

**ISOLATION, CHARACTERIZATION AND STUDY OF  
BIOLOGICAL ACTIVITIES OF FOUR POISONOUS  
MEDICINAL PLANTS OF  
CHITWAN, NEPAL**

**A DISSERTATION  
SUBMITTED FOR THE  
PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR MASTER OF SCIENCE DEGREE IN CHEMISTRY  
BY**

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## **BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL**

This dissertation entitled “Isolation, Characterization and Study of Biological activities of four Poisonous Medicinal Plants of Chitwan, Nepal”, by “*Rama Pokharel*”, under the supervision of “Asst. Prof. Dr. *Surya Kant Kalauni*”, *Central Department of Chemistry*, Tribhuvan University, Nepal, is hereby submitted for the partial fulfillment of the Master of Science (M.Sc.) Degree in Chemistry. This dissertation has not been submitted in any other university or institution previously for the award of a degree.

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## RECOMMENDATION LETTER

This is to certify that the dissertation work entitle” ISOLATION, CHARACTERIZATION AND STUDY OF BIOLOGICAL ACTIVITIES OF FOUR POISONOUS MEDICINAL PLANTS OF CHITWAN, NEPAL” has been carried out by **Mrs. Rama Pokharel** as a partial fulfillment for the requirement of M. Sc. Degree in Chemistry under my supervision. To the best of my knowledge, this work has not been submitted to any other degree in this institute.

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## DECLARATION

I, "*Rama pokharel*", hereby declare that the work presented herein is genuine work done originally by me and has not been published or submitted elsewhere for the requirement of a degree program. Any literature, data or works done by others and cited in this dissertation has not been given due acknowledgement and listed in the reference section.

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## ABSTRACT

Four poisonous medicinal plants species, namely; *Calotropis gigantea*, *Ageratum houstonianum*, *Catharanthus roseus*, *Thevetia peruviana* (seed), and *Thevetia peruviana* (leaves) have been collected from the central Nepal, Chitwan and the crude methanol extract of respective plants were studied for their phytoconstituents and biological activity. Phytochemical screening showed the presence of glycosides, flavonol glycosides and coumarin glycosides as rich components. The Brine-shrimp bioassay of various plants extract showed that *C. gigantea*, *A. houstonianum* and *C.roseus* exhibited high toxicity against brine shrimp nauplii at LC<sub>50</sub> (µg/ml) values of 23.44, 27.54 and 83.17 respectively. In addition to this, study of antimicrobial activity of respective plants extract on methanol showed that, all the four plants species were highly active for *Staphylococcus aureus* and *C. gigantea* is pharmacologically active for other bacteria such *E. coli*, *S. aureus*, *K. oxytoca* and *P. aeruginosa*. Study of anti-cancer activity on human pancreatic cancer cells such as PANC-1 revealed that the preferential cytotoxic activity of crude methanol extract of *C. gigantea* was highest at 100 µg/ml for both NDM and DMEM. Column chromatography of methanol extract of aerial parts of *C. gigantea* has resulted the isolation of two pure compounds CG<sub>21</sub>, and CG<sub>22</sub>. The compound CG<sub>21</sub> was suggested as -sitosterol by comparing TLC, IR and melting point with authentic sample.

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## ACRONYMS

DMSO	:	Dimethyl Sulfoxide
HMBC	:	Heteronuclear Multiple Bond Correlation
HMQC	:	Heteronuclear Multiple Quantum Correlation
LC <sub>50</sub>	:	Lethal Concentration for 50% Mortality
NMR	:	Nuclear Magnetic Resonance
$\lambda_{\text{max}}$	:	Wavelength at maximum absorbance
ppm	:	Parts per Million
R <sub>f</sub>	:	Retention factor
TLC	:	Thin Layer Chromatography
UV	:	Ultra Violet
MIC	:	Minimum Inhibitory Concentration
NDM	:	Nutrient Deprive Medium
DMEM	:	Dulbeco Modified Eagle Medium
PANC-1	:	Pancreatic cancer cell line
PBS	:	Phosphate Buffer