TABLE OF CONTENTS

CHAPTER 1
Introduction

1.1 MEDICINAL PLANTS IN DRUG DISCOVERY
  1.1.1 History
  1.1.2 Medicines from nature: Natural products and drug discovery
  1.1.3 Traditional Wisdom – Ayurveda
    1.1.3.1 Approaches for drug discovery by utilizing Ayurvedic concepts
  1.1.4 Ethnopharmacology
  1.1.5 Chemical diversity
  1.1.6 Standardization
    1.1.6.1 New analytical techniques for standardization
  1.1.7 Problems related to natural product drug discovery and strategies to overcome these problems
    1.1.7.1. Problems
    1.1.7.2. Solutions and strategies

1.2 MALARIA AND ITS TREATMENT
  1.2.1 Malaria Milestones
  1.2.2 Cause and clinical manifestation
  1.2.3 Epidemiology
  1.2.4 Plasmodium life cycle
  1.2.5 Unique aspects of antimalarial drug discovery
  1.2.6 The Prospects

1.3 GENESIS OF THE PROJECT

1.4 OBJECTIVES OF RESEARCH WORK

1.5 STRUCTURE OF THE THESIS

REFERENCES

CHAPTER 2
In vitro cultivation of Plasmodium falciparum

2.1 INTRODUCTION

2.2 DETAILED PROCEDURE FOR IN VITRO CULTIVATION AND MAINTENANCE OF PLASMODIUM FALCIPARUM CULTURE
Plasmodium falciparum

2.2.1.1 RPMI medium

2.2.1.2 Washing medium (Incomplete medium)

2.2.1.3 Serum preparation

2.2.1.4 Complete medium

2.2.2 Preparation of erythrocytes (RBCs) for culture

2.2.3 Continuous culture of Plasmodium falciparum

2.2.3.1 Initiation of culture

2.2.3.2 Monitoring culture growth

2.2.3.3 Subculturing (Passaging)

2.2.4 Synchronization of Plasmodium falciparum

2.2.4.1 Introduction

2.2.4.2 Procedure

2.2.4.3 Results

2.2.5 Freezing and thawing of Plasmodium falciparum

2.2.5.1 Cryopreservation

2.2.5.2 Revival of cryopreserved parasites

REFERENCES

CHAPTER 3
Preliminary Evaluation of Selected Medicinal Plants for Antiplasmodial Activity

3.1 INTRODUCTION

3.1.1 Selection of medicinal plants

3.2 MATERIALS AND METHODS

3.2.1 Chemicals

3.2.2 Collection and authentication of selected medicinal plants

3.2.3 Extraction of plant material

3.2.4 In vitro testing of the antimalarial activity of the selected plants

3.2.4.1 Schizont maturation inhibition assay

3.2.4.2 Lactate dehydrogenase inhibition assay

3.3 RESULTS AND DISCUSSION

3.3.1 Schizont maturation inhibition assay

3.3.2 Lactate dehydrogenase inhibition assay

3.4 CONCLUSION

REFERENCES
CHAPTER 4
Phytochemical and Pharmacological Review of Selected Plants *Adhatoda zeylanica* Nees and *Embelia ribes* Burm.f.

4.1 INTRODUCTION ..... 55

4.2 *ADHATODA ZEYLANICA* Nees ..... 55
   4.2.1 Botanical identity ..... 55
   4.2.2 Distribution ..... 55
   4.2.3 Classical names ..... 55
   4.2.4 Vernacular names ..... 55
   4.2.5 Botanical description ..... 56
   4.2.6 Parts used ..... 56
   4.2.7 Traditional uses ..... 56
   4.2.8 Chemistry ..... 57
   4.2.9 Pharmacology ..... 59
   4.2.10 Therapeutic evaluation ..... 60

4.3 *EMBELIA RIBES* Burm.f. ..... 60
   4.3.1 Botanical identity ..... 60
   4.3.2 Distribution ..... 60
   4.3.3 Classical names ..... 60
   4.3.4 Vernacular names ..... 60
   4.3.5 Botanical description ..... 61
   4.3.6 Parts used ..... 61
   4.3.7 Traditional uses ..... 61
   4.3.8 Chemistry ..... 61
   4.3.9 Pharmacology ..... 63
   4.3.10 Therapeutic evaluation ..... 64

REFERENCES ..... 65

CHAPTER 5
Bioactivity Guided Isolation of Antiplasmodial Potential from Methanolic Extract of the Leaf of *Adhatoda zeylanica* and fruit of *Embelia ribes*

5.1 INTRODUCTION ..... 69

5.2 MATERIALS AND METHODS ..... 69
   5.2.1 Plant material collection and authentication ..... 69
   5.2.2 Bioactivity guided fractionation and isolation of active principle/s
from the methanolic extract of the leaf of *Adhatoda zeylanica* and fruit of *Embelia ribes* .....69

5.2.2.1 Preparation of the methanolic extract .....69

5.2.2.2 Fractionation of the methanolic extract of *A. zeylanica* and *E. ribes* .....70

5.2.2.3 Isolation of vasicine and vasicinone from methanolic extract of *A. zeylanica* leaf .....70

5.2.2.4 Isolation of embelin from methanolic extract of *E. ribes* Fruit .....72

5.2.3 Structure elucidation of the vasicine, vasicinone and embelin .....72

5.2.4 *In vitro* testing of the antiplasmodial activity of vasicine, vasicinone, embelin .....73

5.2.4.1 *Schizont maturation inhibition assay* .....73

5.2.4.2 *Plasmodium falciparum lactate dehydrogenase inhibition assay* .....73

5.2.5 *In vivo* testing of the antiplasmodial activity of vasicine, vasicinone, embelin .....73

5.2.5.1 *Introduction* .....73

5.2.5.2 *Experimental* .....75

5.3 RESULTS AND DISCUSSION .....75

5.3.1 Bioactivity guided fractionation and isolation of active principle/s from the methanolic extract of the *A. zeylanica* leaf and *E. ribes* fruit .....75

5.3.1.1 *Fractionation of the methanolic extract* .....76

5.3.1.2 *Isolation of vasicine and vasicinone* .....77

5.3.1.3 *Isolation of embelin* .....77

5.3.1.4 *Spectral details of vasicine* .....78

5.3.1.5 *Spectral details of embelin* .....84

5.3.2 *In vitro* testing of the antiplasmodial activity of vasicine, vasicinone, embelin .....86

5.3.3 *In vivo* testing of the antiplasmodial activity of vasicine, vasicinone, embelin .....86

5.4 CONCLUSION .....88

REFERENCES .....88

CHAPTER 6

Effect of vasicine, vasicinone and embelin on some important targets of *Plasmodium falciparum*

6.1 INTRODUCTION .....91
6.2 EFFECT OF VASICINE, VASICINONE AND EMBELIN ON SOME IMPORTANT TARGETS OF PLASMODIUM FALCIPARUM

6.2.1 Effect of vasicine, vasicinone and embelin on heme

6.2.1.1 Effect of vasicine, vasicinone and embelin on hemozoin formation inhibition

6.2.1.2 Drug-Heme Interaction Assay

6.2.1.3 GSH-dependent heme degradation, and its inhibition by clotrimazole and embelin

6.2.1.4 Conclusion

6.2.2 Effect of vasicine, vasicinone and embelin on fatty acid biosynthesis (FAB) inhibition

6.2.2.1 Introduction

6.2.2.2 Materials and Methods

6.2.2.3 Results and Discussion

6.2.2.4 Conclusion

6.2.3 Effect of vasicine, vasicinone and embelin on protein kinase inhibition

6.2.3.1 Introduction

6.2.3.2 Materials and Methods

6.2.3.3 Results

6.2.4 Effect of vasicine, vasicinone and embelin on plasmepsin II and IV

6.2.4.1 Introduction

6.2.4.2 Materials and methods

6.2.4.3 Results and discussion

6.2.5 Effect of vasicine, vasicinone and embelin on histidine rich protein-2 (HRP-2)

6.2.5.1 Introduction

6.2.5.2 Materials and methods

6.2.5.3 Results and discussion

6.2.6 Effect of vasicine, vasicinone and embelin on invasion of red blood cells

6.2.6.1 Introduction

6.2.6.2 Materials and methods

6.2.6.3 Results and discussion

6.3 SUMMERISED DISCUSSION

6.4 CONCLUSION

REFERENCES
CHAPTER 7
Synergistic Evaluation of Antiplasmodial Compounds In Vitro

7.1 INTRODUCTION ..... 130
7.2 DEFINING AND PROVING SYNERGY ..... 131
  7.2.1 Construction of isoboles ..... 132
  7.2.2 Interpretation of isobolograms ..... 132
7.3 MATERIALS AND METHODS ..... 134
  7.3.1 Experimental protocol ..... 134
  7.3.2 Sample preparation ..... 134
  7.3.3 Data analysis ..... 134
7.4. RESULTS AND DISCUSSION ..... 135
REFERENCES ..... 138

CHAPTER 8
TLC Densitometric Quantification of the vasicine, vasicinone
and embelin from Adhatoda zeylanica leaves and Embelia ribes fruits

8.1 INTRODUCTION ..... 140
8.2 EXPERIMENTAL ..... 140
  8.2.1 Chemicals ..... 140
  8.2.2 Apparatus ..... 140
  8.2.3 Plant material collection and authentication ..... 141
  8.2.4 TLC analysis of Adhatoda zeylanica leaves
    8.2.4.1 TLC fingerprint profiling ..... 141
    8.2.4.2 Estimation of vasicine and vasicinone using TLC
densitometric method ..... 142
    8.2.4.3 Method validation ..... 143
  8.2.5 TLC analysis of Embelia ribes fruit
    8.2.5.1 TLC fingerprint profiling ..... 143
    8.2.5.2 Estimation of embelin using TLC densitometric method ..... 144
    8.2.5.3 Method validation ..... 145
8.3. RESULTS AND DISCUSSION ..... 145
  8.3.1 TLC fingerprint profiling of Adhatoda zeylanica leaves ..... 145
  8.3.2 Quantification of biomarker vasicine and vasicinone ..... 146
  8.3.3 TLC fingerprint profiling of Embelia ribes fruits ..... 149
  8.3.4 Quantification of biomarker embelin ..... 150
8.4 CONCLUSION

CHAPTER 9
Summary and Conclusion

9.1 INTRODUCTION

9.2 PRELIMINARY ANTIPLASMODIAL SCREENING OF SELECTED MEDICINAL PLANTS FOR ANTIPLASMODIAL ACTIVITY

9.3 BIOACTIVITY GUIDED ISOLATION OF ANTIPLASMODIAL PRINCIPLE/S FROM METHANOLIC EXTRACT OF ADHATODA ZEYLANICA LEAF AND EMBELIA RIBES FRUIT

9.4 EVALUATION OF THE ISOLATED COMPOUNDS FOR ANTIPLASMODIAL ACTIVITY

9.5 EFFECT OF VASICINE, VASICINONE AND EMBELIN ON SOME IMPORTANT TARGETS OF PLASMODIUM FALCIPARUM

9.5.1 Effect of vasicine, vasicinone and embelin on parasite invasion of red blood cells

9.5.2 Effect of vasicine, vasicinone and embelin on hemozoin formation inhibition

9.5.3 Heme-embelin interaction assay

9.5.4 Effect of embelin on GSH (Glutathione reductase)-dependent heme degradation

9.5.5 Effect of vasicine, vasicinone and embelin on Plasmodium fatty acid biosynthesis (FAB) inhibition

9.5.6 Effect of vasicine, vasicinone and embelin on Plasmodium protein kinase inhibition

9.5.7 Effect of vasicine, vasicinone and embelin on plasmepsin inhibition

9.5.8 Effect of vasicine, vasicinone and embelin on Histidine rich protein–2 (HRP-2) inhibition

9.6 SYNERGISTIC EVALUATION OF ANTIPLASMODIAL COMPOUNDS IN VITRO

9.7 QUANTIFICATION OF VASICINE AND VASICINONE IN THE LEAF OF ADHATODA ZEYLANICA LEAF AND EMBELIN IN THE FRUIT OF EMBELIA RIBES

9.7 CONCLUSION

REFERENCES