

TABLE OF CONTENTS

| | Page |
|---------------------------------------|-------------|
| ACKNOWLEDGEMENTS | iii |
| ABSTRACT (THAI) | iv |
| ABSTRACT (ENGLISH) | vi |
| LIST OF TABLES | xii |
| LIST OF FIGURES | xiv |
| ABBREVIATIONS AND SYMBOLS | xvi |
| CHAPTER 1 INTRODUCTION | 1 |
| CHAPTER 2 LITERATURE REVIEW | |
| 2.1 Causes of acne | 3 |
| 2.1.1 Diet | 7 |
| 2.1.2 Hygiene | 8 |
| 2.1.3 Cosmetics | 8 |
| 2.2 Treatments of acnes | 8 |
| 2.2.1 Topical medications | 8 |
| 2.2.2 Oral antibiotics | 9 |
| 2.2.3 Topical antibiotics | 11 |
| 2.2.4 13-cis-retinoic acid (Accutane) | 11 |
| 2.2.5 Vitamin A | 11 |
| 2.2.6 Estrogens | 12 |
| 2.2.7 Steroids | 12 |
| 2.2.8 Surgery | 12 |
| 2.2.9 Cryotherapy | 12 |

| | |
|--|----|
| 2.2.10 Ultraviolet light | 12 |
| 2.2.11 Miscellaneous treatment | 13 |
| 2.2.12 Scar improvement | 13 |
| 2.3 Acne associated bacteria | 13 |
| 2.3.1 <i>S. aureus</i> | 13 |
| 2.3.1.1 Morphology | 13 |
| 2.3.1.2 Biochemical characteristic | 13 |
| 2.3.2 <i>P. acnes</i> | 14 |
| 2.3.1.1 Morphology | 14 |
| 2.3.1.2 Biochemical characteristic | 14 |
| 2.4 Plants | 16 |
| 2.4.1 <i>Garcinia mangostana</i> Linn. | 16 |
| 2.4.2 <i>Psidium guajava</i> Linn. | 17 |
| 2.4.3 <i>Cinnamomum verum</i> J. Presl | 18 |

CHAPTER 3 MATERIALS AND METHODS

| | |
|---|----|
| 3.1 Equipments and materials use in experiment | 19 |
| 3.1.1 Apparatus and Instruments | 19 |
| 3.1.2 Plant extracts | 19 |
| 3.1.3 Microorganisms | 20 |
| 3.1.4 Media | 20 |
| 3.1.5 Animals | 20 |
| 3.1.6 Chemicals | 20 |
| 3.2 Antibacterial activity test | 21 |
| 3.2.1 Culture Media | 21 |
| 3.2.2 Inoculum preparation | 21 |
| 3.2.2.1 Preparation and standardization of the inoculums | 21 |
| 3.2.2.2 Preparation of antibacterial drug and extracts | 21 |
| 3.2.3 Antibacterial activity by agar cup diffusion method | 22 |
| 3.2.4 Determination of minimum inhibitory concentration | 22 |

| | |
|---|----|
| 3.2.5 Determination of minimum bactericidal concentration | 23 |
| 3.3 Experiments | 24 |
| 3.3.1 Solubility test of the MG-2 extract | 24 |
| 3.3.2 Solubility in acid-base test | 24 |
| 3.4 Formulation and stability test of gel base | 24 |
| 3.4.1 Formulation of gel base | 24 |
| 3.4.2 Characterization of Gel base | 25 |
| 3.4.2.1 Visualization of physical appearances | 25 |
| 3.4.2.2 Spreadability and feel on skin | 25 |
| 3.4.2.3 pH | 25 |
| 3.4.2.4 Stability test of gel base | 25 |
| 3.5 Formulation and stability test of MG-2 gel | 25 |
| 3.6 skin irritation test in Rabbits | 26 |
| 3.7 Skin irritation test in Volunteers | 27 |
| 3.7.1 Subjects of study | 27 |
| 3.7.2 Test substance application protocol | 28 |
| 3.8 Clinical Study of MG-2 gel for anti-acnes | 29 |
| 3.8.1 Subjects of study | 29 |
| 3.8.2 Test substance application protocol | 30 |
| 3.8.3 Clinical evaluation | 30 |
| 3.8.4 Statistical analyses | 30 |

CHAPTER 4 RESULTS AND DISCUSSIONS

| | |
|--|----|
| 4.1 Antibacterial activity test | 31 |
| 4.1.1 Antibacterial activity by agar cup diffusion method | 31 |
| 4.1.2 Minimum inhibitory concentration (MIC) and Bactericidal activity | 33 |
| 4.2 Experiments | 34 |
| 4.2.1 Solubility test | 34 |

| | |
|--|----|
| 4.2.2 Solubility in acid-base test | 34 |
| 4.3 Formulation and stability test of gel base | 38 |
| 4.4 Formulation and stability test of MG-2 gel | 39 |
| 4.5 Antibacterial activity of MG-2 gel | 43 |
| 4.6 Skin irritation test | 47 |
| 4.6.1 Skin irritation test in rabbits | 48 |
| 4.6.2 Skin irritation test in volunteers | 50 |
| 4.7 Clinical study of MG-2 gel for anti-acnes | 51 |
| CHAPTER 5 CONCLUSION | 58 |
| REFERENCES | 60 |
| APPENDICES | 64 |
| APPENDIX A | 65 |
| APPENDIX B | 67 |
| CURRICULUM VITAE | 69 |

LIST OF TABLES

| Table | Page |
|--|------|
| 2.1 Some biochemical characteristics of <i>P. acnes</i> | 15 |
| 3.1 Modified Draize-FHSA Model used in this research | 26 |
| 3.2 Draize-FHSA Scoring System | 27 |
| 4.1 Antibacterial activity of commercial plant extracts | 31 |
| 4.2 Determination of MIC and MBC of the commercial plant extracts. | 33 |
| 4.3 The stability of MG-2 at various pH and temperature | 36 |
| 4.4 The physical properties and pH of gel base Formula I and Formula II before and after heating-cooling cycling method | 39 |
| 4.5 The physical properties and pH of MG-2 gel before and after heating-cooling cycling method The chemical and physical properties of MG-2 gel at 2-8°C at various storage time | 40 |
| 4.6 The chemical and physical properties of MG-2 gel at 2-8°C at various storage time | 42 |
| 4.7 The chemical and physical properties of MG-2 gel at room temperature (dark) at various storage time | 42 |
| 4.8 The chemical and physical properties of MG-2 gel at room temperature (light) at various storage time | 43 |
| 4.9 The chemical and physical properties of MG-2 gel at 45°C at various storage time | 43 |
| 4.10 Antibacterial activity against <i>S.aureus</i> of MG-2 gel after storage at various temperature for 6 months | 44 |
| 4.11 Antibacterial activity against MRSA of MG-2 gel after storage at various temperature for 6 months | 45 |
| 4.12 Antibacterial activity against <i>P. acnes</i> of MG-2 gel after storage at various temperature for 6 months | 46 |

| | |
|---|----|
| 4.13 The scores (Erythema and Edema) of MG-2 extract, | 49 |
| gel base and MG-2 gel | 49 |
| 4.14 The value of primary dermal irritation index (PDII) of | 50 |
| MG-2 extract, gel base and finishing MG-2 gel | |
| 4.15 The conclusion value of primary dermal irritation index (PDII) | 50 |
| of gel base, finishing MG-2 gel and 1% sodium lauryl sulfate in | |
| volunteer (assessment at 0, 1, 7 days after occlusion period) | |
| 4.16 The decreasing value of acnes and oily (Treated and Placebo) | 51 |
| 4.17 The percentage of satisfaction on MG-2 gel | 56 |

LIST OF FIGURES

| Figure | Page |
|---|------|
| 3.1 Two fold dilution method for MIC (Minimum inhibitory concentration) and Determination of MBC (Minimum bactericidal concentration) | 23 |
| 3.2 Profile of test site on patch test | 28 |
| 3.3 The profile of test side on volunteer | 30 |
| 4.1 Antibacterial activity of commercial plant extracts | 32 |
| 4.2 The solubility test of extracts in various soluble | 34 |
| 4.3 Stability of MG-2 at various pH and temperature | 37 |
| 4.4 Physical appearance gel base before and after storage at various temperatures | 38 |
| 4.5 MG-2 gel at before and after heating-cooling cycling | 40 |
| 4.6 Stability of MG-2 gel at 2-8°C at various storage time | 41 |
| 4.7 Stability of MG-2 gel at room temperature (dark) at various storage time | 41 |
| 4.8 Stability of MG-2 gel at room temperature (light) at various storage time | 41 |
| 4.9 Stability of MG-2 gel at 45°C at various storage time | 42 |
| 4.10 Antibacterial activity against <i>S.aureus</i> of MG-2 gel after storage at various temperature for 6 months | 45 |
| 4.11 Antibacterial activity against MRSA of MG-2 gel after storage at various temperature for 6 months | 46 |
| 4.12 Antibacterial activity against <i>P.acnes</i> of MG-2 gel after storage at various temperature for 6 months | 47 |
| 4.13 Skin irritation test of MG-2 extract, MG-2 gel and gel base on various sites of rabbit skin (closed patch test) | 48 |

| | |
|---|----|
| 4.14 Skin irritation test of MG-2 gel in rabbits (assessment at 1, 24, 48, 72 hours after occlusion period) | 48 |
| 4.15 Profile of test site on patch finn chamber (untreated site, gel base, MG-2 gel and 1% sodium lauryl sulfate; SLS) | 50 |
| 4.16 The decreasing of acne compared with volunteers appearance at the begining of treatment | 52 |
| 4.17 % Effectiveness of acne reduction compared with the appearance beginning of treatment in 21 volunteers | 52 |
| 4.18 The decreasing of oiliness compared with volunteers appearance at the begining of treatment | 53 |
| 4.19 % Effectiveness of oiliness reduction compared with the appearance at the beginning of treatment in 21 volunteers | 54 |
| 4.20 The overall changes of facial acne in subjects compared to the appearance at the beginning of the study | 55 |
| 4.21 The percentage of satisfaction on MG-2 gel | 57 |

ABBREVIATION AND SYMBOLS

| | |
|--------|------------------------------------|
| ATCC | American Type Culture Collection |
| A.R. | analytical grade |
| ° C | degree Celsius |
| CFU/mL | colony forming unit per milliliter |
| g | gram |
| hr | hour |
| mL | milliliter |
| mg | milligram |
| mg/mL | milligram/ milliliter |
| pH | $-\log [H]^+$ |
| R.S.D | relative standard deviation |
| S.D | standard deviation |
| v/v | volume by volume |
| w/v | weight by volume |
| µg | microgram |