

“Antimicrobial activity of Madagascar Periwinkle (*Catharanthus roseus* L) against pathogenic bacteria”

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ABSTRACT

Catharanthus roseus (periwinkle) is an important medicinal plant for novel pharmaceuticals since most of the bacterial pathogens are developing resistance against many of the currently available anti microbial drugs. Plants have proved to be significant natural resources for effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. The antimicrobial activity has been checked against microorganisms like *Pseudomonas aeruginosa*, *E.coli*. The *Catharanthus roseus* L. plant leaf extracts were prepared by using solvents such as acetone, ethanol and chloroform and tested against pathogenic microorganisms to determine their antimicrobial potential.

Keywords: *Catharanthus roseus* L., solvents, pathogenic microorganisms.

I. INTRODUCTION

Medicinal plant products could prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health. *Catharanthus roseus* (L.) which is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases. *C. roseus* also possess good antioxidant potential. There are about two common cultivars of *C. roseus* which is named on the basis of their flower color that is the pink flowered ‘Rosea’ and the white flowered ‘Alba’.

The plants are having capacity to produce a large number of organic chemicals so called secondary metabolites ; which are divided into different categories based on their mechanism of function like Chemotherapeutic, bacteriostatic, bactericidal and antimicrobial agent. Plants have been used in the preparation of traditional medicine for a long time and most of these folk medicines were prepared from locally grown wild plants. The other scientific name for “*Vincarosea*” is *Catharanthus roseus* .Vincristine is Vinca Alkaloids from *Catharanthus roseus*.

II. MATERIALS AND METHODS

Collection of plant

Healthy leaves of *Catharanthus roseus* were collected from Talegaon Bk, Akola district, Maharashtra, India. The freshly plant material like leaves firstly were collected. Then dried for 4 to 5 days after that the soaked leaves are grinded by grinder. Powder sample were collected. The powder sample were stored in polythene bags. This was used as a raw material for the extraction of antimicrobial compounds against the pathogenic microorganisms.

Collection of sample

Multiple urine, blood samples were collected from GMC Akola. The urine, blood, pus samples are collected are specifically from the patient suffering from various types of infection.

Collection of pathogenic microorganisms

The pathogenic microorganisms obtained are,

- E.coli*
- Pseudomonas aeruginosa*
- Klebsiella pneumonia*
- Salmonella typhi*
- Proteus vulgaris*

The clinical isolates were confirmed by using Gram staining, sugar fermentation, biochemical test and also inoculating them on selective media. The microorganisms were maintained on nutrient agar slant at 4°C.

Perform Biochemical test

To confirm that the microorganisms is *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi*, *Proteus vulgaris*, various test are performed. The results obtained by performing various biochemical tests will be cross checked with that of the results from Bergey’s Manual of Systemic Bacteriology. This will result in identification of the isolate which is being tested.

Sugar fermentation test

All the bacteria are not able to ferment sugars. Therefore sugar fermentation tests are used to identify the types of organism to be examined. Along with the ability to ferment sugar, acid production is also tested .

Extraction of plant- leaves

Solvent extraction

Take extract of leaves powder then 10 gm of these powder were dissolved in 100 ml of acetone, ethanol and chloroform. These solution were refrigerate for 48 hrs. Filter these solution through (Whatman filter paper no.1). The dried extract were dissolved in DMSO (Dimethylsulfoxide) for testing it’s antimicrobial potential.

Antimicrobial activity by well diffusion method

The MHA (Mueller Hinton Agar) media was sterilized at 121⁰ C under 15 lbs pressure for 30 mins. After cooling the media at 65⁰ C, 25 ml of it was poured in petridish. The plates are stored at

4⁰ C room temperature for solidification. Bacterial culture was spread over the MHA plates by using spreader. In the medium the wells were made by using 7mm cork borer. In DMSO the dried plant extract was dissolved to final extract of 10 mg/ml. Every wells in plate was filled with 40 µl of plant extract. In one of the plates DMSO was use as negative control. The plates were incubated along with negative control for 24 to 48 hrs at 37⁰ C. After the incubation period zone of inhibition were measured to the milimeter.

III. RESULTS AND DISCUSSION

Confirmation of the pathogenic microorganisms

The pathogenic microorganisms were confirmed E.coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi and Proteus vulgaris on the basis of Gram staining, microscopy and sugar fermentation, biochemical tests and their results are given in table

Sugar fermentation:-

| Name of Organisms | Glucose | | Lactose | | Mannitol | |
|-------------------------------|---------|-----|---------|-----|----------|-----|
| | Acid | Gas | Acid | Gas | Acid | Gas |
| <i>Escherichia coli</i> | +ve | +ve | +ve | +ve | +ve | +ve |
| <i>Klebsiella pneumonia</i> | +ve | +ve | +ve | +ve | +ve | +ve |
| <i>Pseudomonas aeruginosa</i> | +ve | -ve | +ve | -ve | +ve | -ve |
| <i>Salmonella typhi</i> | +ve | +ve | +ve | +ve | +ve | +ve |
| <i>Proteus vulgaris</i> | +ve | +ve | +ve | -ve | +ve | +ve |

Biochemical test:-

| Name of Organisms | Indole | Methyl Red | VogesProskauer | Citrate |
|------------------------|--------|------------|----------------|---------|
| Escherichia coli | +ve | +ve | -ve | -ve |
| Klebsiella pneumonia | -ve | -ve | +ve | +ve |
| Pseudomonas aeruginosa | -ve | -ve | -ve | +ve |
| Salmonella typhi | -ve | +ve | -ve | -ve |
| Proteus vulgaris | +ve | +ve | -ve | +ve |

Antimicrobial activity of Catharanthus roseus leaf extracts (mm)

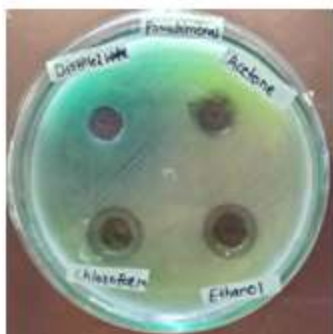
- Antimicrobial activity of Catharanthus roseus leaf extracts against pathogenic microorganisms result shows that Pseudomonas aeruginosa and E.coli was

studied under in vitro condition with different solvent extracts.

- Among the extract tested Chloroform highest and ethanol extract intermediary recorded highest antimicrobial activity against the pathogenic microorganisms.

Table:- Antimicrobial activity of solvents

| Name of Organisms | Chloroform | Ethanol | Acetone |
|-------------------------------|------------|---------|---------|
| <i>Pseudomonas aeruginosa</i> | 19 | 18 | 10 |
| <i>Escherichia coli</i> | 18 | 16 | 13 |



Pseudomonas aeruginosa



E.coli

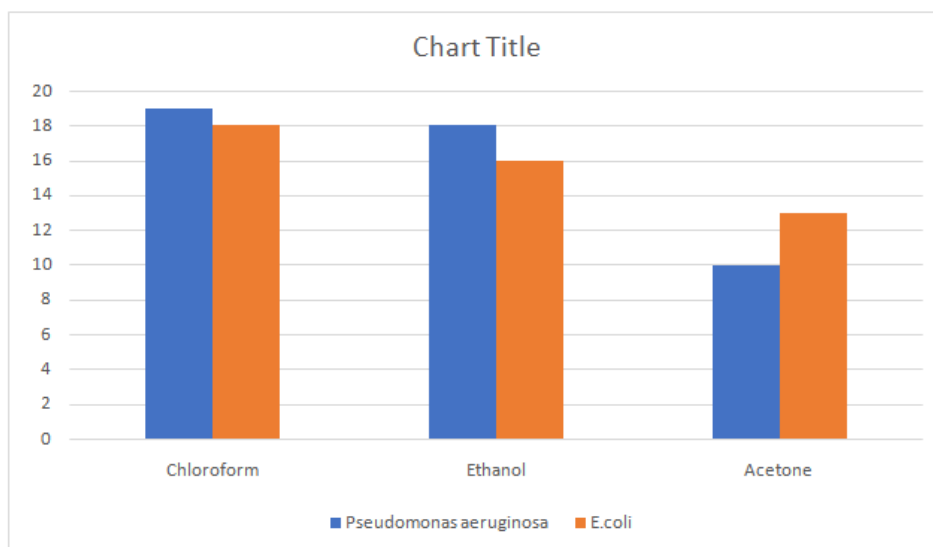
Fig:- Antimicrobial activity of Catharanthus roseus leaf extracts against two pathogenic microorganisms viz., Pseudomonas aeruginosa and E.coli

The results are described as follows :-

- The two isolates were susceptible to the solvent extracts when compared to other pathogenic microorganisms.
- Pseudomonas aeruginosa were found to be more susceptible to the plant extracts viz., Catharanthus roseus leaf extracts, since their growth was inhibited at relatively lower concentration E.coli.

IV. DISCUSSION :

The antimicrobial activity of Catharanthus roseus leaf extract was tested against two pathogenic bacteria, Pseudomonas aeruginosa and E.coli. The tested extract showed very strong antimicrobial activity against these pathogenic microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition. The strongest inhibition activity of the leaf extract was observed against Pseudomonas aeruginosa (19mm zone) at 100 mg/ml of leaf extract followed by E.coli which showed (18mm) inhibition zone at 100 mg/ml leaf extracts.



Graphical representation:- Antimicrobial activity of *Catharanthus roseus* leaf extracts

V. CONCLUSION

All the tested pathogenic microorganisms were highly effective against the *Catharanthus roseus*. If the phytoactive components are purified and adequate dosage determined for proper administration then antimicrobial activity can be enhanced. As the global scenario is now changing towards the use of nontoxic plant products heaving traditional medicinal use, development of modern drug from *Catharanthus roseus* should be emphasized for the control of pathogenic microorganisms.

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