TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT (THAI)	v
ABSTRACT (ENGLISH)	vii
LIST OF TABLES	xv
LIST OF FIGURES	xvii
ABBREVIATIONS	xix
CHAPTER 1 INTRODUTION	1
CHAPTER 2 LITERATURE REVIEW	5
2.1 Polysaccharides and their fundamental analyses	5
2.1.1 Fundamental analyses of polysaccharides	6
2.1.1.1 Chemical composition analysis	6
2.1.1.2 Monosaccharide analysis	6
2.1.1.3 Structural analysis of polysaccharides	7
2.1.1.4 Physical properties of polysaccharides	7
2.1.2 Classification of polysaccharides	. 9
2.1.2.1 Classification of polysaccharides by structures	9
2.1.2.2 Classification of polysaccharides by charges	10
2.1.2.2.1 Anionic polysaccharides	10
2.1.2.2.2 Cationic polysaccharides	10
2.1.2.2.3 Nonionic polysaccharides	11
2.1.2.2.4 Amphoteric polysaccharides	11
2.1.2.2.5 Hydrophobic polysaccharides	12
2.2 Skin structure and functions	12
2.2.1 Skin structure	13
2.2.2 Skin functions	15
2.3 Moisture in skin	16

	Page
2.4 Dry skin	16
2.4.1 Potential causes of dry skin	16
2.4.1.1 External causes	17
2.4.1.2 The skin's ability to retain moisture	17
2.4.2 The visible and tactile characteristics of dry skin	17
2.5 Moisturizers	18
2.5.1 Principal ingredients for preserving the moisture of the skin	18
2.5.2 Action of moisturizers on the skin	20
2.5.3 Hydrating substances /Moisturizing agent s	21
2.6 Free radicals	22
2.6.1 Sources of free radical	23
2.6.2 Classification of free radicals	25
2.6.3 Antioxidant defenses	29
2.6.4 In vitro methods to determine antioxidant activities	30
2.6.4.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl radical)	30
radical scavenging assay	
2.6.4.2 Trolox equivalent antioxidant capacity (TEAC)	32
assay (ABTS ⁺⁺ decolorization assay)	
2.6.4.3 Thiobarbituric acid-reactive substances (TBARS)	33
2.7 Phenolic compounds	35
2.8 Algae materials	36
CHAPTER 3 MATERIALS AND METHODS	39
3.1 Chemicals	39
3.2 Algae materials	40
3.3 Instruments	40
3.4 Research designs	42
3.5 Collection and identification	43
3.6 Extraction of the algae	43
3.7 Determination of antimicrobial activities	43

	Page
3.8 Determination of antioxidant activities	44
3.8.1 Determination of antioxidant activity by free radical	44
scavenging activity on 2,2'-diphenyl-1-picrylhydrazyl	
(DPPH [•])	
3.8.2 Determination of antioxidant activity by free radical	45
scavenging activity on 2,2'-azino-bis3-ethylbenzothiazoline-	
6-sulfonic acid (ABTS*+)	
3.8.3 Determination of antioxidant activity by inhibition of lipid	46
peroxidation using the thiobarbituric acid reaction	
substances (TBARS) assay	
3.9 Determination of total phenolic content in R. hieroglyphicum	46
extract	
3.10 Content analysis of RW extract	47
3.10.1 Phenol-sulfuric acid method	47
3.10.2 Protein analysis	47
3.10.2.1 Bradford protein assay	47
3.10.2.2 Amino acid analysis	47
3.10.3 Polysaccharide analysis	48
3.10.3.1 Fourier transform infrared (FT-IR) spectroscopy	48
3.10.3.2 Determination of the molecular weight by	48
size exclusion chromatography (SEC) mass/molar	
3.10.3.3 Sugar analysis	48
3.11 Physical and physicochemical properties	50
3.11.1 Morphology and observation of algae extracts using	50
scanning electron microscope (SEM)	
3.11.2 Textural properties	50
3.11.3 Gelling and melting temperature	50
3.11.4 Solubility test	51
3.11.5 Acid-base solubility test	51
3.12 Primary skin irritation testing on animal	51

	Page
3.13 Skin moisturizing tests	53
3.13.1 Moisturizing test on pig skin	53
3.13.2 Moisturizing test on human skin	54
3.14 Formulation and stability test of the cream base	54
3.14.1 Formulation of the cream base	54
3.14.2 Stability test of the cream base	55
3.15 Formulation and stability test of moisturizing cream	55
3.16 Clinical evaluation	55
3.16.1 Skin irritation testing in human volunteers	55
3.16.2 Subjects of the study	56
3.16.3 Test substance application protocol	57
3.17 Performance test and satisfaction of volunteers	57
3.17.1 Volunteers of the study	58
3.18 Statistical analysis	58
CHAPTER 4 RESULTS	59
4.1 Identification of freshwater macroalga	59
4.2 Extraction of R. hieroglyphicum	60
4.3 Antimicrobial activities of R. hieroglyphicum extracts	61
4.4 Antioxidant activities of R. hieroglyphicum extracts	62
4.5 Content analysis of RW extract	65
4.5.1 Phenol-sulfuric acid method	65
4.5.2 Protein and amino acid analysis	65
4.5.3 Polysaccharide analysis	67
4.5.3.1 FT-IR spectroscopy	67
4.5.3.2 Sugar analysis and molecular weight	68
4.5.3.3 SEC profiles of RW extract	69
4.6 Physicochemical properties of the RW extract	71
4.6.1 Morphology and observation of the R. hieroglyphicum	71
extracts using scanning electron microscope (SEM)	
4.6.2 Textural properties	71

	Page
4.6.3 Gelling and melting temperatures	74
4.6.4 Solubility tests	75
4.6.5 Acid-base tolerance and stability test	80
4.7 Primary skin irritation testing on animal	85
4.8 Moisturizing tests	86
4.8.1 Moisturizing test on pig skin	86
4.8.2 Moisturizing test on human skin	87
4.9 Formulation and stability test of the cream base	89
4.9.1 Formulation of the cream base	89
4.9.2 Stability test of selected cream bases	90
4.10 Formulation and stability test of moisturizing creams	91
4.11 Clinical evaluation	94
4.11.1 Skin irritation test	94
4.11.2 Skin moisturizing test	94
4.11.3 Satisfaction of RW cream and test creams by volunteers	97
CHAPTER 5 CONCLUSION	102
REFERENCES	105
APPENDICES	128
APPENDIX A Certificate of ethical clearance	129
APPENDIX B Information sheet	131
APPENDIX C Informed consent sheet	137
APPENDIX D Satisfaction questionnaire	139
APPENDIX E Bradford protein assay	143
APPENDIX F Texture profile analysis	145
CURRICULUM VITAE	147

LIST OF TABLES

Table		Page
2.1	Members of polysaccharides found in Algae	6
2.2	Types of free radicals	25
3.1	Preparation of standard sugar solutions A and B	49
3.2	Draize-FHSA Scoring System	52
3.3	Classification of skin irritation	53
4.1	IC ₅₀ , standard equivalent of antioxidant activities and total phenolic	63
	content of R. hieroglyphicum and S. platensis	
4.2	Amino acid composition and % of total amino acid value of the	66
	RW extract	
4.3	Gelling properties of the RW extract compared to kappa-carrageenan	74
4.4	Dissolution and visual appearance of the R. hieroglyphicum	78
	extract (RW)	
4.5	Acid-base stability test of the RW extract (10% w/v in H ₂ O) before	81
	and after 1 month storage at various conditions	
4.6	Acid-base stability test of the RW extract (5% w/v in H ₂ O) before	82
	and after 1 month storage at various conditions	
4.7	Acid-base stability test of the RW extract (2% w/v in H ₂ O) before	83
	and after 1 month storage at various conditions	
4.8	Primary dermal irritation index (PDII) and skin irritation reaction	86
	in rabbits	
4.9	Physical appearances of five cream base formulations	89
4.10	Physical appearances of A cream base after stability test for	90
	1 month	
4.11	Physical appearances of active formulations and their cream base	91
4.12	pH and viscosity of test creams after various storage conditions	93
	for 6 months and the heating/cooling condition of 8 cycles.	

xvi

4.13	Primary dermal irritation index (PDII) and skin irritation reaction	94
	in 30 volunteers	
4.14	The percentage of satisfaction on CRW and CHA cream	99
4.15	The percentage of satisfaction on CG and CPG cream	100
4.16	The percentage of satisfaction on CB cream	101

LIST OF FIGURES

Figure		Page
2.1	Structure and layers of skin	12
2.2	Layers of the epidermis	13
2.3	Major sources of free radicals in the body and the consequences	24
	of free radical damage	
2.4	Antioxidants defenses against free radicals attack	30
2.5	Reaction for DPPH radical scavenging assay	31
2.6	Reaction for ABTS** decolorization assay	32
2.7	Pathways of lipid peroxidation	34
3.1	Application sites of moisturizing test	58
4.1	The morphology of R. hieroglyphicum (C.Agardh) Kützing	59
4.2	The appearance of the R. hieroglyphicum extract	61
4.3	GC-MS chromatogram of the R. hieroglyphicum extract (RW)	65
4.4	FT-IR spectrum of the R. hieroglyphicum extract (RW)	68
4.5	The TLC plate shows the oligosacchrides of fraction 2 compared	69
	with standards	
4.6	SEC profiles of RW extract before $\hfill\Box$ and after Δ MeOH treatment	70
4.7	Molecular weight distributions from SEC-mass/molar: distribution	70
	of mass fractions and distribution of molar fractions	
4.8	SEM images of RW extracts powder	71
4.9	Texture profile analysis of the R. hieroglyphicum extract (RW)	72
4.10	Texture profile analysis of the kappa-carrageenan	73
4.11	Visual appearance of the kappa-carrageenan and RW gels	74
4.12	Characteristic solubility of the R. hieroglyphicum extract (RW)	76
	at room temperature	
4.13	Characteristic solubility of the R. hieroglyphicum extract (RW)	77
	at 60 °C	

xviii

4.14	Visual appearance of the RW extract at pH 2-9	84
4.15	Rabbit skin primary irritation test of test substances	85
4.16	Moisture increasing on pig skin after application of the test	87
	substances for 5, 15 and 30 min	
4.17	Moisture increasing on human skin after application of the test	88
	substances for 10, 15 and 30 min	
4.18	Moisture increasing after application of the test cream for	95
	15, 30 min and1 hour	
4.19	Moisture increasing after application of the tested cream for 1 week	96
4.20	Satisfaction of volunteers on moisturizing effects of the test creams	98