

## ANTIMICROBIAL POTENTIAL OF *CATHARANTHUS ROSEUS* EXTRACTS AGAINST PATHOGENIC BACTERIAL AND FUNGAL STRAINS

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**ABSTRACT:** In the present investigation, the antimicrobial activity of *Catharanthus roseus* (leaves and stem) was evaluated. The plant material was extracted through maceration method and the results were recorded against different bacterial (*Bacillus subtilis* and *Pseudomonas fluorescense*) and fungal (*Aspergillus flavus* and *Fusarium culmorum*) strains. The zones of inhibition were measured against these strains and compared with commercially available standard antimicrobial discs including ampicillin (10ug) against *B. subtilis*, sulphomethoxazole (30ug) against *P. fluorescense*, fuconazole (250mg/625ml) against *A. flavus* and Ketoconazole (30ug) against *F. culmorum*. Maximum zone of inhibition was observed for methanol leaf extract against *B. subtilis* ( $19 \pm 2.08$ )<sup>a</sup> while, the minimum for methanol stem extract against *F. culmorum* ( $5.33 \pm 0.57$ ). Standard antimicrobial discs produced maximum zones of inhibition ampicillin  $32.93 \pm 1.60$  against *B. subtilis*, sulphomethoxazole  $25.91 \pm 0.5$ , fuconazole  $33.21 \pm 0.5$  and ketoconazole  $46.55 \pm 0.5$ .

**Key words:** *Catharanthus roseus*, Antibacterial activity, Zone of inhibition, Antifungal activity, Standards.

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### INTRODUCTION

Many plants particularly the edible ones are mainly consumed for their nutritional values without much consideration given to their medicinal importance (Awoyemi *et al.*, 2012). Various efforts have focused on identifying plants and their active components against a wide array of pathogens, including viruses, bacteria, and protozoa (Monzole *et al.*, 2014). Plants have antimicrobial activity and can be used to treat various diseases. This has become the need of the hour to elaborate capacities of the plants regarding their potential in medical science (Sudhir *et al.*, 2012). The biologic therapies have proven to be highly successful and effective in majority of the diseases in the past and now a day this trend is growing day by day (Mc Alindon *et al.*, 2014). *Catharanthus roseus* is a novel plant, belongs to *Apocynaceae* which is very much famous for treatment of cancer and leukemia. Furthermore, used for treatment of diabetes mellitus, wound healing, hypertension, bleeding gums, throat and nose bleeding treatment, cystitis and antidiuretic, etc. (Gajalakshmi *et al.*, 2013).

Medicinal plants are valuable and used for the synthesis of numerous drugs. Indigenous plants are traditionally used against various ailments. Present work is based on the research conducted on methanolic crude plant extracts of three medicinal plants of family Solanaceae i.e. leaves of *Datura innoxia* Mill., *Withania somnifera* (L.) Dunal and *Solanum surrattense* were screened to investigate the biological activities i.e. antibacterial, antifungal, antioxidant, cytotoxic and

antitumor activities. Antibacterial activity was performed against *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive), *Vibrio cholera*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Agrobacterium tumefaciens*, *Escherichia coli* (Gram negative) by following the disc diffusion method. Considerable biological activity was exhibited by each methanolic extract of plants. MIC exhibited by three plants was 15 mg/ml (Mehmood *et al.*, 2012).

Sidambaram *et al.*, 2011 concluded that acetone and methanol extracts of leaves and seeds of *Solanum xanthocarpum* were subjected to antibacterial, antifungal and cytotoxic studies. The results indicated significant antibacterial activity of the extracts at 50µg/ml concentration on *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli* and *Salmonella typhi* but no inhibition of *Pseudomonas aeruginosa* and *Vibrio cholerae*. The extracts showed marked growth inhibition of *Candida albicans*, *Aspergillus niger* and *Trichophyton mentagrophytes*.

Pirbalouti *et al.*, 2011 Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments as they contain antimicrobial properties. Antifungal activities of essential oil of four Iranian herbs including, *Thymus daenensis* var *daenensis* Celak, *Zataria multiflora* Boiss, *Thymbra spicata* var. *spicata* L. and *Bunium persicum* (Boiss.) K.-Pol. were investigated against *Aspergillus niger*,

*Aspergillus fumigates*, *Aspergillus flavus* and *Aspergillus parasiticus* by agar disc diffusion assay. Some of the essential oils showed relatively antifungal activity against the tested fungal.

Keeping in view the above facts, the present study was conducted to determine the antimicrobial potential of medicinal plants found in Sialkot, Pakistan.

## MATERIALS AND METHODS

The current investigation was carried out in Ethnobotany and Taxonomy laboratory, Department of Botany, Lahore College for Women University, (LCWU) Lahore. The analysis was proposed to investigate the antimicrobial activity of *Catharanthus roseus* according to the following plan of work.

First of all, the plant material (leaves and stem) was collected from Sialkot, Punjab, Pakistan. The voucher specimen was submitted and authenticated by Plant Herbarium, LCWU, Lahore. The material was cut into small pieces, dried and ground. The extraction was done through maceration method in a series of solvents starting from petroleum ether, chloroform, methanol and ending with water.

Test organisms used during experiment were both bacterial and fungal strains including *Pseudomonas fluorescence*, *Bacillus subtilis*, *Aspergillus flavus* and *Fusarium culmorum*.

### Antimicrobial evaluation

➤ Antimicrobial evaluation was done by following the methodology of Alzorky and Nakhara 2003. Bacterial strains were cultured according to following recipe: Nutrient Broth: 4g, Agar: 7g by mixing in 500ml Double distilled water and the pH was adjusted at 7.4 under perfect sterilization conditions (121°C for 15 min with 15 lb/sq inch pressure).

➤ The fungal strains were cultured on potato dextrose agar medium by mixing 3.9g PDA in 250ml Distilled water and the pH was maintained at 5.5 (Dorman and Deans, 2000).

**Experimental design:** The whole experiment was designed on the basis of Random Complete Split Block Design (RCD) in triplicate fashion. The petri plates were prepared by Pour plate method then inoculated and the plant extracts were introduced through disc diffusion method by making a hole of 0.5mm. The whole set-up was incubated at 37°C for 24 hrs in case of bacteria whereas at 33°C for minimum 48hrs in case of fungi. A uniform hole of 0.5mm was made with the help of cork

borer No. 2. For negative control the zone of inhibition were recorded against all the extraction solvents whereas the positive control was done by using commercially available standard discs against respective strains i.e. Ampicillin 10ug (*B. subtilis*), Sulphomethoxazole 30ug (*P. fluorescence*), Fluconazole 250mg/625ml (*A. parasiticus*) and Ketoconazole 30ug (*F. culmorum*). All the measurements were recorded by using Vernier calliper

**Statistical analysis for antimicrobial activity:** Mean and standard deviation values were calculated through Microsoft excel. The statistical analysis was done by using the computer software Costat cs6204 W.exe. by applying ANOVA (Analysis of Variance) Duncan's Multiple Range Test at 5% level of significance.

## RESULTS AND DISCUSSION

Against *B. subtilis*, methanol and chloroform stem extracts showed maximum value for zone of inhibition against *B. subtilis* i.e.  $19 \pm 2.08^a$  and  $18 \pm 4.16^a$  respectively. The petroleum ether extract of stem exhibited the minimum value for zone of inhibition i.e.  $7.33 \pm 0.57^b$  against *B. subtilis*.

In case of *Pseudomonas fluorescence*, *C. roseus* chloroform stem extract showed the highest value for zone of inhibition i.e.  $10 \pm 4.04^a$  whereas the methanol extract of leaves exhibited the lowest value for zone of inhibition i.e.  $7 \pm 1^{ab}$ .

Against fungal strains, the extracts also showed the potent values. As in case of, *Aspergillus flavus* the chloroform extract of leaves and methanol extract of stem showed the maximum values for zone of inhibition i.e.  $8 \pm 1^a$  and  $8 \pm 3.46^a$  respectively. The water extract of leaves produced the lowest zone of inhibition i.e.  $5.33 \pm 0.57^b$ . As far as the *Fusarium culmorum* was concerned, the highest zone of inhibition value was shown by P. ether leaf extract i.e.  $8.33 \pm 1.52^a$  whereas the methanol extract exhibited lowest value of zone of inhibition i.e.  $5.33 \pm 0.57^b$ .

Bacterial strains were also run against all the solvents and the potential values for zone of inhibition were observed. Ampicillin B against *B. subtilis* formed zone of inhibition as  $32.93 \pm 1.60^b$ . Sulphomethoxazole against *P. fluorescence* produced zone of inhibition as  $25.91 \pm 0.5^c$  whereas Fluconazole against *Aspergillus flavus* showed zone of inhibition i.e.  $33.21 \pm 0.5^b$ . Ketoconazole against *Fusarium culmorum* formed zone of inhibition as  $46.55 \pm 0.5^a$ .

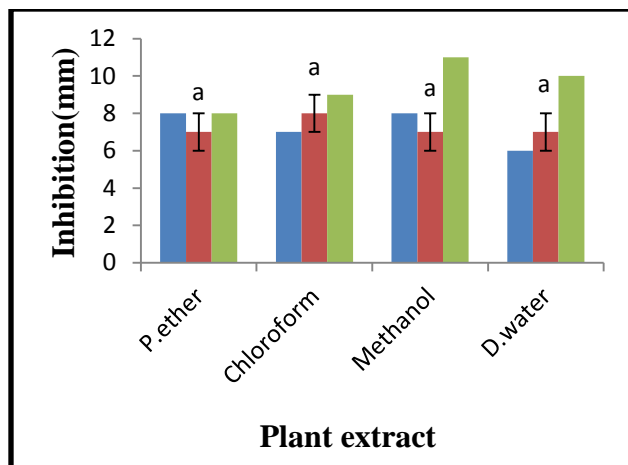


Fig 1: Inhibitory Zone (mm) produced by *C. roseus* leaf extracts against *B. subtilis*

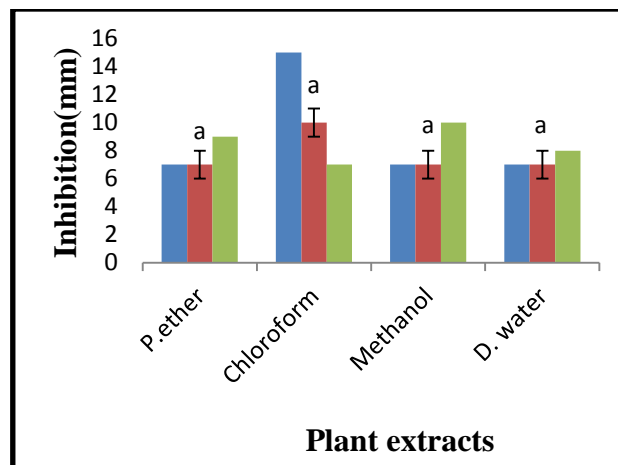


Fig 4: Inhibitory Zone (mm) produced by *C. roseus* stem extracts against *P. fluorescense*

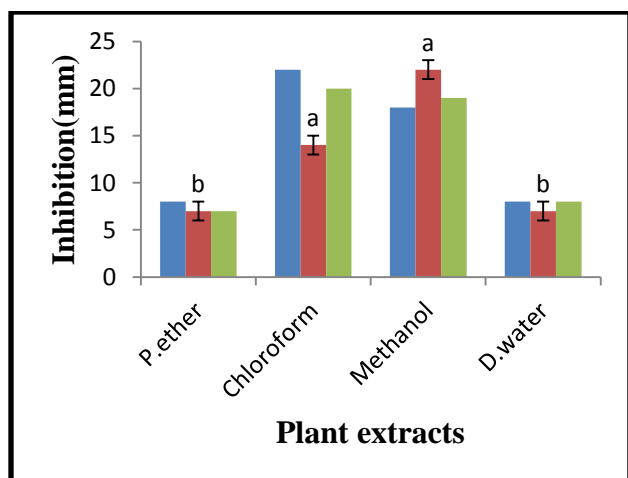


Fig 2: Inhibitory Zone (mm) produced by *C. roseus* stem extracts against *B. subtilis*.

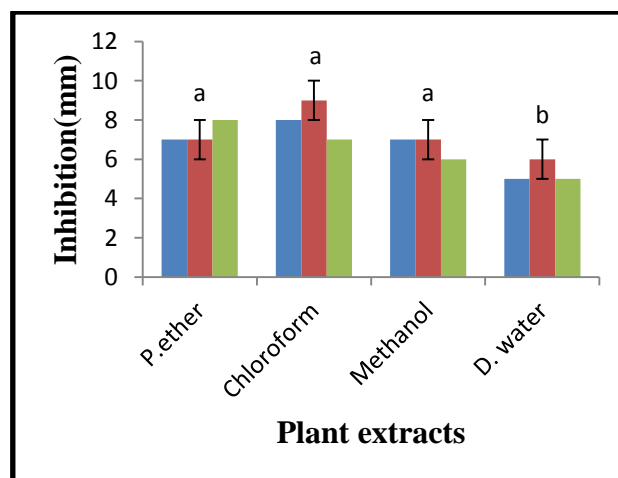


Fig 5: Inhibitory Zone (mm) produced by *C. roseus* leaf extracts against *A. flavus*

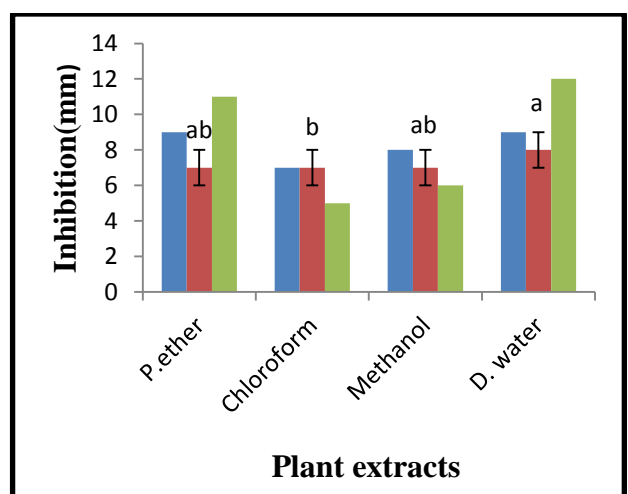


Fig 3: Inhibitory Zone (mm) produced by *C. roseus* leaf extracts against *P. fluorescense*

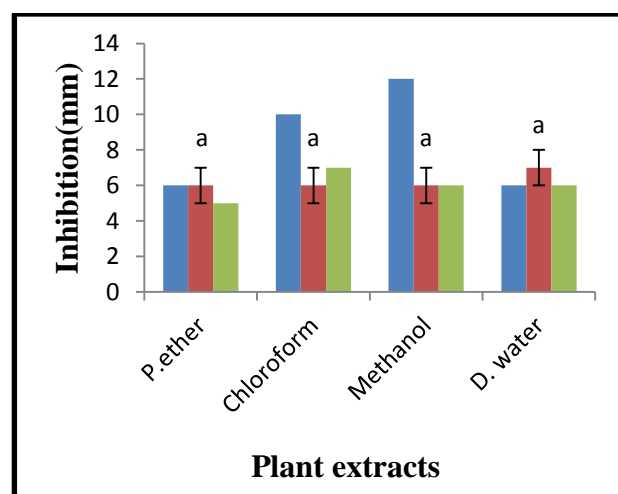


Fig 6: Inhibitory Zone (mm) produced by *C. roseus* stem extracts against *A. flavus*

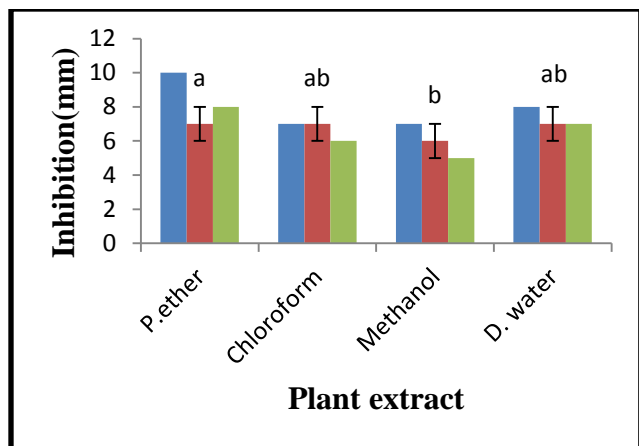


Fig 7: Inhibitory Zone (mm) produced by *C. roseus* leaf extracts against *F. culmorum*

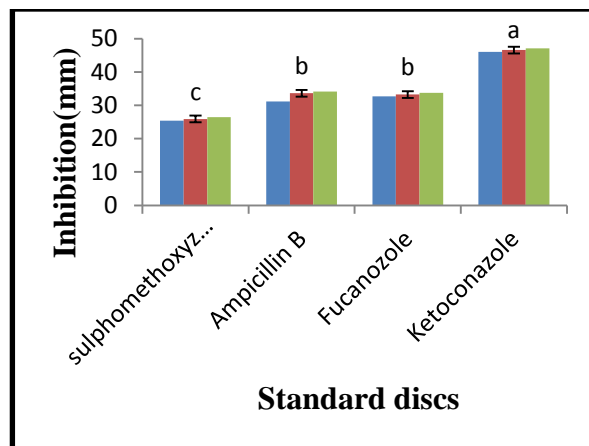


Fig 9: Positive Control (Zone of inhibition produced by standard discs against respective strains)

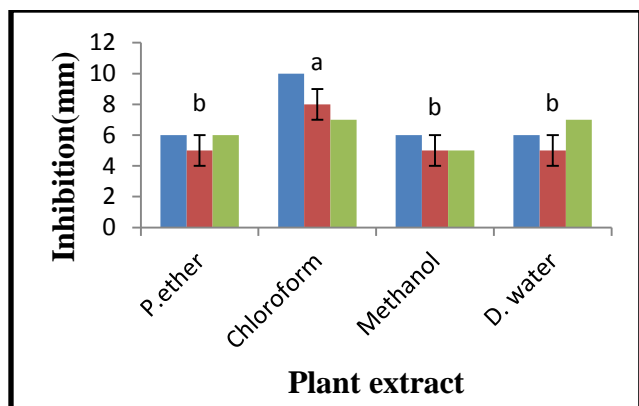


Fig 8: Inhibitory Zone (mm) produced by *C. roseus* stem extracts against *F. culmorum*

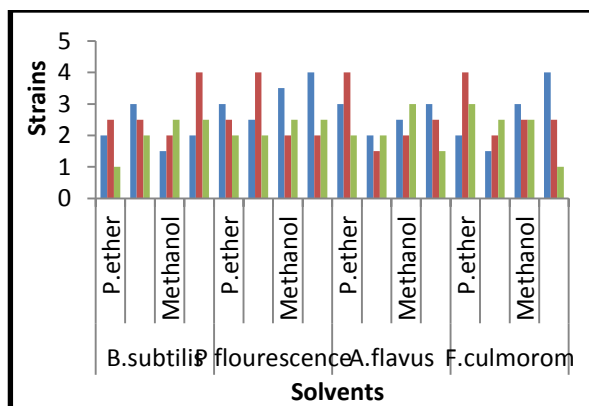


Fig 10: Negative Control (Zone of inhibition produced by solvents against bacterial and Fungal strains)



Plate 2: Zone of inhibition (mm) produced by *B. subtilis* against Ampicillin B



Plate 3: Zone of inhibition (mm) produced by *P. fluorescence* against sulpho methoxyzole



Plate 4: Zone of inhibition (mm) produced by *A. flavus* against Fuconazole

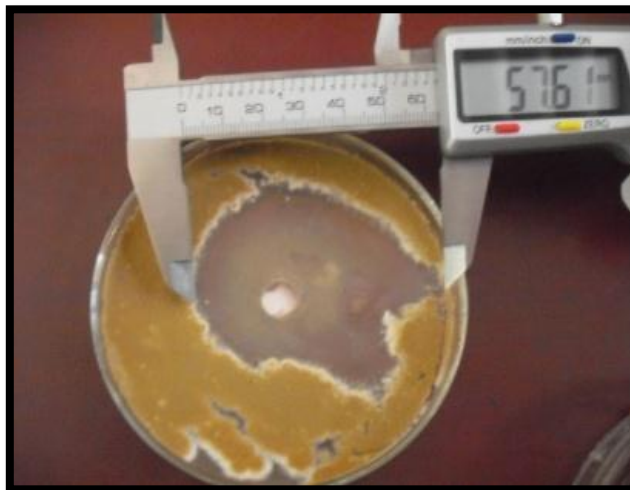


Plate 5: Zone of inhibition (mm) produced by *F. Culmorum* against Keto conazol

The results indicated that *C. roseus* was antimicrobial in nature, as it produced the zone of inhibition against bacterial and fungal strains, while some of the extracts were comparatively weaker for antimicrobial potential.

Methanolic and chloroform extracts of underground part of *C.roseus* showed maximum zone of inhibition ( $19 \pm 2.08$  and  $18 \pm 4.16$ ) respectively against *B. subtilis* among all bacterial strains. The same work was done by Govindasamy and Srinivasan (2012) on antibacterial activity of *C. roseus* against different bacterial strains and determined the same results. They observed that the methanolic extract showed the maximum zone against *S. typhi* and minimum zone of inhibition against *E.coli*. The results of present work resemble with their works. Another very similar work was conducted by Al Sieni (2013) while performing antimicrobial activity of *Salvadora persica* (miswak) and *Commiphora agileadensis* and confirmed that methanolic extract was more effective as compared to water extracts.

In a study Shalini and Sampathkumar (2012) investigated the antimicrobial activity of plant extracts for disease management. The antimicrobial activity for both methanol and aqueous plant extracts were evaluated. The antifungal activity of methanolic extracts showed maximum value for zone of inhibition against *Curvularia* sp. These results supported the results of the present investigation i.e. the methanol extract of stem showed the maximum value of zone of inhibition against *B. subtilis* i.e.  $19 \pm 2.08^a$ .

Chloroform extract of stem showed the maximum value for zone of inhibition against *P. fluorescence* i.e.  $10 \pm 4.04^a$  and the minimum value of zone of inhibition by methanol extract of leaves against same bacteria i.e.  $7 \pm 1^{ab}$ . Hassan *et al.* (2011) investigated the antimicrobial potential of crude chloroform, hexane and

ethanol extracts of leaves, stem, fruits and seeds from *Citrulluslanatus* var. *citroides* against bacterial and fungal strains and noticed the supportive results for current work.

**Conclusion:** Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria and fungi lead to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment. However, further studies are needed for further effective improvement of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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