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Short Communication

Evaluation of the antimicrobial potential of *Catharanthus roseus* extract

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Catharanthus roseus L. (Apocyanaceae), also known as Madagascar periwinkle, is an evergreen herb with therapeutic qualities. The present study investigated the antibacterial activity of *C. roseus* leaf ethanol extract (*Cr*-LEE) against *E. coli.* The result indicated significant growth inhibitory activity of *Cr*-LEE on *E. coli*. The effects were dose-dependent. From our earlier research, GC/MS (Gas Chromatography-Mass Spectrometry) was also used to determine the significant phytoconstituents in the extract. The secondary metabolites present in the extract may individually or synergistically be responsible for the antibacterial action of *C. roseus*.

Keywords: *Catharanthus roseus*, *Escherichia coli*, Growth inhibition, Leaf extract

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Introduction

Ayurveda is a traditional medicine system in India that focuses on the medicinal potential of plants and natural products. *Catharanthus roseus* L. (Apocynaceae), also known as Madagascar periwinkle or Sadabahar, is a well-recognized herbal medicinal plant[1,2]. It is widely distributed in South Africa and Australia and grows well in the United States and Southern Europe. In India, it is available throughout the country. Due to cultivation through slashes and burns, this plant is endangered in the wild. 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[3].

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E. coli, a gram-negative bacteria, can be grown on nutrient agar or other similar media. After 18 hours of incubation at 37°C, it forms big (2–3 mm in diameter), round, low convex, colourless, opaque, or slightly translucent colonies. The bacteria are common inhabitants of the human gut. Often, it causes extraintestinal illness, urinary tract infection, abdominal and pelvic infection, pneumonia, bacteremia, etc., in humans[4]. The *E. coli* is also a suitable model organism for molecular and microbiological studies.

The extensive use of antibiotics in different sectors has raised its levels in the ecosystem, leading to antibiotic resistance[5,6]. The development of resistance to pathogens is very rapid and is appalling to the healthcare system[7,8]. The discovery of new effective antibiotics against deadly pathogens is a big challenge to the scientific community[9,10]. On the contrary, plants have a plethora of active phytoconstituents that can be utilised for antibacterial activities[11]. Therefore, this study aimed to screen the antimicrobial potential of *C. roseus* leaf ethanol extract *(Cr*-LEE) against *E. coli*.

Materials and Methods

Plant sample collection and identification

Leaves of *Catharanthus roseus* were collected in April 2023 from Deshbandhu College, Kalkaji, New Delhi, India. This plant is commonly grown in the botanical garden of the College for academic purpose. The leaves were washed, shade-dried, and ground to make a fine powder. About 40 g of this powder was taken and extracted with 400 mL of ethanol at 45°C using the Soxhlet apparatus. The extract was filtered and concentrated by Rotary Evaporator (Buchi). The extract was then dissolved in Dimethyl Sulfoxide (DMSO), and different concentrations (0.5, 1, and 2%) of plant extract were prepared for experimental purposes.

Antibacterial activity

Pure cultures of *E. coli* (DH5α) were grown on Luria–Bertani broth (LB) nutrient medium at 37ºC for 24 hours in a BOD incubator. All the inoculations were performed in a biosafety hood. For liquid culture, after inoculation in 1.5% LB broth, the tubes were kept in a shaker at 37°C and 120 rpm for 24 hours. To study the effect of *Cr*-LEE, 1 mL of

extracts of 0.5, 1, and 2.0% concentrations were added to 4 mL of LB broth, respectively. This mixture was inoculated with 20 µL of overnight-grown culture. In control, 1 mL of DMSO was added in place of extract. All the experimental tubes were kept in a shaker overnight condition. The cell growth in each tube was studied by measuring turbidity by spectrophotometry at 600 nm (Model: Cary UV-Vis). The OD (Optical Density) was converted to cell density by Agilent Genomics: Tool-BioCalculators (https://www.chem.agilent.com/store/biocalculators/calcODBacterial.jsp)[12].

For phytoconstituents present in the extract, GC/MS data were also studied from our previous studies[13].

Results and Discussion

The results presented in Table 1 indicate that *Cr*-LEE has significant growth inhibitory activities against *E. coli*. In the control tubes, significant bacterial growth was recorded after 24 hours of incubation, for which the density of *E. coli* cells was $1.90x10^9$ cells/mL. In the experimental tubes, the growth of *E. coli* was significantly less than the control. The maximum growth inhibition was observed at 2% of *Cr*-LEE with a density of *E. coli* cells $0.80x10^8$ cells/mL. In the 1 and 0.5% *Cr*-LEE treatments, the bacterial cell count was $1.57x10^9$ and $1.56x10^9$ cells/mL, respectively. This concludes that the growth inhibitory effect of *Cr*-LEE was dose-dependent. At higher concentrations, i.e. (2%), the total bacterial cell density became lesser, which shows a significant difference between control and treated samples.

Table 1 — Effect of *C. roseus* leaf ethanol extract on the growth of *E. coli* DH5α

Concentration of *Cr*-LEE	Total Bacterial cell density (Cell/mL) (Mean±SE)
Control	$1.90^{a}x10^{9}$±0.44
0.5%	1.56^{ab} $x10^{9}$±0.18
1%	1.57^{ab} $x10^{9}$±0.11
2%	0.80^{b} $x10^{8}$±0.06
F value	3.497 (3,8)
p-value	0.02

Means followed by the same letter in a column are not significantly different (P <0.05) computed by one-way ANOVA followed by Tukey's all pairwise multiple comparison test

C. roseus has also been reported by many researchers. For example, the growth inhibitory activity of aqueous extracts of *C. roseus* roots, stems, and flowers was reported against specific bacterial species, including *Staphylococcus sp*. and *Bacillus sp*[14]. Similar antibacterial effects were observed when petroleum, ether, acetone, and methanol extracts of leaves and flowers of *C. roseus* were used with higher effects in methanol extracts[15]. Similarly, the growth of *Citrobacter freundii* was highly inhibited when flowering extracts of *C. roseus* were used[16]. The *Catharanthus* plant aqueous extracts had favourable findings against bacteria such as *E. coli*, *Staphylococcus aureus*, and *Pseudomonas sp*. However, the study found that the extracts (75%) had minimal inhibition zone against *E. coli* Muller Milton agar, which was 5.20 mm[17,18], and it is coherent with the antibacterial effects of *C. roseus* as observed in the current study. Similar results were reported where the aqueous extracts of leaves showed a more potent ability to kill Gram-negative bacteria *E. coli* compared to Gram-positive bacteria *S. aureus*. Furthermore, the antibacterial ability is more pronounced in methanol as compared to the hexane extracts[19]. The methanolic leaf extract of *C. roseus* had shown a significant zone of inhibition as well when a 20 µL concentration of the extract was tested in Dulbecco's modified eagle's medium against *E. coli* and *S. aureus*[20].

The current study finds that *Cr*-LEE has antibacterial properties against the *E. coli* strain. Our previously performed GC-MS analysis showed the presence of important potential antimicrobial or antibacterial agents in *Cr*-LEE (Table 2). The GC-MS analysis of *Cr*-LEE showed the presence of many secondary metabolites such as Phytol (Fig. 1a); Ethyl oleate (Fig. 1b); Hexacosane (Fig. 1c); Tetracontane (Fig. 1d); Stigmasta-5,22-dien-3-ol (Fig. 1f); Stigmast-5-en-3-ol, (3.beta) (Fig. 1g); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Fig. 1h). These phytochemicals may individually or synergistically play a crucial role in the inhibition of bacterial growth. The results obtained from the present study indicate that further study can be conducted to isolate, purify, and characterise several other potential phytoconstituents that can be utilised as potent drugs against such pathologically important strains without the threat of resistance development.

Table 2 — GC-MS analysis of *C. roseus* leaf ethanol extract showing compounds having antimicrobial activity[28]

S. No	R/T*	Peak Area (%)	Name of the Compound	Molecular Weight	Molecular Formula	Compound Nature	Activity**	References
1	20.769	2.15	Phytol	296	$C_{20}H_{40}O$	Diterpene	Antioxidant, antimicrobial, anticancer, anti-inflammatory, and diuretic	21,22,23
2	21.357	2.29	Ethyl oleate	310	$C_{20}H_{38}O_2$	Ethanolic fatty acid oleic	Antibacterial	24
3	31.367	1.05	Hexacosane	366	$C_{26}H_{54}$	Hydrocarbon	Pesticide, insecticide, Antimicrobial	25
4	34.260	2.64	Tetracontane	562	$C_{40}H_{82}$	Long chain n-alkane	Antimicrobial, antitumor, and anticancer	26
5	38.332	1.86	Hexatriacontane	506	$C_{36}H_{74}$	n-alkanes	Antioxidant	26
6	37.917	1.11	Stigmasta-5,22-dien-3-ol	412	$C_{29}H_{48}O$	Steroidal	Antioxidant, antibacterial activity, anti-inflammatory, antiarthritic diuretic	27
7	39.350	4.81	Stigmast-5-en-3-ol, (3. beta.)	414	$C_{29}H_{50}O$	Steroidal	Antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma, diuretic, antioxidant	27
8	46.697	2.68	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial	22

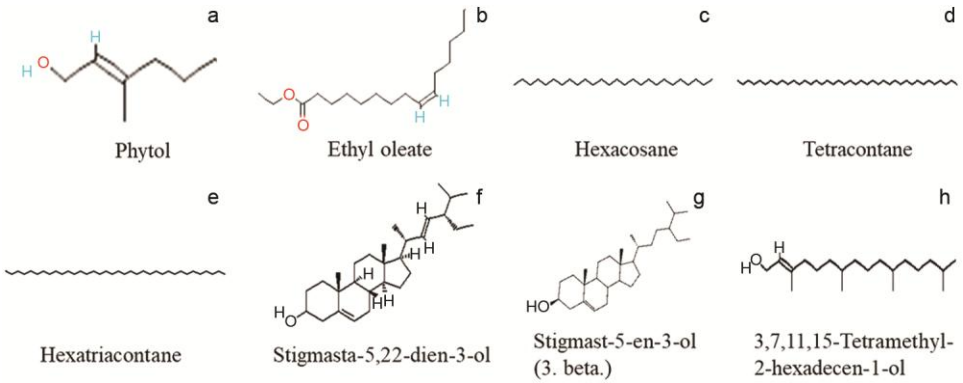

Fig. 1 — Molecular structure of compounds present in *C. roseus* leaf ethanol extract having antimicrobial activity. a) Phytol; b) Ethyl oleate; c) Hexacosane; d) Tetracontane; e) Hexatriacontane; f) Stigmasta-5,22-dien-3-ol; g) Stigmast-5-en-3-ol, (3. beta.); and h) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol.

Conclusion

A dose-dependent growth inhibition was observed in *E. coli* in response to *Cr*-LEE. The secondary metabolites such as Phytol; Ethyl oleate; Tetracontane; Stigmasta-5,22-dien-3-ol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Stigmast-5-en-3-ol, (3.beta.) has been found in the GC-MS analysis of *Cr*-LEE. These secondary metabolites individually or synergistically impacted the growth of *E. coli*. Further study can be conducted to see the impact of these

secondary metabolites against *E. coli* and other deadly pathogens.

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Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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