## CONTENTS

Acknowledgement i
Declaration iii
Certificate iv
Abstract vi
Contents vi
List of tables vi
List of figures vi
Abbreviations vi

### Chapter 1

1. INTRODUCTION 1
   1.1. Overview 1
   1.2. Objectives 7

### Chapter 2

2. REVIEW OF LITERATURE 8
   2.1. Foodborne diseases and diagnosis 8
   2.2. PCR and its detection of pathogens 9
   2.3. *Escherichia coli* 11
   2.4. *Salmonella* spp. 13
   2.5. *Vibrio cholerae* 15
   2.6. PCR application in detection and identification of target pathogens 18
   2.7. Bio-prospection and biological property of *Cleistanthus collinus* 27
   2.8. Medicinal plants in Nanotechnology 32
   2.9. Phytochemical wealth of medicinal plants 35
   2.10. Biological property of botanicals 40
Chapter 3

3. MATERIALS AND METHODS

3.1. Detection and Identification of Selected Pathogens

3.1.1. Primer designing for detection of target bacterial pathogens

3.1.2. Chemicals

3.1.3. Bacterial strains and culturing

3.1.4. Media used for bacterial culture and their composition

3.1.5. Selection of food samples (Fish)

3.1.6. Isolation of target bacterial pathogens from fish tissue

3.1.7. DNA Extraction from target bacteria

3.1.8. Target gene amplification by Polymerase Chain Reaction

3.1.9. Determination of specificity of PCR for detection of target bacterial Pathogens

3.1.10. Identification of Limit of Detection (LOD)

3.1.11. PCR detection of target bacterial pathogens in food and environmental samples

3.1.12. DNA sequencing of amplified PCR product for confirmation

3.2. Bio-prospecting of Cleistanthus collinus

3.2.1. Collection of plant sample

3.2.2. Preparation of plant extracts

3.2.3. Phytochemical screening of water, ethanol, ethyl acetate and methanol crude extracts of C. collinus leaf crude extracts

3.2.4. Thin Layer Chromatography analysis of C. collinus crude extracts

3.2.5. GC- MS Analysis of C. collinus crude extracts

3.2.6. Collection of bacterial pathogens for antibacterial activity assay

3.2.7. Determination of antimicrobial activity of C. collinus
crude extracts

3.2.8. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *C. collinus* crude Extracts

3.3. Comparative analysis of *Cleistanthus collinus* aqueous leaf extract and fractions for its antibacterial potential 55

3.3.1. Preparation of *C. collinus* leaf aqueous extract and fractions 55

3.3.2. Phytochemical screening and UV-Vis spectrophotometer analyses of *C. collinus* extracts and its fractions 56

3.3.2. Collection of bacterial pathogens for antibacterial activity 56

3.3.3. Estimation of antibacterial activity and assessment of MIC and MBC of *C. collinus* aqueous extracts and its fractions 56

3.4. Isolation of botanicals from *Cleistanthus collinus* extract and its antibacterial activity 57

3.4.1. Chemicals 57

3.4.2. Separation of the botanicals from *C. collinus* crude extracts by Thin Layer Chromatography (TLC) 57

3.4.3. Fractionation of botanicals from crude extracts of *C. collinus* by column chromatography 58

3.4.4. Estimation of antibacterial activity of fractioned compound CM, HE and TE 59

3.4.5. Determination of MIC and MBC of fractioned compound TE 60

3.4.6. Effect of fractioned compound TE on biofilm formation 60

3.4.7. Assessment of cytotoxicity property of fractioned compound TE 60

3.4.8. Evaluation of genotoxicity of fractioned compound TE 61
3.5. Biosynthesis of silver nanoparticles using *Cleistanthus collinus* extract, compound TE and assessment antibacterial potential

3.5.1. Chemicals

3.5.2. Preparation of plant extract and fractionation of compound TE

3.5.3. Bio synthesis of silver particles from extract and fraction of *C. collinus*

3.5.4. Characterization of Nanoparticles

3.5.5. Collection of bacterial pathogens for antibacterial activity

3.5.6. Evaluation of antibacterial activity of synthesized SNPs from ethanol extract of *C. collinus* and compound TE

3.6. Spectroscopic studies of biologically active purified compound TE

3.6.1. Chemicals

3.6.2. Ultraviolet Visible spectral analysis of fractioned compound TE

3.6.3. Infrared (IR) spectral analysis of fractioned compound TE

3.6.4. Nuclear Magnetic Resonance (NMR) spectroscopy analysis of fractioned compound TE

3.6.5. Liquid Chromatography and Mass Spectrometry (LC-MS) of fractioned compound TE

3.6.6. Elemental Analysis of fractioned compound TE

3.6.7. Structure elucidation and name of compound TE

Chapter 4

4. RESULTS AND DISCUSSION

4.1. Detection and Identification of Selected Pathogens

4.1.1. Primer designing for detection of target bacterial pathogens
4.1.2. Selection of food samples (fish) and isolation of target bacterial pathogens from fish tissue 75
4.1.3. PCR Detection and Identification of target pathogens 77
4.1.4. Determination of specificity and Limit of Detection (LOD) of designed primer 79
4.1.5. Detection and Identification of target bacterial pathogens from food samples 80
4.1.6. Effect of incubation time on detection and identification of the target pathogens from fish and environmental samples 83
4.1.7. Molecular confirmation of amplified product 86

4.2. **Bio-prospecting of Cleistanthus collinus** 90

4.2.1. Collection of plant sample 90
4.2.2. Preparation of plant extracts 91
4.2.3. Preliminary phytochemical screening and Thin-Layer Chromatography (TLC) from *C. collinus* extracts 92
4.2.4. GC-MS Analysis of *C. collinus* extracts 95
4.2.5. Determination of antibacterial activity of *C. collinus* extracts 100

4.3. **Comparative analysis of Cleistanthus collinus aqueous leaf extract and fractions for its antibacterial potential** 104

4.3.1. Preparation of *C. collinus* leaf aqueous extract and fractions 104
4.3.2. Phytochemical screening and UV-Vis spectrophotometer analyses of *C. collinus* extracts and its fractions 104
4.3.3. Estimation of antibacterial activity and assessment of MIC and MBC of *C. collinus* aqueous extracts and its fractions 107

4.4. **Isolation of botanicals from Cleistanthus collinus us extracts and its antibacterial activity** 113

4.4.1. Fractioned compounds from *C. collinus* extracts 113
4.4.2. Estimation of antibacterial activity and assessment of 114
MIC and MBC of fractioned compound against selected pathogens

4.4.3. Effect of fractioned compound TE on biofilm formation 123
4.4.4. Assessment of cytotoxicity property of fractioned compound TE 124
4.4.5. Evaluation of genotoxicity of fractioned compound TE 126

4.5. Biosynthesis of silver nanoparticles using *Cleistanthus collinus* extract, compound TE and assessment antibacterial potential

4.5.1. Ultraviolet- Visible Spectroscopy of SNPs 128
4.5.2. Fourier Transform Infrared Spectroscopy (FTIR) Measurements of SNPs 129
4.5.3. X-ray Diffraction (XRD) analyses of SNPs 131
4.5.4. Scanning Electron Microscope (SEM) analysis of SNPs 132
4.5.5. Evaluation of antibacterial activity of synthesized SNPs from ethanol extract of *C. collinus* and compound TE 133

4.6. Spectroscopic studies of biologically active purified compound TE

4.6.1. Ultraviolet Visible spectral analysis of fractioned compound TE 138
4.6.2. Fourier Transform Infrared (FTIR) spectral analysis fractioned compound TE 139
4.6.3. $^1$H NMR spectral studies of compound TE 140
4.6.4. $^{13}$C NMR spectral studies of compound TE 142
4.6.5. Mass Spectrum analysis of compound TE 143
4.6.7. Elemental analysis of compound TE 144
4.6.8. Structure elucidation and name of compound TE 144

**Chapter 5**

5. SUMMARY AND CONCLUSION 148

References

List of Publications