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BIO-ASSAY GUIDED FRACTIONATION BASED ISOLATION AND IDENTIFICATION OF ANTI-BACTERIAL ACTIVITY THE STEM AND ROOT OF *CATHARANTHUS ROSEUS* IN ETHANOL EXTRACT

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Abstract: *Catharanthus roseus* commonly known as evergreen herb is one of the famous medicinal herb in the field of cancer treatment. Then the plants were shade dried to avoid the loss of bioactive compounds. 100g of each plant part were Extract in different organic and aqueous solvents which have varying polarity (Petroleum ether, EtOAc, EtOH, H_2O). The extract were tested bacteria cultures were grown nutrient in broth medium at 37 °C for 24 hrs. Results of the present study indicate that antibacterial activity of the extracts varied significantly depending upon the plant part used viz., Stem, Root. Data indicate that extracts prepared from root exhibited better anti bacterial activities than those extracts prepared from other parts of the plant. The root extracts exhibited maximum inhibitions. Ethanol was found to be a more suitable solvent for the maximum extraction of active metabolites. Antibacterial activities were dose-dependent. However, the efficacies of plant extracts were less than the standard.

Key Words: *Catharanthus roseus* , Staphylococcusaureus.MTCC9542, Antibacterial Susceptibility Assay

INTRODUCTION

Catharanthus roseus, commonly known as bright eyes, Cape periwinkle, graveyard plant, Madagascar periwinkle, old maid, pink periwinkle, rose periwinkle,[1] is a species of flowering plant in the family Apocynaceae. It is native and endemic to Madagascar, but grown elsewhere as an ornamental and medicinal plant. It is a source of the drugs vincristine and vinblastine, used to treat cancer.[2] It was formerly included in the genus *Vinca* as *Vinca rosea*.

Catharanthus roseus commonly known as evergreen herb is one of the famous medicinal herb in the field of cancer treatment. Many famous phytochemicals such as vincristine and vinblastine were isolated from this medicinal plant. It has many pharmacological properties such as anti-oxidant, anti-microbial, anti-diabetic, wound healing, anti-ulcer, hypotensive, antidiarrhoeal, hypo lipidemic and memory enhancement. Alkaloids

are one of major phytochemicals responsible for its anti-cancer properties followed by phenolic compound ssuch as flavonoids. The purpose of the current study is to document updated data about its traditional uses, isolated bioactive compounds and pharmacological activities reported.

MATERIALS AND METHODS

Plant collection

Catharanthus roseus was identified and mature plants were collected in the garden of A.P.A. College of Arts and Culture, Palani in the month of March. The plants were washed thoroughly with tap water to avoid dust. Then the plants were shade dried to avoid the loss of bioactive compounds. After complete drying, each part of the plant were subjected to mechanical grinding and collected in an air tight container.

Preparation of dried *Catharanthus roseus*

In previous studies, various drying methods, such as freeze drying, air drying, low-temperature drying, infrared drying or drying under the shade have been applied to prepare dried *catharanthus roseus* for further recovery of bio-active compounds . *Catharanthus roseus* material bio-active compounds are sensitive to heat, light and oxygen. Freeze drying has been reported as a prominent and effective drying method in terms of the retention of bio-active compounds.50 ◦C was suggested for drying the leaf and the flower which contained high levels of phenolics and flavonoids.

Extract preparation

100g of each plant part were Extract in different organic and aqueous solvents which have varying polarity (Petroleum ether, EtOAc, EtOH, H2O). Each 250ml of the solvent were used, with the help of soxhlet apparatus the extract was prepared and stored in clean beakers.

Bio-assay fractionation of ethanol extract of *catharanthus roseus*

There have been numerous extraction techniques used for this method including conventional extraction, ultrasonic-assisted extraction and Soxhlet extraction using the common solvents petroleum ether, ethyl acetate, ethanol and water. Soxhlet extraction was used to prepare the *catharanthus roseus* hairy root extract. Although this method is simple, well established and inexpensive. Further, it occurs at the boiling point of solvents over a long period that may cause the thermal degradation of bio-active compounds.

BacterialStrains

1. Staphylococcusaureus.MTCC9542
2. BacillussubtilisMTCC9706
3. KlebsiellapneumoniaeMTCC10309
4. Pseudomonasaureginosa.MTCC944

Antibacterial Susceptibility Assay

The bacteria cultures were grown nutrient in broth medium at 37 °C for 24 hrs. The assay medium Muller Hinton agar was prepared and sterilized at 121^{0C} for15 minutes at15lb pressure. 15 ml of sterile nutrient

agar was transferred to the petridishes. The cultures were inoculated on the solid surface of Mueller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated with different concentration of the drugs were placed on surface of each inoculated plate. The plates were incubated at 37 °C for 24 h. After this period, it was possible to observe inhibition zone. The results obtained were compared with the zone of inhibition observed in the standard drugs.

Results and Discussion

ANTI-BACTERIAL ASSAY

Table 1. Bacterial strains used in the present study.

S.No	Bacterial strain	Gram (+/-)
1.	Staphylococcus aureus.MTCC9542	+
2.	Bacillus subtilis MTCC9706	+
3.	Escherichia coli MTCC1687	-
4.	Pseudomonas aureginosa. MTCC944	-

Table 2. Anti-microbial activity of *C. roseus* petroleum ether extracts.

S.No	Bacterial strain	Plant Part	
		Stem	Root
1.	Staphylococcus aureus.MTCC9542	+	+
2.	Bacillus subtilis MTCC9706	+	+
3.	Escherichia coli MTCC1687	-	-
4.	Pseudomonas aureginosa. MTCC944	-	-

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 3. Anti-microbial activity of *C. roseus* Ethyl acetate extracts.

S.No	Bacterial strain	Plant Part	
		Stem	Root
1.	Staphylococcus aureus.MTCC9542	+	+
2.	Bacillus subtilis MTCC9706	+	+
3.	Escherichia coli MTCC1687	-	-
4.	Pseudomonas aureginosa. MTCC944	-	-

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 4. Anti-microbial activity of *C. roseus* Ethanol extracts.

S.No	Bacterial strain	Plant Part	
		Stem	Root
1.	Staphylococcus aureus.MTCC9542	-	-
2.	Bacillus subtilis MTCC9706	++	-
3.	Escherichia coli MTCC1687	++	++
4.	Pseudomonas aureginosa. MTCC944	++	++

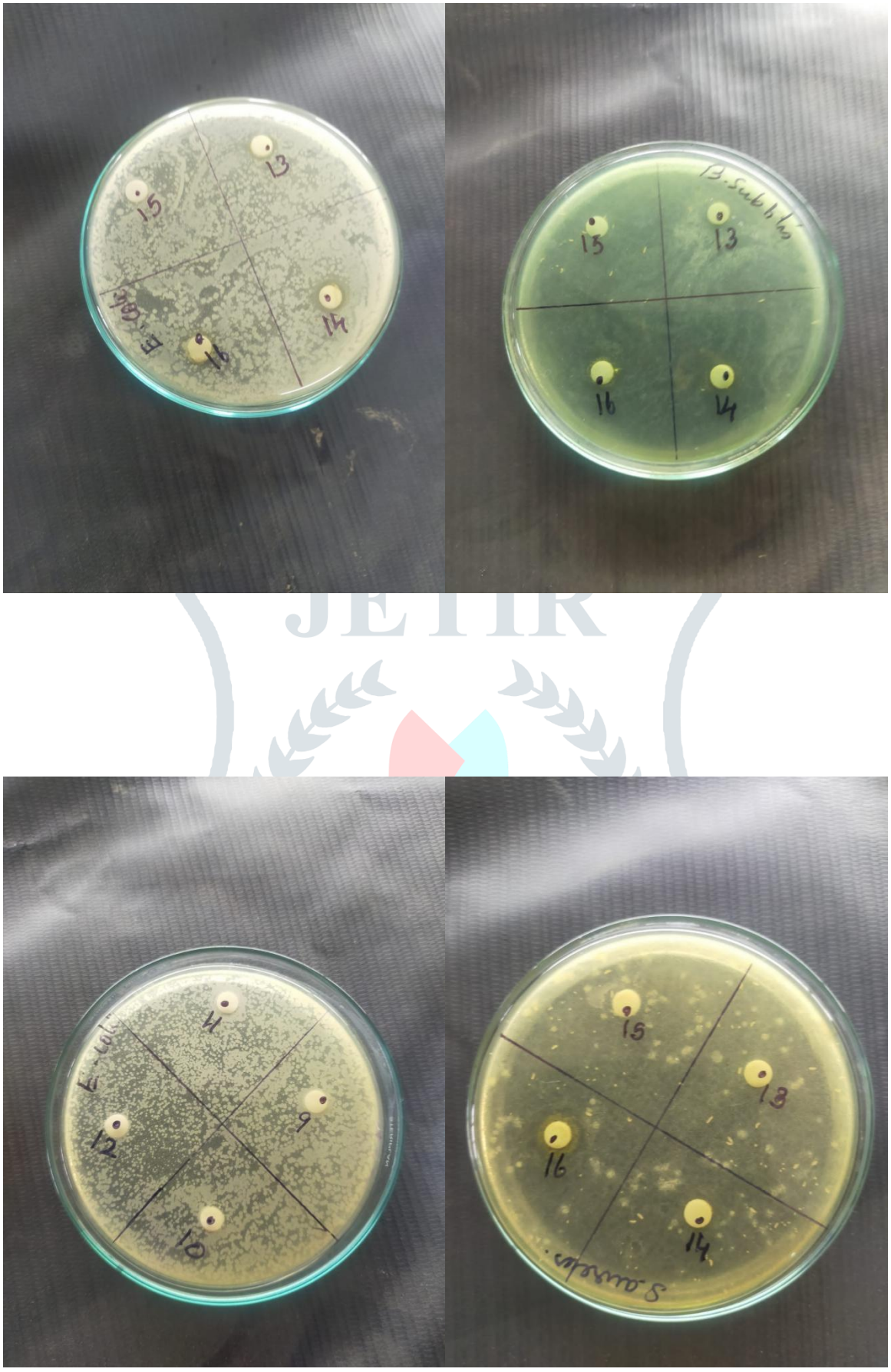
(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 5. Anti-microbial activity of *C. roseus* water extracts.

S.No	Bacterial strain	Plant Part	
		Stem	Root
1.	Staphylococcus aureus.MTCC9542	-	++
2.	Bacillus subtilis MTCC9706	+	+
3.	Escherichia coli MTCC1687	-	++
4.	Pseudomonas aureginosa. MTCC944	+	++

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Results of the present study indicate that antibacterial activity of the extracts varied significantly depending upon the plant part used viz., Stem, Root. Further, data obtained demonstrates that the antibacterial activity of plant parts depends largely upon the extraction procedure, type of solvent used for extraction, and the bacterial strains tested. Data indicate that extracts prepared from root exhibited better anti bacterial activities than those extracts prepared from other parts of the plant. Almost all parts of the plant showed significant antibacterial activity (Table 2-5). The root extracts exhibited maximum inhibitions. However, floral extract were comparatively inactive towards the microbial strains tested. Phytochemical extracts were found to be inhibitory than their respective aqueous extracts. Ethanol was found to be a more suitable solvent for the maximum extraction of active metabolites. Gram-positive bacteria were found more susceptible as compared to Gram-negative species. Antibacterial activities were dose-dependent. However, the efficacies of plant extracts were less than the standard.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E. coli
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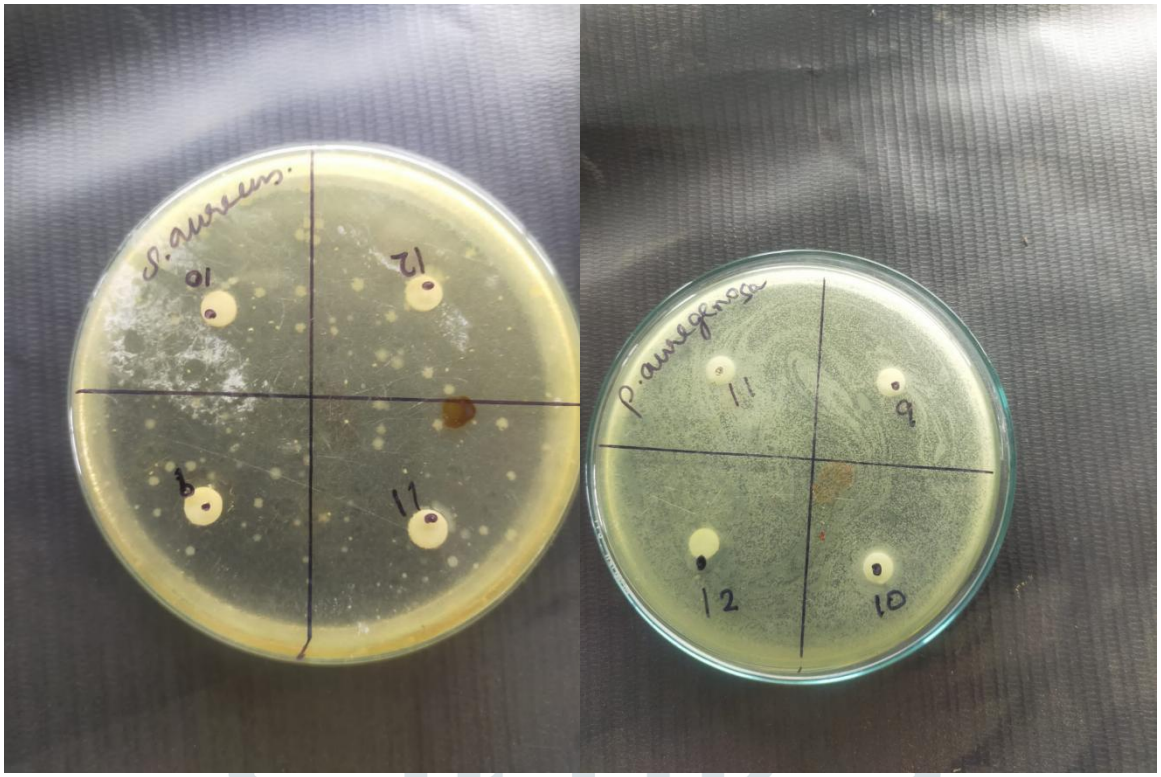
S. aureus


S. aureus.
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P. aureginosa
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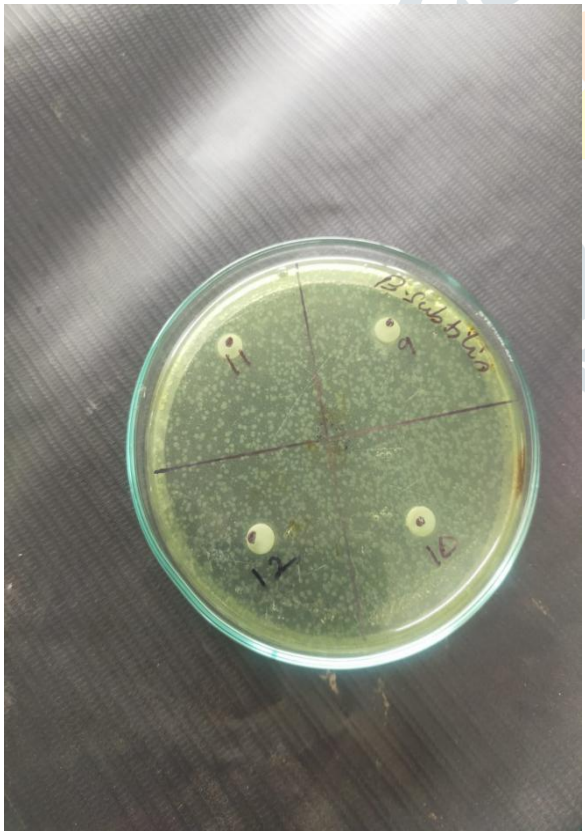
B. subtilis
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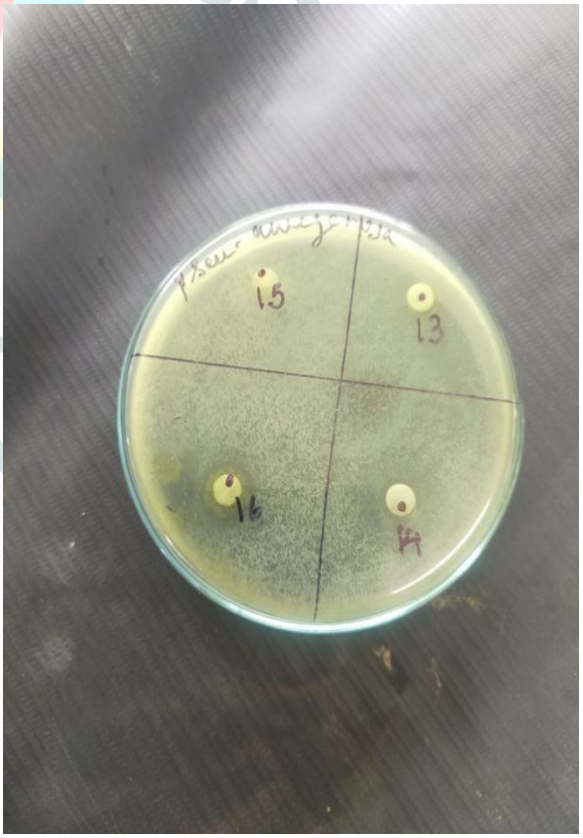
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Discussion

As can be seen from the literature survey that this plant has been mostly studied with respect to its anticancer properties and its anti diabetic properties. Till date, very little studies have been done on the anti microbial properties of the plant extracts. Therefore, this study focuses on the anti microbial properties of the root and stem extracts. These extracts may not find a therapeutic use in immediate future but definitely it can be used as a prophylactic agent in regions where certain diseases can occur as endemic if not in pandemic scale. It would go a long way to remove the stress on specific vaccine production to protect such population from the pathogen, which changes its invasive strategies by varying it antigenic nature. It can be seen from the results above that the root extract contained many indole alkaloids, and some phenolic compounds. The phenolic compounds are known for their antimicrobial properties. The significance of these compounds are that these can substitute long term antibiotic therapy like in case of chronic kidney infection, bacterial endocarditis, carrier conditions of typhoid. These compounds having minimum side effect can be easily substituted for antibiotics. Actually the indole alkaloids do not show direct antimicrobial actions but strengthens the immune system and it is this system that takes care of the pathogens.

Conclusion

It can be concluded from the present findings that, the ethanolic extract of C. roseus collected from the Palani region was showed antimicrobial activity source for various infects. The anti bacterial studies showed the anti microbial activity against Pseudomonas aeruginosa , Staphylococcs aureus and Bacillus subtiIis among the pathogen used. Further, studies is need to be confirm identify the particular compounds to use as a drug as main ingredient in the traditional medicine.

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