

## TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	<b>ABSTRACT</b>	iii
	<b>LIST OF TABLES</b>	iv
	<b>LIST OF FIGURES</b>	v
	<b>LIST OF SYMBOLS</b>	xiii
<b>1</b>	<b>INTRODUCTION</b>	1
<b>2</b>	<b>LITERATURE OVERVIEW</b>	8
	2.1 Marine Natural Products	8
	2.2 Red Algae	14
	2.3 <i>Gelidiella acerosa</i>	15
	2.4 Cancer	16
	2.5 Molecular mechanism of metastasis	18
	2.5.1 Tumor cell disintegration and EMT.	18
	2.5.2 Invasion and cell migration	18
	2.5.3 Anoikis	18
	2.5.4 Angiogenesis	19
	2.5.5 Outgrowth of secondary tumors	19
	2.5.6 Metastatic cancer stem cells	19
	2.5.7 Contribution of the microenvironment	20
	2.6 Apoptosis	20
	2.6.1 The mitochondrial pathway	21
	2.6.2 NF $\kappa$ B	21
	2.6.3 PI3K/Akt/GSK3B signaling cascade	22
	2.7 Lung cancer	22
	2.7.1 The PI3K signaling cascade	23
	2.8 Chemoprevention	24

	2.8.1 Mechanism of chemoprevention	24
	2.8.2 Phytochemicals as Chemo preventive agents	24
	2.9 Molecular Docking	25
	2.10 Animal models in drug research.	26
	2.10.1 Zebrafish as animal model	26
	2.11 Tissue chip technology	28
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>30</b>
	3.1 Seaweed collection and processing	30
	3.2 Extraction of the algae	30
	3.3 Phytochemical screening	30
	3.4 Quantification of Polyphenols	34
	3.5 Quantification of Flavonoids	34
	3.6 Characterization of crude extracts	34
	3.6.1 Fourier transform infrared (FT-IR) spectrometry	34
	3.6.2 High-performance liquid chromatography (HPLC)	35
	3.6.3 Determination of antioxidant activity	35
	3.7 Isolation and characterization and identification of compounds from Crude ethyl acetate extract (GAE)	36
	3.7.1 Characterization of compounds <sup>1</sup> H and <sup>13</sup> C Nuclear Magnetic Resonance spectroscopy analysis of GAE compounds.	36
	3.7.2 GC-MS analysis of GAE and GACs	37
	3.8 <i>In silico</i> studies	37
	3.8.1 Preparation of Protein	37
	3.8.2 Preparation of ligand	38
	3.8.3 Molecular docking	38
	3.8.4 Post-docking analysis	38
	3.9 Determination of Toxicity	39

3.9.1	Acute and chronic toxicity analysis in Zebrafish	39
3.9.2	Novel Tissue-Chip for toxicity screening	39
3.10	Determination of anticancer activity under <i>in vitro</i> conditions	40
3.10.1	Cell lines and maintenance	40
3.10.2	Analysis of cell viability (MTT assay)	40
3.10.3	Analysis of Apoptosis	41
3.10.3.1	Cell biology studies	41
3.10.3.2	Flow cytometry analysis	42
3.10.3.3	Immunoblot analysis of apoptosis and cell survival cascade	42
3.11	Determination of antimetastatic activity	43
3.11.1	Scratch assay	43
3.11.2	Clonogenic assay	43
3.11.3	Immunoblot analysis of MMP expression	44
3.12	Determination of anti-inflammatory activity	44
3.12.1	Analysis of NFkB activity	44
3.12.2	Analysis of anti-inflammatory marker	45
3.12.3	Analysis of gene expression by Real-Time PCR	45
3.12.3.1	Isolation of RNA	45
3.12.3.2	Synthesis of cDNA	46
3.12.3.3	Real-Time PCR	46
3.13	Determination of anticancer activity under <i>in vivo</i> conditions	48
3.14	Statistical analysis	48

<b>4</b>	<b>EXTRACTION, ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM <i>G.ACEROSA</i></b>	<b>49</b>
	4.1 Introduction	49
	4.2 Methods	49
	4.3 Results	49
	4.3.1 Qualitative screening of phytochemicals in <i>G.acerosa</i>	49
	4.3.2 Quantitative screening of phytochemicals	51
	4.3.3 HPLC analysis of algal extracts	52
	4.3.4 FT-IR analysis of algal extracts	55
	4.3.5 Open column chromatography of ethyl acetate extract from <i>G.acerosa</i>	59
	4.3.6 Structural analysis of GAC 1	60
	4.3.7 Structural analysis of GAC 2	61
	4.4 Discussion	64
	4.4.1 Extraction of <i>G.acerosa</i>	64
	4.4.2 Qualitative screening of phytocompounds in <i>G.acerosa</i>	64
	4.4.3 Quantitative screening of phytocompounds in <i>G.acerosa</i>	65
	4.4.4 HPLC analysis of <i>G.acerosa extracts</i>	65
	4.4.5 FT-IR analysis revealed the presence of various functional groups in <i>G.acerosa</i>	66
	4.4.6 Isolation and characterization of pure compounds from <i>G.acerosa</i>	67
	4.4. 6.1 Open column chromatography of ethyl acetate extract (GAE)	67
	4.4.6.2 NMR and GC-MS analysis of GACs	67

	4.5 Conclusion	68
<b>5</b>	<b>EVALUATION OF INTERACTION OF GACS WITH PROTEINS INVOLVED IN APOPTOTIC, CELL SURVIVAL AND ANTI-INFLAMMATORY PATHWAYS (BY <i>IN SILICO</i> METHODS)</b>	<b>69</b>
	5.1 Introduction	69
	5.2 Methods	69
	5.3 Interaction of GACs with apoptotic proteins	69
	5.3.1 Interaction of GACs with caspase 3	69
	5.3.2 Interaction of GACs with caspase 8	72
	5.3.3 Interaction of GACs with Bax	74
	5.3.4 Interaction of GACs with Bcl2	76
	5.3.5 Interaction of GACs with Bcl-XL	78
	5.4 Interaction of GACs with proteins regulating cell survival	81
	5.4.1 Interaction of GACs with PI3K.	81
	5.4.2 Interaction of GACs with Akt	83
	5.4.3 Interaction of GACs with GSK3 $\beta$	85
	5.4.4 Interaction of GACs with PTEN	87
	5.5 Interaction of GACs with anti-inflammatory protein complex	91
	5.6 Discussion	93
	5.7 Conclusion	96
<b>6</b>	<b>EVALUATION OF THE ANTIOXIDANT PROPERTY OF THE RED ALGAL EXTRACT (GAE) AND THE ISOLATED COMPOUNDS (GAC 1- 4) UNDER <i>IN VITRO</i> CONDITION</b>	<b>97</b>
	6.1 Introduction	97
	6.2 Methods	97
	6.3 Results	97
	6.3.1 Antioxidant activity of crude algal extracts	97

	6.3.2 Antioxidant activity of GACs	99
	6.3.3 GAE and GACs enhanced SOD and POX activities	100
	6.4 Discussion	102
	6.4.1 <i>G. acerosa</i> exhibited antioxidant activity	102
	6.4.2 GAE and GACs induced SOD and POX activities	102
	6.5 Conclusion	103
<b>7</b>	<b>EVALUATION OF THE ANTICANCER AND ANTI-INFLAMMATORY PROPERTIES OF THE RED ALGAL EXTRACT (GAE) AND THE ISOLATED COMPOUNDS (GAC 1- 4) UNDER <i>IN VITRO</i> CONDITION</b>	<b>104</b>
	7.1 Introduction	104
	7.2 Methods	104
	7.3 Results	104
	7.3.1 GAE and GACs are cytotoxic under <i>in vitro</i> conditions	104
	7.3.2 GAE and GACs induced apoptosis	109
	7.3.3 GAE and GACs activated the intrinsic pathway of apoptosis	112
	7.3.4 Effect of GAE on cell migration	121
	7.3.5 GAE treatment suppressed colonization	123
	7.3.6 GAE and GACs suppressed MMP2 level	123
	7.3.7 GAE and GACs modulated NFκB expression	126
	7.4 Discussion	131
	7.4.1 GAE and GACs induced apoptosis	132
	7.4.2 GAE and GACs activated the intrinsic pathway of apoptosis	133
	7.4.3 GAE and GACs activated GSK3β	135

	7.4.4 GAE and GACs affected PI3K/Akt expression	135
	7.4.5 GAE inhibited cell migration, colonization through MMP2	137
	7.4.6 GAE and GACs affect NFκB expression	138
	7.5 Conclusion	140
<b>8</b>	<b>EVALUATION OF THE TOXICITY, ANTICANCER AND ANTI-INFLAMMATORY PROPERTIES OF THE RED ALGAL EXTRACT (GAE) AND ISOLATED COMPOUNDS (GAC 1- 4) UNDER <i>IN VIVO</i> CONDITIONS IN TUMOR MODELS OF ZEBRAFISH</b>	<b>142</b>
	8.1 Introduction	142
	8.1.1 Acute Toxicity analysis	142
	8.1.2 Chronic Toxicity analysis	143
	8.2 Methods	143
	8.3 Results	144
	8.3.1 GAE is not toxic in animals	144
	8.3.2 GAE and GACs inhibited lung tumor proliferation <i>in vivo</i>	147
	8.3.3 GAE and GACs inhibited PI3K/Akt/NFκB pathway	151
	8.4 Discussion	158
	8.5 Conclusion	160
<b>9</b>	<b>DISCUSSION</b>	<b>161</b>
<b>10</b>	<b>CONCLUSION</b>	<b>170</b>
<b>11</b>	<b>SCOPE FOR FURTHER WORK</b>	<b>170</b>
	<b>REFERENCES</b>	<b>172</b>
	<b>TECHNICAL BIOGRAPHY</b>	<b>199</b>

