

## TABLE OF CONTENTS

	<b>Page</b>
ACKNOWLEDGEMENTS	iii
ABSTRACT (ENGLISH)	v
ABSTRACT (THAI)	viii
LIST OF TABLES	xxi
LIST OF FIGURES	xxvii
ABBREVIATIONS AND SYMBOLS	xxxv
CHAPTER 1 INTRODUCTION	1
1.1 Statement and significance of the problem	1
1.2 Objectives	3
1.3 Scope of study	3
1.4 Literature reviews	6
1.4.1 Aging skin	6
1.4.1.1 Causes of aging skin	7
1.4.1.2 Mechanisms of aging skin	9
1.4.1.3 Types of aging skin	11
1.4.1.4 Treatments of aging skin	14
A. Medical treatments	14
B. Cosmeceutical treatments	18
1.4.2 Anti-aging agents/products	18

1.4.2.1 Synthetic antioxidants	18
1.4.2.2 Natural antioxidants	20
1.4.2.3 Anti-aging products in the markets	23
1.4.3 Long Kong ( <i>Lansium domesticum</i> Correa)	25
1.4.3.1 Botanical characteristics	25
1.4.3.2 Bioactive constituents	27
1.4.3.3 Applications and researches on Long Kong	33
A. Pharmaceuticals	33
B. Cosmetics	36
C. Foods and food supplements	37
D. Agriculture	38
1.4.4 The extraction processes of natural products	39
1.4.4.1 Crude extracts	40
A. Ultrasound extraction (Sonication)	41
B. Hot extraction	41
C. Supercritical carbon dioxide fluid extraction (scCO <sub>2</sub> )	42
1.4.4.2 Semi-purified extracts	42
1.4.4.3 Discolor subfractions from the semi-purified extracts by partition technique	43
1.4.5 Characteristic analysis of the extracts from the natural products	44
1.4.5.1 Phytochemical screening test	44
1.4.5.2 Specification test	54

1.4.5.3 HPLC fingerprint profile	56
1.4.6 Topical pharmaceutical and cosmetic formulation development	56
1.4.6.1 Types of formulations	56
1.4.6.2 Formulation development	58
A. Chemical stability	59
B. Physical stability	60
1.4.7 <i>In vitro</i> anti-aging assays	61
1.4.7.1 DPPH radical scavenging assay	61
1.4.7.2 Ferrous metal chelating activity assay	62
1.4.7.3 Lipid peroxidation inhibition by ferric-thiocyanate complex activity assay	62
1.4.7.4 Tyrosinase inhibition assay	63
1.4.7.5 Gelatinolytic activity on MMP-2 inhibition (Zymography) in human skin fibroblasts	64
A. Types of collagen in the skin	65
B. Gelatinase A (MMP-2) in aging skin	65
C. MMP inhibitors for anti-aging skin	67
D. Zymography assay	68
1.4.7.6 Cytotoxicity by Sulphorhodamine B (SRB) assay in human skin fibroblasts	70
1.4.8 <i>In vivo</i> anti-aging biological assays	71
1.4.8.1 Irritation test in rabbit	71

1.4.8.2 Performance test in human volunteers	73
A. Skin elasticity measurement	73
B. Skin roughness measurement	75
C. Skin hydration measurement	76
D. Skin erythema and melanin measurements	77
<b>CHAPTER 2 MATERIALS AND METHODS</b>	<b>79</b>
2.1 Materials and equipments	79
2.1.1 Chemicals	81
2.1.2 Cell lines	81
2.1.3 Animals	81
2.1.4 Equipments	81
2.2 Methods	83
Part 1: Preparation and <i>in vitro</i> anti-aging activities of the crude extracts from leaves of Long Kong	84
1.1 Selection of the crude extract from eight parts of Long Kong	84
1.2 Preparation of the crude extracts	85
1.3 Characteristics of the crude extracts	85
1.3.1 Phytochemical test of the crude extracts	85
1.3.2 Determination of the total phenolic and flavonoid contents of the crude extracts	87
1.3.3 <i>In vitro</i> anti-aging activities of the crude extracts determination	88
1.3.4 Tyrosinase inhibition assay	90

1.3.5 Cytotoxicity of the crude extracts on human skin fibroblasts by sulforhodamine B (SRB) assay	91
1.3.6 Gelatinolytic activity on MMP-2 inhibition zymography of the crude extracts on human skin fibroblasts	92
1.4 Selection of the crude extract	93
Part 2: Preparation and <i>in vitro</i> anti-aging activities of the semi-purified extracts	93
2.1 Preparation of the semi-purified extracts from the selected crude extract	93
2.2 Characteristics of the prepared semi-purified extracts	94
2.2.1 Phytochemical tests of the semi-purified extracts	95
2.2.2 Determination of the total phenolic and flavonoid contents of the semi-purified extracts	95
2.2.3 <i>In vitro</i> anti-aging activities of the semi-purified extracts determination	95
2.2.4 Specification of the selected semi-purified extracts	95
2.3 Selection of the semi-purified extract	98
Part 3: Discoloration and <i>in vitro</i> anti-aging activities of the selected semi-purified extracts	99
3.1 Discoloration of the selected semi-purified extract	99
3.2 Characteristics of the discolored semi-purified fractions	99
3.3 Selection of the discolored semi-purified fractions	99
3.4 <i>In vivo</i> rabbit skin irritation by Draize test of the ethyl acetate semi-purified extract and the discolored water fraction	99

Part 4: Development of the cosmetic base formulations	100
4.1 Preparation of the cosmetic base formulations	100
4.2 Physical stability determination of the cosmetic base formulations by heating cooling cycles	103
4.3 <i>In vivo</i> rabbit skin irritation by Draize test	103
4.4 Selection of the cosmetic base formulations	103
Part 5: Development of the cosmetic formulations containing the extract from leaves of Long Kong	104
5.1 Preparation of the cosmetic formulations containing the selected extract from leaves of Long Kong	104
5.2 Physico-chemical stability investigation of the cosmetic formulations containing the selected extract from leaves of Long Kong	105
5.2.1 Physical stability determination	105
5.2.2 Chemical stability determination	105
5.3 Selection of the cosmetic formulations containing the extract from leaves of Long Kong	107
Part 6: <i>In vivo</i> anti-aging evaluation of the cosmetic formulations containing extract from leaves of Long Kong	108
6.1 <i>In vivo</i> irritation on rabbit skin by Draize test of the cosmetic formulations containing the semi-purified extract from leaves of Long Kong	108

6.2 <i>In vivo</i> anti-aging activities in human volunteers of the cosmetic formulations containing the selected extract from leaves of Long Kong	111
6.2.1 Subjects and study protocol	108
6.2.2 Skin elasticity measurement	109
6.2.3 Skin roughness measurement	110
6.2.4 Skin hydration measurement	111
6.2.5 Skin erythema and melanin measurements	111
6.2.6 Satisfactory questionnaires of the cosmetic formulations	111
CHAPTER 3 RESULTS AND DISCUSSION	113
Part 1: Preparation and <i>in vitro</i> anti-aging activities of the crude extracts from leaves of Long Kong	113
1.1 Preparation of the crude extracts	113
1.1.1 Percentages yield of the crude extracts	113
1.2 Characteristics of the crude extracts	114
1.2.1 Phytochemical constituents of the crude extracts	114
1.2.2 Total phenolic and flavonoid contents of the crude extracts	115
1.2.3 <i>In vitro</i> anti-aging activities of the crude extracts	116
1.2.4 Tyrosinase inhibition activity of the crude extracts	118
1.2.5 Cytotoxicity on human skin fibroblast of the crude extracts	119
1.2.6 Gelatinolytic activity on MMP-2 inhibition activity of the crude extracts	120
1.3 Selection of the crude extract	121



Part 2: Preparation and <i>in vitro</i> anti-aging activities of the semi-purified extracts	124
2.1 Percentages yield of the semi-purified extracts prepared from the selected crude extract	124
2.2 Characteristics of the prepared semi-purified extracts	124
2.2.1 Phytochemical analysis of the semi-purified extracts	124
2.2.2 Total phenolic and flavonoid contents of the semi-purified extracts	125
2.2.3 <i>In vitro</i> anti-aging activities of the semi-purified extracts	126
2.2.4 Tyrosinase inhibition activity of the semi-purified extracts	128
2.2.5 Cytotoxicity on human skin fibroblast of the semi-purified extracts	129
2.2.6 Gelatinolytic activity on MMP-2 inhibition activity of the semi-purified extracts	130
2.2.7 Specification of the semi-purified extract	132
2.3 Selection of the semi-purified extract	136
Part 3: Discoloration and <i>in vitro</i> anti-aging activities of the semi-purified extract	139
3.1 Percentages yield of the discolored fractions	139
3.2 Characteristic of the discolored fractions	139
3.2.1 Phytochemical tests of the discolored fractions	139
3.2.2 Determination of the total phenolic and flavonoid contents of the discolored fractions	140
3.2.3 <i>In vitro</i> anti-aging activities of the discolored fractions determination	141



3.2.4 Tyrosinase inhibition activity of the discolored fractions	143
3.2.5 Cytotoxicity on human skin fibroblasts of the discolored fractions	144
3.2.6 Gelatinolytic activity on MMP-2 inhibition activity of the discolored fractions	145
3.2.7 Specification of the selected discolored fractions	146
3.3 Selection of the discolored semi-purified fraction	150
3.4 <i>In vivo</i> rabbit skin irritation by Draize test of the ethyl acetate semi-purified extract and the discolored water fraction	151
Part 4: Development of cosmetic base formulations	153
4.1 Preparation of the cosmetic base formulations	153
4.2 Physical stability determination of the cosmetic base formulations by using heating cooling cycles	153
4.3 <i>In vivo</i> rabbit skin irritation by Draize test of the cosmetic base formulations	156
4.4 Selection of the cosmetic base formulations	157
Part 5: Development of cosmetic formulations containing the selected semi-purified extract prepared from the hot water crude extract from leaves of Long Kong	157
5.1 Preparation of the cosmetic formulations containing the semi-purified extract at various concentrations	157
5.2 Physico-chemical stability of the cosmetic formulations containing the semi-purified extract at various concentrations	158

Part 6: <i>In vivo</i> anti-aging activities on human volunteers of the cosmetic formulations containing the extract from leaves of Long Kong	165
6.1 <i>In vivo</i> rabbit skin irritation by Draize test of the cosmetic formulations containing the semi-purified extract from leaves of Long Kong	165
6.2 <i>In vivo</i> anti-aging activities in human volunteers of the cosmetic formulations containing the semi-purified extract from leaves of Long Kong	166
6.2.1 Skin elasticity	166
6.2.2 Skin roughness measurement	171
6.2.3 Skin hydration	173
6.2.4 Skin erythema and melanin	175
6.2.5 Satisfactory questionnaires on physical appearances of the cosmetic formulations	177
CHAPTER 4 CONCLUSION	178
REFERENCES	188
APPENDICES	215
APPENDIX A	216
APPENDIX B	218
APPENDIX C	220
APPENDIX D	225
APPENDIX E	227
CURRICULUM VITAE	230

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
1	The comparison between features of intrinsic and extrinsic factors of aging skin	8
2	Criteria used for distinguish types of aging according to their characteristics by Procter & Gamble beauty researchers	12
3	The laser beam used for aging skin treatments	17
4	The chemical structures of synthetic antioxidants used in cosmetics	19
5	Anti-aging compounds from natural resources	22
6	The global anti-aging market growth rate	23
7	Examples of anti-aging or wrinkle products in the markets	24
8	Chemical constituents of the essential oil from pulp of <i>L. domesticum</i>	32
9	Peak areas and quantities of organic acids in pulp of <i>L. domesticum</i>	33
10	Anti-malarial activity of natural compounds isolates from seeds of <i>L. domesticum</i>	34
11	Percentages of inhibitory effect on EBV-EA activation on Raji cell inducing with TPA of seventeen derivatives of 3-oxo-24-cycloarten-21-oic acid isolated from leaves of <i>L. domesticum</i> extract	35
12	Anti-bacterial activities of nine compounds isolated from ethanolic extract from the twigs of <i>L. domesticum</i>	36
13	cosmetic properties of extracts from various parts of <i>L. domesticum</i>	37
14	Food value per 100 g of edible portion of <i>L. domesticum</i>	38

15	Examples of the natural crude extracts and essential oil prepared from various plants for cosmetic purposes	39
16	Advantages and disadvantages of the popular extraction methods for crude extract preparation from plants	43
17	Phytochemical screening tests of the plant extracts	45
18	Examples of specification for standardized Ginkgo extract	55
19	Collagen family in the skin and other organs	66
20	Classification system for skin reactions	73
21	Response categories of irritation in rabbit	73
22	Definitions of skin elasticity indices by Cutometer	75
23	Parameters used in the assessment of skin visioscan® VC98	77
24	The descriptive term used for explaining solubility of the substance	96
25	Reagents used for the chemical resistant test	97
26	The descriptive definition of the chemical stability test	98
27	Compositions of the gel and serum base formulations	101
28	Compositions of the cream base formulations	102
29	Descriptions of the samples in skin anti-aging evaluation in human volunteers	109
30	The description of five levels of Likert's rating scale of the satisfactory score estimation of volunteers Amounts of the composition in the prepared nanovesicles	112
31	Percentages yield of the crude extracts from leaves of Long Kong prepared by six different processes	114

32	Phytochemical constituents of the crude extracts from leaves of Long Kong prepared by six different processes	115
33	Comparison of the antioxidant activities of the crude extract from leaves of Long Kong prepared by six different processes	117
34	Comparison of the <i>in vitro</i> tyrosinase inhibition of the six crude extracts from the leaves of Long Kong	119
35	Comparison of the percentages cell viability on human skin fibroblast of the crude extracts from leaves of Long Kong	120
36	Percentages yield of the semi-purified extracts prepared from the hot water crude extract from leaves of Long Kong	124
37	Phytochemical constituents of the semi-purified extract prepared from the hot water crude extract of leaves from Long Kong	125
38	Comparison of the <i>in vitro</i> antioxidant activities (DPPH radical scavenging, metal ion chelating and lipid peroxidation inhibition) of the three semi-purified extracts prepared from the hot water crude extract of leaves from Long Kong	127
39	Comparison of the <i>in vitro</i> tyrosinase inhibition of the three semi-purified extracts prepared from the hot water crude extract of leaves from Long Kong	129
40	Comparison of the percentages cell viability on human skin fibroblasts of the semi-purified extract prepared from the hot water crude extract of leaves from Long Kong	130
41	Physical appearances and pH values of the semi-purified extracts (0.1% dissolved in distilled water)	133

42	Solubility of the three semi-purified extracts prepared from the hot water crude extract of leaves from Long Kong	134
43	Chemical stability of the three semi-purified extracts prepared from the hot water crude extract of leaves from Long Kong	134
44	Percentages of the gallic acid contents in the hot water crude extract and its semi-purified extracts from leaves of Long Kong	135
45	Percentages yield of the discolored water and chloroform fractions	139
46	Phytochemical constituents of the discolored fractions prepared from the ethyl acetate semi-purified extract	140
47	Antioxidant activity of the discolored fractions prepared from the ethyl acetate semi-purified extract	142
48	Tyrosinase inhibition activity of the discolored fractions prepared from the ethyl acetate semi-purified extract	144
49	Cytotoxicity on the human skin fibroblasts of the discolored fractions prepared from the ethyl acetate semi-purified extract	145
50	Physical appearances and pH values of the 0.1% discolored fractions dissolved in distilled water	147
51	Solubility of the discolored water and chloroform fractions prepared from the ethyl acetate semi-purified extract of leaves from Long Kong	148
52	Chemical stability of the discolored fractions prepared from the ethyl acetate semi-purified extract from leaves of Long Kong	149
53	Percentages of the gallic acid contents of the discolored water fraction prepared from the ethyl acetate semi-purified extract	150



54	Comparison of the <i>in vitro</i> anti-aging activities of the ethyl acetate semi-purified extract and the discolored water fraction prepared from leaves of Long Kong	151
55	Primary irritation index (PII) and category of irritation based on PII of the ethyl acetate semi-purified extract and the discolored water fraction prepared from leaves of Long Kong	152
56	Physical appearances and characteristics (color, odor, texture and pH value) of the nine cosmetic base formulations kept at the heating and cooling cycle ( $4\pm 2$ and $45\pm 2^{\circ}\text{C}$ ) for 6 cycles (12 days)	154
57	Primary irritation index (PII) and category of the irritation based on PII of various cosmetic base formulations	155
58	The physical stability of the various cosmetic formulations containing the ethyl acetate semi-purified at 0.1 and 0.5% kept at $4\pm 2$ , $27\pm 2$ and $45\pm 2^{\circ}\text{C}$ at the initial and after stored for 3 months	158
59	The order of reactions, half lives and shelf lives of various cosmetic formulations stored at various temperatures ( $27\pm 2$ , $4\pm 2$ and $45\pm 2^{\circ}\text{C}$ )	161
60	Primary irritation index (PII) and category of irritation based on PII of various samples containing the ethyl acetate semi-purified extract at 0.1, 0.3 and 0.5%	165
61	Percentages of the parameter changes (%) of before application and after 4 weeks of application of various formulations and the untreated area	166
62	Percentages changes (%) of the skin roughness, skin hydration and skin melanin of before and after 4 weeks of application of various formulations and the untreated area	171



63	Percentages of the satisfaction parameter scale of the human volunteers on the cream formulations containing the semi-purified extract at 0.1, 0.3 and 0.5%	177
E1	General information summaries of human volunteers who have assessed for <i>in vivo</i> anti-aging activities and satisfaction evaluations	229
E2	Number out of 20 volunteers in the satisfaction evaluation of the physical appearance and organoleptic evaluation on the cream formulations containing the ethyl acetate semi-purified extract at 0.1, 0.3 and 0.5% (w/w)	229

## LIST OF FIGURES

Figure		Page
1	Comparison of the skin cross-sections of the younger and older skin	6
2	A schematic cross-section through layers of the different aged skin	10
3	The difference between line, wrinkle and fold of the facial aging skin base on the depth of crevices in each skin layer	11
4	Histological features of the healthy young skin (a), old skin (b) and photodamaged aged skin (c) Upper panels are 5 mm hematoxylin and eosin-stained sections (X160). Lower panels (X240)	14
5	The major injectable locations of the facial dermal filler treatments	16
6	Chemical structure of the nordihydroguariaretic acid (NDGA), a natural antioxidants extracted from leaves of <i>L. divaricata</i> which were used in drugs and cosmetics	21
7	The appearance of tree (a), leaves (b), flowers (c), young fruit (d), ripening fruit (e) and seeds (f) of <i>L. domesticum</i> Corr.	26
8	Chemical structures of the compounds exist in the seeds of <i>L. domesticum</i>	27
9	The structure of tetranortriterpenoids found in seed of <i>L. domesticum</i>	28
10	The chemical structure of onoceranoic triterpenes were isolated from the fruit peels of <i>L. domesticum</i> together with two known triterpenoids (methyl lasionate and lansic acid which exhibited mild toxicity against brine shrimp ( <i>Artemia salina</i> ))	29

11	The chemical structure of lansioside A and its derivatives obtained from dry peel ethanolic extract of <i>L.domestiucm</i>	30
12	The chemical structure of 3-oxo-24-cycloarten-21-oic which is a minor constituent of leaves of <i>L. domesticum</i>	30
13	Nine new onoceranoid type triterpenoids were isolated from the ethanolic extract of twigs of <i>L. domesticum</i>	31
14	Chemical derivatives of 3-oxo-24-cycloarten-21-oic acid isolated from leaves of <i>L. domesticum</i> extract which were further studied on <i>in vivo</i> anti-skin tumor in mouse skin	35
15	Structures of triterpenes isolated from <i>L. domesticum</i> which has insect antifeedant activity against rice weevil except 3-ketolansiolic acid	39
16	The UV/Vis spectroscopy data of <i>A. thaliana</i> extract removal chlorophyll using chloroform extraction. The spectrum of the extract before chlorophyll removal exhibited two maximum peak of chlorophyll (A), while the upper (B) and lower (C) layer which were aqueous phase and chloroform phase respectively showing the broad spectrum	44
17	Chemical structures of some common alkaloids	46
18	Chemical structures of flavonoid classes	47
19	Chemical structures of saponins	48
20	Common condensed tannin and hydrolysable tannin in plants	48
21	chemical structure of the reducing sugars which are qualitatively determined in the plant extracts by Fehling's solution test	49

22	Major anthocyanins containing in the purple corn	50
23	Common anthocyanins visible in various color edible plants	51
24	Chemical structure of carotenoids presence in all- <i>trans</i> and <i>cis</i> isomers of zeaxanthin and $\beta$ -carotene	52
25	Major steroids determined in plants such as ouabain from <i>Digitalis purpurea</i> and Proscillaridin A from <i>Urginea maritime</i> , and in animals such as marinobufagenin from <i>Bufo marinus</i>	53
26	Example of tetracyclic and pentacyclic triterpenoids	54
27	HPLC chromatograms of mixture of the marker compounds valerenic acid and acetoxyvalerenic acid (upper) and valerian tablets (lower)	57
28	The process of pharmaceutical and cosmetic formulation development	59
29	Reaction of the DPPH radical in the presence of the antioxidant during the DPPH assay	61
30	Melanogenesis pathway	64
31	Type of MMPs divided by substrate and catalytic domain	67
32	Zymograms of MMP-2 and -9 which exhibited distinguish molecular weight of pro- and active- enzymes	69

- 33 Skin deformation curve obtained with Cutometer. Cutometer parameters:  $U_e$  is the elastic deformation of the skin due to the application of stress (vacuum or torque) by the instrument;  $U_v$  is the visco-elastic creep occurring after the elastic deformation;  $U_f$  is the total extensibility of the skin;  $U_r$  is the elastic deformation recovery due to stress removal;  $U_a$  is the total deformation recovery at the end of the stress-off period;  $R$  is the amount of deformation not recovered by the end of the stress-off period 74
- 34 Examples of skin surface profiles of women evaluated with the surface evaluation of living skin method at week 0 (A) and after 12 weeks of consuming high flavanol cocoa beverages (B) The corresponding top view of the skin is shown in the lower Photographs 76
- 35 The reflectance of the simplified three-layer skin model. In the epidermis (layer 1), light is strongly absorbed by melanin and the non-absorbed part reaches the hemoglobin-rich papillary dermis (layer 2). The remaining part of the light is then diffusely reflected by dermal collagen (layer 3) 78
- 36 Scheme of the scope of the study 83
- 37 Scheme of purification of the water crude extract from old leaves of Long Kong which gave the three semi-purified extracts including ethyl acetate, butanol and water soluble fractions (in frames) 94

- 38 Comparison of the total phenolic and flavonoid contents of the crude extracts from leaves of Long Kong prepared by six different processes 116
- 39 The gelatinolytic activity of pro (A) and active (B) MMP-2 inhibition (% of the control) of the six crude extracts prepared by the six different processes of Long Kong leaves and ascorbic acid at 0.01, 0.1 and 1 mg/ml 122
- 40 Zymograms of pro and active MMP-2 inhibition of the six crude extracts prepared by the six different processes from leaves of Long Kong and ascorbic acid at 0.01, 0.1 and 1 mg/ml 123
- 41 Comparison of the phenolic and flavonoid contents of the three semi-purified extracts prepared from the hot water crude extract of leaves from Long Kong 126
- 42 The gelatinolytic activity of pro (A) and active (B) MMP-2 Inhibition (% of the control) from the three semi-purified extracts prepared from the hot water crude extract of leaves of Long Kong and ascorbic acid at 0.01, 0.1 and 1 mg/ml. 131
- 43 Zymograms of pro and active MMP-2 inhibition of the semi-purified fractions partitioned from the hot water crude extract from leaves of Long Kong and vitamin C at 0.01, 0.1 and 1 mg/ml and the negative control (5% DMSO) 132

- 44 Chromatograms of the gallic acid (1 mg/ml) at the retention time of 3.235 min and the hot water crude extract of leaves from Long Kong (20 mg/ml) which gave the gallic acid peak at the retention time of 3.188 min 137
- 45 Chromatograms of the water, butanol and ethyl acetate semi-purified extracts prepared from the hot water crude extract of the leaves from Long Kong showed the gallic acid peak at the retention time of 3.012, 3.147 and 3.272 min, respectively. 138
- 46 Total phenolic and flavonoid contents of the discolored fractions prepared from the ethyl acetate semi-purified extract 141
- 47 Comparison of the percentages of pro and active MMP-2 inhibition of the discolored water and chloroform fractions prepared from the ethyl acetate semi-purified extract of leaves from Long Kong 146
- 48 Chromatograms of the gallic acid and the discolored water fraction indicating the gallic acid peak at the retention time of 3.377 and 3.415 min, respectively 149
- 49 Physical appearance of serum No.1, gel No.1 and cream No.1 155
- 50 Propylene glycol base (A), the ethyl acetate semi-purified extract at 0.1 (B), 0.3 (C) and 0.5% (D) dissolved in propylene glycol, the commercial product (E), cream base (F) and cream formulations containing the ethyl acetate semi-purified extract at 0.1 (G), 0.3 (H) and 0.5% (I) 157



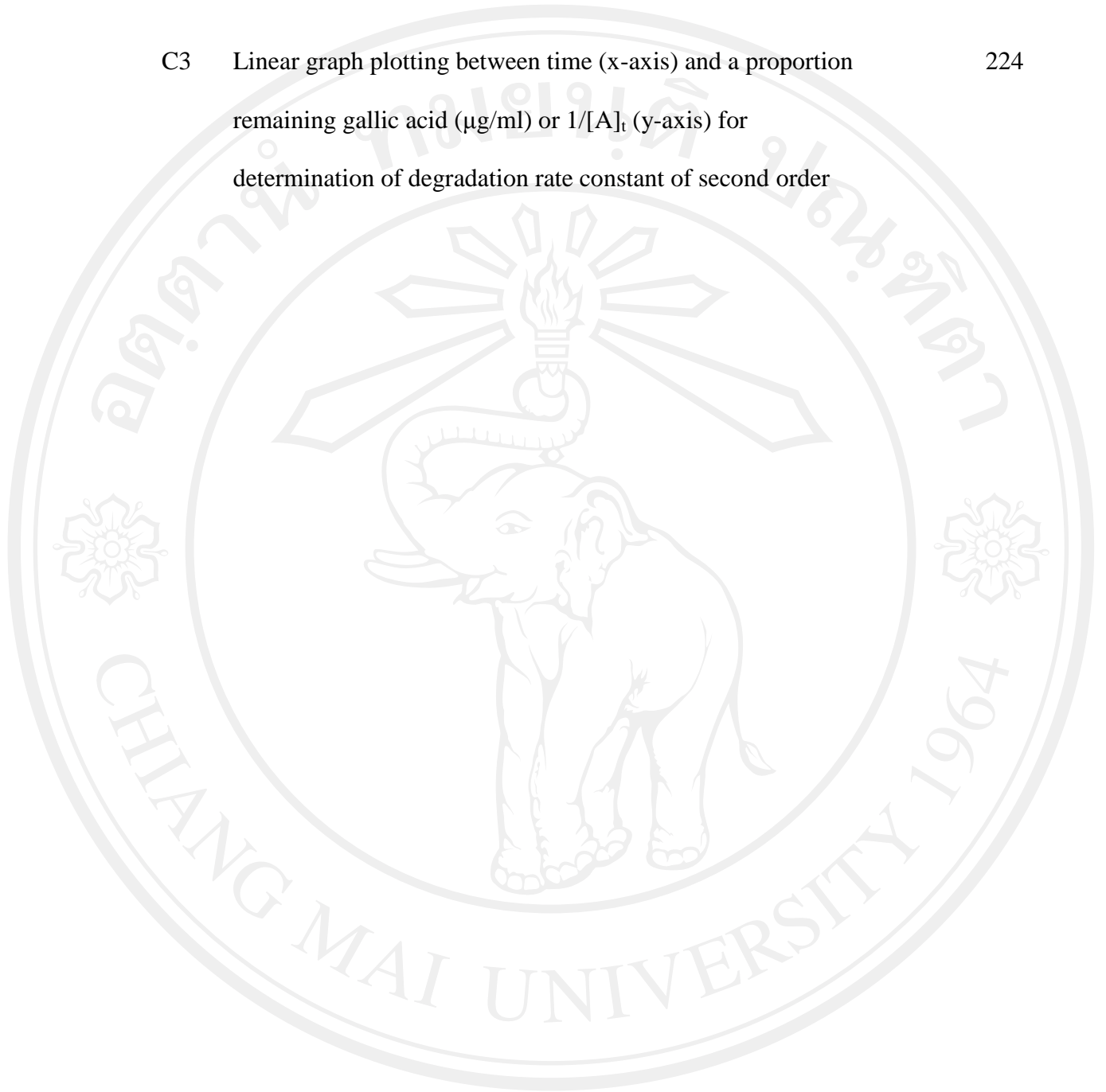
51	The percentages of gallic acid remaining in various cosmetic formulations at different storage temperatures ( $27\pm 2$ , $4\pm 2$ and $45\pm 2^\circ\text{C}$ ) for 3 months	160
52	Comparison of the skin elastic extension values (A) and the skin elastic recovery values (B) of the skin area treated between the cream formulations and propylene glycol solutions containing the extract at various concentrations, commercial product and the untreated area at before and after application for 1, 2,3 and 4 weeks	167
53	Comparison of the skin roughness of skin area treated with the various formulations at before, after applications and after 1 week of the wash-out period	171
54	Comparison of the skin hydration of the skin area treated with various formulations before application, after application and after 1 week of the wash-out period	173
55	Comparison of the skin melanin index of the various formulations before application, after applications and after 1 week of the wash-out period	175
C1	Linear graph plotting between time (x-axis) and remaining gallic acid ( $\mu\text{g/ml}$ ) or $[A]_t$ (y-axis) for determination of degradation rate constant of zero order	223
C2	Linear graph plotting between time (x-axis) and remaining gallic acid ( $\mu\text{g/ml}$ ) or (y-axis) for determination of degradation rate constant of first order	223

C3 Linear graph plotting between time (x-axis) and a proportion

224

remaining gallic acid ( $\mu\text{g/ml}$ ) or  $1/[A]_t$  (y-axis) for

determination of degradation rate constant of second order



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University  
All rights reserved

## ABBREVIATIONS AND SYMBOLS

CC <sub>50</sub>	chelating concentration at 50% activity
cm	centimeter
cm <sup>2</sup>	square centimeter
DI	deformability index
DLS	dynamic light scattering
D-MEM	dulbecco's modified eagle's medium
DMSO	dimethyl sulfoxide
DPPH	1, 1-Diphenyl-2-picrylhydracyl
EDTA	ethylenediaminetetraacetic acid
FBS	fetal bovine serum
g	gram
h	hour
HPLC	high performance liquid chromatography
IC <sub>50</sub>	tyrosinase inhibition concentration at 50% activity
IPC <sub>50</sub>	inhibition peroxidation concentration at 50% activity
kDa	kilodalton
kg	kilogram
M	molar
mg	milligram
ml	milliliter
mm	millimeter
MMP	matrix metalloproteinase

mV	millivolt
MW	molecular weight
nm	nanometer
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate-buffered saline
PII	primary irritation index
SC <sub>50</sub>	scavenging concentration at 50% activity
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	scanning electron microscope
SLS	sodium lauryl sulfate
SRB	sulphorodamine B
t <sub>50</sub>	half life
t <sub>90</sub>	shelf life
TEMED	N,N,N',N'-tetramethyl ethylenediamine
TFA	trifluoroacetic acid
Tween 20	polyoxyethylene sorbitan monolaurate
UV	ultraviolet
vs	versus
w/v	weight by volume
°C	celcius degree
µg	microgram
µl	microliter
µm	micromiter