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Antibacterial activity of methanolic and ethanolic extracts of leaves of *Catharanthes roseus* L.

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Abstract
Catharanthes roseus L. plant belongs to Apocynaceace family. Mostly all the members of this family having alkaloid content in their phytochemicals. In this study antibacterial activity of ethanolic and methanolic leaves extract of *Catharanthes roseus* L. were assessed against *E-coli* bacteria. Ethanolic leaves extract showed positive results against *E-coli* bacteria from 5 mg to 20 mg concentration. At 5 mg concentration 1.5 mm, 10 mg concentration 2.0 mm, 15 mg concentration 4.5 mm and at 20 mg concentration 8.5 mm zone of inhibition were observed. Ethanolic and methanolic both extract showed positive results. So, *Vinca* leaves having antibacterial property because of the presence of different phytochemical constituents. Further research can provide the data of specific phytochemical which is responsible for the activity.

Keywords: *Catharanthes roseus* L., Antibacterial activity, *E- coli*

Introduction
***Catharanthes roseus* L.**
Co mmon Names: Cayenne Jasmine, Old maid, Periwinkle, Sadabahar, Sadaphul, Sadaphuli, Nityakalyani, Rasna, Sadampushpa, Sadapushpi, Cutukattuppu, Bilaganneru, Barmasi, Noyontara, Ganesha nahoo.

Distribution
The plant is native to Indian Ocean Island of Madagascar. It is also found in Australia and South Africa. In India, it is mostly found in Tamil Nadu, Karnataka, Gujarat, Odisha, West Bengal and Andhra Pradesh. In the United States, the plant grows well in South Carolina, Texas, Mississippi, Florida and Georgia. It also grows in Southern Europe. It is an endangered plant in wild because of slashes and burn cultivation. This herb is now co mmon in many tropical and subtropical regions.

Systematic position-*Catharanthes roseus* L. (var. major)
Kingdom: Plantae (Plants)

Division: Angiosperms

Class: Dicotyledon

Sub-class: Gamopetalae

Series: Bicarpellatae

Order: Gentialales

Family: Apocynaceae (Dogbane family)

Genus: *Catharanthus* (Periwinkle)

Species: *roseus*

(According to Bentham and Hooker)
Catharanthus roseus L. is a fleshy perennial growing to 32in (80 cm; around 1 to 2 feet) high. The plant spreads around 1 to 2 feet area. Leaves are glossy dark green, oval leaves (1-2 inches long), opposite-subopposite, simple, entire and leaf blade length 2 to 4 inches. Flowering occurs throughout the year and pink, purple, Lavender. Fruit: Pod, 0.5 to 1 inch, dry or hard and green in colour.

E-coli
Systematic position of *E-coli*
Kingdom: Bacteria

Phylum: Proteobacteria

Class: Ga mma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *coli*

Escherichia coli is a co mmensal bacterium lives har mless in intestinal microflora in variety of animals including man, however sometimes they cause fatal diseases in humans, ma mmals and birds. It is gram negative, non-spore forming and straight rod measuring 1-3 × 0.4-0.7 µm. it is motile by peritrichous flagella and facultative anaerobe. It can be grown on media like nutrient agar forming large (2-3 mm in diameter), circular, low convex, colourless, opaque or partially translucent colonies after 18 hrs incubation at 37 ºC. Urinary tract infection, pyogenic infections, diarrhoea and septicaemia are four main types of clinical syndromes caused *E-coli*.

Materials and Methods
Maintenance of Pure Culture
Pure cultures of bacteria were grown on nutrient broth at 37 ºC and 28 ºC for 48 hrs and were maintained on respective agar plates at 4 ºC in refrigerator.

Experimental Design
For all experiments two replicates and control were kept. Medium was prepared by dissolving desired quantity of ingredients in distilled water. Both solid and liquid media were used for the study were sterilized by autoclaving (Shivam Company) at 121 ºC, 15 lb pressure for 5 minutes and pH of the medium was set according by digital pH meter (Elico made). Microbial inoculation was done with nicrome wire loop under aseptic condition of stericlean horizontal laminar air flow bench (Sun Instruments). Digital balance was used for weighing of materials. Glassware used for the study was sterilized by dry heat method in hot air oven (Ambassy).

Collecting and Drying of plant-materials
The leaves of selected ornamental Apocynaceae species were collected from the Gujarat University Campus, washed, Air dried under open shade, powdered and then stored in paper-bags at room tempreture. These plant powders were used for Extract preparation.

Extract preparation method
Eight conical flasks were selected and weighed leaves powder transferred into these conical flasks. Solvents Methanol and Ethanol were added (In 1:10 amount, so if 1 gm powder material is there we have to add 10 ml of Ethanol or Methanol). These processes done for all eight flasks four methanolic extracts and four ethanolic extracts. Cover the flasks with aluminium foil. These all sets were kept on shaker for 24hrs. After that, all extracts separately filtered with the help of Whatmann filter paper no.1. After filtration, transferred it into petriplates and allow it open for 24 hrs for solvent evaporation. After 24 hrs all the extracts were ready.

Preparation of Liquid Extracts Series for Anti-microbial tests
Crude extracts were weighed with the help of weighing balance (mg) and solvent were added in appropriate proportions i.e., 5 mg extract/2 ml Solvent, 10 mg/2 ml solvent, 15 mg/2 ml solvent, 20 mg/2 ml solvent. Here two solvents namely Ethanol and Methanol were used for Liquid extract preparation. Hence ethanol alone was used as control for ethanolic extract series and methanol alone was tested as control for methanolic extract series.

Antibacterial Activity
Agar Well Diffusion method for antibacterial activity was selected for study. 25-30 ml of nutrient agar media was poured in sterilized petri-plates and allowed it to solidify at room temperature. 24 hrs broth culture of test bacteria was used as inoculums under sterile conditions. The flashy prepared 100µl or 0.1 ml (1×10^6 cells/ ml) of organisms were spread with sterile L shaped bent glass-rod. Using cork-borer several wells of 6 mm in diameter were punched. 100µl of the extract was poured in each well. The plates were incubated for optimum growth conditions at 35ºC and 1 day. Inhibition zone was measured with zone scale of 1 mm or more was considered positive inhibition.

Results and Discussion
Antibacterial activity of different extracts of *Catharanthes roseus* L. against *E-coli*
Antibacterial activity of ethanolic extract
The results obtained shows that 5 mg concentration of extract results in 8.5 mm zone of inhibition, 10 mg concentration of extract results in 9.5 mm zone of inhibition, 15 mg concentration of extract results in 11 mm zone of inhibition and 20 mg concentration of extract results in 12.5 mm zone of inhibition.

Antibacterial activity of methanolic extract
The results obtained shows that 5 mg concentration of extract results in 7.5 mm zone of inhibition, 10 mg concentration of extract results in 9 mm zone of inhibition, 15 mg concentration of extract results in 11 mm zone of inhibition and 20 mg concentration of extract results in 13 mm zone of inhibition.

Table 1: Showing antibacterial activity of Ethanolic and Methanolic extracts of *Catharanthes roseus* L.

Extracts	Extract concentration (mg/2 ml)					
	Control	5 mg/2 ml	10 mg/2 ml	15 mg/2 ml	20 mg/2 ml	mm= milli-meter
Ethanol	0.0 mm	8.5 mm	9.5 mm	11.0 mm	12.5 mm	
Methanol	0.0 mm	7.5 mm	9.0 mm	11.0 mm	13.0 mm	

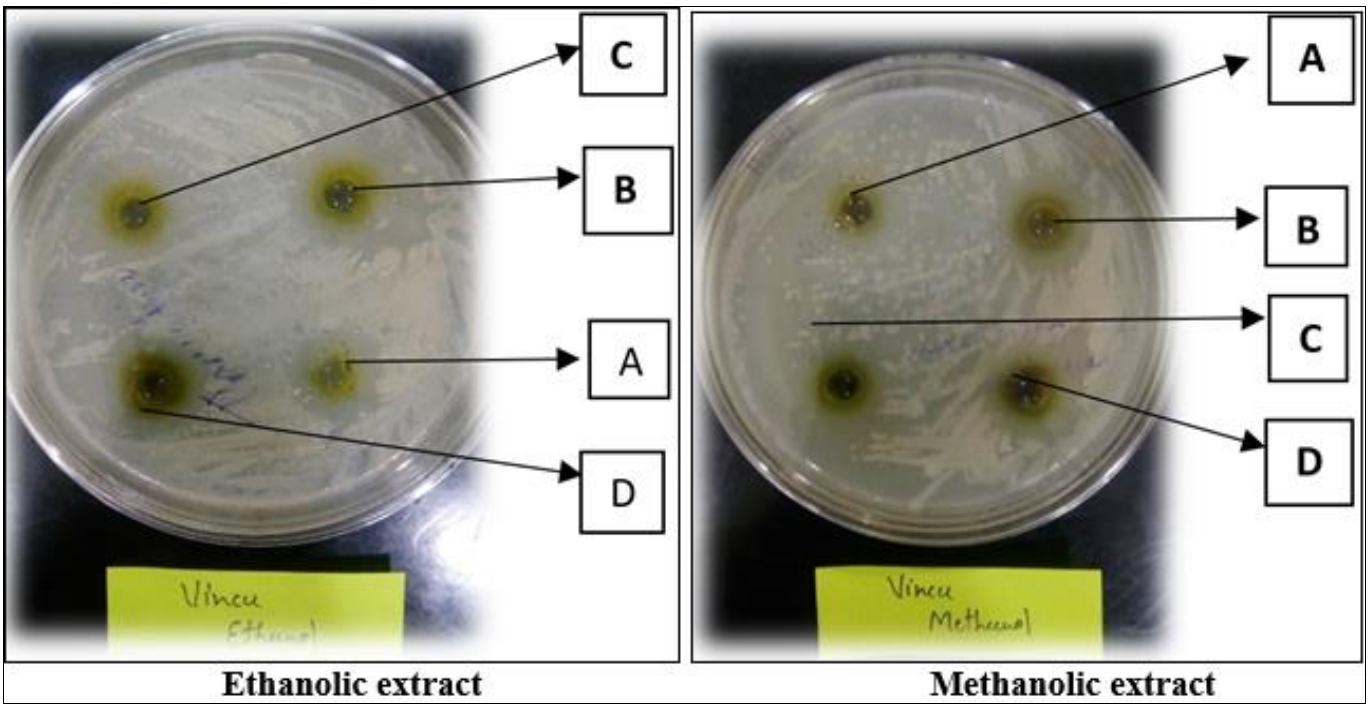

Plate 1: Antibacterial activity of *Catharanthes roseus* L. against *E-coli*

Discussion

Goyal *et al*., 2008 [5] analysed that aqueous extracts of roots, stem and flowers of *Catharanthus roseus* L. has capacity to supress the growth of certain bacterial species like *Bacillus* sp., *Staphylococcus* sp. etc. Govindaswamy and Srinivasan, 2012 [6] analysed the antibacterial activity of aqueous extracts of the different parts of the plant in which aqueous extracts of all parts showed positive results against *E- coli*, *Staphylococcus aurous*, *Pseudomonas* sp. etc. But the minimum zone of inhibition they showed of extraction against *E-coli* was found around 5.20 mm. Here the research data reviles that not only the extracts of root, stem and flowers but leaves also has antibacterial activity against *E-coli* bacterial strain and minimum zone of inhibition was about 8.00 mm against *E-coli*.

Conclusion

Catharanthus roseus L. has potent plant which has many of the pharmaceutical applications because of the presence of potent phytochemicals and antibacterial activity against different bacterial strains. In future, another plant pathogenic bacterial strains can be cross checked so, would be fruitful for the bio-pesticide preparation.

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