

ANTIBACTERIAL ACTIVITIES OF METHANOLIC EXTRACTS OF AERIAL PARTS OF *Delphinium uncinatum* Hook's and Thoms

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Abstract

Methanolic extracts of aerial parts of *Delphinium uncinatum* Hook's and Thoms were analysed for its antibacterial activities aligned to two gram positive bacterial strains, *Bacillus cereus* and *Staphylococcus aureus* and two gram negative bacterial strains, *Escherichia coli* and *Klebsilla pneumonia*, using Agar Well Diffusion method. Bactericidal activity was observed against both gram negative and gram positive bacterial strains. Extracts used were more active against *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* at all prepared concentrations but lesser against *Klebsilla pneumonia*.

Key words: *Delphinium uncinatum*, Aerial part, Methanolic extract, Antibacterial.

Introduction

In various countries of the world, heady and prevailing drugs obtained from medicinal plants are symbolised as affluent antimicrobial agents (Srivastava et al., 1996). Extracts obtained from roots, flowers/exudates, fruits and stems of plants are used as raw drugs for possessing medicinal values. Traditionally, these raw drugs are collected by community in smaller quantity for local use, while herbal industries are utilising them on commercial scale (Uniyal et al., 2006).

Recently, antibiotic resistance is a hot issue globally as infectious diseases are the main cause of mortality, particularly, in developing countries. Testing and development of medicinal products from natural source can be utilised for multiple curative purposes (Korosechviz and Howe-Grant, 1992). Still a large area has been unexplored in higher plants and very low percentage has been subjected to photochemical analysis and pharmacological selection. For development of drugs, screening and development of new organic compounds and different active constituents from plants is need of the day.

In the current study *Delphinium uncinatum* Hook's and Thoms methanolic extracts were examined for their antimicrobial activities, by following Agar Well Diffusion method.

Materials and Methods

D. uncinatum plant was collected and identification was done at Botany Department, University of Malakand, Chakdara, Dir. The collected plant was first washed with tap water and then with de-ionised water. Leaves and stem were separated and shade dried. The shade dried leaves

and stem were chopped and then powdered with electrical grinder. Powdered material (100gm) was added to 90% methanol in a plastic can. It was kept for one week with constant stirring. Then it was filtered and the filtrate was dried, using rotary evaporator. The crude extract obtained was air dried and shifted to an air tight glass container.

The average viable count per ml of stock cultures having *E. coli*, *K. pneumonia*, *B. cereus* and *S. aureus* was carried out using surface viable count technique. Aliquots of approximately 10^8 - 10^9 colony-forming unit (CFU) per ml were used and each time fresh stock suspension was made (Sofowara, 1993).

Using Agar Well Diffusion method (Sofowara, 1993) extracts were evaluated for antimicrobial potentials. Calibrated bacterial inoculums (10^8 - 10^9 CFU/ml) were mixed with 60ml sterile nutrient agar maintained at 55° C and then transferred aseptically to sterilised petri plates that were left for a while to solidify in laminar flow hood. Next, using a sterile cork borer approximately 7 mm wells were punched equidistantly from each other. Using sterile micropipette, wells were filled to top with 10 mg/ml, 50 mg/ml and 100mg/ml strength extracts of stem and leaves. Plates were kept in refrigerator for 45 minutes in order to allow the extracts to diffuse in seeded nutrient agar plates. The plates were incubated at 37° C overnight in upright position. Controls included standard antibiotics as well as the solvent used for extraction. The tests were done in triplicate and average zones of inhibitions were measured in millimeters (Chouhan et al., 2002).

Results and Discussion

Antibacterial activity of the extract against gram positive bacteria

Average diameters of zone of inhibition of 8.66, 7.00 and 7.00mm were observed for 10, 50 and 100 mg/ml solutions respectively. The zone of inhibitions for positive control, ciprofloxacin were 53.33, 50.32 and 50.32 mm at concentration of 10, 50 and 100 mg/ml, respectively, against *S.auerus*.

Table 1. Antibacterial activity of methanolic extract of *Delphinium uncinatum* against Gram positive bacteria *Staphylococcus aureus*.

<i>Delphinium uncinatum</i> Hook. Methanolic extract		Ciprofloxacin	
Concentration of plant extract (mg/ml)	Diameter of zone of inhibition (mm) (Mean \pm SD*)	Concentration of ciprofloxacin (mg/ml)	Diameter of zone of inhibition (mm) (Mean \pm SD*)
10	8.667 \pm 1.282	10	53.333 \pm 57.735
50	7.000 \pm 1.732	50	50.320 \pm 40.000
100	7.000 \pm 4.359	100	50.320 \pm 40.000

*Standard Deviation.

Table 2. Antibacterial activity of ethanolic extract of *Delphinium uncinatum* against gram positive bacteria *Bacillus cereus*.

<i>Delphinium uncinatum</i> (Methanolic extract)		Ciprofloxacin	
Concentration of plant extract (mg/ml)	Diameter of zone of inhibition (mm) (Mean \pm SD*)	Concentration of ciprofloxacin (mg/ml)	Diameter of zone of inhibition (mm) (Mean \pm SD*)
10	6.667 \pm 3.512	10	20.000 \pm 0.000
50	3.667 \pm 3.500	50	25.000 \pm 1.000
100	4.667 \pm 1.285	100	27.130 \pm 2.900

*Standard Deviation.

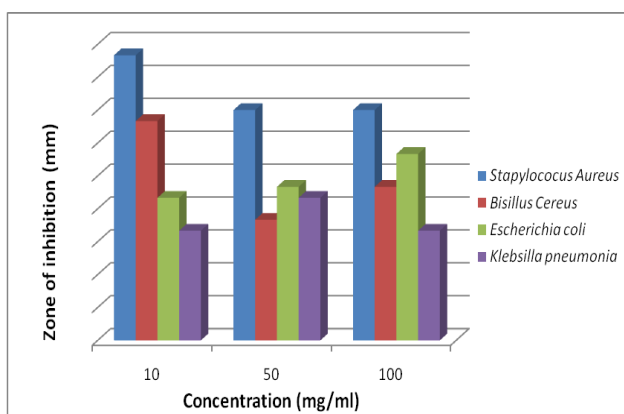


Fig. 1. Graphical representation of *Delphinium uncinatum* against bacterial strains.

Antibacterial activity of the extract against Gram negative bacteria

The crude extract inhibited *E. coli* with the inhibition rate of 4.33, 4.66 and 5.66 mm at 10, 50 and 100 mg/ml concentrations, respectively, in comparison to Ciprofloxacin (positive control) that has activities of (56.66, 50.9 and 40.51 mm). The data is tabulated (Table 3) as mean \pm standard deviation of three recordings. It is evident from the

For *B. cereus* inhibition obtained was 6.66, 3.66 and 4.66 mm respectively, in comparison to positive control, i.e., 20.0, 25.0 and 27.13 mm, respectively, at similar test concentrations. Increase in concentration resulted in increase in activities, apparent from the widening of zones of inhibitions. The results are expressed as mean of three recordings and standard deviation (Tables 1 and 2) as well as graphically represented in Figs. 1 and 2.

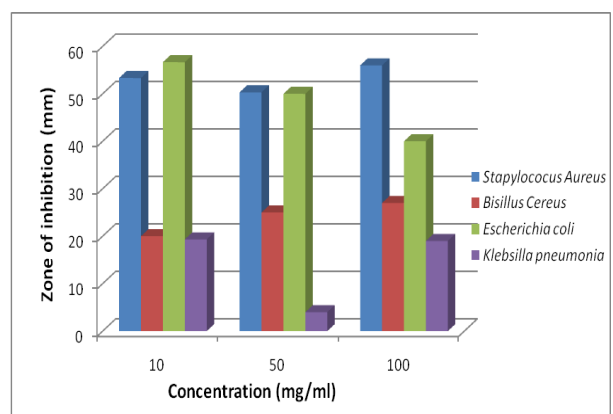


Fig. 2. Graphical representation of ciprofloxacin against bacterial strains.

results that the dose increase does not significantly affect activity. However, *K. pneumnonia* zones remained to be 3.33, 4.33 and 3.33mm at 10, 50 and 100 mg/ml concentration, respectively, in comparison to standard antibiotic that had 19.33, 4.40 and 19.44mm zone of inhibition. The results are tabulated in Table 4 and represented graphically in Figs. 1 and 2, which show that an increase in the dose has no significant impact on the efficacy of the extract.

Our findings correlate with Mahesh and Satish (2008), who stated that the methanol extracts of leaves of *Acacia nilotica* showed significant antibacterial activity against, *E. coli* (15 mm zones of inhibition). These findings are also in correlation with Abalaka et al. (2010) who stated that *E. coli* was the most susceptible to ethanolic extracts of leaves of *Ziziphus mauritiana* L. and *Ziziphus*

spinachristi L. Activities against *B. cereus* at all used concentrations were also observed.

All concentrations of extracts used showed activity against tested strains of bacteria. All tested samples showed activity against used strains that was comparable with the control Ciprofloxacin.

Table 3. Antibacterial activity of methanolic extract of *Delphinium uncinatum* against Gram negative bacteria *Escherichia coli*.

<i>Delphinium uncinatum</i> Hook. Methanolic extract		Ciprofloxacin	
Concentration of plant extract (mg/ml)	Diameter of zone of inhibition (mm) (Mean ± SD*)	Concentration of ciprofloxacin (mg/ml)	Diameter of zone of inhibition (mm) (Mean ± SD*)
10	4.333 ± 1.155	10	56.667 ± 54.857
50	4.667 ± 2.517	50	50.900 ± 34.001
100	5.667 ± 3.055	100	40.513 ± 14.002

*Standard Deviation.

Table 4. Antibacterial activity of methanolic extract of *Delphinium uncinatum* against Gram Negative bacteria *Klebsillapneumonia*.

<i>Delphinium uncinatum</i> (methanolic extract)		Ciprofloxacin	
Concentration of plant extract (mg/ml)	Diameter of zone of inhibition (mm) (Mean ± SD*)	Concentration of ciprofloxacin (mg/ml)	Diameter of zone of inhibition (mm) (Mean ± SD*)
10	3.333 ± 1.528	10	19.333 ± 1.155
50	4.333 ± 2.517	50	4.104 ± 0.000
100	3.33 ± 1.155	100	19.440 ± 1.560

*Standard Deviation.

Conclusions

From the current study, we conclude that the tested plant has medicinal importance, especially, against Gram positive bacteria. Furthermore, this study also confirms the classical application and medicinal value of *D. uncinatum* against infections caused by various micro-organisms. Finally, it can be concluded that the tested plant can be used as a good source against Gram positive bacteria, like, *S. aureus*.

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