

**STUDY ON ANTIMICROBIAL ACTIVITIES OF ACTINOMYCETES  
ISOLATED FROM SOILS OF DIFFERENT PARTS OF KHUMBU REGION**

A

Dissertation

Submitted to the Central Department of Microbiology  
Tribhuvan University

In Partial Fulfillment of the Requirement for the Award of the Degree of  
Masters of Science in Microbiology  
(Environment and Public Health)

**By**

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## RECOMMENDATION

This is to certify that **Ms. Junu Koirala** has completed this dissertation work entitled **“Study on Antimicrobial activities of Actinomycetes isolated from soils of different parts of Khumbu region”** as a partial fulfillment of M. Sc. Degree in Microbiology under our supervision. To our knowledge this thesis work has not been submitted to any other degree.

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## ABSTRACT

The present study has been carried out at the laboratory of National Academy science and Technogy between 2006 October to 2007 September.

The soil samples (N=4) collected from different places of 'Khumbu' region, Nepal (2500-5050m altitude) were processed to isolate antifungal and antibacterial Actinomycetes.

Altogether 22 isolates of Actinomycetes were isolated. In all isolates, 18 isolates were identified to genus level of which 11 isolates belonged to genus *Streptomyces*, three isolates belonged to *Micromonospora*, and two isolates belonged to *Nocardia* and one each belonging to *Actinomadura* and *Streptoverticillium*.

All the eighteen isolates were screened for their antimicrobial activities against target cultures viz; plant pathogenic fungi: *Alternaria* spp., *Fusarium oxysporum*, *Fusarium moniliforme*, *Geotricum* spp., *Gloeosporium sporioides*, *Helminthosporium* spp. and *Sclerotinia* spp. and bacterial pathogens: *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Citrobacter* spp., *Salmonella paratyphi A* and *Shigella* spp.

In all Actinomycetes isolates, 14 isolates which showed antimicrobial activity, 5 isolates showed activity only against gram positive bacteria while 5 showed broad host range showing activity against both gram positive and gram negative bacteria.

However, only two isolates X1 and C4 belonging to *Streptomyces* spp. were active against all the target fungal cultures.

Out of all potential antifungal and antibacterial isolates, X1 and C4 and one isolate Ch9 belonging to *Actinomadura* spp. were chosen for the antimicrobial compound production and extraction of bioactive compound.

The fermentation was carried out for 21 days using starch casein broth and the cell free supernatant was subjected for solvent extraction. The extracted residue also showed broad spectrum antibacterial and antifungal activity against target organisms.

The bioactive residues (crude extracts) were dissolved in chloroform and run in silica gel precoates separately using chloroform: methanol (40:1) as the solvent system. The crude extract of isolate X1 gave two spots having  $R_f$  0.58 and 0.66 and that of Ch9 and C4 each gave single spot having  $R_f$  0.79 and 0.66 respectively.

**Key words:** Actinomycetes, Antimicrobial activities, bioactive secondary metabolite, Khumbu, *Streptomyces* spp.

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## LIST OF ABBREVIATIONS

cm	centimeter
° C	Degree Centigrade
cfu	Colony forming unit
DMSO	Dimethyl sulfoxide
D/W	Distilled water
GAA	Glycerol Asparagine agar
gm	gram
hrs	hours
L	Liter
mg	milligram
MHA	Muller Hinton Agar
ml	milliliter
mm	millimeter
µl	micro liter
NA	Nutrient Agar
NAST	Nepal Academy of Science and Technology
No.	Number
PDA	Potato Dextrose Agar
R <sub>f</sub>	Retardation factor
rpm	Revolution per minute
spp.	species
TLC	Thin Layer Chromatography
UV	Ultraviolet
v/v	volume/volume
wt.	weight
w/v	weight/volume
ZOI	Zone of Inhibition