytochemicals:

Phytochemical screening in crude extracts of *C. roseus* was carried out using standard methods with minor modifications [10].The major secondary metabolites classes such as flavonoids, alkaloids terpenoids, glycosides,tannins and saponins were screened according to the common phytochemical methods described by Harborne1998[11].

Flavonoid's detection:

A few drops of sulphuric acid was added to 1 mL of methanol extract. The presence of flavonoids has been indicated by orange color appearance.

Alkaloid's detection:

1 mL HCl was added to 1 mL of crude extract of *C. roseus*. One or two drops of Mayer's reagent was added, formation of a yellow coloured precipitate indicating the existence of alkaloids.

Terpenoids detection :

1 mL of Plant extract was dissolved in 2 mL of methanol and then evaporated to dryness followed by the addition of 3 mL of conc. sulphuric acid, formation of reddish brown color.

Glycosides detection:

To 1 mL of Plant extract, 2 mL of chloroform was added, followed by the 2 mL of concentrated sulphuric acid, Formation of reddish brown colored steroidal ring indicating the presence of glycosides.

Phenol's detection:

One or two drops of Ferric chloride ($FeCl_3$) solution was heated with 2 mL crude extract of *C. roseus*, resulting in a blue-black coloration, indicating the presence of phenols.

Carbohydrate's detection:

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:

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:

2 mL crude extractof *C. roseus* was combined with 2 mL dist. water and thoroughly shaken. Saponins were detected by the creation of a 1cm layer of foam.

Tannins detection:

10% alcoholic ferric chloride was added to 2-3 mL of methanolic plant extract,formation of dark blue colour of the solution indicated presence of tannins.

2.4. Antibacterial activity

Bacterial strains were obtained from Microbial Clinical Laboratory, Christian Medical College Hospital, Vellore, India.Four Gram negative strainsMCLCMC 443 (*Escherichia coli*), MTCC 109 (*Klebsiellapneumoniae*), MTCC 450 *(Schigellaflexneri)*, MCLCMC 441 (*Proteus vulgaris*), and four Gram positive strainsMTCC 441 (*Bacillus subtilis*), MCLCMC 96 (*Staphylococcus aureus*), MCLCMC 1457 *(Clostridium perfringens)* MCLCMC 1538 (*Micrococcus luteus*) were used in the present study as test organismsfor investigating antibacterial activity.

The antibacterial activity of *C. roseus* was investigated using well diffusion assay against bacterial strains [12]. Nutrient agar was prepared and poured in the sterile petri dishes and allowed to solidify. 24 h growing bacterial cultures (*Escherrichia coli, Proteus vulgaris, Klebsiella pneumonia, Staphylococcus aureus, Micrococcus luteus,* and *Bacillus subtillis*) were swabbed on it. Then, 5 wells (8mm diameter) were made by using a sterile cork borer. The 4 different concentrations (250µg, 500µg, 750µg and 1000µg) of the plant extracts were loaded in the wells. Sterile distilled water served as negative control. To allow the solutions to diffuse, the plates were held at room temperature for 30 minutes. The plates were then incubated at 37ºC for 24h. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. After incubation the inhibition diameter was measured [13].

2.5. Minimum Inhibitory Concentration

MIC test was done on selectively against eight organisms that were found susceptible in agar diffusion assay. Periodontal Pathogens such as *Escherichia coli,Klebsiellapnemoniae, Proteus vulgaris,Schigellaflexneri, Micrococcus luteus, Bacillus subtilis,Clostridium perfringens* and *Staphylococcus aureus*were used. MIC values also showed that extracts were able to inhibit the bacterial strains at lower concentrations.

2.6. Antioxidant activity assays

The radical scavenging activity of different extracts was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity estimation according to Chang *et al* (2008)[14].Antioxidant activity of *C. roseus* leaf extract at various concentrations (20 to 200 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.1 mL of 0.1mM DPPH solution in ethanol was added to various volumes of *C. roseus*root extract (20, 40, 60, 80, 100, 120, 140, 160, 180, 200 µg/ml).The setup was left at dark in room temperature andthe solution mixture was incubated for 30 min.The absorption was monitored after 30 minutes. Ascorbic acid was used as references. The absorbance was measured at 517nm by UV-Visible spectrophotometer. The decrease in absorbance of DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.960 µl of

0.1mm methanolic DPPH solution mixed with 20 to 200µg/ml of plant extract and vortexes thoroughly. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample} * 100}{\text{Abs. control}}$$

Abs. control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + plant extract. Measurements were performed in triplicates. Absorbance values were corrected for radicals decay using blank solutions.IC_{50} values have done by Broth dilution assay [15].

3.0. Results and discussion

3.1. PreliminaryPhytochemical Analysis

The leaf extracts *of C. roseus* prepared in five different solvents were evaluated for the presence of different phytochemicals and the result obtained are presented in **Table 1**, perusal of the results revealed that all the five extracts, showed the presence of alkaloids, terpenoids, flavonoids and phenols. Methanolic extract showed the maximum number of phytoconstituents, followed by the hexane, chloroform, ethyl acetate and aqueous extract. Glycosides were detected in all the extracts, except petroleum ether extract. Terpenoids were detected in both methanolic and chloroform extracts. Tannins were detected in both methanolic and aqueous extract chloroform extracts. Saponins were detected in the aqueous extract only.Similarly, the preliminary phytochemical screening tests for the root extracts of *C.roseus* root revealed the presence of flavonoids, alkaloids, terpenoids, glycosides, phenolics, saponins and tannins are observed in **Table 2.** Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-bacterial activity of the plant.

Table1. Phytochemical analysis of *C. roseus* leaf extracts

S.No	Phytochemicals	Method applied	Leaf extract				
			Hexane	chloroform	ethylacetate	methanol	aqueous
1	Flavonoids	Sulphuric acid test	+	+	+	+	+
2	Alkaloid	Mayer's test test	+	+	+	+	+
3	Terpenoids	Sulphuric acid test	+	+	+	+	+
4	Glycosides	Salkowski	+	_	_	+	_

		test					
5	Phenol	Ferric chloride test	+	+	+	+	+
6	Saponins	Foam test	_	_	_	_	+
7	Tannins	Braemer's test:	_	_	_	+	+

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

Table2. Phytochemical analysis of *C. roseus*root extracts

S.No	Phytochemicals	Method applied	Root extract				
			Hexane	chloroform	ethylacetate	methanol	aqueous
1	Flavonoids	Sulphuric acid test	+	+	+	+	+
2	Alkaloid	Mayer's test test	+	+	+	+	+
3	Terpenoids	Sulphuric acid test	+	+	+	+	+
4	Glycosides	Salkowski test	_	_	_	_	_
5	Phenol	Ferric chloride test	+	+	+	+	+
6	Saponins	Foam test	+	+	+	+	+
7	Tannins	Braemer's test:	+	+	+	+	+

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

3.2. Antibacterial Susceptibility Assay of *C.roseus*

In the present study, the five different solvents of *C.roseus*root extracts were selected for antibacterial activity on eight – different organisms in fourdifferent concentrations are given in **Table 3**. Among the five differentorganic solvents in root extractof*C.roseus*, chloroformextract shows highest antibacterial activity against *Bacillus subtillis,Micrococcusluteus*and *Staphylococcus aureus*andwith the zone of inhibition 17, 18 and 19mm respectively.

The strongest inhibition activity of the leaf extract of *C. roseus*in ethanol extract was observed against *Staphylococcus aureus* (16 mm zone) at 1000 µg/mL followed by *E.coli* which shows(12 mm inhibition zone) at 1000 µg/mL[16].Methanol extract was found to be more suitable solvent and shows highest antibacterial activity againstpathogens.

3.3.Minimum Inhibitory Concentration of *C.roseus*

MIC of *C. roseus*results were revealed the chloroform and ethanol extracts should be more effective than other extracts. MIC values also showed that extracts were able to inhibit the bacterial strains at lower concentrationsobserved **Table 4**.The lowest MIC for chloroform and methanol extracts were reduced against the five organisms. The MIC technique is used to determine the efficacies of antimicrobial agents. In this study, the MIC values tends to support the results obtained in the antibacterial screening by agar diffusion method in chloroform and ethanol extracts were more potent than other solvents.

Name of the extract	Conc.of root extract (µg)	Zone of Inhibition (mm)							
		Micro organisms							
		E.c	*K.p*	*P.v*	*S.f*	*B.s*	*M.l*	*S.a*	*C.p*
Hexane	250	-	-	-	-	11	-	-	-
	500	-	-	-	-	12	-	-	-
	750	-	-	-	-	14	10	-	10
	1000	-	-	-	-	15	11	-	11
Chloroform	250	-	10	-	10	-	10	10	10
	500	10	11	10	12	11	11	12	11
	750	11	13	11	13	13	12	13	13
	1000	13	15	13	14	14	13	14	14
Ethylacetate	250	-	-	-	-	10	10	-	10
	500	9	11	10	10	11	11	10	11
	750	10	13	11	12	13	12	12	12
	1000	11	15	12	12	14	13	13	13
Methanol	250	-	-	-	-	-	-	-	-
	500	10	10	-	10	10	14	10	14
	750	11	11	-	11	15	16	11	16
	1000	12	12	-	12	17	18	19	16
Aqueous	250	-	-	-	-	-	--	-	-
	500	-	-	-	-	-	-	-	-
	750	-	-	-	-	14	13	-	13
	1000	-	-	-	-	15	14	-	14

Table 3. Antibacterial activity of crude root of *C.roseus*

Ec: Escherichia coli, Kp: Klebsiella pnemoniae, Pv: Proteus vulgaris, Sf:Schigella flexneri, Ml: Micrococcus luteus, Bs: Bacillus subtilis, Cp:Clostridium perfringens, Sa:Staphylococcus aureus.

Table 4. Minimum inhibitory concentration of *C.roseus*

Name of the Organism	Minimum Inhibitory Concentration (mg/mL)				
	Aqueous	Methanol	Ethylacetate	Chloroform	Hexane
Escherichia coli	-	0.5	0.5	0.5	NA
Klebsiella pnemoniae	-	0.5	0.5	0.25	NA
Proteus vulgaris	-	NA	0.5	0.5	NA
Micrococcus luteus	0.75	0.5	0.25	0.25	NA
Bacillus subtilis	0.75	0.5	0.25	0.5	0.25
Staphylococcus aureus	-	0.5	0.5	0.25	NA
Schigella flexneri	-	0.5	0.5	0.25	NA
Clostridium perfringens	0.75	0.5	0.25	0.25	NA

NA – No activity

3.4. Antioxidant activity of *C.roseus*

The antioxidant activity of *C.roseus* evaluated by DPPH assay at different concentrations and the results of the antioxidant activity of best screened chloroform extracts of root and leaf of *C.roseus*byDPPH assays at different concentrations are observed**Table 5**.The IC_{50} values of the root extracts, which were found to be, vary from 19.85 to 70.65for chloroform extractand leaf extract vary from 21.65 to 72.10formethanol extract were determined **Table 6**. In the present investigation, the obtained data shows that chloroformand methanol extracts are free radical scavengers and may act as primary antioxidants which can react with free radicals by donating hydrogen.

Antioxidant activity of *C. roseus* leaf extract at various concentrations (100 to 1000 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,terpenoids and phenols. **Figure 2** shows a dosedependent increase in the antioxidant activity, with roughly 70.65% inhibition of root extract and 72.10 % inhibition of leaf extract at1000 µg/mL, which is equivalent to the degree of inhibition found with normal ascorbic acid.

According to the findings, root extract of *C. roseus* contains different secondary metabolites such as flavonoids, alkaloids, terpenoidsas well as high phenolic content, indicating that it has a high antioxidant potential. Furthermore, the findings revealed that secondary metabolites found in leaf and root extracts have significant antibacterial activity was observed.

Thus in this study leaf extracts exhibited maximum inhibition, followed by roots.Chloroform and methanol were found to be a more suitable solvents for the maximum extraction of active metabolites.However, organic solvents extracts were found to be inhibitory than their respective aqueous extracts.The leaf extracts of*C. roseus* has significant antibacterial activity against Gram-positive and Gram-negative bacteria species. Furthermore this study revealed that a Gram-positive bacteria were more susceptible to this extract as compared to Gram-negative bacteria species [17]. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms [18].Antibacterial activities were dose-dependent. However, the efficacies of plant extracts were less than the standard. This study showed that the extraction of antimicrobial substances by organic solvents is better as compared to aqueous extracts [19]. The polarity of bio compounds make them more readily extracted by organic solvents. It has been shown using organic solvents crucial for their bioactivity against pathogenic bacteria species.

Table 5. Radical Scavenging Activity (RSA) of the selected plant extract

Concentration (µg/mL)	Root	Leaf	Standard
20	73.46	75.52	19.52
40	75.12	77.20	36.25
60	76.90	79.15	48.65
80	79.65	81.40	52.40
100	83.46	84.17	56.10
120	85.12	87.19	58.15
140	87.62	90.15	67.35
160	89.15	92.20	73.82
180	92.36	94.65	75.45
200	93.80	95.70	85.50

Table 6. IC_{50}Determination – broth dilution Assay

Concentration (µg/mL)	Inhibition (%)	
	Root	Leaf
100	19.85	21.65
200	22.45	24.50
300	28.15	30.45
400	33.20	35.75
500	39.50	42.65
600	45.25	50.45
700	51.10	53.10
800	58.45	59.20
900	64.20	67.55
1000	70.65	72.10

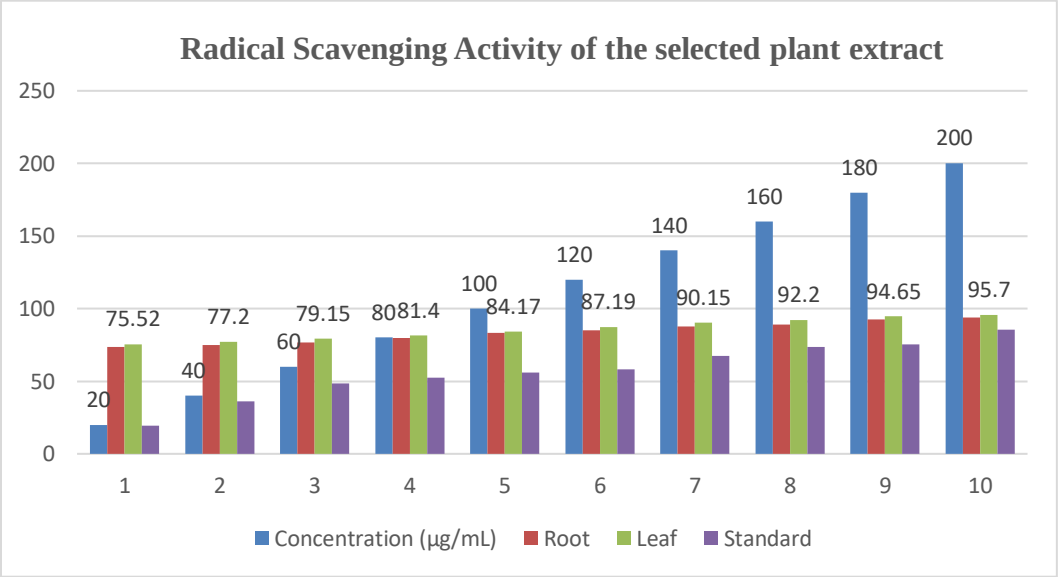

Figure 1. Radical Scavenging Activity (RSA) of the selected plant extract

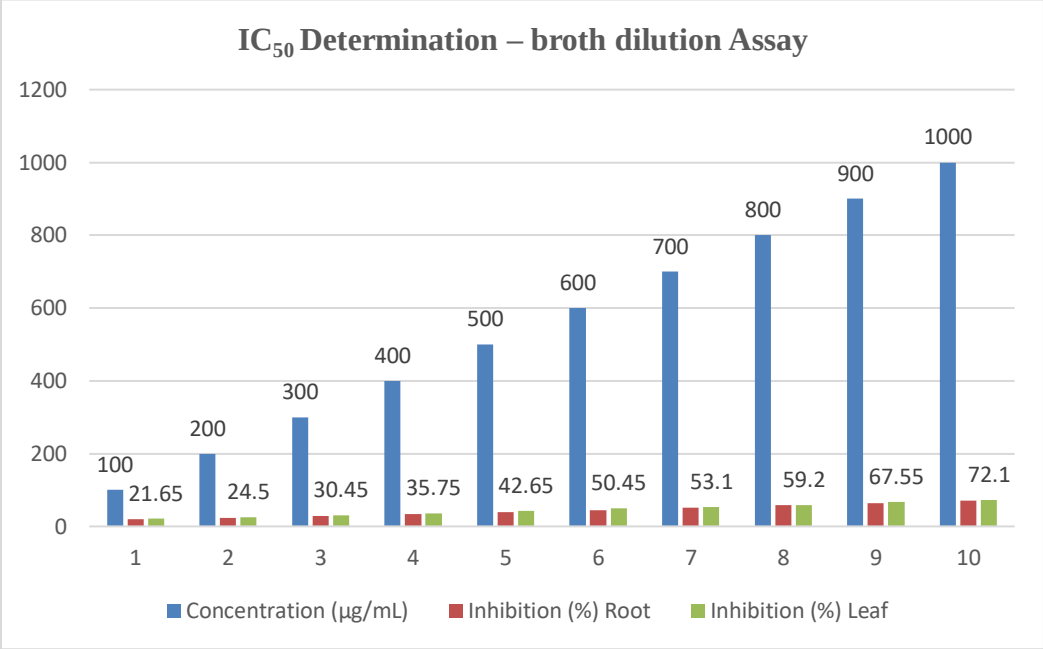

Figure 2. IC_{50} Determination – broth dilution Assay

4.0.Conclusion

In this present study five plant extracts prepared in various solvents from leaves and roots of *C. roseus* were analyzedfor their*in vitro* antioxidant potential and antibacterial activity.Among the five extracts, methanol and chloroform extracts of *C. roseus* showed maximum antibacterial properties.Thehighest inhibition zone of 17, 18 and 19 mm was observed by *C.roseus*against *Bacillus subtillis. Micrococcusluteus*and*Staphylococcusaureus*whencompared to other microorganisms. According to the findings, *C. roseus* contains highest phenolic alkaloids and flavonoids content, indicating that it has a high antioxidant potential. The antioxidant potential of the extract was also found to be highest as it showed minimum IC50 values in DPPH assay method.Furthermore, secondary metabolites found in root and leaf extracts have significant antibacterial activity. This study supports the concept of these medicinal plants might be useful as an antioxidant and antimicrobial agents.The study needs further investigation, to isolate the active bio compounds present in *C. roseus*for formulation of new drugs against various bacterial infections.

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