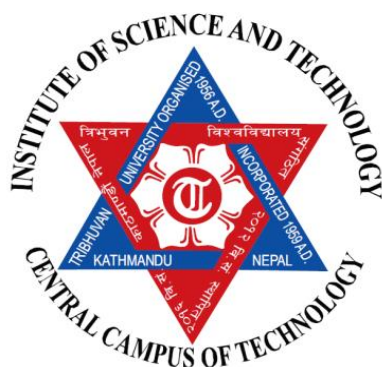


**ANTI-PHYTOPATHOGENIC EVALUATION OF
SYNERGESTICALLY FORMULATED AQUEOUS
EXTRACT AND COW URINE EXTRACT OF
SELECTED PLANTS**



A
Dissertation Submitted to the
Department of Microbiology, Central Campus of Technology,
Tribhuvan University, Dharan, Nepal
In Partial Fulfillment of the Requirements for the Award of Degree
of Masters of Science in Microbiology
(Agriculture)

By:
Bidhya Dhungana
T.U.Regd.No.5-2-459-0002-2011
Roll No.:MB429/072
Dharan
2019
©Tribhuvan University

RECOMMENDATION

This is to certify that **Miss Bidhya Dhungana** has completed this dissertation work entitled “**Anti-phytopathogenic evaluation of synergistically formulated aqueous extract and cow urine extract of selected plants**” as a partial fulfillment of the requirement of M.Sc. degree in Microbiology (Agriculture) under my supervision. To our knowledge, this work has not been submitted for any other degree.

.....

Hemanta Khanal
Supervisor
Assistant Professor
Department of Microbiology
Central Campus of Technology
Hattisar, Dharan, Nepal

Date:/...../.....

CERTIFICATE OF APPROVAL

On the recommendation of **Asst. Professor Mr. Hemanta Khanal** this dissertation work of **Miss Bidhya Dhungana** entitled “**Anti-phyto-pathogenic evaluation of synergistically formulated aqueous extract and cow urine extract of selected plants**” has been approved for the examination and is submitted for the Tribhuvan University in Partial fulfillment of the requirements for M.Sc. degree in Microbiology (Agriculture).

.....
Mr. Shiv Nandan Sah

Asst. Professor
Head of Department
Department of Microbiology
Central Campus of Technology
Tribhuvan University,
Dharan

.....
Mr. Hemanta Khanal

Asst. Professor
M.Sc. Microbiology
Programme Coordinator
Department of Microbiology
Central Campus of Technology
Tribhuvan University,
Dharan

Date:/...../.....

BOARD OF EXAMINERS

Recommended by:

.....

Asst. Professor Mr. Hemanta Khanal

Supervisor

Approved by:

.....

Asst. Professor Mr. Siv Nandan Sah

Head of Department

Examined by:

.....

()

Internal Examiner

.....

()

External Examiner

Date:/...../.....

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my respected supervisor **Asst. Professor Mr. Hemanta Khanal** for his continuous support, guidance and encouragement throughout my research work. It would not have been possible to complete this dissertation work successfully without his valuable help.

I am very much obliged to my Campus Chief **Professor Dr. Dhan Bahadur Karki**, **Asst. Professor Shiv Nandan Sah**; Head of Department of Microbiology, Central Campus of Technology for providing me with the required facilities and instructions for the dissertation work.

I am also thankful to all the teachers especially **Asst. Professor Mr. Suman Rai** and laboratory staffs **Ain Bahadur Karki**, **Prajwal Bhandari**, librarian **Mr. Om Khatiwada** and library staffs for their great cooperation and help.

Additionally, I would express my gratitude to my classmates, especially **Mr. Bijay Kumar Shrestha**, **Mr. Jenish Shakya**, **Mrs. Sushila Khadka**, **Mrs. Romika Shrestha**, **Ms. Manita Subba** and **Ms. Jyoti Limbu** for their help and support.

Finally, I would like to convey my regards to my family members for motivating and supporting me during the thesis work.

.....
Bidhya Dhungana

Date:/...../.....

ABSTRACT

Plants have been classified as an essential source of medicinal agents and a huge number of novel drug components have been isolated from natural plant sources. Plants have evolved secondary biochemical pathways that allow them to synthesize a raft of secondary metabolites, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks, or nutrient deprivation. Plants produced secondary metabolites (phytochemicals); have demonstrated their potential as antimicrobial when used alone and as synergists or potentiates of other antibacterial agents. The aim of the study is to investigate the medicinal properties of *Azadirachta indica*, *Allium sativum* and *Capsicum annum* by performing phytochemical screening and evaluating their antibacterial and antifungal properties. All the selected plants were extracted in water and cow urine by maceration, infusion or decoction method. The antibacterial properties were evaluated by agar cup-well diffusion method and antifungal properties were evaluated by poison food technique method. Minimum Inhibitory concentrations of all the extracts were evaluated by serial broth dilution method. Preliminary qualitative phytochemical screening shows the presence of tannins, saponins, alkaloids and flavonoids, carbohydrate and amino acids. The ZOI was more with 50 µg/ml concentration than the 25µg/ml concentration by all the plant extracts where the cow urine extract of neem at 50µg/ml showed largest ZOI (14mm) against *X. axonopodis* pv *citri*. The mycelial growth was more inhibited by cow urine extract of neem with highest mycelia growth inhibition of 92% against *F. oxysporum* f.sp *cubense* at 50µg/ml concentration. The best antimicrobial actions were observed in the Neem in combination with other plant extract against the selected plant pathogens. Among cow urine and aqueous, cow urine extract of *A. indica* + *A. sativum* and showed the better result among the bacterial and fungal plant pathogens with lowest MIC value of 1250µg/ml for *X. oryzea* pv *oryzea*, 312.5µg/ml for *X. axonopodis* pv *citri*, 2500µg/ml for *B. oryzea* and 312.5µg/ml for *F. oxysporum* f.sp *cubense* respectively. Thus, from the overall comparison of plant extract cow urine and aqueous plant extract were found to be significant with $p < 0.05$.

Key Words: Antibacterial, antifungal, phytochemical, metabolites.

TABLES OF CONTENTS

Title Page	i
Recommendation	ii
Certificate of Approval	iii
Boards of Examiners	iv
Acknowledgements	v
Abstract	vi
Table of Contents	vii-ix
List of Abbreviations	x
List of Tables	xi
List of Figures	xii
List of Photographs	xiii
List of Appendices	xiv
CHAPTER I: INTRODUCTION AND OBJECTIVES	1-5
1.1 Background	1-4
1.2 Objectives	5
1.2.1 General objectives	5
1.2.2 Specific objectives	5
CHAPTER II: LITERATURE REVIEW	6-19
2.1 Natural Antimicrobials from Plants	6
2.2 Botanical Plants	7-8
2.2.1 History of botanical plants	7
2.2.2 General uses of botanicals	7-8
2.3 Active components of plant extract	8-10
2.3.1 Alkaloids	9
2.3.2 Flavonoids	9
2.3.3 Terpenoids	9
2.3.4 Tannins	9-10

2.4 Significance of Antimicrobial Susceptibility Testing	10-11
2.5 Extraction Technique of Plant Extract	11-12
2.6 Medicinal Plants under the Study	12-16
2.6.1 <i>Azadirachta indica</i>	12-14
2.6.2 <i>Allium sativum</i>	14-15
2.6.3 <i>Capsicum annum</i> (Chili pepper)	16-17
2.7 Phytopathogens under study	17-19
2.7.1 Plant Pathogenic Bacteria	17-18
2.7.2 Plant Pathogenic Fungi	18-19
CHAPTER III: MATERIALS AND METHODS	20-27
3.1 Materials	20
3.2 Methods	20
3.2.1 Study site	20
3.2.2 Sampling site	20
3.2.4 Preparation of Plant Extract	20-21
3.2.5 Calculation of Percentage Yield of Extract	21
3.2.6 Phytochemical Screening of Plant Extract	21-23
3.2.7 Anti-phytopathogenic Evaluation	23-26
3.2.8 Quality Control for Tests	26
3.2.8 Data Analysis	26
3.3 Flow Diagram of the Study	27
CHAPTER IV: RESULTS	28-37
4.1 Physical Characteristics of Sample	28
4.2 Qualitative Phytochemical Screening of Selected Plants	29
4.3 Microbial Sensitivity Test	30-38
4.3.1 Antibiotic Sensitivity Pattern of Selected Plants	30

4.3.2 Antimicrobial Activities of Plant Extracts	31-38
CHAPTER V: DISCUSSIONS	39-44
CHAPTER VI: CONCLUSION AND RECCOMENDATIONS	45-46
6.1 Conclusion	45
6.2 Recommendations	46
REFERENCES	47-63
APPENDICES	i-vii

LIST OF ABBREVIATIONS

DMSO	-	Dimethyl sulfoxide
MA	-	MacConkey Agar
MIC	-	Minimum Inhibitory Concentration
MHA	-	Muller Hinton Agar
MIC	-	Minimum Inhibitory Concentration
MR	-	Methyl Red
NA	-	Nutrient Agar
NB	-	Nutrient Broth
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
PV	-	Pathovar
PPB	-	Plant Pathogenic Bacteria
PPF	-	Plant Pathogenic Fungi
VP	-	Voges-Proskauer
ZOI	-	Zone of Inhibition

LIST OF TABLES

Table No	Title of Table	Page No
Table 2.6.1	Classification of <i>Azadirachta indica</i> (Neem)	13
Table 2.6.2	Classification of <i>Allium sativum</i> (Garlic)	14
Table 2.6.3	Classification of <i>Capsicum annum</i> (Chili pepper)	16
Table 4.1	Physical Characteristics of Plants Extracts	28
Table 4.2	Phytochemical Screening of Samples	29
Table 4.3.1	AST of <i>X. oryzea</i> pv <i>oryzea</i> and <i>X axonopodis</i> pv <i>citri</i>	30
Table 4.3.2.1	ZOI of plant extract (aqueous) against bacterial plant pathogens.	31
Table 4.3.2.2	ZOI of plant extract (cow urine) against bacterial plant pathogens.	32
Table 4.3.2.2	Mycelial growth inhibition (in percentage) by the crude aqueous extract of selected plants against fungal plant pathogens.	33
Table 4.3.2.2	Mycelial growth inhibition (in percentage) by the crude cow urine extract of selected plants against fungal plant pathogens.	34
Table 4.3.2.5	MIC of crude aqueous plant extract against <i>X. oryzea</i> pv <i>oryzea</i> and <i>X. axonopodis</i> pv <i>citri</i>	35
Table 4.3.2.6	MIC of crude cow urine plant extract against <i>X. oryzea</i> pv <i>oryzea</i> and <i>X. axonopodis</i> pv <i>citri</i>	36
Table 4.3.2.7	MFC of crude aqueous plant extract against plant pathogenic fungi <i>Fusarium oryспорum</i> f.sp <i>ubense</i> and <i>Bipolaris oryzea</i>	37
Table 4.3.2.8	MFC of crude cow urine plant extract against plant pathogenic fungi <i>Fusarium oryспорum</i> f.sp <i>ubense</i> and <i>Bipolaris oryzea</i>	38

LIST OF FIGURES

- Fig 1 Flow diagram of the study
- Fig 2 Molecular structure of Azadirachtin

LIST OF PHOTOGRAPHS

- Photograph 1: Working in Laboratories and Morphological view of fungal pathogens.
- Photograph 2: Cultural and Microscopic views of bacteria
- Photograph 3: ZOI by plant extract against *X. axonopodis* pv *citri*
- Photograph 4: Determination of MIC of aqueous extract of plant extract against fungal plant pathogens.

LIST OF APPENDICES

- APPENDIX A:** Materials Used in the study
- APPENDIX B:** Test organisms and biochemical test
- APPENDIX C:** The collection site and Parts of selected plants for the study
- APPENDIX D:** Zone Size Interpretative Chart for Antibiotic Sensitivity
Test of Selected Antibiotics Discs
- APPENDIX E:** Statistical analysis