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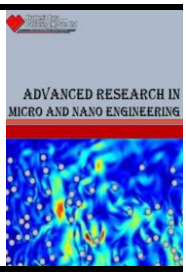

Anticancer Potential of Bioactive Compounds from Microalgae. A Review

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ABSTRACT

In this review, we discussed the important breakthroughs in the field of cancer treatment involving algae and microalgae. The continuous development and commercialization of marine drugs has been a rewarding scientific endeavour. The pipeline of marine-derived anticancer agents provides promising treatment options for patients with certain types of cancer. With the approval of four drugs and eighteen agents in late development, the pipeline of marine-derived anticancer drugs is one of the most robust in the industry. Natural products containing bioactive components have been increasingly popular in recent years Bioactive chemicals from marine organisms have been isolated after extensive research. About 9% of bioactive chemicals generated from marine organisms are found in algae and microalgae. Microalgae have been found to contain a variety of new metabolites with remarkable properties, comprising sterols, fatty acids, phenolic compounds, and carotenoids. Anticancer, antibacterial, antioxidant, and anti-inflammatory effects have been demonstrated for these substances.

1. Introduction

In the year of 2023, an estimation of a total 1,958,310 new cancer cases and 609,820 deaths caused by cancer are going to occur in the United States according to the American Cancer Society [1]. The rise of cancer patients worldwide is significant, as significant improvements in stroke and coronary heart disease rates. This puts immense pressure on people, families, and health systems, especially in low- and middle-income nations. Healthcare organizations struggle to handle this challenge, and many patients have limited access to prompt, efficient diagnosis and treatment [2,3].

Mutations in cancer cells may cause uncontrollable division, leading to uncontrollable cell division. Studying the basic theory of cancer formation is crucial for developing effective drugs. Mutations can cause cells to divide more than normal, creating dangerous groups that may harm the host. Continuous cycles of mutation and natural selection contribute to cancer [4]. Furthermore,

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cancer occur when the cell loss its function or their controlled process is breakdown for examples cell differentiation, proliferation and programmed cell death (apoptosis) and the regulatory feedback mechanisms were involved resulting extremely high rate of cell growth [5].

Health style, including tabaco smoking, UV ray exposure, alcohol consumption, and no breastfeeding, may contribute to cancer risk, potentially increasing the risk of breast cancer [6]. Treatment available and practice nowadays include radiation therapy, surgery, chemotherapy and hormone therapy. 50% of cancer patients undergo radiation therapy. Radiation treatment leads to 40% of cancer treatment [7]. Current cancer therapy strategy improves with single molecular anomalies or cancer pathways, but targeting a single hallmark or route remains challenging [8].

Metastatic spreading and hallmark identification are crucial for cancer biology understanding, as they support tumor growth and provide a strong fundamental for understanding cancer biology. This progression helps rationalize human tumor pathogenesis and prevents malignant growth [9]. Bioactive compound (polysaccharides, proteins, peptides, lipids, and carotenoids) extracted from the microalgae was claimed to be effective for treating the cancer cell and malignant tumor or responding as agents for chemoprevention [10].

Despite several substances accumulated in microalgae cells having a high economic value, high extraction and purification processes cost, causing commercialization to struggle [11]. Microalgae study in Malaysia have widely conducted in academic and industry application. The application of microalgae is more focusing on environmental application such as wastewater treatment, biofuel production and carbon dioxide fixation [12,13]. In fact, a lot of literature found was using variety of microalgae species for biodiesel production. The study of using microalgae as new source alternative in producing biofuels is very widespread [14]. Oncological study reveals nowadays cancer treatment uses chemotherapeutic drugs, causing side effects like appetite loss, hair loss, diarrhea, and immunosuppression. Thus, new natural ingredients and metabolites discovered in microorganisms, animals, and plants exhibit cytotoxic activity on tumor cells without harming normal cells [15].

Studies show plants as an important source of anticancer drugs, offering a useful treatment for various tumor types with lower side effects. Algae contain natural antitumor amalgams, but limited research on microalgae-derived bioactive compounds [16]. Algae, including microalgae, contribute to global yield up to 40%. These autotrophic plants have diverse body structures, ranging from small to multicellular organisms. They are found in freshwater lakes, ponds, and marine environments, influencing human health and physiology [17]. Scientifically algae classified into red, green, and brown algae, are categorized by size, with macroalgae visible to the naked eye, while microalgae are small, single-celled algae [16]. Microalgae offers high potential in human health, enhancing cardiovascular health, anticancer, anti-inflammatory, anticoagulant, antiviral, antibacterial, and antifungal properties the extracts boost the immune system, lower cholesterol levels, and eliminate toxic substances from the body [5,18] which contributed to the improvement of medicinal industry.

2. Microalgae as Bioactive Compounds

H. cuneiformis extract exhibits anti-cancer properties, triggering DNA denaturation on cells, causing apoptosis and cell cycle arrest, highlighting its potential in enhancing cancer prevention [19]. Ethanolic extracts of *Chloromonas* sp significantly inhibit the growth of HeLa and A375 in dose dependant manner [20]. Dichloroacetic acid (DCA), Oximes and L-α-terpinol that were found in ethanolic extract of green algae collected from Arabian gulf, *Chaetomorpha sp.* have elucidated anticancer effect on MDA-MB-31 cancer cell lines as supported by other studies that claim L-α-terpinol antiproliferative effect through suppressing NF-kb signalling on several breast cancer line [19-22].

2.1 Phytosterols

The most types of phytosterols found synthesised by microalgae are β-sitosterol, campesterol, brassicasterol, stigmasterol and ergosterol. Some microalgae species have reported to produce of phytosterols derived, biosynthetic sterol which are *P. tricornutum*, *Nannochloropsis oceanica*, and *Chlamydomonas reinhardtii*. Carbon phytosterols for example brassicasterol, campesterol, ergosterol, β-sitosterol and stigmasterol are the most familiar phytosterol group in microalgae few studies [23-25] shows four different microalgae oil extracts from *Isochrysis galbana*, *Nannochloropis gaditana*, *Nannochloropsis* sp. and *Phaeodactylum tricornutum*, there are around 0.7% to 3.4% off phytosterol found depending on the solvent use and extraction method. Significant result of the effect of stigmasterol isolated from microalgae *Navicula incerta* towards hepatocarcinoma (HepG2) cells have been reported. It shows that the extract is capable to induce apoptosis by upregulate gene Bax and p53 and downregulate anti-apoptotic gene Bcl-2.

The highest producer of phytosterol microalgae reported are *Diacronema lutheri (syn.Pavlova lutheri*), *Tetraselmis sp*. and *Nannochloropsis sp* which composed of around 0.4% to 2.6% dry weight biomass, and reaching 5.1%, depending on nutrients, salinity and cultivation duration [26]. In the study done by Park *et al.,* [27], inhibition of cell proliferation and apoptosis activity was reported involving phytosterol derivative, β-sitosterol in which it helps increasing the ratio of Bax/Bcl-2 and activation of capcase-3 in leukaemia cells. On that matter, in G0/G1 phase of cell cycle, phytosterol was proven to induce cell apoptosis of MDA-MB-231 cells. The study demonstrate mitochondrial membrane depolarization potential can be trigger and increasing of Bax/Bcl-2 ratio by exposing the human breast cancer with β-sitosterol [28].

2.2 Carotenoids

Marine algae are excellent natural sources of antioxidants and anticancer compounds, with phenolic and carotenoid compounds preventing free radicals and minimizing DNA damage. These algae also contain phycobilin protein, a chemotherapeutic agent not found in terrestrial plants [27,28]. Phenolics compound have elucidate antioxidants potential [29]. ROS regulate cellular signalling, affecting gene expression and signalling pathways. Carotenoids can prevent damage to proteins and DNA and act as pro-oxidants by promoting phenolic acid production. The conversion of carotenoid from antioxidant to pro-oxidant depends on concentration and oxygen partial pressure [30-32].

2.2.1 Astaxanthin

In recent years, microalgae-based carotenoid development has been studied comprehensively, for examples, *Dunaliella salina* β-carotene, *Synechocystis* zeaxanthin, *Chlorella protothecoides* lutein, *Chlorella protothecoides* lutein, *Haematococcus pluvialis* and *Chlorella zofingiensis* astaxanthin. The main natural producer of astaxanthin are green microalgae *Haematococcus pluvialis* and *Chlorella zofingiensis*. *H. pluvialis* was considered a great astaxanthin manufacturer potential due to its high intracellular astaxanthin content [33].

Through the result experiment conducted by, Kavitha *et al.,* [34] on the hamster buccal pouch (HBP) carcinogenesis model by observing and analyse its capability to inhibit NF-κB and Wnt signalling pathways and support the activation of apoptosis by downregulation of the main IKKβ and GSK-3β regulatory enzymes. The findings present definitive proof that astaxanthin, extracted from *Haematococcus pluvialis*, shows antiproliferative effects by inhibiting transcription factor

phosphorylation, triggering kinases, and promoting cell death through downregulating Bcl-2 expression and upregulating Bax expression [35].

2.2.2 Fucoxanthin

Important group of marine microalgae, fucoxanthin-containing diatoms, account for 40% of marine primary productivity and 20%-25% of worldwide net primary production. Due to the conjugated carbonyl, a 5,6-monoepoxide and an acetyl group, it known for their unique structure of molecule which may attribute to their anticancer property [36]. *Dunaliella salina*, *Chlorella zofingiensis*, and *Chlorella pyrenoidosa* exhibit higher carotenoid production compared to higher plants, resulting in significant production of β-carotene, canthaxanthin, lutein and many others [37]. Several microalgae were reported to have generate fucoxanthin as their main carotenoid. Microalga *P. tricornutum* shows high potential of producing higher quantity of fucoxanthin comparing to other microalgae and poses the possibility of serving as a commercial source of fucoxanthin [38].

Peng *et al.,* [39] reported that fucoxanthin exerted antiproliferation effect on tumour cells human promyelocytic leukaemia HL-60 cell line and may induce apoptosis mechanism of HL-60 [40]. Apoptosis induction was mentioned cause by fucoxanthin facilitated mitochondrial membrane permeabilization and caspase-9 and caspase-3 activation [41]. A study shows that it could also inducing cell cycle arrest at G1 phase and apoptosis to minimise the tumour cells proliferative activity [42].

Fucoxanthin also significantly decreased endothelial cell migration by inhibiting the phosphorylation of fibroblast growth factor 2 (FGF-2) mediated by intracellular signalling proteins along with extracellular signal-regulated kinases (ERK1/2) and protein kinase B (Akt). Not only that, fucoxanthin extracted from *Undaria pinnatifida* and *Codium fragile* also supressing its receptor (FGFR-1) along with the trans-activation factor, EGR-1. In human cancer cells, fucoxanthin has promoting the inhibitory of cancer cell proliferation by increasing gap junction intercellular communication, possibly contributing to increased intracellular signalling, which promotes cell cycle arrest and apoptosis. Fucoxanthin has a lot of potential as a cancer chemo preventive and/or chemotherapeutic agent. The factors involve fucoxanthin's anticancer effect has been proposed to be its free radical scavenging activity [15,43,44].

2.2.3 β-carotene

The major type of provitamin A, β-carotene, is known for its ability to scavenge toxic oxygen and nitrogen radicals. β-Carotene has a wide range of uses in food, medicines, and cosmetics due to its beneficial characteristics. β-Carotene has been demonstrated to protect against UV and oxidative damage, and β-carotene supplement intake (30-180 mg daily) will inhibit UV-induced erythema in humans [15,45,46]. The discussion of β-carotene function in reducing a few types of cancer and deteriorating diseases in humans was found in Ranga Rao *et al.,* [47].

China study claims β-carotene, vitamin E, and selenium combination as potential anticancer agents. Nishino *et al.,* [48] reported that β -carotene, astaxanthin, canthaxanthin, and zeaxanthin were found to assist the reduction size and quantity of liver neoplasia in vivo investigation. However, the comparison of β-carotene and astaxanthin with fucoxanthin, a xanthophyll type of carotenoids demonstrates that fucoxanthin has the greater anti-tumour activity [40].

According to Jayappriyan *et al.,* [49] among general species of *Dunaliella*, most highly β-carotene quantities accumulation is *D. salina*, *D. bardawil* and *D. parva* which is more than 10 to 12% of their dry weight. HPLC examination reveals 9-cis-beta-carotene, synthesized by *Dunaliella*, is 10 times

effective in cancer prevention compared to normal carotene. *D. salina* strain produces high β-carotene, affecting cell death in PC-3 human prostate adenocarcinoma cell line.

Meanwhile, in the comparison of three microalgae species, *Chlorella sp*., *Dunaliella sp*. and *Isochrysis sp*, the highest carotenoids content according to Ahmad *et al.,* [50] was *Dunaliella sp*. As result of the study shows that β-carotene has a high toxicity effect on MCF-7 cancer cells, reducing viability and inhibiting inflammatory signalling. It can reduce iNOS and COX-2 synthesis, thereby deactivating ROS and promoting gastric healing.

2.2.4 Lutein

Lutein and zeaxanthin are important pigments in the macula lutea of the human eye retina. They can be found in both terrestrial plants and green algae. Lutein protects photoreceptors by filtering blue light, which can limit the amount of light that damages the retina by up to 40% [51,52]. Lutein also protects endothelial cells in vitro and has anticancer properties [53]. In a study done by Rafi *et al.,* [54] found that lutein enhances the antiproliferative and apoptotic effects of chemotherapy medications, as well as inhibiting cell cycle progression in prostate cancer cell lines when used alone or in combination with chemotherapy drugs. Furthermore, lutein inhibits the expression of biomarker genes linked to prostate cancer growth and survival. Antiproliferation activity on breast cancer cell line through increasing intracellular ROS levels and promoting apoptotic cell death by downregulating Bcl2 genes and upregulating pro-apoptotic genes, as well as by improving the p53 signalling pathway [55]. This is well support in the study conducted by Chang *et al.,* [56] where lutein also reported shows anti-breast cancer via triggering the NrF2/ARE pathway and disabling the NF-κB signalling pathway. Alongside downregulated expression of Bcl-2 and poly-ADP ribose polymerase to induce apoptosis on breast cancer line [57].

2.3 Secondary Metabolite Bioactive Compounds

Anti-carcinogenic activity of phlorotannin obtain from Brown algae which have the phenolics character compound was proven by clinical trials which demonstrate that certain phlorotannins are capable of preventing the progression and development of cancer [58]. Effects of dieckol extract on the release and gene expression of various cyclooxygenase 1 enzymes were studied. It was concluded that dieckol inhibited the growth of various tumor cells [59]. Dieckol exhibited a protective effect against UVB radiation-induced cell damage. It increased the survival rate of fibroblast cells and decreased the toxicity of the cells [60]. Dieckol, extracted from *E. stolonifera*, demonstrated anticancer activities in a dose-dependent method against human Hep3B hepatocellular carcinoma cells, limiting tumor growth and activating key genes. Phlorotannin eckol decreased stemness and carcinogenesis in glioma stem-like cells in another investigation [61].

3. Conclusions

Algae and microalgae are rich sources of bioactive compounds with great potential in the healthcare industry. Recent studies show that phytochemicals may have significant roles in treating and preventing chronic diseases like diabetes, cancer, and heart diseases. Marine-derived anticancer agents are expected to translate into the discovery of new drug targets. While algae bioactive compounds have been studied well, other potential aspects like metabolic activity, cell cycle regulation, apoptosis, and cell differentiation have not yet been thoroughly investigated. These investigations are expected to aid in understanding the functions and applications of phytochemicals

extracted from microalgae, facilitating the development of novel approaches to diagnosis, prevention, treatment, and management of diseases, particularly cancer.

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References
[1] Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. "Cancer statistics, 2018." *CA: a cancer journal for clinicians* 68, no. 1 (2018): 7-30. https://doi.org/10.3322/caac.21442
[2] Sung, Hyuna, Jacques Ferlay, Rebecca L. Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." *CA: a cancer journal for clinicians* 71, no. 3 (2021): 209-249. https://doi.org/10.3322/caac.21660
[3] Chhikara, Bhupender S., and Keykavous Parang. "Global Cancer Statistics 2022: the trends projection analysis." *Chemical Biology Letters* 10, no. 1 (2023): 451-451.
[4] Fior, Rita, and Rita Zilhão. *Molecular and cell biology of cancer*. Springer International Publishing, 2019. https://doi.org/10.1007/978-3-030-11812-9
[5] Levine, Ira A. "Algae: A way of life and health." In *Microalgae in health and disease prevention*, pp. 1-10. Academic Press, 2018. https://doi.org/10.1016/B978-0-12-811405-6.00001-3
[6] Blackadar, Clarke Brian. "Historical review of the causes of cancer." *World journal of clinical oncology* 7, no. 1 (2016): 54. https://doi.org/10.5306/wjco.v7.i1.54
[7] Baskar, Rajamanickam, Kuo Ann Lee, Richard Yeo, and Kheng-Wei Yeoh. "Cancer and radiation therapy: current advances and future directions." *International journal of medical sciences* 9, no. 3 (2012): 193. https://doi.org/10.7150/ijms.3635
[8] Zugazagoitia, Jon, Cristiano Guedes, Santiago Ponce, Irene Ferrer, Sonia Molina-Pinelo, and Luis Paz-Ares. "Current challenges in cancer treatment." *Clinical therapeutics* 38, no. 7 (2016): 1551-1566. https://doi.org/10.1016/j.clinthera.2016.03.026
[9] Hanahan, Douglas. "Hallmarks of cancer: new dimensions." *Cancer discovery* 12, no. 1 (2022): 31-46. https://doi.org/10.1158/2159-8290.CD-21-1059
[10] Talero, Elena, Sofía García-Mauriño, Javier Ávila-Román, Azahara Rodríguez-Luna, Antonio Alcaide, and Virginia Motilva. "Bioactive compounds isolated from microalgae in chronic inflammation and cancer." *Marine drugs* 13, no. 10 (2015): 6152-6209. https://doi.org/10.3390/md13106152
[11] Ventura, S. P. M., B. P. Nobre, F. Ertekin, M. Hayes, M. Garciá-Vaquero, F. Vieira, M. Koc, L. Gouveia, M. R. Aires-Barros, and A. M. F. Palavra. "Extraction of value-added compounds from microalgae." In *Microalgae-based biofuels and bioproducts*, pp. 461-483. Woodhead Publishing, 2017. https://doi.org/10.1016/B978-0-08-101023-5.00019-4
[12] Rajkumar, R., and M. Sobri Takriff. "Prospects of algae and their environmental applications in Malaysia: a case study." *J Bioremediation Biodegrad* 7, no. 01 (2016): 1-12. https://doi.org/10.4172/2155-6199.1000321
[13] Alishah Aratboni, Hossein, Nahid Rafiei, Raul Garcia-Granados, Abbas Alemzadeh, and José Rubén Morones-Ramírez. "Biomass and lipid induction strategies in microalgae for biofuel production and other applications." *Microbial Cell Factories* 18 (2019): 1-17. https://doi.org/10.1186/s12934-019-1228-4
[14] Hossain, Nazia, M. H. Hasan, T. M. I. Mahlia, A. H. Shamsuddin, and A. S. Silitonga. "Feasibility of microalgae as feedstock for alternative fuel in Malaysia: A review." *Energy Strategy Reviews* 32 (2020): 100536. https://doi.org/10.1016/j.esr.2020.100536
[15] Moghadamtousi, Soheil Zorofchian, Hamed Karimian, Ramin Khanabdali, Mahboubeh Razavi, Mohammad Firoozinia, Keivan Zandi, and Habsah Abdul Kadir. "Anticancer and antitumor potential of fucoidan and fucoxanthin, two main metabolites isolated from brown algae." *The Scientific World Journal* 2014 (2014). https://doi.org/10.1155/2014/768323
[16] Abd El-Hack, Mohamed E., Sameh Abdelnour, Mahmoud Alagawany, Mohamed Abdo, Moustafa A. Sakr, Asmaa F. Khafaga, Samir A. Mahgoub, Shaaban S. Elnesr, and Manar G. Gebriel. "Microalgae in modern cancer therapy: Current knowledge." *Biomedicine & pharmacotherapy* 111 (2019): 42-50. https://doi.org/10.1016/j.biopha.2018.12.069
[17] Borowitzka, Michael A. *Algal physiology and large-scale outdoor cultures of microalgae*. Springer International Publishing, 2016. https://doi.org/10.1007/978-3-319-24945-2_23

[18] de Jesus Raposo, Maria Filomena, Rui Manuel Santos Costa de Morais, and Alcina Maria Miranda Bernardo de Morais. "Health applications of bioactive compounds from marine microalgae." *Life sciences* 93, no. 15 (2013): 479-486. https://doi.org/10.1016/j.lfs.2013.08.002

[19] Desai, Ankur R., Andrew D. Richardson, Antje M. Moffat, Jens Kattge, David Y. Hollinger, Alan Barr, Eva Falge et al. "Cross-site evaluation of eddy covariance GPP and RE decomposition techniques." *agricultural and forest meteorology* 148, no. 6-7 (2008): 821-838. https://doi.org/10.1016/j.agrformet.2007.11.012

[20] Suh, Sung-Suk, Eun Jin Yang, Sung Gu Lee, Ui Joung Youn, Se Jong Han, Il-Chan Kim, and Sanghee Kim. "Bioactivities of ethanol extract from the Antarctic freshwater microalga, Chloromonas sp." *International journal of medical sciences* 14, no. 6 (2017): 560. https://doi.org/10.7150/ijms.18702

[21] Haq, Samina Hyder, Ghaida Al-Ruwaished, Moudhi Abdullah Al-Mutlaq, Sundus Ali Naji, Maha Al-Mogren, Sarah Al-Rashed, Qura Tul Ain, Abir Abdullah Al-Amro, and Adnan Al-Mussallam. "Antioxidant, anticancer activity and phytochemical analysis of green algae, Chaetomorpha collected from the Arabian Gulf." *Scientific reports* 9, no. 1 (2019): 18906. https://doi.org/10.1038/s41598-019-55309-1

[22] Hassan, Saadia Bashir, Hala Gali-Muhtasib, Hanna Göransson, and Rolf Larsson. "Alpha terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling." *Anticancer Research* 30, no. 6 (2010): 1911-1919.

[23] Fabris, Michele, Michiel Matthijs, Sophie Carbonelle, Tessa Moses, Jacob Pollier, Renaat Dasseville, Gino JE Baart, Wim Vyverman, and Alain Goossens. "Tracking the sterol biosynthesis pathway of the diatom P haeodactylum tricornutum." *New Phytologist* 204, no. 3 (2014): 521-535. https://doi.org/10.1111/nph.12917

[24] Mohammady, Nagwa Gamal. "Total, free and conjugated sterolic forms in three microalgae used in mariculture." *Zeitschrift für Naturforschung C* 59, no. 9-10 (2004): 619-624. https://doi.org/10.1515/znc-2004-9-1002

[25] Luo, Xuan, Peng Su, and Wei Zhang. "Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications." *Marine drugs* 13, no. 7 (2015): 4231-4254. https://doi.org/10.3390/md13074231

[26] Ryckebosch, Eline, Charlotte Bruneel, Romina Termote-Verhalle, Koenraad Muylaert, and Imogen Foubert. "Influence of extraction solvent system on extractability of lipid components from different microalgae species." *Algal Research* 3 (2014): 36-43. https://doi.org/10.1016/j.algal.2013.11.001

[27] Park, Cheol, Dong-Oh Moon, Chung-Ho Rhu, Byung Tae Choi, Won Ho Lee, Gi-Young Kim, and Yung Hyun Choi. "β-Sitosterol induces anti-proliferation and apoptosis in human leukemic U937 cells through activation of caspase-3 and induction of Bax/Bcl-2 ratio." *Biological and Pharmaceutical Bulletin* 30, no. 7 (2007): 1317-1323. https://doi.org/10.1248/bpb.30.1317

[28] Vundru, Shanthi Sri, Raosaheb K. Kale, and Rana P. Singh. "β-sitosterol induces G1 arrest and causes depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells." *BMC complementary and alternative medicine* 13 (2013): 1-9. https://doi.org/10.1186/1472-6882-13-280

[29] Muniyappan, Gayathry, Norfatihah Mohd Adenam, Muhamad Yuzaini Azrai MY, Nurul Hijanah MH, Daruliza Kernain, and Hasyiya Karimah Adli. "Phytochemical Screening of Muntingia Calabura Fruit for Antioxidant and Cytotoxic Activities." *Journal of Advanced Research in Applied Sciences and Engineering Technology* (2022).

[30] Britton, George, Synnøve Liaaen-Jensen, and Hanspeter Pfander. "Editors' Introduction: A Healthy Debate." In *Carotenoids: Volume 5: Nutrition and Health*, pp. 1-6. Basel: Birkhäuser Basel, 2009. https://doi.org/10.1007/978-3-7643-7501-0_1

[31] Okuyama, Yusuke, Kotaro Ozasa, Keiichi Oki, Hoyoku Nishino, Sotaro Fujimoto, and Yoshiyuki Watanabe. "Inverse associations between serum concentrations of zeaxanthin and other carotenoids and colorectal neoplasm in Japanese." *International journal of clinical oncology* 19 (2014): 87-97. https://doi.org/10.1007/s10147-013-0520-2

[32] Tan, Chen, Jin Xue, Xiaowei Lou, Shabbar Abbas, Yu Guan, Biao Feng, Xiaoming Zhang, and Shuqin Xia. "Liposomes as delivery systems for carotenoids: comparative studies of loading ability, storage stability and in vitro release." *Food & Function* 5, no. 6 (2014): 1232-1240. https://doi.org/10.1039/c3fo60498e

[33] Liu, Jin, Zheng Sun, Henri Gerken, Zheng Liu, Yue Jiang, and Feng Chen. "Chlorella zofingiensis as an alternative microalgal producer of astaxanthin: biology and industrial potential." *Marine drugs* 12, no. 6 (2014): 3487-3515. https://doi.org/10.3390/md12063487

[34] Kavitha, K., J. Kowshik, T. Kranthi Kiran Kishore, Abdul Basit Baba, and S. Nagini. "Astaxanthin inhibits NF-κB and Wnt/β-catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic apoptosis in a hamster model of oral cancer." *Biochimica et Biophysica Acta (BBA)-General Subjects* 1830, no. 10 (2013): 4433-4444. https://doi.org/10.1016/j.bbagen.2013.05.032

[35] Palozza, Paola, Cristiana Torelli, Alma Boninsegna, Rossella Simone, Assunta Catalano, Maria Cristina Mele, and Nevio Picci. "Growth-inhibitory effects of the astaxanthin-rich alga Haematococcus pluvialis in human colon cancer cells." *Cancer letters* 283, no. 1 (2009): 108-117. https://doi.org/10.1016/j.canlet.2009.03.031

[36] Xia, Song, Ke Wang, Linglin Wan, Aifen Li, Qiang Hu, and Chengwu Zhang. "Production, characterization, and antioxidant activity of fucoxanthin from the marine diatom Odontella aurita." *Marine drugs* 11, no. 7 (2013): 2667-2681. https://doi.org/10.3390/md11072667

[37] Gong, Mengyue, and Amarjeet Bassi. "Carotenoids from microalgae: A review of recent developments." *Biotechnology advances* 34, no. 8 (2016): 1396-1412. https://doi.org/10.1016/j.biotechadv.2016.10.005

[38] Kim, Sang Min, Suk-Woo Kang, O-Nam Kwon, Donghwa Chung, and Cheol-Ho Pan. "Fucoxanthin as a major carotenoid in Isochrysis aff. galbana: Characterization of extraction for commercial application." *Journal of the Korean Society for Applied Biological Chemistry* 55 (2012): 477-483. https://doi.org/10.1007/s13765-012-2108-3

[39] Peng, Juan, Jian-Ping Yuan, Chou-Fei Wu, and Jiang-Hai Wang. "Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health." *Marine drugs* 9, no. 10 (2011): 1806-1828. https://doi.org/10.3390/md9101806

[40] Miyashita, Kazuo, Sho Nishikawa, Fumiaki Beppu, Takayuki Tsukui, Masayuki Abe, and Masashi Hosokawa. "The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds." *Journal of the Science of Food and Agriculture* 91, no. 7 (2011): 1166-1174. https://doi.org/10.1002/jsfa.4353

[41] Kim, Kil-Nam, Ginnae Ahn, Soo-Jin Heo, Sung-Myung Kang, Min-Cheol Kang, Hye-Mi Yang, Daekyung Kim et al. "Inhibition of tumor growth in vitro and in vivo by fucoxanthin against melanoma B16F10 cells." *Environmental Toxicology and Pharmacology* 35, no. 1 (2013): 39-46. https://doi.org/10.1016/j.etap.2012.10.002

[42] Satomi, Yoshiko, and Hoyoku Nishino. "Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and gadd45 expression by the carotenoid fucoxanthin in human cancer cells." *Biochimica et Biophysica Acta (BBA)-General Subjects* 1790, no. 4 (2009): 260-266. https://doi.org/10.1016/j.bbagen.2009.01.003

[43] Martin, Luc J. "Fucoxanthin and its metabolite fucoxanthinol in cancer prevention and treatment." *Marine drugs* 13, no. 8 (2015): 4784-4798. https://doi.org/10.3390/md13084784

[44] Ganesan, Ponesakki, Kiminori Matsubara, Tatsuya Sugawara, and Takashi Hirata. "Marine algal carotenoids inhibit angiogenesis by down-regulating FGF-2-mediated intracellular signals in vascular endothelial cells." *Molecular and cellular biochemistry* 380 (2013): 1-9. https://doi.org/10.1007/s11010-013-1651-5

[45] Shaish, Aviv, Ami Ben-Amotz, and Mordhay Avron. "[41] Biosynthesis of β-carotene in Dunaliella." In *Methods in enzymology*, vol. 213, pp. 439-444. Academic Press, 1992. https://doi.org/10.1016/0076-6879(92)13145-N

[46] Stahl, Wilhelm, and Helmut Sies. "β-Carotene and other carotenoids in protection from sunlight." *The American journal of clinical nutrition* 96, no. 5 (2012): 1179S-1184S. https://doi.org/10.3945/ajcn.112.034819

[47] Rao, A. Ranga, Vallikannan Baskaran, Ravi Sarada, and Gokare Aswathanarayana Ravishankar. "In vivo bioavailability and antioxidant activity of carotenoids from microalgal biomass—A repeated dose study." *Food research international* 54, no. 1 (2013): 711-717. https://doi.org/10.1016/j.foodres.2013.07.067

[48] Nishino, Hoyoku, Michiaki Murakoshi, Tsunehiro Ii, Manabu Takemura, Masashi Kuchide, Motohiro Kanazawa, Xiao Yang Mou et al. "Carotenoids in cancer chemoprevention." *Cancer and Metastasis Reviews* 21 (2002): 257-264. https://doi.org/10.1023/A:1021206826750

[49] Jayappriyan, K. R., R. Rajkumar, V. Venkatakrishnan, S. Nagaraj, and R. Rengasamy. "In vitro anticancer activity of natural β-carotene from Dunaliella salina EU5891199 in PC-3 cells." *Biomedicine & Preventive Nutrition* 3, no. 2 (2013): 99-105. https://doi.org/10.1016/j.bionut.2012.08.003

[50] AHMAD, AHMAD SHAMSUDDIN, YONG JULIUS FU SIONG, DESY FITRYA SYAMSUMIR, NOR ATIKAH MOHAMED ZIN, SITI AISHA MOHD RADZI, MURNI NUR ISLAMIAH KASSIM, MOHD ARIFF MUZAMEL, MOHD RIDZUAN YUSOF, and THIRUKANTHAN CHANDRA SEGARAN. "The potential of carotenoids from marine tropical microalgae in the healing process of gastritis." (2015).

[51] San Millán, Coral, Beatriz Soldevilla, Paloma Martín, Beatriz Gil-Calderón, Marta Compte, Belén Pérez-Sacristán, Encarnación Donoso et al. "β-Cryptoxanthin synergistically enhances the antitumoral activity of oxaliplatin through Δ NP73 negative regulation in colon cancer." *Clinical Cancer Research* 21, no. 19 (2015): 4398-4409. https://doi.org/10.1158/1078-0432.CCR-14-2027

[52] Ylönen, Katriina, Georg Alfthan, Leif Groop, Carola Saloranta, Antti Aro, Suvi M. Virtanen, and Botnia Research Group. "Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study." *The American journal of clinical nutrition* 77, no. 6 (2003): 1434-1441. https://doi.org/10.1093/ajcn/77.6.1434

[53] Stahl, Wilhelm, and Helmut Sies. "Bioactivity and protective effects of natural carotenoids." *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1740, no. 2 (2005): 101-107. https://doi.org/10.1016/j.bbadis.2004.12.006

[54] Rafi, Mohamed M., Saravanan Kanakasabai, Sarita V. Gokarn, Eric G. Krueger, and John J. Bright. "Dietary lutein modulates growth and survival genes in prostate cancer cells." *Journal of medicinal food* 18, no. 2 (2015): 173-181. https://doi.org/10.1089/jmf.2014.0003

[55] Gong, Xiaoming, Joshua R. Smith, Haley M. Swanson, and Lewis P. Rubin. "Carotenoid lutein selectively inhibits breast cancer cell growth and potentiates the effect of chemotherapeutic agents through ROS-mediated mechanisms." *Molecules* 23, no. 4 (2018): 905. https://doi.org/10.3390/molecules23040905

[56] Chang, Jingzhi, Yuxia Zhang, Yichuan Li, Kun Lu, Yongjie Shen, Yali Guo, Qingfeng Qi, Mingchen Wang, and Shanfeng Zhang. "NrF2/ARE and NF-κB pathway regulation may be the mechanism for lutein inhibition of human breast cancer cell." *Future Oncology* 14, no. 8 (2018): 719-726. https://doi.org/10.2217/fon-2017-0584

[57] Kavalappa, Yogendra Prasad, Sowmya Shree Gopal, and Ganesan Ponesakki. "Lutein inhibits breast cancer cell growth by suppressing antioxidant and cell survival signals and induces apoptosis." *Journal of Cellular Physiology* 236, no. 3 (2021): 1798-1809. https://doi.org/10.1002/jcp.29961

[58] Erpel, Fernanda, Raquel Mateos, Jara Pérez-Jiménez, and José Ricardo Pérez-Correa. "Phlorotannins: From isolation and structural characterization, to the evaluation of their antidiabetic and anticancer potential." *Food Research International* 137 (2020): 109589. https://doi.org/10.1016/j.foodres.2020.109589

[59] Li, Yong-Xin, Isuru Wijesekara, Yong Li, and Se-Kwon Kim. "Phlorotannins as bioactive agents from brown algae." *Process biochemistry* 46, no. 12 (2011): 2219-2224. https://doi.org/10.1016/j.procbio.2011.09.015

[60] Heo, Soo-Jin, Seok-Chun Ko, Seon-Heui Cha, Do-Hyung Kang, Heung-Sik Park, Young-Ung Choi, Daekyung Kim, Won-Kyo Jung, and You-Jin Jeon. "Effect of phlorotannins isolated from Ecklonia cava on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation." *Toxicology in vitro* 23, no. 6 (2009): 1123-1130. https://doi.org/10.1016/j.tiv.2009.05.013

[61] Venkatesan, Jayachandran, Kishor Kumar Keekan, Sukumaran Anil, Ira Bhatnagar, and Se-Kwon Kim. "Phlorotannins." *Encyclopedia of food chemistry* (2019): 515. https://doi.org/10.1016/B978-0-08-100596-5.22360-3