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(Research Article)

An investigation of Antibacterial activity of *Pedilanthus tithymaloides* on Different Strains of Bacteria

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ABSTRACT

Over the past few decades, extensive research has been carried out on the evaluation of the different plants extracts for antimicrobial activities. The present study is carried out to examine the antibacterial activity of Pedilanthus tithymaloides. It is a medicinal plant, which is found to posses varied medicinal activities including anti-inflammatory, wound healing along with antioxidant property. In the present work, methanol, n-butanol, chloroform, ethereal, ethyl acetate extracts of Pedilanthus tithymaloides were selected. The in vitro antimicrobial activity of these extracts was carried out against various gram positive and gram-negative bacteria. This was carried out with the help of disc plate method. The zone of inhibition formed against these microorganisms was measured for the determination of the antibacterial efficacy of the different plant extracts. Of the selected extracts, n-butanol extracts showed significant antibacterial potential against Bacillus subtilis, Proteus mirabilis, Streptococcus pyogens, Aeromonas, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. In addition, petroleum ether extracts showed activity against Aeromonas species. The ethyl acetate and chloroform extracts showed antibacterial activity only against Klebsiella pneumonia.

Key Words: *Pedilanthus tithymaloides*, Euphorbiaceae, Antibacterial screening, Disc plate method.

INTRODUCTION

Over the past few decades, many different varieties of plants have been screened for various medicinal properties. Many of the plants have shown excellent anti-inflammatory, antioxidant, wound healing and antimicrobial properties to name a few. *Pedilanthus tithymaloides* (Family: Euphorbiaceae) is one such medicinal plant which is found to posses varied medicinal activities. including anti-inflammatory, wound healing along with antioxidant property [1, 2].

Also, there have been growing incidences of resistance of microbes to many of the synthetic antimicrobial drugs. Antibiotic resistance refers to a situation where the micro-organisms adapt itself and are able to survive the exposure of antibiotic drugs. There are genes which are responsible for this antibiotic resistance. Such genes can be transferred from one bacteria to other through various processes like transduction, conjugation or transformation. For this very reason, many pharmacognosy and botanical laboratories across the world are engaged in screening various parts of the plant for their antibacterial and antifungal properties[3, 4].

In the present work, methanol, n-butanol, chloroform, ethereal, ethyl acetate extracts of *Pedilanthus tithymaloides* were selected for performing *in vitro* antimicrobial activity against various gram positive and gram negative bacteria.

MATERIAL AND METHODS

Collection of Plant Material

The leaves of *Pedilanthus tithymaloides* were collected from, Thane district, Maharashtra (India) during December, 2009.The herbarium specimen of the plant is preserved in Dr.L.H.Hiranandani College of Pharmacy,Ulhasnagar. Collected leaves were washed with sterile water to remove any dirt or filthy particles present on the surface. They were then further subjected to rinsing with distilled water for five times and were then subsequently air dried.

Preparation of the Extracts

The dried leaves were powdered using a mortar pestle and passed through 40# mesh. 10g of powdered sample was weighed and mixed with 50 ml of different solvents (methanol, n-butanol, chloroform, ethereal, ethyl acetate) for 72 hrs. in a 100 ml conical flask. Whatman No. 1 filter papers were utilized for the filtration process of the above prepared mixtures. The filtrate was then collected in a separate beaker. The extract was obtained on evaporating the solvents. The extracts obtained were used at a concentration of 50mg/ml and were subjected to antibacterial evaluation [5].

Maintainance of Culture

The stock cultures of *Bacillus subtilis, Streptococcus pyogens, Aeromonas, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* were procured from Dr. L. H. Hiranadani Hospital, Powai, Mumbai. The stock culture was then subcultured in nutrient media 24 hrs. prior to the experiment and was used for the bioassay. The slants preparation was carried out from the pure cultures, which were subjected to storage in refrigerator at 4^0C for further use.

Inoculation

Nutrient agar media was selected as the nutrient medium and was subsequently prepared. Autoclaving technique was chosen for the sterilisation of the prepared nutrient agar media for 30 minutes. 10 ml of the nutrient agar media was transferred into each of the sterilised petriplates. After the media solidified, they were inoculated with 200µl of *Proteus mirabilis, Bacillus subtilis, Streptococcus pyogens, Aeromonas, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* aseptically. They were spread uniformly over the gelled agar plated with the help of the cotton swabs.

Disc Diffusion Method

Disc diffusion method / Petri plate method was selected as the method for evaluation of the antibacterial potential of the leaves of *Pedilanthus tithymaloides*. The discs of Whatmann® paper were dipped in the different extract solutions and were utilised for the antibacterial bioassay. The negative discs dipped in respective solvents were also taken into account. Standard antibiotic discs were used as a positive control. The discs of the extracts and the standard antibiotic were placed carefully with adequate spacing between each other. These petri plates were incubated at 37^0c for 24 hrs. The annular radius of the zone of inhibition was measured using a metric ruler, recorded and the antibacterial activity was compared with the standard antibiotic disc [6,7].

RESULTS AND DISCUSSION

In the current work, antibacterial activity of *Pedilanthus tithymaloides* was evaluated against *Proteus mirabilis, Bacillus subtilis, Streptococcus pyogens, Aeromonas, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* by disc diffusion method. The methanol, n-butanol, chloroform, ethereal, ethyl acetate extracts of the leaves of *Pedilanthus tithymaloides* were prepared and utilized for the antimicrobial activity determination. The gelled agar plate swabbed with microbes showed a zone of inhibition, which demonstrated the antibacterial activity of the plant. In the study, Ciprofloxacin was used as a positive control while the respective solvents were used as a negative control.

The observations of the experiment are indicated in the table 1. Of the selected extracts, n-butanol extracts showed significant antibacterial potential against *Bacillus subtilis, Proteus mirabilis, Streptococcus pyogens, Aeromonas, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*. In addition, petroleum ether extracts showed activity against *Aeromonas* species. The ethyl acetate and chloroform extracts showed antibacterial activity only against *Klebsiella pneumonia*

Table 1: Antimicrobial Activity of the plant extracts against test bacteria

Micro-organism	Zone of Inhibition (mm) in different solvent ectracts				
	Chloroform	Petroleum Ether	Methanol	n-Butanol	Ethly Acetate
Aeromonas	ND	8	ND	10	ND
Bacillus subtilis	ND	ND	ND	8	ND
Escherichia coli	ND	ND	10	8	ND
Klebsiella pneumoniae	10	ND	8	8	7
Pseudomonas aeruginosa	ND	ND	7	9	ND
Proteus mirabilis	ND	ND	ND	8	ND
Staphylococcus aureus	ND	ND	ND	10	ND
Streptococcus pyogens	ND	ND	ND	8	ND

ND: Antibacterial activity not detected
Zone of inhibition includes the diameter of disc (06mm)

CONCLUSION

The crude extracts of the leaves of *Pedilanthus tithymaloides* showed good antimicrobial potential against various microorganisms under study.Further research needs to be carried out for the identification of specific phytochemical constituent reponsible for its antimicrobial potential.

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