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IN-VITRO ANTIMICROBIAL ACTIVITY OF N-CHLOROPICOLINAMIDE

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ABSTRACT

The present study describes the antimicrobial activity of N-Chloropicolinamide (NCP). The antimicrobial activities were assessed by measuring the diameter of the inhibition zones. The bacterial strains like gram positive bacteria *staphylococcus aureus* and *bacillus subtrilis*, gram negative bacteria *Escherichia coli* and three strains of fungi *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* were used. NCP was screened for antimicrobial activity against bacteria and fungi at various concentrations using disc diffusion method. The synthesized N-Chloropicolinamide compounds exhibited good antimicrobial activity.

Keywords: N-Chloropicolinamide, Antimicrobial activity, Bacteria and Fungi, N-halo Compounds

INTRODUCTION

N-Chloro compounds have been used as versatile reagents in kinectic study and organic synthesis. This compound offers many advantages like easy method of synthesis, low cost, easy handling, low toxicity and mild nature with appreciable stability **[1]**. The antimicrobial activities of N-Chloro compounds have been already reported **[2-8]**. N-halo compounds are being used in kinetics, analytical and organic structural investigation and in synthesizing organic substrates. The compound has been established as an effective source of positive halogen. NCP is a biologically important

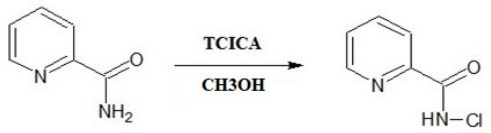
oxidizing agent, due to its reported antimicrobial activity by disc diffusion method **[9-12]**.

The present work deals with testing the N-Chloropicolinamide for its antimicrobial activity against bacteria *staphylococcus aureus, bacillus subtrilis* and *herichia coli* and three strains of fungi *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*.

MATERIALS AND METHODS

SYNTHESIS AND CHARACTERISATION OF N-CHLOROPICOLINAMIDE:

A simple method was developed for the preparation of N-Chloropicolinamide (NCP). In a 250ml round bottom flask 3.932g of picolinamide was dissolved in methanol. 1.371g of trichloroisocyanuric acid (TCICA) was added and cyanuric acid was precipitated. After stirring for 1 hour the mixture was vaccum filtred and the solid was washed with methylene chloride. Solvents were removed by using a rotary evaporator and the final residue of N-Chloropicolinamide (92% yield) was obtained. Recrystalization of NCP was done with ether solvent **[6]**.



The melting point was found to be 138^{o}C with molecular formula as $C_6H_5ON_2Cl$. NCP was found to be soluble in water, acetic acid, DMSO, DMF, sparingly soluble in ethanol, ethyl acetate, chloroform and insoluble in benzene, acetonitrile, dioxane and DCM.

ANTIMICROBIAL ACTIVITY

TEST ORGANISMS:

The bacterias: *staphylococcus aureus, bacillus subtilis* and *Escherichia coli* and the fungus *candida albicans, aspergillus flavus* and *aspergillus niger* were procured from the microbial type of culture collection (MTCC) at the institute of microbial technology (IMTECH) chandigarh, India.

PREPARATION OF DISC

The sample was dissolved in distilled water to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg). Standard solution of chloramphenicol (bacteria) and Flulonazole (fungi) (25mg\ ml distilled water-30µl) were used to compare with the test solution. Whatman's filter paper (N0.1) was used to prepare the disc of approximately 6mm in diameter, which are placed in hot air for sterilization. The discs were kept under refrigerated condition unless they were used for the experiment.

ANTIMICROBIAL ASSAY

Antibiogram was done by disc diffusion method **[13, 14]** using sample. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab was dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* species of bacteria were spread on Nutrient agar plates and *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* species were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample (50µl, 100µl and 150µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

The antimicrobial activity of NCP along with some of its precursors were evaluated by antibiogram assay against three bacterial strains and three fungal strains selected on the basis of their relevance as human pathogens. The diameter of zone of inhibition obtained against NCP by disc diffusion method was also compared to those obtained against standard antibiotics such as chloromphenical (for bacteria) and fluconazole (for fungai). The results of these bacterial bioassays were given in **Table 1**. This antibacterial assay revealed that out of the three different bacteria, the NCP posses' highest antibacterial activity against *Escherichia coli* and lowest antibacterial activity against *Staphylococcus aureus and Bacillus subtilis.*

The results of these fungal bioassays were given in **Table 2**. NCP was tested for zone of inhibition against fungal strains containing *Candida albicans, Aspergillus flavus* and *Aspergillus niger* .The assay revealed that the N-halo compound NCP exhibited the highest antifungal activity against *Aspergillus niger* and moderate antifungal activity against *Candida albicans, Aspergillus flavus*.

Table 1: Antibacterial activity of N-Chloropicolinamide

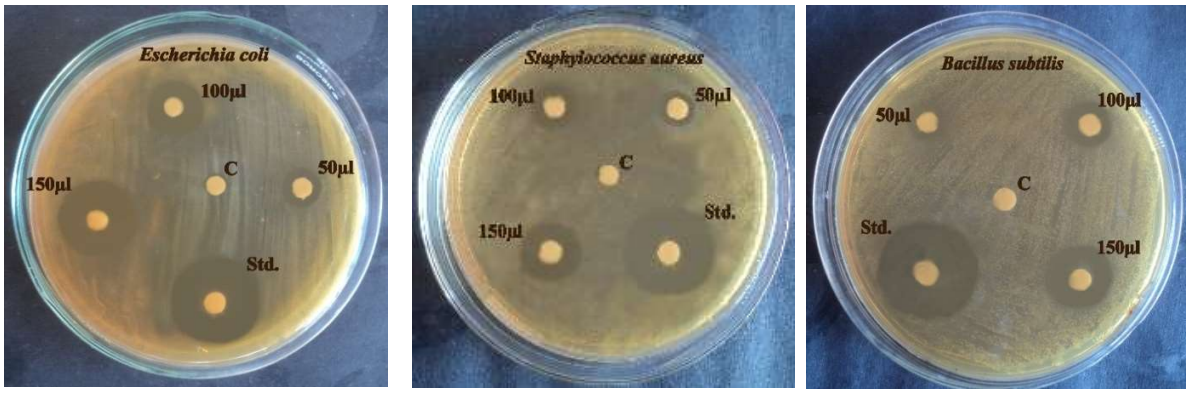
Zone of Inhibition for the Microorganisms	Concentrations(µl/ml)			Standard (30µl/ml)	Control (30µl/ml)
	50µl	100µl	150µl		
Escherichia coli (mm)	1.70±0.12	4.80±0.34	7.40±0.52	9.50±0.66	0.20±0.01
Staphylococcus aureus (mm)	0.80±0.6	3.10±0.22	6.30±0.44	9.70±0.68	0.10±0.01
Bacillus subtilis (mm)	1.10±0.08	3.50±0.25	6.70±0.47	9.60±0.67	0.10±0.01

Values were expressed as Mean ± SD; Bacterial standard: Chloramphenicol; Control: DMSO

Table 2: Antifungal activity of N-Chloropicolinamide

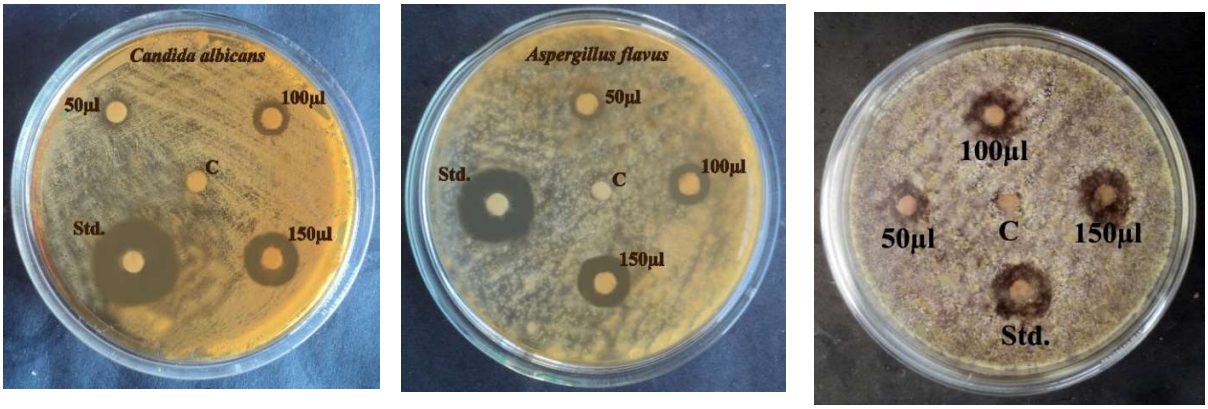
Zone of Inhibition for the Microorganisms	Concentrations (µl/ml)			Standard (30µl/ml)	Control (30µl/ml)
	50 µl	100 µl	150 µl		
Candida albicans (mm)	0.30±0.02	1.80±0.13	4.40±0.31	8.80±0.62	0.10±0.01
Aspergillus flavus (mm)	0.50±0.04	2.30±0.16	4.60±0.32	8.60±0.60	0.20±0.01
Aspergillus niger (mm)	5.30 ± 0.37	7.20± 0.50	9.80 ± 0.68	10.10 ± 0.70	0.50 ± 0.03

Values were expressed as Mean ± SD; Fungal standard: Fluconazole; Control: DMSO



Escherichia coli *Staphylococcus aureus* *Bacillus subtilis*

Figure: 1 Antibacterial activity of N-Chloropicolinamide



Candida albicans *Aspergillus flavus* *Aspergillus niger*

Figure 2: Antifungal activity of N-Chloropicolinamide

CONCLUSION

N-Chloropicolinamide was synthesized and characterized by spectral analysis. The synthesized N-Chloropicolinamide was found to have highest antibacterial activity against *Escherichia coli* and highest antifungal activity against *Aspergillus niger* at the concentration 150 (µl/ml).

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