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ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF BARK OF *PRUNUS CORNUTA*

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ABSTRACT: *Prunus cornuta* (family Rosaceae) commonly known as 'Himalayan Bird Cherry', is a deciduous tree growing to 15 m (49ft 3in) at elevations of 2100-3500 meters. It is hardy to zone (Uttarakhand) 5. It is in flower in May, and the seeds ripen from July to October. The flowers are hermaphrodite and are pollinated by insects and birds. It can grow in semi-shade (light woodland) or no shade. It prefers moist soil. Plant extract (sample named PC-1) of *Prunus cornuta* bark was prepared in methanol and the prepared extract was subjected to Phytochemical screening which shows the presence of steroids, terpenoids, alkaloids, tannin, saponin, carbohydrate, protein etc. The methanolic extract (sample named PC-1) of powdered bark of *Prunus cornuta* were examined for their antimicrobial activity using agar well diffusion method against different pathogenic bacterial stains like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*. Thus minimum inhibitory concentration (MIC) of resulting extract were also checked. The sensitivity of the pathogens was also checked with standard antibiotics erythromycin, Fluconazole, and N-saline. The present study was intended to evaluate the qualitative analysis of *P. cornuta* methanolic extract and also to examine their antimicrobial activity against different pathogenic bacteria.

INTRODUCTION: The Genus *Prunus* belongs to the family Rosaceae and consists of about 430 species of deciduous, evergreen trees and shrubs growing mainly in the temperate regions of the Northern hemisphere. About nineteen *Prunus* species grow wildly in India in the Himalayan regions, the majority of these species are of considerable horticultural importance^{1, 2}. Quite a few of species find applications for their medicinal values³.

Prunus species have been reported as an antipyretic, a refrigerant, a thirst quencher and for the treatment of Leprosy and Leucoderma⁴. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are as a source of many potent and powerful drugs⁵. Antimicrobial agents disrupt microbial process that differ from those of the host. They may damage pathogens and they are widely employed to cure bacterial diseases. Antimicrobial agents that reversibly inhibit growth of bacteria are called bacteriostatic whereas those with irreversible lethal action on bacteria are called as bactericidal^{6, 7}.

Prunus cornuta (or Himalayan Bird Cherry) belong to a family Rosaceae is a deciduous medium-sized tree with grey-brown to brown bark.

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Leaves are oblong to lance-shaped, 8 - 15 cm long, long-pointed, with finely toothed margin. Small white flowers are borne in long drooping clusters, 10 - 15 cm long. Flowers are up to 1cm across, with round petals and blunt sepals. Fruits are round cherries, about 8 mm, in a long raceme, initially red, maturing to dark purple and black.

The fruit often gets infected by an Insect, and becomes long and horn-like, from which comes its species name *cornuta*, meaning horn-like. The fruit is edible, and the leaves are used for fodder.

Himalayan Bird Cherry is found in the Himalayas, at altitudes of 2100 - 3500 meters. Flowering: April-June. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity from *P.cornuta*. *Prunus* species have been reported as antipyretic, refrigerant, useful for thirst, leprosy, and leucoderma.

MATERIALS AND METHODS:

Collection of Plant Materials: The bark of *P.cornuta* was collected from Lata Village of Joshimath block, District Chamoli, Uttarakhand. The collected bark of *P.cornuta* was washed with distill water and then dried under shade. The air-dried bark was pulverized into powdered form by using heavy duty blander.

Preparation of Extract: The powdered sample was extracted with methanol solvent by using Soxhlet extractor for 72 hrs. After complete extraction, the methanol was evaporated through the help of Rotatory Evaporator under reduced pressure to obtain a methanolic crude extract. The obtained crude extract was examined for qualitative analysis and antimicrobial activity.

Antimicrobial activity: Extracts from *P.cornuta* (bark) showed a moderate activity against some selected bacteria and the methanolic extract showed good activity against some studied bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida albicans*).

Agar-Well diffusion method and paper disc diffusion methods were employed to determine the antimicrobial activities for methanolic extracts^{8,9}.

In vitro Antimicrobial Activity Studies:

- **Determination of antimicrobial activity:**
- **Culture Media:** For antibacterial test, Soyabean Casein Digest agar/broth and Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India were used for antifungal test.
- **Inoculum:** The bacteria were inoculated into Soyabean Casein Digest broth and incubated at 37 °C for 18 h and suspension was checked to provide approximately, 10⁵ CFU/ml. The same procedure was done for fungal strains and there strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48 - 72 h.
- **Microorganisms Used:** The pure cultures of test organisms viz. *Pseudomonas aeruginosa* ATCC 25619, *Staphylococcus aureus* (Local Isolated Culture), *Candida albicans* (Local Isolated Culture) and *Aspergillus niger* (Local Isolated Culture) were obtained from National Centre of Fungal Taxonomy (NCFT), New Delhi, India.
- **Determination of Antimicrobial Activity by Well Diffusion Method:** The agar well diffusion method (Perez et al., 1993) was modified. Soyabean Casein Digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with extracts (200 µg/ml), positive controls and solvent blanks. Conventional antibiotic viz. Erythromycin (1 mg/ml) was used as standard positive control and antibacterial agent and Flucanazole (1 mg/ml) was used as standard positive control and antifungal agent. The plates were then incubated at 37 °C for 18 h for determination of antibacterial activity and 28 °C for 48 h-72 h for determination of antifungal

activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism^{10,11}.

➤ **Determination of Minimum Inhibitory Concentration (MIC):** MIC value of potent extracts was determined by the method adopted by Vollekova *et al.*, 2001 and Usman *et al.*, 2007, with some modifications. The potent extracts were prepared in highest concentration (200 µg/ml) in sterile distilled water and is serially diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial/fungal suspension was added to different test tubes and incubated for 48 h. The inhibition of turbidity

appeared in the minimum dose at which total growth of bacteria/fungus gets killed is known as minimum lethal concentration (MLC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria/fungus is known as Minimum inhibitory concentration (MIC)¹².

RESULTS AND DISCUSSION: The results obtained in the present study showed that methanolic extract of bark of *P.cornuta* had significant antimicrobial activity against different pathogenic gram positive and gram negative bacteria. Zone of inhibition and agar well diffusion method determined the antimicrobial activity of extract.



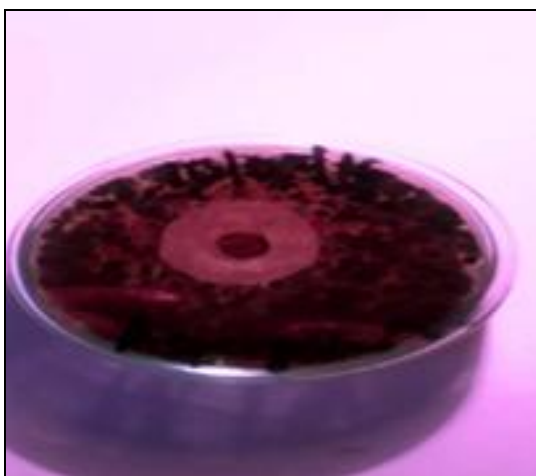
Antibacterial activity of PC 1 against *pseudomonas aeruginosa*



Antibacterial activity of PC 1 against *Staphylococcus aureus*



Antifungal activity of PC 1 against *Candida albicans*



Antifungal activity of PC 1 against *Aspergillus niger*

FIG. 1: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY AGAINST DIFFERENT PATHOGENIC BACTERIA

TABLE 1: ANTIMICROBIAL ACTIVITY OF EXTRACTS AGAINST PATHOGENS

Extracts/Compounds/Controls	Diameter of zone of inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
PC1	27.0	10.0	30.0	11.0
Erythromycin (1 mg/ml)	35.0	45.0	NT	NT
Flucanazole (1 mg/ml)	NT	NT	26.0	28.0
N-saline	NA	NA	NA	NA

*NA, No activity; NT, Not tested

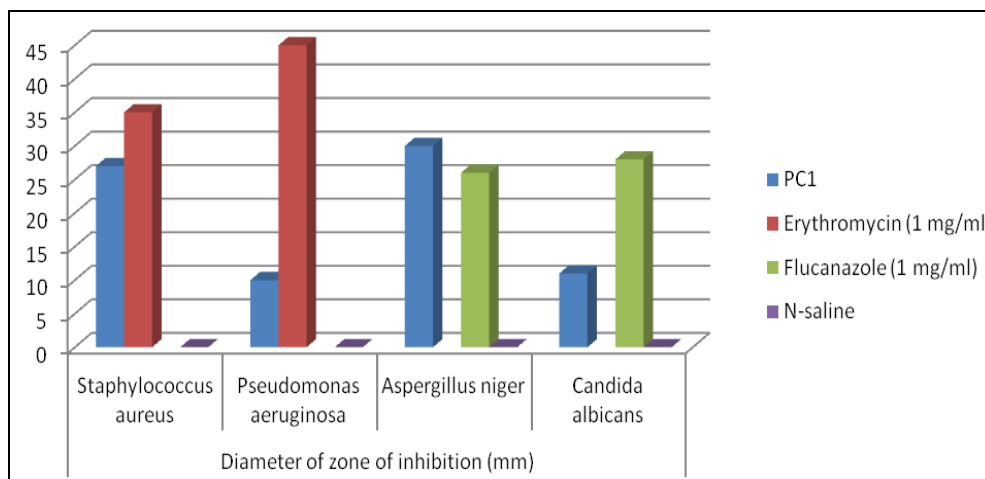


FIG. 2: ANTIMICROBIAL ACTIVITY OF EXTRACTS AGAINST PATHOGENS

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM LETHAL CONCENTRATION (MLC) OF THE EXTRACT/COMPOUND

Extracts/Compounds/Controls	MIC & MLC (µg/ml)							
	<i>Staphylococcus aureus</i>		<i>Pseudomonas aeruginosa</i>		<i>Aspergillus niger</i>		<i>Candida albicans</i>	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
PC1 (200 µg/ml)	50	75	100	150	35	50	100	150
Erythromycin (1 mg/ml)	12	15	08	10	NT	NT	NT	NT
Flucanazole (1 mg/ml)		NT		NT	15	20	13	16
N-saline	NA	NA	NA	NA	NA	NA	NA	NA

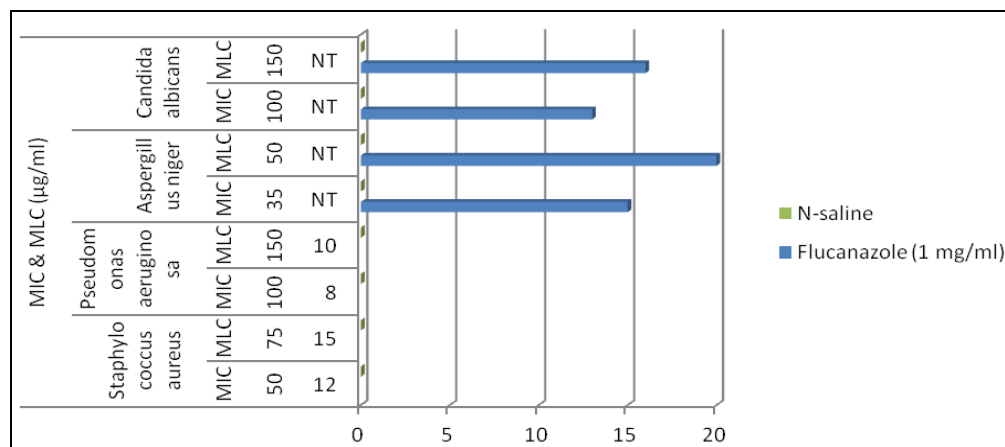


FIG. 3: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM LETHAL CONCENTRATION (MLC) OF THE EXTRACT/COMPOUND

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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