

Microalgae-Derived Peptides Targeting Lifestyle-Related Diseases: Discovery, Mechanisms, Structure-Activity Relationships and Structural Modifications

Mohammed S. Qoms¹, Sok Kuan Wong², Norsyahida Mohd Fauzi³, Khairana Husain³,
Suzana Makpol¹, Jen Kit Tan^{1,*}

¹Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

²Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

³Centre for Drug and Herbal Development, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

***Corresponding author**

Jen Kit Tan

E-mail: jenkittan@ukm.edu.my

Table S1: Antioxidant peptides derived from different microalgae species discovered in the past ten years

Peptide source/sequence	MW (Da)	PCL	Assay model/ Effective concentration	Key findings/mode of action	Ref.
			Cell-free & molecular docking		
<i>Haematococcus pluvialis</i>					
GVFHPPGF IDPGTWRPL APIIGKPAGFK	856 1053 1194	8 9 12	DPPH, ABTS, HRS, FRAP 0.33- 1.10 mg/mL 0.14- 0.89 mg/mL 0.25- 0.78 mg/mL	- Strong radical scavenging and reducing power activity - Interacted with Keap1 through hydrogen bonds and hydrophobic interactions - interacted with DPPH and ABTS via hydrogen bonds	[105]
<i>Auxenochlorella pyrenoidosa</i>					
AGWACLVG YPLDL IDLAY	776 620 594	8 5 5	DPPH 200 µg/mL	- Strong DPPH radical scavenging activity ranging between 81.11-89.31% - IC ₅₀ = 53.45 µg/mL (68.88 µM) only reported for AGWACLVG	[89]
<i>Schizochytrium limacinum</i>					
PYK	406	3	DPPH, HRS 0.10-1.00 mg/mL	- Comparable radical scavenging to the positive control L-glutathione (GSH)	[110]
<i>Arthrospira platensis</i>					
VTAGLVGGGAGK	986	12	ABTS, HRS, FIC 1.08-1.35 mg/mL	- Highly stable radical scavenging under thermal processing (25-100°C) and pH conditions (pH 3-11) as well as during <i>in-vitro</i> gastrointestinal digestion	[48]
<i>Synechococcus</i> sp. VDW					
AILQSYSAGKTK ALNKTHLIQT IPDAHPVK LLVHAPVK VVVLRDGAVQQLGTPR	1266 1266 876 876 1708	12 11 8 8 16	NO 200 µg/mL	- Moderate NO scavenging activity (10.38-20.89%).	[111]
<i>Tetrademus obliquus</i>					
WPRGYFL SDWDRF	937 824	7 6	DPPH, ABTS 4.70-13.97 µM	- Dose-dependent radicals scavenging activity with relatively low IC ₅₀ .	[109]
			Cell model		
<i>Arthrospira platensis</i>					
INSSDVQGKY	1109	10	H ₂ O ₂ -stressed HaCaT cells 100-400 µg/mL	- Good <i>in-vitro</i> ORAC (0.32 mmol Trolox equivalent/g) ↓ ROS levels in HaCaT cells Non-toxic to HaCaT cells	[49]
NPLSTQDDVAASL LGLDVWEHAYYL GGTCVIRGCVPKKLM	1330 1478 1561	13 12 15	H ₂ O ₂ -stressed leucocytes 7.8-250 µM 6.25-200 µM 6.25-200 µM	- Dose-dependent radical scavenging activity (DPPH, ABTS, HRS, SARS and NO) ↓ ROS production in the leucocytes - Non-toxic to human blood and Vero cells	[99–101]
EYFDALA GMCCSR	828 655	7 6	AAPH-stressed erythrocytes 1.2-120 µM 1.5-150 µM	- Dose-dependent DPPH (IC ₅₀ =5.80 & 16.94 µM) and ABTS (IC ₅₀ 13.40 & 36.93 µM) radical scavenging activity, comparable to the positive control vitamin C ↓ Oxidative haemolysis of erythrocytes ↓ Lipid peroxidation (MDA) ↑ Antioxidative enzymes (SOD, CAT, GPx) - Non-toxic to human erythrocytes and L929 cells	[96,102]
<i>Isochrysis zhanjiangensis</i>					
IIAVEAGC IIAVE AGC	774 543 249	8 5 3	H ₂ O ₂ -stressed SH-SY5Y cells 20-100 µM	↓ ROS and mitochondrial damage ↑ Antioxidant enzymes (HO-1 and SOD) ↑ Nrf2 expression pathway ↓ PI3K, MAPK and NF-κB signalling pathways - Non-toxic to SH-SY5Y cells	[94]
AYAPE	549	5	H ₂ O ₂ -stressed HUVEC and SH-SY5Y cells 20-100 µM	↑ Antioxidant enzymes (HO-1 and GPx1) ↑ Anti-apoptotic (↓ expression of P53 and cytochrome c proteins) ↑ Anti-inflammatory (↓ pro-inflammatory factors IL-6 and IL-1β)	[107]

				↓ NF-κB and MAPK signalling pathways (↓ phosphorylation of JNK and P38 proteins, and ↓ P65, P50 and Iκβ-α) ↑ Nrf2/Keap1 pathway - Non-toxic to HUVEC and SH-SY5Y cells	
DAPTMGY	753	7	LPS-stressed HUVEC 1-50 μM	↓ Mitochondrial oxidative stress ↓ ROS production ↑ Antioxidative enzymes (SOD, GPx, HO-1) ↓ Inflammatory proteins (COX-2/ iNOS) ↓ NF-κB and MAPK signalling pathways - Non-toxic to HUVEC cells	[108]
NDAEYGICGF	1088	10	Ethanol-stressed HepG2 cells 10-100 μM	↓ ROS production ↑ Antioxidant enzyme SOD ↓ GGT - Non-toxic to HepG2 cells	[59]
<i>Chlorella pyrenoidesa</i>					
AGYSPIGFVR VLDELTAR LFDPVYLFQDG	1066 1029 1313	10 9 11	AAPH-stressed HepG2 cells 0.04 mg/mL 0.09 mg/mL 0.41 mg/mL	↓ ROS production Non-toxic to HepG2 cells	[87]
<i>In-vivo</i>					
<i>Haematococcus pluvialis</i>					
KFTPAP	659	6	<i>Caenorhabditis elegans</i> (Nematode) 100 μM	↑ Antioxidant enzymes SOD, CAT ↓ Lipid peroxidation (MDA) - Non-toxic to nematodes	[106]
<i>Arthrospira platensis</i>					
VKYVSPTCGPCH	1290	12	H ₂ O ₂ -stressed zebrafish and cardiomyocytes cells 6.25-200 μM	- Dose-dependent <i>in-vitro</i> radical scavenging activity (DPPH, ABTS, HRS and SARS) ↓ ROS production in zebrafish and cardiomyocytes cells - Non-toxic to human blood and H9c2 cells	[103]
<i>Arthrospira platensis</i>					
TGCPNGCARPYMAEL GIAVRAGHHCAQP	1582 1316	15 13	H ₂ O ₂ -stressed zebrafish larvae and leucocytes 7.8-125 μM 10-80 μM	- Dose-dependent <i>in-vitro</i> radicals scavenging activity (DPPH, ABTS, SARS and HRS) ↓ ROS production of leucocytes and larvae ↑ Antioxidant enzymes (SOD, CAT) - Non-toxic to blood cells and zebrafish larvae	[93,104]
<i>Arthrospira platensis</i>					
GGGAFSGKDPTKVDR	1490	15	H ₂ O ₂ -stressed zebrafish larvae and MDCK cells 5-45 μM	- Dose-dependent <i>in-vitro</i> radicals scavenging activity (ABTS, SARS, HRS and NO) ↓ Oxidative stress and apoptosis of MDCK cells and zebrafish larvae ↑ Antioxidant enzymes (SOD, CAT) ↑ mRNA antioxidant enzymes gene expression (GPx, GST, GCS) - Non-toxic to MDCK and zebrafish larvae	[95]

AAPH: 2,2'-Azobis(2-amidinopropane) dihydrochloride; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); CAT: catalase; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; GCS: glutamyl cysteine synthetase; GGT: gamma-glutamyl transferase; GPx: glutathione peroxidase; GST: glutathione S-transferase; H₂O₂: hydrogen peroxide; H9c2: embryonic rat cardiomyocytes cells; HaCaT: human immortal keratinocyte line; HepG2: human liver cancer cells; HO-1: heme oxygenase-1; HRS: hydrogen radical scavenging; HUVEC: human umbilical vein endothelial cells; L929: normal mouse fibroblasts; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinases; MDA: malondialdehyde; MDCK: Madin-Darby canine kidney cells.; MW: molecular weight; NF-κB: nuclear factor κB; NO: nitric oxide; Nrf2: nuclear erythroid 2-related factor 2; ORAC: oxygen radical antioxidant capacity; PCL: peptide chain length; SARS: superoxide anion radical scavenging; SH-SY5Y: Human neuroblastoma cells; SOD: superoxide dismutase; Vero cells: African green monkey kidney fibroblast

Table S2: Antihypertensive peptides derived from different microalgae species discovered in the past ten years

Peptide source/sequence	MW (Da)	PCL	IC ₅₀ /Model/Dose	Inhibitory pattern/key findings	Ref.
<i>Arthrospira platensis</i>			Cell-free ACE inhibition (IC₅₀) & molecular docking		
ILLYR	676	5	10.54 µM	- Non-competitive inhibitor - Interacted with ACE via hydrogen bonds, and bound to C12 binding site - Relatively stable under gastrointestinal conditions (IC ₅₀ = 23.35 µM)	[124]
VTY	381	3	23.39 µM	- VTY interacted with ACE active pockets (S1' & S2') through hydrogen bonds, while LGVP formed fewer hydrogen bonds with S1 active pocket	[114]
LGVP	384	4	45.76 µM		
IRDLDDYY	957	7	1748 µM	- Interacted with ACE active pocket via hydrogen bonds - Non-toxic to Vero and human dermal fibroblast cells	[85]
PTGNPLSP	781	8	1971.50 µM	- High stability under temperature (25-100°C), pH (3-11) and gastrointestinal digestion	[48]
<i>Chlorella pyrenoidosa</i>					
LKKAP	555	5	36.19 µM	- Non-competitive inhibitors - Interacted with ACE active pockets S1, S2 and/or S' via hydrogen bonds and salt bridges - High stability under pH (pH 2-12) and temperature (20-100°C) - High gastrointestinal stability and released new di/tri-peptides with low IC ₅₀ (AP=230 µM, VAK=85.43 µM, PGL=13.93 µM and RP=15.20 µM.	[20]
LVAKA	500	5	26.66 µM		
PGLRP	538	5	44.78 µM		
<i>Chlorella sorokiniana</i>					
VW	303	2	580 nM	- Potent ACE inhibitory activity, particularly with those containing tryptophan at the C-terminal	[51]
IW	317	2	500 nM		
LW	317	2	1.11 µM		
WV	303	2	307.61 µM		
<i>Isochrysis galbana</i>					
YMGLDLK	839	7	36.10 µM	- Non-competitive inhibitor - Highly stable under gastrointestinal digestive enzymes	[126]
<i>Tetrademus obliquus</i>					
GPDRPKFLGPF	1230	11	5.73 µM	- Exhibited a dose-dependent ACE inhibitory activity with low IC ₅₀	[109]
WYGPDRPKFL	1278	10	820 nM		
<i>Nannochloropsis oculata</i>					
NKFPYTTQ	998	8	1.00 µM	- Most of the peptides showed strong ACE inhibitory activity with low IC ₅₀ of 1-1.65 µM	[128]
LVGADAHALGVICS	1325	14	1.17 µM		
VVGAVGAADLL	984	11	1.01 µM		
GDVGLF	606	6	1.65 µM		
KGGGSGANGGRL	1030	12	481.64 µM		
AGDVGFDPGLGF	1094	11	913.92 µM		
VYNKFPYTTQ	1260	10	793.39 µM		
YANDLLCMPI	1152	10	867.76 µM		
			Cell/in-vivo models & molecular docking		
<i>Isochrysis zhanjiangensis</i>					
FEIHCC	750	6	- Cell-free ACE inhibition (IC ₅₀) 61.38 µM - Cell model Ang II-induced HUVEC 10-100 µM	- Non-competitive inhibitor - Interacted with ACE and VCAM-1 through hydrophobic interaction and hydrogen bonds, and bound to ACE with C12 binding site (Arg522) ↓ Ang II-induced vascular dysfunction (↓ICAM-1, VCAM-1, MCP-1, iNOS & ET-1) and ROS ↓ Inflammation and apoptosis through NF-κB, Nrf2, MAPK, and Akt signalling pathways - Non-toxic to HUVEC	[127]
EMFGTSSET	988	9	- Cell-free ACE inhibition (IC ₅₀)	- Non-competitive inhibitor - Strongly bound to ACE and VCAM-1 active pockets	[122]

			15.08 μ M - Cell model Ang II-induced HUVEC 10-100 μ M - <i>In-vivo</i> SHR 10 mg/kg rat body weight	through hydrogen bonds, with major contribution from Ser $\downarrow$ Ang II-induced vascular dysfunction ($\downarrow$ ROS, IL-8, IL-1 β , TNF- α , iNOS, COX-2, ET-1, ATR1, ICAM-1 & VCAM-1) $\downarrow$ Ang II-induced inflammation and apoptosis through NF- κ B, MAPK and Akt signalling pathways ($\downarrow$ endothelial damage) - Oral administration of the peptide induced significant reductions in systolic blood pressure, and the activity was maintained for 10 h, like the Captopril treatment at 10 mg/kg Non-toxic to HUVEC	
<i>Arthrospira</i> sp.					
TVLYEH LQAGGLF	760 704	6 7	- Cell-free ACE inhibition (IC ₅₀) 2.88 μ M 66.83 μ M - <i>In-vivo</i> SHR 10-20 mg/kg rat body weight	- Competitive inhibitors - Strong binding affinity to ACE active pockets through hydrogen bonds, hydrophobic interactions and electrostatic forces - Stable under pH (2-12) and temperatures (0-100°C) - TVLYEH was more stable under gastrointestinal digestion - Oral administration significantly decreased SBP and DBP - Long-lasting blood pressure reduction until 8 hours after oral administration, while Captopril maintained its effect within 2 hours	[83]
TMEPGKP	759	7	- Cell-free ACE inhibition (IC ₅₀) 132 μ M - Cell model Ang II-induced HUVEC 62.5-250 μ M	- Non-competitive inhibitor - Interacted with ACE and Ang II via hydrogen and van der Waals interactions $\downarrow$ Ang II-induced vascular dysfunction ($\downarrow$ iNOS & ET-1) and ROS $\downarrow$ MAPK pathway - Non-toxic to HUVEC	[123]
<i>Arthrospira platensis</i>					
IQP VEP	356 440	3 3	- Cell-free ACE inhibition (IC ₅₀) 5.77 μ M 27.36 μ M - <i>In-vivo</i> SHR 10 mg/kg rat body weight/day	- Non-competitive inhibitors - Long-term oral intake (8 weeks) significantly decreased SBP, DBP, left ventricular mass index and right ventricular mass index - Regulated the main components of RAAS in myocardium and kidney ($\downarrow$ ACE, Ang II & ATR1) ACE-Ang II-ATR1 axis $\uparrow$ ATR2, ACE2, Ang-(1-7) and Mas receptor (ACE2-Ang-(1-7)-Mas axis) - Non-toxic to SHR	[119,120]
GIVAGDVTP1	941	10	- <i>In-vivo</i> SHR 10 mg/kg rat body weight	- Oral administration significantly decreased SBP (~43 mmHg) between 2 and 8 hours - NO-dependent mechanism by activating PI3K/AKT/eNOS pathways	[125]
<i>Chlorella pyrenoidosa</i>					
LRAKA	557	5	- Cell-free ACE inhibition (IC ₅₀) 350 nM - <i>In-vivo</i> SHR 20 mg/kg rat body weight	- Modified peptide from its precursor peptide (LVAKA) based on alanine scanning and saturated mutations (by substituting Val2 with Arg) - The peptide showed a potent ACE inhibitory activity with a very low IC ₅₀ (nanomolar), ranking it in the top 1% ACE-inhibitory peptides - Reduced SBP (-20.50 mmHg) and DBP (-17.70 mmHg) in SHR within 12 hours at 20 mg/kg body weight.	[20]
<i>Chlorella vulgaris</i>					
TTW VHW	406 441	3 3	- Cell-free ACE inhibition (IC ₅₀) 610 nM 910 nM - <i>In-vivo</i> SHR 5 mg/kg rat body weight	- Non-competitive inhibitors - Strong binding to ACE active pockets through hydrogen bonds - Stable under gastrointestinal digestion conditions and ACE hydrolysis - Oral administration significantly decreased the SBP and DBP, comparable to Lisinopril	[86]

ACE: angiotensin-converting enzyme; Akt: serine/threonine kinase; Ang II: Angiotensin II; COX-2: cyclooxygenase-2; DBP: diastolic blood pressure; ET-1: endothelin-1; ICAM-1: intracellular adhesion molecule 1; IL-1 β : interleukin 1 β ; IL-8: interleukin 8; iNOS: inducible nitric oxide synthase; MAPK: mitogen-activated protein kinases; MW: molecular weight; NF- κ B: nuclear factor κ B; NO: nitric oxide; Nrf2: nuclear erythroid 2-related factor 2; PCL: peptide chain length; SBP: systolic blood pressure; SHR: spontaneously hypertensive rats; TNF- α : tumour necrosis factor- α ; VCAM-1: vascular cell adhesion molecule 1.

Table S3: Antidiabetic and anti-obesity peptides derived from microalgae discovered in the past ten years

Peptide source/ sequence	MW (Da)	PCL	Inhibitory of diabetic-related enzymes (IC ₅₀ / Activity)			Inhibition pattern/ Mechanism/Key findings	Ref.
Antidiabetic peptides							
<i>Chlorella vulgaris</i>			DPP-IV	α - glucosidase	α - amylase		
VPW IPR VPA IPL	400 384 285 341	3 3 3 3	348.60 μ M 376.60 μ M 503.50 μ M 466.00 μ M	-	-	- Competitive inhibitors (VPW & IPR) - VPW and IPR bound with DPP-IV active pockets S1 or S2 through hydrogen bonds, Van der Waals and hydrophobic interactions - High gastrointestinal stability, and good DPP-IV inhibitory in mouse serum (IC ₅₀ =2.5-3.5 mM)	[140]
Arthrospira sp.							
GPNYASSER TPLGMVSML SLPLGGN SFVAGTM VMAPPAP GIPISTFIG ASGGSMP	980 948 656 711 681 904 605	9 9 7 7 7 9 7	358 μ M 397 μ M 415 μ M 808 μ M 953 μ M >5000 μ M >5000 μ M	-	-	- Inhibited the activity of DPP-IV in a concentration-dependent manner, except for GIPISTFIG and ASGGSMP which showed the highest IC ₅₀ of >5000 μ M	[28]
Chlorella sorokiniana							
VW IW LW WV	303 317 317 303	2 2 2 2	27.70%** 15.5%** 23.80%** 12.70%**	26.70%** 33.80%** 27.90%** 57.70%**	-	- Relatively higher α -glucosidase inhibitory activity than DPP-IV - Interacted with α -glucosidase at the pocket near the N-terminal β -sheet domain and behind the β -strands of the catalytic domain - VW, IW, LW were located within the catalytic domain (Ser630, Asp708, His740) of DPP-IV - WV was positioned far from the catalytic site of DPP-IV, which had the lowest DPP-IV activity	[84]
Arthrospira platensis							
GVPMPNK RNPVFVAPTLTVAAR LRSELAAWSR	741 1772 1187	7 16 10	259.51 μ M 102.26 μ M 140.94 μ M	204.45 μ M 92.83 μ M 113.06 μ M	318.76 μ M 608.13 μ M 264.20 μ M	- Strong concentration-dependent inhibitory activity - Their inhibitory activity (%) towards α -amylase and DPP-IV was almost similar with acarbose and sitagliptin, while 2-fold higher than acarbose against α -glucosidase	[91]
Anti-obesity peptides							
Chlorella pyrenoidosa							
Cell-free							
SISISVAGGGR KQTALVELVK KNGAPAEK SRQLTLYPGAER LLVVYPWTQR SDDPHTFGQGTK	1003 1128 814 1390 1274 1289	11 10 8 12 10 12	- Pancreatic lipase inhibition - Bile salts adsorption capacity - Cholesterol-lowering capacity - Gut modulation in high cholesterol colonic fermentation model - 5-20 mg/mL			- SISISVAGGGR (99.49%) and LLVVYPWTQR (63.03%) had the highest pancreatic lipase inhibitory activity - SISISVAGGGR (10.49%) had the highest bile salts adsorption capacity - SISISVAGGGR (28.99%) and SRQLTLYPGAER (23.60%) had the highest cholesterol-lowering effect and gastrointestinal stability - SISISVAGGGR modulated gut microbiota: $\uparrow$ beneficial bacteria (<i>Lactobacillus</i> & <i>Akkermansia</i>) and $\downarrow$ harmful bacteria (<i>Escherichia-Shigella</i>)	[54]
Arthrospira platensis							
Cell model							
CANPHELPNK	1122	10	- Lipid inhibition in 3T3-L1 cells			$\downarrow$ preadipocytes differentiation	[57]

NPVWKRK NALKCCHSCPA LNNPSVCDCDCMMK AAR	927 1146 1871	7 11 17	- 0.60-2.00 mg/mL	(32-60%) at 0.2 mg/mL - CANPHELPNK and NPVWKRK reduced the accumulation of TG by 19.50 and 23.70%, at 0.60 mg/mL - Non-toxic to L-O2 cells	
<i>Chlorella pyrenoidosa</i>			Cell model		
LLVVYPWTQR	1274	10	- Pancreatic lipase inhibition - Lipid inhibition in 3T3-L1 cells - 0.20-0.60 mg/mL	- Interacted with pancreatic lipase through hydrogen bonds at the catalytic sites (Ser153, Asp177 and His 264) ↓ Pancreatic lipase by 47.95% ↓ TG by 27.90% at 0.60 mg/mL, comparable to simvastatin (24.10%) ↓ Fat accumulation and fatty acid synthesis in adipocyte cells by activating the ↑AMPK pathway and ↓ adipogenic-specific proteins (C/EBPα, SREBP-1c and PPARγ)	[56]
<i>Chlorella pyrenoidosa</i>			In-vivo		
SISISVAGGGR	1003	11	- High-fat diet induced obesity in C57BL/6J male mice - 100-600 mg/kg mice body weight/d	↓ Body weight, epididymal fat weight, heart weight index, hepatic lipid accumulation and prevented liver inflammation ↓ Fasting glucose level and improved insulin resistance ↓ Serum levels of TG and LDL, while ↑HDL ↓ Firmicutes/Bacteroidetes ratio ↓ Obesogenic and proinflammatory bacteria such as <i>Acetatifactor</i> , <i>Dorea</i> and <i>Ruminococcus gnavus</i> ↑ Beneficial bacteria such as <i>Bacteroides</i> , <i>Parabacteroides</i> and <i>Alistipes</i> ↑ Regulation of pathways involved in carbohydrates metabolism, amino acids metabolism and membrane transport - The highest concentration has better anti-obesity effects	[54,55]
<i>Haematococcus pluvialis</i>			In-vivo		
KFTPAP	659	6	- Wild-type <i>C. elegans</i> strain N2 nematodes - 100 μM	↓ Fat content (12.97%), TG (19.62%), lipid droplets (22.75%) ↑ Ratio of oleic acid (C18:1n9) to stearic acid (C18:0), promoting the desaturation of C18:0 to C18:1n9 ↑ Energy expenditure by increasing locomotor activity and shortening the defecation cycle ↑ fat-6 and fat-7 genes (Fat-6/Fat-7 Pathway), which are involved in lipid metabolism ↑ Activated NHR-49/PPARα pathway, enhancing fatty acid β-oxidation and promoting lipolysis ↑ Activated AAK-2/AMPK pathway, which is involved in regulating energy balance and lipid mobilisation	[147]

AAK-2: AMP-activated protein kinase catalytic subunit alpha-2; AMPK: adenosine monophosphate-activated protein kinase; C/EBPα: CCAAT/enhancer binding protein alpha; DPP-IV: dipeptidyl peptidase-4; HDL: high-density lipoprotein; LDL: low-density lipoprotein; L-

O2: normal liver cells; NHR-49; nuclear hormone receptor 49; PPAR γ : peroxisome proliferator-activated receptor gamma; SREBP-1c: sterol regulatory element-binding protein 1c; TG: triglyceride.

** Activity was tested at peptide concentration of 0.25 $\mu\text{g/mL}$.

Table S4: Anti-ageing peptides derived from different microalgae species discovered in the past ten years

Peptide source/ sequence	MW (Da)	PCL	Model/Dose	Inhibition mechanism/Key findings	Ref.
			Cell-free		
<i>Arthrospira platensis</i>					
DER	418	3	-Tyrosinase (diphenolase) inhibitory activity	- Interacted with tyrosinase through hydrogen bonds with the key residues His85, His244, His259 and Asn260 - Strong inhibitory activity (IC_{50} = 1.04 mM)	[92]
			Cell model		
<i>Arthrospira platensis</i>					
AFGRFR	752	6	- <i>In-vitro</i> tyrosinase	- SPSWY (IC_{50} =12.1 μM) and MAACLR (86.9 μM) showed the highest monophenolase inhibitory activity	[155]
MAACLR	663	6	(monophenolase	- Only MAACLR and RCLNGRL showed both	
RCLNGRL	831	7	& diphenolase)	monophenolase (IC_{50} = 86.9 μM & 116.20 μM) and	
RYVTYAVF	1018	8	inhibitory	diphenolase (IC_{50} =990.3 & 1069.8 μM) inhibitory activity	
SPSWY	638	5	activity	- Only SPSWY, GRF and AADQRGKDKCARDIGY had	
GRF	378	3	- Melanoma cell: tyrosinase inhibitory activity and melanin production	cellular diphenolase inhibitory (IC_{50} =34.2-363.2 μM) - All the peptides reduced cellular melanin synthesis by 47.05-75.54% at 200 $\mu\text{g/mL}$, except RYVTYAVF which increased melanin synthesis up to 3-fold (potential pigmentation agent) - Non-toxic to melanoma cells up to 200 μM	
AADQRGKDKCAR DIGY	1766	16			
GMCCSR	655	6	- UVB (80 mJ/m^2)-induced HSF 15.20 μM	$\uparrow$ Proliferation of human skin fibroblasts and restored collagen production by 16.5% (similar to original level) after UVB irradiation - Non-toxic to human erythrocyte and normal mouse fibroblasts	[102]
<i>Isochrysis zhanjiangensis</i>					
DAPTMGY	753	7	- UVB (40 mJ/m^2)-Irradiated HaCaT cells 20-100 μM	$\uparrow$ Cell viability after UVB exposure and anti-apoptotic protein Bcl-2 $\downarrow$ Pro-apoptotic proteins (Bax, caspase-8/-3) and intracellular ROS $\uparrow$ Antioxidant enzymes (SOD, CAT and GPx) $\downarrow$ Collagen-degrading enzymes MMP-1 and MMP-3 $\uparrow$ Collagen synthesis via increased procollagen I expression $\downarrow$ Suppressed photoageing by inhibiting phosphorylation of MAPK pathway proteins (ERK, JNK and p38), AP-1 and NF- κB signalling pathway ($\downarrow$ p-p65 & p-I κB) - Non-toxic to human keratinocyte cells up to 200 μM	[152]
AYAPE	549	5	- UVB (40 mJ/m^2)-irradiated HaCaT cells 10-50 μM - H_2O_2 (600 μM)-induced senescence in BJ cells 10-50 μM	$\uparrow$ Viability of stressed human keratinocyte and dermal fibroblasts - Non-toxic to human keratinocyte and dermal fibroblasts cells up to 150 μM UVB-induced human keratinocyte cells (HaCaT): $\downarrow$ Intracellular ROS and DNA damage $\downarrow$ Collagen-degrading enzyme MMP-1 $\uparrow$ Anti-apoptotic effect ($\uparrow$ Bcl/Bax ratio, $\downarrow$ nuclear P53, $\downarrow$ cleaved caspase-3/-9) $\downarrow$ MAPK/AP-1 pathway ($\downarrow$ p-p38, p-ERK, p-JNK, p-c-Jun) $\downarrow$ NF- κB signalling ($\downarrow$ p-p65, iNOS, COX-2) Human dermal fibroblast cells (BJ): $\downarrow$ Senescence markers ($\downarrow$ p53, p21 & p16) and p-Rb phosphorylation ($\downarrow$ AKT activation) $\downarrow$ Cellular ageing (fewer β -galactosidase-positive cells)	[153]
EMFGTSSET	988	9	- UVB (40 mJ/m^2)-irradiated	$\uparrow$ Viability of stressed human keratinocyte and dermal fibroblasts - Non-toxic to keratinocyte (200 μM) and dermal fibroblasts	[151]

			HaCaT cells 50-200 μ M - H ₂ O ₂ (600 μ M)-induced senescence in BJ cells 50-200 μ M	cells (300 μ M) - Comparable anti-ageing effect to vitamin C UVB-induced human keratinocyte cells (HaCaT): $\downarrow$ ROS through $\uparrow$ expression of Nrf2/HO-1 signalling pathway $\uparrow$ Autophagosomes, lysosomes and autophagy-related proteins ($\uparrow$ LC3, Beclin 1, ATG5-ATG12) by $\uparrow$ expression of phosphorylated AMPK and $\downarrow$ phosphorylated mTOR (mTOR/AMPK signalling pathway) $\uparrow$ Mitochondrial potential ($\uparrow$ Bcl-2/Bax ratio, $\downarrow$ p53, $\downarrow$ caspase-3/-9) Human dermal fibroblast cells (BJ): - Regulated senescence-related factors ($\downarrow$ p53, $\downarrow$ p21, $\downarrow$ p26, $\uparrow$ phosphorylated Rb) $\uparrow$ Collagen generation: $\uparrow$ TGF- β 1 and $\downarrow$ phosphorylated Smad2/3 (TGF- β 1/Smad pathway)	
			In-vivo model		
<i>Arthrospira platensis</i>					
Ac-GMCCSR-NH ₂	655	6	- UVB (35 mJ/m ²)-induced HaCaT cell model 10 mg/mL - UVB (60 mJ/m ²)-induced mice dorsal skin 10 mg/mL	$\uparrow$ Proliferation of UVB-irradiated HaCaT cells - Normalised epidermal thickness and restored skin moisture; $\downarrow$ inflammation and vacuolar degeneration in irradiated mice $\uparrow$ Antioxidant enzyme activities ($\uparrow$ CAT, GPx) and $\downarrow$ collagen-degrading enzymes (MMP-1, MMP-3) and $\downarrow$ MDA level in skin tissue of mice - Proteomics revealed regulation of 60 proteins and involvement of HIF-1, glycolysis, and keratinisation pathways - Non-toxic to HaCaT cells and safe to mice	[154]
<i>Synechococcus</i> sp. VDW					
AILESYSAGKTK	1267	12	- Cell-free tyrosinase inhibitory activity (monophenolase & diphenolase) - Melanoma cells model 6.25-100 μ M - Zebrafish embryos 6.25-100 μ M	- Strong cell-free monophenolase (IC ₅₀ =489.71 μ M) and diphenolase (IC ₅₀ =765.57 μ M) inhibitory activity - Competitively inhibited tyrosinase through binding with the active site via hydrogen bonds (His244, Arg268, Cys83) Pi-alkyl (His263, Phe264, Val248) and Van der Waals interactions $\downarrow$ Melanin content in melanoma cells and zebrafish embryos in a concentration-dependent manner $\downarrow$ Melanogenesis-related genes (TYR, TRP-1 & TRP-2) $\uparrow$ MITF - Non-toxic to melanoma cells up to 400 μ M - Safe to zebrafish embryos up to 100 μ M	[157]
<i>Haematococcus pluvialis</i>					
KFTPAP	659	6	- Wild-type <i>C. elegans</i> strain N2 nematodes (normal and stress-induced ageing models) 100 μ M	$\uparrow$ Lifespan of nematodes under normal, oxidative and thermal stress conditions $\uparrow$ physiological functions of nematodes by $\downarrow$ ageing pigment (lipofuscin) and $\uparrow$ locomotion and pharyngeal pumping activity without impairing reproduction $\uparrow$ SOD and CAT activity and $\downarrow$ MDA level $\uparrow$ Genes expression related to stress resistance (skn-1, hsp-16.2) and antioxidant activity (sod-3 and ctl-2) and IIS pathway (daf-16, daf-2, ins-18 and sir-2.1) - IIS pathway was critical to the peptide's anti-ageing mechanism, through the regulation of subcellular localisation of DAF-16	[156]

AMPK: adenosine 5'-monophosphate (AMP)-activated protein kinase; ATG12: autophagy-related 12; ATG5: autophagy-related 5; B16-F10: melanoma cells; Bcl-2: B-cell lymphoma 2; Beclin 1: Bcl-2 interacting protein 1; BJ cells: human dermal fibroblasts; CAT: catalase; GPx: glutathione peroxidase; HaCaT cells: human keratinocyte; HO-1: heme oxygenase-1; HSF: human skin fibroblasts; IIS: insulin/insulin-like growth factor signalling; LC3: microtubule-associated protein 1 light chain 3; MAPK: mitogen-activated protein kinases; MDA: malondialdehyde; MITF: microphthalmia-associated transcription factor; MMP: metalloproteinases; mTOR: mammalian target of rapamycin; NF- κ B: nuclear factor κ B; Nrf2: nuclear erythroid 2-related factor 2; SOD: superoxide dismutase; TGF- β 1: transforming growth factor β 1; TRP: tyrosinase-related protein; TYR: tyrosinase; UVB: ultraviolet B radiation.