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SCREENING OF ANTIBACTERIAL ACTIVITY OF *NEPETA CILIARIS* BENTH. AGAINST RESPIRATORY TRACT PATHOGENS

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ABSTRACT
The aim of present study was to evaluate the antibacterial potential of various extracts (petroleum ether, acetone, methanol and aqueous) of *Nepeta ciliaris* against selected respiratory tract pathogens. The extracts from the aerial parts of *N. ciliaris* at concentration of 200 mg/ml were screened against three gram-positive (*Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655 and *Streptococcus pyogenes* MTCC 442) and one gram-negative (*Pseudomonas aeruginosa* MTCC 2474) bacterial pathogens. The agar well diffusion method was adopted to examine antibacterial and minimum inhibitory concentration (MIC) values of most effective extracts against the susceptible bacteria. Erythromycin was used as positive control to determine the sensitivity of the strains. Out of the four bacterial species tested, *S. pneumoniae* was the most susceptible. The acetone extract exhibited maximum activity against all the tested microorganisms while methanol extract showed activity against *P. aeruginosa.* The MIC values ranged from 40 to 50 mg/ml for all the organisms. The *N. ciliaris* is potentially a good source of antimicrobial agents.

Keywords: Antibacterial activity, antimicrobial agents, minimum inhibitory concentration, respiratory tract pathogens

INTRODUCTION
Comparative study between antibiotics and pathogen resistance concluded that antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. But, overuse of antibiotics has become the major factor for the emergence of serious infections due to multi-drug resistant (MDR) strains poses a treatment challenge [1]. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents [2]. Research and scientific data supports the antimicrobial activity of extracts and biologically active compounds isolated from medicinal plants [3].

Most respiratory tract infections are caused by viral and bacterial pathogens responsible for higher morbidity and mortality [4]. Respiratory diseases including allergies, asthma and chronic obstructive pulmonary disease (COPD) are a major public health burden worldwide. The leading causes of noncommunicable disease deaths in 2008 due to respiratory diseases were 3.9% and 4.2 million deaths were reported due to asthma and COPD globally [5].

The plant *Nepeta ciliaris* Benth. used in this study, belongs to the family Lamiaceae (Labiatae) the mint family and the genus *Nepeta* comprises about 250 species. Locally, plant is known as Nueet [6] and Jufa yabis (Punjabi) [7]. The plant is subshrub, perennial, around 40-70 cm tall. *N. ciliaris* used for preparation of joshandah, extensively used by the masses in India for treatment of common cold, catarrh, cough and associated respiratory distress and fever [8]. This study looks into the *in vitro* antibacterial activity of *N. ciliaris* against four respiratory pathogens that usually cause upper and lower respiratory tract infections.

MATERIALS AND METHODS

Plant material

The plant material was collected in October, 2011 from China hill, Nainital, Uttarakhand. The China hill is lying between 29°24'03.15" N and 79°26'32.89" E. The plant was authenticated at Botanical Survey of India, Northern Regional Center, Dehradun where a herbarium voucher specimen (Acc. No. 113419) was deposited. The whole plant (aerial parts) is included in this study. Collected plant material was washed in fresh water and dried under shade at room temperature. The plant was crushed to small pieces using pestle and motar and powdered in an electric grinder.

Preparation of extract

The plant extracts were prepared by immersing 200 gm of dried powdered material in 600 ml of solvents i.e. petroleum ether, acetone, methanol and water using the Soxhlet apparatus. Crude extracts were obtained by removing the solvent in vacuum evaporator at 30°C and stored in sterile bottles at 4°C until further use. The extracts were dissolved in the same solvent with which it has been extracted (petroleum ether, acetone, methanol and water) to a final concentration of 200 mg/ml for agar well diffusion method.

Microorganisms tested

The bacterial strains of respiratory infection bacteria used in this study were *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655 and *Streptococcus pyogenes* MTCC 442. All the bacterial strains were grown and maintained on nutrient agar slants at 4°C.

Antibacterial testing

The antibacterial activity of different extracts was determined by agar well-diffusion method according to Ahmed et al [9]. 0.1 ml of 12-16 hrs incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. Selected solvents (i.e. petroleum ether, acetone, methanol and water) were used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 hrs in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimetre (mm) as observed from the clear zones surrounding the wells.

Determination of minimum inhibitory concentrations (MICs)

The MIC of most effective extract (acetone) was performed by agar well diffusion method for all selected test pathogens (Table 2). Concentration of 200, 100, 75, 50, 40, 30, 25 and 20 mg/ml of the crude acetone extract were prepared separately and dissolved in 1 ml of fresh acetone. The MIC of the extract for each test microorganism was regarding as the lowest concentration that inhibited visible growth of the test organisms on the agar plate after 24 hrs incubation at 37°C.

RESULTS AND DISCUSSION

The plant showed broad spectrum antibacterial activity (Table 1). The acetone, water, methanol and petroleum ether extracts were active against all the selected respiratory

pathogens. The *N. ciliaris* extracts were found to be less effective as compared to erythromycin. In case of *S. aureus*, *S. pneumoniae* and *S. pyogenes* acetone extract exhibited the highest degree of antimicrobial activity as compared to aqueous, methanol and petroleum ether extracts. The maximum inhibition by acetone extract was found against *S. pneumoniae* and *S. aureus* were 17 mm and 14 mm respectively. While in case of *P. aeruginosa* the methanol extract was most active and showed maximum inhibition (15 mm) following by aqueous, acetone and petroleum ether. Further MIC was determined against the four respiratory pathogens that showed positive inhibition and found that the lowest value was exhibited by *S. aureus*, *S. pneumoniae* and *S. pyogenes* at 40 mg/ml and *P. aeruginosa* at 50 mg/ml concentrations.

The study showed that the crude extract of the *N. ciliaris* was found active against all four tested bacteria. Our findings were also supported by its medicinal properties reported by other researchers. The decoction of leaves and seeds is taken in fever [6]. *N. ciliaris* is used as antipyretic and antitussive agent [10, 11]. The pharmaceutical cough syrups and drugs use it as principle ingredient. The liquid extract (Araq-e-Zuufaa) and squash (Sharbat-e-Zuufaa) prepared from *N. ciliaris* is prescribed when phlegm is thick and sticky and chest is congested [12].

The tested plant extracts have good potential as antibacterial compounds and can be used in the treatment of respiratory infections caused by microbes.

Table 1: The inhibition zones diameters of various extracts of *Nepeta ciliaris* (aerial parts)

Pathogens	*Diameters of the inhibition zone (mm)				Positive Control (Erythromycin)
	Pt. Ether	Acetone	Methanol	Aqueous	
P. aeruginosa	9	13	15	14	17
S. aureus	9	14	12	13	31
S. pneumoniae	10	17	13	13	20
S. pyogenes	9	13	12	12	26

*Values are mean of three replicates, Cork borer diameter: 6 mm.

Table 2: Determination of MIC value of Acetone extract of *Nepeta ciliaris* against tested respiratory pathogens

Concentration (mg/ml)	Test Pathogens			
	S. aureus	*P. aeruginosa*	*S. pneumoniae*	*S. pyogenes*
200	-	-	-	-
100	-	-	-	-
75	-	-	-	-
50	-	-	-	-
40	-	+	-	-
30	+	+	+	+
25	+	+	+	+
20	+	+	+	+

(-): Absence of growth, (+): Presence of growth

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