The role of microalgal extracts and their combination with tamoxifen in the modulation of breast cancer immunotherapy (Review)

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Abstract. Cancer is one of the deadliest health menaces humans have ever witnessed. It is a leading cause of human mortality. Today, it remains a main leading cause of death globally primarily due to lifestyle changes and population ageing. A total of ~12.7 million cancer cases and 7.6 million cancer deaths were reported in 2008. In developing countries, cancer accounted for 56% of cases and 64% of deaths. Tamoxifen is the most reputable and recommended specific oestrogen receptor modulator drug used for the treatment of breast cancer. In the past decade, algae have demonstrated remarkable potency for advanced life applications. They can remain a focus of interest in the coming decades because they are one of the most diverse organisms in the entire ecosystem with immense bio nutritional benefits. Algae and their extracts play a pivotal role in the pharmaceutical industry as bioactive compounds and new drugs and nutraceutical industry as probiotics and antioxidants. However, a broad range of the health benefits of these organisms remains to be explored. The present review highlights the applications and co-application of microalgal crude extracts with tamoxifen for breast cancer immunotherapy. Given that recent studies have suggested that tamoxifen is an essential and primary treatment for breast cancer, the present review focused on the identification of a new treatment approach involving the co-application of tamoxifen and microalgal extracts to provide promising anticancer activity with few side effects on normal cells. The present review includes a general background and blueprint for the use of microalgal extracts as potential and affordable treatments or adjuncts for breast cancer management.

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Contents

1. Introduction

Cancer incidence. Globally, cancer is amongst the most prevalent diseases and the second most common cause of mortality after cardiovascular diseases likely due to changing lifestyles, genetics and population ageing. In 2008, 12.7 million cancer cases and 7.6 million cancer deaths were reported worldwide, of which 56 and 64% were reported in developing countries, respectively (1). Between 2007 and 2008, 23% of the 500,000 deaths reported in the United States were cancer-related (2). Leukaemia is the most common cause of death amongst those <40 years old, whereas lung cancer is the most common cause of death amongst those aged ≥40 (3). The most common cancer amongst women is breast cancer, which is most prevalent amongst women in the age group of 20-59 years-old, whereas leukaemia and lung cancers are common in age groups <20 and >60 years old, respectively. Globally, breast cancer accounts for 23% of all reported cancer cases and is responsible for 14% of all cancer-related deaths in women worldwide (the highest amongst cancer-related deaths) (4).

Several risk factors for breast cancer have been extensively investigated and commonly include early menarche age, late menopausal age, short breastfeeding sessions, late-early-full-term pregnancy, nulliparity and low parity (5). However, studies have been conducted mainly in developed countries in the Western world. Although a handful of studies in Asia have reported similar findings as those in the West, they were primarily conducted in cities and urban areas wherein lifestyles are somewhat comparable to Western lifestyles; therefore, they not represent a considerable part of the Asian population (6,7). Hence, an extensive and all-inclusive study with a large sample size is warranted to determine whether risk factors play the same role amongst Asian populations in third-world countries as those in the West. Such data will substantially contribute to a suitable strategic approach for improving breast cancer awareness and management in Asia (8).

Cancer is an unregulated proliferation of cells leading to excessive cell division (9). The growth stages of cancer cells from normal to malignant are demonstrated in Fig. 1. Cancer cells exhibit six essential changes in their physiology that lead to progression and metastasis: i) Insensitivity to growth-inhibiting signals; ii) self-sufficiency in the presence of various growth signals; iii) limitless replicative potential; iv) tissue invasion and metastasis; v) sustained angiogenesis; and vi) evasion of programmed cell death (apoptosis) (10,11). Cancer can be divided into carcinomas, lymphomas, sarcomas and leukaemia. Carcinomas are the most commonly diagnosed cancers in the skin, lungs, pancreas, breasts and in other different organs or glands (12,13). Lymphomas are related to the lymphocytes of the body's immune system, and sarcomas arise from muscle, bone, cartilage, or fat and are uncommon cancer types (14). Leukaemia is the cancer of the blood, bone marrow and bone; metastasis is the distribution and growth of cancer in different locations or organs in the body (15). The stages of development common to almost all cancer types are illustrated in Fig. 1. Firstly, the cancer starts in the innermost lining (stage 1). Later, the cancer moves to the inner wall of tissue and then distributes outside tissue to the nearest tissues (extravasation) but not to the lymph nodes (stage 2). It then spreads specifically to the closest lymph nodes; however, not to other parts of the body (stage 3). Finally, the cancer metastasises to other vital organs of the body, such as the lungs and liver (stage 4) (16).

Breast cancer. Breast cancer is the most common cancer in women worldwide (17). The age-adjusted breast cancer incidence in Malaysia is 47.4/100,000, which is ~50% of that in North America. The Chinese have the highest incidence of breast cancer (59.9/100,000), followed by Indians (54.2/100,000) and Malays (34.9/100,000) (18). The estimated incidence and death rates of all cancer types and breast cancer worldwide in 2020 as reported by the WHO is demonstrated in Fig. 2.

According to GLOBOCAN 2012, breast cancer is the second most prevalent cancer and fifth most common cause of cancer-related deaths worldwide, with an age-standardised incidence rate of 43.3 per 100,000 women per year and a worldwide mortality rate of 12.9% in 2012 (19,20). In the USA, the cancer mortality rate decreased by 1.8% per year in male patients and by 1.6% in female patients between 2004 and 2008, and the age-standardised cancer death rate reduced by 1.5% (175.8 per 100,000). The cancer incidence rates in men (by 0.6% per year) are declining as a result of medical development and health care, whereas those in women have remained the same. Between 1990 and 2008, the cancer-related death rate decreased by ~22.9% in male patients and 15% in female patients (21,22).

In 2016, the global cancer prevalence ranged from 0.2-2% and was roughly similar as reported in numerous previous studies, which revealed that breast cancer had the highest incidence amongst all cancers in the world (~0.12%) with a total of 8.16 million cases (23,24). Between 2008 and 2017, breast cancer prevalence rates worldwide increased by 20% (25). In 2002, the AVON Breast Cancer Foundation reported that >39,600 breast cancer-related deaths occurred in a population of American women (26). Furthermore, in 2018, breast cancer was reported to be the second most widespread cancer in the world, accounting for 2 million cases, and the most predominant cancer in women (27).

In Malaysia, breast cancer is an important cause of death amongst the population regardless of the sex; however, is more prominent in women than in men (28). Colorectal cancer is more dominant in male patients than in female patients (29). Lung cancer is the third most common cancer in the Malaysian population and second most common cancer amongst male patients; it is responsible for 29% of cancer-related deaths in men and 26% of all cancer-related deaths in women worldwide (30). The National Cancer Registry (NCR) reported data upholding the latter finding. Specifically, data from the NCR revealed that >2,000 reported cases of lung cancer exhibited male predisposition with the M:F ratio of 1,445:603. Lung, prostate, bronchus and colorectal cancers accounted for the highest proportion of cancer-related deaths amongst both sexes. In terms of predisposition, the most prominent cancers amongst female patients are breast, lung, bronchus and colorectal cancers, whereas those amongst male patients are lung, prostate, bronchus and colorectal cancers (31).

Cancer treatment pathways. Chemotherapy and surgery are the most common therapies for cancer. However, chemotherapeutic agents are associated with several side effects, including digestive problems, DNA damage, hair loss, non-specific targets and leukopenia. Moreover, the development of drug resistance decreases the efficiency of drugs in chemotherapy (32-34). Anticancer therapeutic pathways work through numerous mechanisms of action. They include invasion, metastasis, induction of apoptosis in cancer cells and inhibition of cancer cell growth (35). Apoptosis may be initiated by a mitochondrial-mediated (intrinsic) pathway or by a death receptor-mediated (extrinsic) pathway (36,37); each of these strategies involves the activation of caspases (38).

Numerous cancer therapies that have been developed have been associated with intrinsic drug resistance, which reduces the likelihood that patients with cancer will have promising survival times. Amongst all cancer mechanisms, genetic alterations or aberrations have been designated as the primary culprit in cancer development and metastasis (39). Radiotherapy and anticancer agents/drugs may target various signalling pathways in the cell cycle. This effect activates

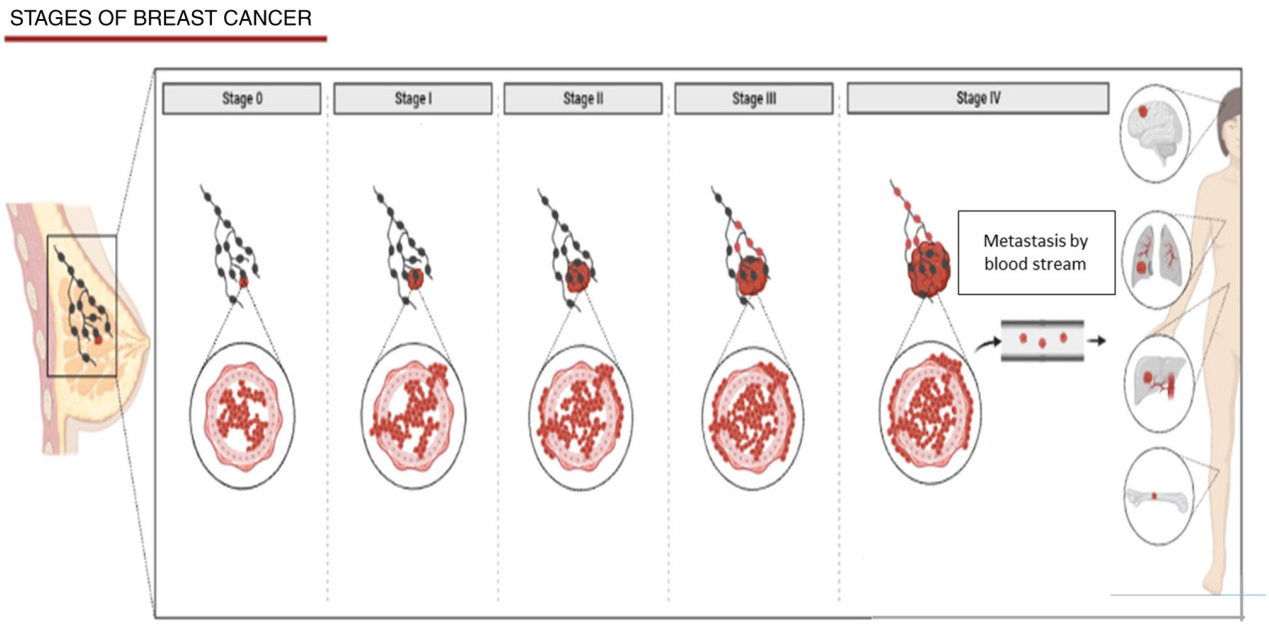

Figure 1. Cancer growth stages.

the apoptotic machinery (programmed cell death) of cancer cells towards cell survival pathways (40). The ratios and percentages of survival and apoptotic pathways result in the overall efficacy of anticancer therapy and are all activated by therapeutic agents (41). In cancer cells, caspases result in apoptosis by inducing caspase-cascade signals. Proapoptotic proteins/enzymes, such as caspases 8, 9 and 3, may be down-regulated in various malignancies (42).

A protein known as TP53 contains tumour protein P53, a tumour-suppressor gene. Its primary function in cell cycle transitions involves regulating and managing damage reactions (DNA damage detection) and apoptosis (43). The association between P53 and drug resistance has been thoroughly investigated in several clinical trials and studies, and most works have established strong associations between P53 variants and chemoresistance, especially in ovarian and lung carcinomas (44). However, a solution to these issues has yet to be found due to the multifactorial characteristics of symptoms.

Protein kinase B (PKB), also known as AKT, is present in a broad range of cancers and plays a vital role in cancer progression along with downstream receptors that control cancer cell progression (45,46). AKT prevents apoptosis by inactivating numerous proapoptotic factors, such as mammalian rapamycin target, the Bcl-2-associated death promoter Poor and caspase-9, hence contributing to cancer cell survival and progression (47). Tumour metastasis is another complicated process involving: i) The detachment of cells from the primary tumour, (ii) invasion into the surrounding normal tissue, (iii) further intravasation into the blood circulatory system and (iv) extravasation and growth in other organs. The cytoskeleton plays a crucial role in promoting the colonisation of metastatic tumour cells. Rho GTPases are one of the important families of GTPases, which regulate the actin cytoskeleton 12; Rho enhances tumour cell metastasis by increasing cell movement (16).

Natural products from microalgae. Algae are organisms that can perform photosynthetic activity; worldwide, they naturally grow in freshwater and wastewater environments that are mainly located near the sea (48). They have the potential for advanced life applications, at least in the coming five decades, because they are one of the most diverse groups of organisms in the ecosystem; their roles depend on their supply and demand, as well as technology (49). Although algae and their extracts are being extensively used in the pharmaceutical industry as bioactive compounds and new drugs and in the nutraceutical industry as probiotics and antioxidants, they have not yet not been explored entirely and may have several applications that require investigation (48).

Algae can be classified into different sizes on the basis of their natural visualisation. Macroalgae can be visualised naturally (for example, seaweeds), whereas microalgae require microscopy aids to be visualised (48). Microalgae contain vitamins, amino acids, proteins, pigments, polyunsaturated fatty acids, minerals and antioxidants. They possess a wide range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, antiviral, antiallergy and antitumoral activities (50,51). They have also been found to exhibit antibacterial, antifungal and antineoplastic properties (52). Marine algae can decrease the risk of cancer because they may exert potential antioxidant and anti-inflammatory effects and are rich in dietary fibre (53). The role of marine microalgae in anticancerous activity may reduce the dangerous side effects of radiation therapy and chemotherapeutic drugs (51).

As aforementioned, microalgae are photosynthetic and can produce hydrocarbons and lipids through the consumption of carbon dioxide and solar energy, thus behaving as a source of energy for the environmental ecosystem (54). They contain various components that could be utilised for human health and provide protection against chronic diseases (55). Microalgae have been described as a rich source of carbon. Therefore, they have a wide range of applications, including

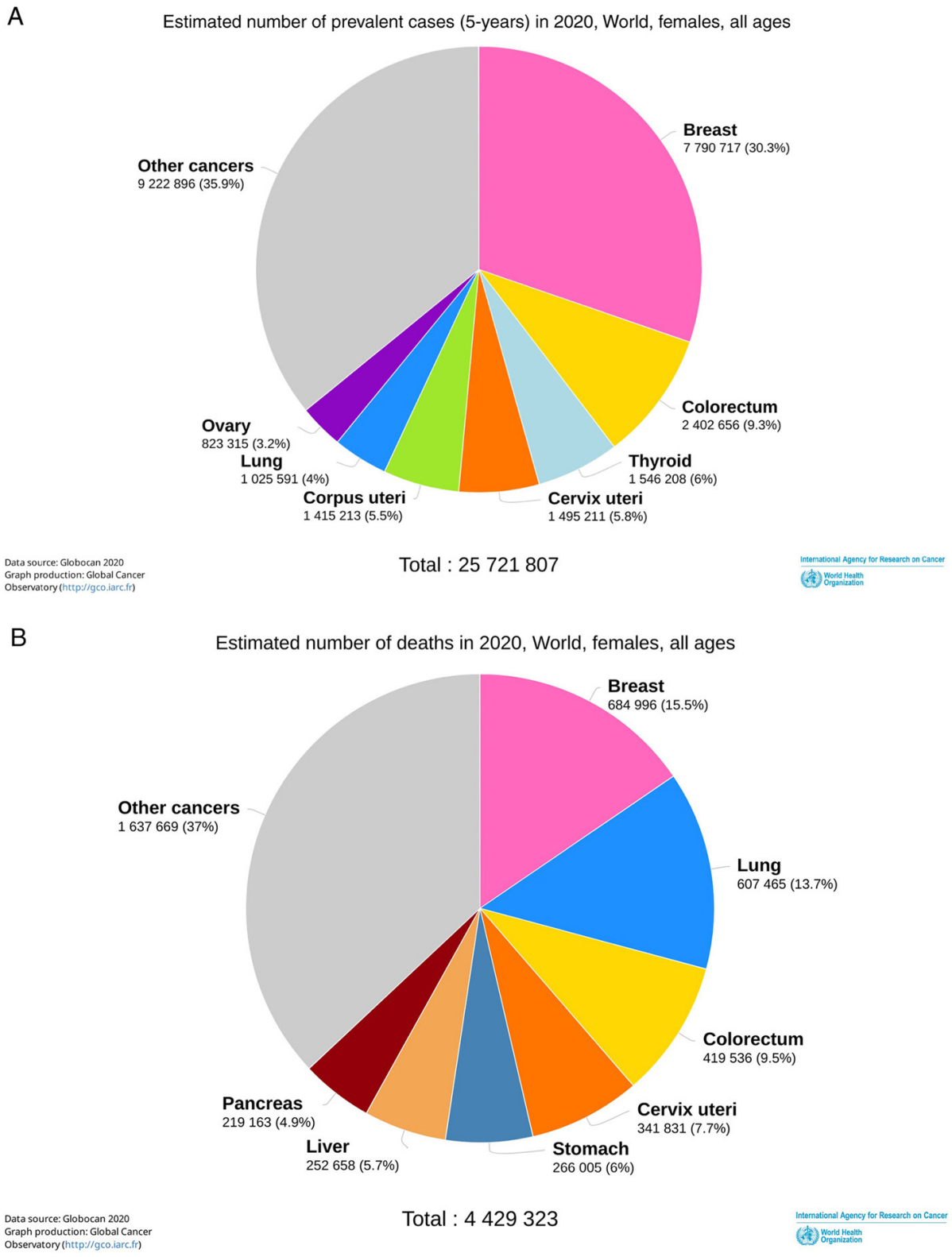

Figure 2. (A) Estimated number of breast cancer cases and (B) death rates.

pharmaceuticals, health supplements, cosmetics and biofuels (56). They have also been employed in wastewater remediation (57). Microalgae produce several high-value products (HVPs), such as food, agar, alginates, carotenoids, fatty acids and pigments, which are beneficial for wild and marine creatures and have several applications in the pharmaceutical, industrial and nutraceutical sectors. They contain long-chain polyunsaturated fatty acids, such as omega-3 and omega-6, which are beneficial for the cardiac and neurological development of the human body (58). *Spirulina* and *Chlorella* species are leading the worldwide microalgae market because they are gaining acceptance in food and health-related supermarkets

Table I. Microalgal pharmaceutical significance.

First author/s, year'	Disease	Type of microalgae	Composition	Significance	(Refs.)
Cha *et al*, 2008	Colon carcinoma	*Chlorella ellipsoidea*	Carotenoid extract	Significant	(66)
Kusaikin *et al*, 2010	Colorectal adenocarcinoma	*Synedra acus*	Polysaccharide	Revealed a dose-time dependent trend	(67)
Pasquet *et al*, 2011	Breast cancer	*Dunaliella tertiolecta*	Violaxanthin	Apoptosis induction in MCF-7 cells	(68)
Erfani *et al*, 2015	Breast adenocarcinoma	*Cladophoropsis sp*.	Ethanol extract	Has high cytotoxic effects	(69)
Srivastava *et al*, 1988	Skin melanoma	*Amphidinium carterae*	Hexane fraction	Prognostic significance in intermediate thickness melanomas	(70)
Kim *et al*, 2014	Liver hepatocellular carcinoma	*Navicula incerta*	Stigmasterol (phytosterol)	Revealed potent apoptosis inductive effects	(71)

and stores (48). Compared with traditional protein sources, such as fish, eggs and soybean, microalgae provide more substantial contributions to protein supply, making them an improved source of natural protein in terms of quality and quantity (48).

Microalgae are classified into four main groups on the basis of their colours: Green (chlorophytes), red (rhodophytes), blue green (cyanobacteria) and other colours (chromophytes) (48). Each group consists of hundreds of species, and every species has thousands of strains (59). The carotenoids, polysaccharides and sterols obtained from marine microalgae are natural sources of drugs. They exhibit potential anticancer activities against different cancer types, as illustrated in Table I (60). Microalgae are the primary source of various valuable products, such as fibre, enzymes, protein, oil, carbohydrates and minerals (calcium, magnesium, iron, potassium and iodine) and are considered as nutritional supplements and health products for humans (61). They also have a remarkable vitamin content. Land-grown vegetables lack vitamin B12, whereas microalgae are a valuable source of this vitamin, along with vitamins C, B1 and B2 (62).

Asia, Australia and the USA were the first to start the production of microalgae in the 1980s (63). Previous technological advancements and progress in biotechnology have enabled the production of high-value nutrients, such as fatty acids, phycobilin, carotenoids, polysaccharides, poly-hydroxy-alkanoates and sterols, from microalgae through the management of culture systems (64). Microalgae could survive in aquatic and non-aquatic ecosystems (65) and tolerate a wide range of parameters; for example, temperature, salinity and pH. Microalgal bioactive components play an essential role in disease-inhibiting and health-promoting products. The pharmaceutical value of microalgae is revealed in Table I.

2. Applications of microalgae

Algae have been used as a source of food and therapeutics for different illnesses in Japan, Taiwan, China and Australia for ≥2,000 years (48). Historically, microalgae have supplied nutrients to humans; for example, *Nostoc*, a genus of cyanobacteria, has been consumed by the Chinese for 20 centuries (66). The microalgal environment is surrounded by various factors that contribute to the production of numerous compounds, such as carbohydrates, lipids, proteins and other secondary metabolites of medicinal value (67). The most common algal products include hydrocolloids, carotenoids and polyunsaturated fatty acids and have wide applications in food, feed, pharmaceuticals, cosmetics and chemicals (48).

The global application of alga-based products in different sectors (for example, pharmaceuticals) has increased (68). Various products in the pharmaceutical industry are derived from microalgae and used as antiviral, antimicrobial and antifungal drugs and therapeutic proteins. The pharmaceutical products derived from microalgae include omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), β-carotene and astaxanthin (69). Algal products are categorised into three types on the basis of their market value, namely, high-, medium- and low-value products (48). Microalgae and cyanobacteria are marketable resources of HVPs (for example, β-carotene, astaxanthin, pigments and algal extracts), which are used in cosmetics and pharmaceutical industries (48).

EPA and DHA derived from algae participate in the inhibition of numerous diseases, including thrombosis, atherosclerosis and arthritis (48). Omega-3 and omega-6 are the two most important fatty acids extracted from microalgae (70), wherein they are found in high concentrations (48). In contrast to humans, the microalgal species *Crypthecodinium*, *Thraustochytrium* and *Schizochytrium* contain the omega-3 fatty acid DHA, and *Phaeodactylum*, *Chlorella* and *Monodus* contain EPA (48).

Astaxanthin is one of the most prominent and active carotenoid molecules present in various types of microalgae. It is the most abundant pigment in nature and isolated from microalgae through extraction (71). The global astaxanthin market has been estimated to be US $257 million (48). Aquaculture utilises the majority of astaxanthin produced

from microalgae specifically in fish coloration. The market size of astaxanthin was last assessed in 2016 and found to be US $555.4 million (48). Astaxanthin is used in the salmon feed industry due to its antioxidant activity (48). It is produced commercially; however, it is distributed naturally in several different species of microalgae. It is most abundant in *Hematococcus pluvialis*. Astaxanthin has been used widely in the nutraceutical and pharmaceutical industries due to its antioxidant and fortification activities (72). β-Carotene is another molecule that is abundant in microalgae. It provides health benefits to humans because it is a favourable source of vitamin A and has antioxidant effects (59). The carotenoid content of most algae is 0.1-2%. However, *Dunaliella*, if cultured under the appropriate conditions of high salinity and light intensity, could yield up to 14% of β-carotene (73). The prospective product areas of algae include nutrient-rich food, bioenergy, bioactive medicine, novel enzymes, special chemicals, bio-fertilisers and bioremediation.

3. Microalgae types in the literature

Chlorella species are non-flagellate autotrophic green microalgae composed of single spherical cells with diameters of ~2-10 μm (73). They contain chlorophylls a and b in their chloroplasts, which help them in photosynthesis. They require CO_2, water, sunlight and a small amount of minerals for rapid growth and can be grown commercially in large circular tanks, photobioreactors and ponds. Initially, *Chlorella* is grown indoors in small culture flasks and then inoculated into outdoor tanks and ponds for large-scale production. *Chlorella* is harvested through centrifugation or auto-flocculation; the harvested biomass is then dried, and the obtained powder is used for different purposes. *Chlorella* contains high amounts of various biologically active compounds and is also used as a source of food, feed and medicine. The biochemical composition of *Chlorella* is 11-58% protein, 12-28% carbohydrates and 2-46% lipids. *Chlorella* also contains various vitamins, such as provitamin A, β-carotene, vitamin E, thiamine B1, riboflavin B2, niacin B3, vitamin B6, inositol, vitamin B12, biotin, folic acid and pantothenic acid (74-77). *Chlorella* has been found to have favourable environmental remediation effects and bioenergy generation ability (51,78-80).

Microalgae are a reliable source of feedstock because of their broad accessibility and can be a favourable source of oil. A study investigated the calorific values of *Chlorella* strains, including four freshwater strains (*Chlorella sorokiniana*, *Chlorella protothecoides*, *Chlorella emerson* and *Chlorella vulgaris*) and one marine strain (*C. minutissima*) and suggested that *Chlorella* strains might be suitable for use as a diesel replacement (81). The utilisation of *C. protothecoides* as a biodiesel source (82,83).

The role of iron in microalgae growth has been reported in numerous studies. Through fluorescence methods and estimations assumed over 12 years, it was determined that iron has a prominent role in managing and directing phytoplankton biomass under high nitrate low-chlorophyll conditions and in oligotrophic waters close to the equator and further south (84,85). However, the response of some biochemical compounds, for example, lipids, in microalgae when the deficiency of 'bioavailable' iron is the primary factor restricting algal biomass production has rarely been reported (86,87). *Nitzschia communis* (88), *Dunaliella* (89), *Botryococcus braunii* (90) and the diatom *Chaetoceros muelleri* (91) were also found to be valuable biofuel sources. Previous investigations have revealed that the lipid content of microalgae might change under different conditions, such as fluctuating salinity (92), silicon deficiency (88), nitrogen deficiency (89), phosphate limitation (90) and excessive cadmium (91), and alternately co-immobilised over alginate globules by the bacterium *Azospirillum brasilense* (90,93). Dry biomass oil levels of 20-50% can be obtained and might reach 80%.

Chlorella strains have great potential to be used as a tool for biodiesel development because of their straightforward cultivation and fast growth. Under general growth conditions, the lipid content of *Chlorella* reaches ~14-30% by dry biomass weight (85,94), which cannot satisfy the commercial criteria for biodiesel output. As already stated, iron is the main factor in improving phytoplankton abundance in ocean waters. In addition, some cultivation conditions may increase lipid quantities. The effect of iron on the production and lipid composition of the marine strain *C. vulgaris* has been studied to determine whether iron may facilitate biomass productivity or lipid aggregation under laboratory conditions. Studies on microalgae and their associations are demonstrated in Table II. These studies demonstrated that the final cell density and lipid content of the marine strain *C. vulgaris* increased by introducing chelated iron into cultures in the late exponential growth stage and *C. vulgaris* increased considerably when its cells were reinoculated into new media with high iron concentrations (95).

The Antimicrobial Stewardship Programme studied the autotrophic growth of microalgae. Autotrophic growth provides several benefits. For example, i) microalgae can convert and produce energy from the sun at the cost of inexpensive natural resources (for example, H_2O and CO_2) (96), leading to global CO_2 reduction; and ii) microalgae may flourish in areas wherein salty water, excessive solar exposure and lack of vital nutrients prevent certain crops from developing (97,98). The concept of the autotrophic growth of microalgae for biodiesel development is fascinating, enticing and technically feasible. Nevertheless, this culture style makes achieving high microalgal biomass densities difficult because light absorption is inversely proportional to cell density (85). Cell coverage can also induce insufficiency, resulting in the low productivity of algal lipids and biomass (99). Additionally, the insufficient production of biomass often increases the cost of biomass processing (100,101).

Microalgae can be grown in heterotrophic systems for developing cost-effective algal oil production wherein organic compounds, such as organic acids and sugars, act as carbon sources. This culture style removes the need for light and thus offers the possibility of drastically increasing cell density, efficiency and productivity (102). Some microalgae can proliferate heterotrophically (103). The heterotrophic algal growth system has been documented to produce not only high algal biomass productivity but also high cellular oil contents (104,105). The heterotrophic growth of *C. protothecoides* on corn compound hydrolysate resulted in a 3.4-fold higher biomass yield than the autotrophic growth system and increased lipid content by 4.2-fold.

Compared with that during autotrophic growth, the production of biomass and lipids during heterotrophic growth

Table II. Studies on microalgae and pharmaceutical significance.

First author/s, year	Disease	Microalgae type, extract or active compound	Type of cell lines	Significance	(Refs.)
Renju *et al*, 2014	Prostate cancer	*Chlorella marina* carotenoids (lycopene), Dose 50 μM	PC-3 cell line	Significant	(101)
Saad *et al*, 2006	Liver cancer	Chlorella vulgaris Hot water extract, 1,600 μg/ml	HepG2	Significant	(102)
Wu *et al*, 2005	Liver fibrosis	Spirulina and Chlorella water extract	HepG2 and HSCs cells	Significant	(103)
Yusof 2010	Hepatoma	Hot water extract of *Chlorella vulgaris* 1.6 mg/ml	HepG2 and WRL68 normal liver cells	The anticancer mechanism of C. Vulgaris in hepatoma cells (HepG2) is by inhibiting DNA synthesis, triggering DNA damage and inducing apoptosis	(104)
Pugh *et al*, 2001	Immunostimulatory activity	Polysaccharides from *Spirulina platensis* (Immulina), *Aphanizomenon flos-aquae* (Immunon) and *Chlorella pyrenoidosa* (Immurella)	THP-1 human monocytes	Microalgal polysaccharides have the main role in immunotherapy in the treatment of cancer and infectious diseases	(105)
Jayshree *et al*, 2016	Breast cancer	*C. vulgaris* and *C. reinhardtii* methanol extract	MCF-7	Significant	(106)
Cha *et al*, 2011	Human colon cancer	Carotenoids extracted from *Chlorella ellipsoidea* and *Chlorella vulgaris*	HCT116	xanthophylls of *C. ellipsoidea* significant and more effective than *Chlorella vulgaris* extracts	(66)
Sedighi *et al*, 2016	Breast cancer	*Chlorella vulgaris* peptides 50 mg/ml	Breast cancer cells	Significant	(107)
Yasukawa *et al*, 1996	Tumor promotion mouse skin	Sterols from *Chlorella vulgaris*	*In vivo* mice	Significant	(108)

is higher; however, it remains not as cost-effective because it requires a higher amount of organic carbon (acetate or glucose) than that of all other nutrients. A cheap tool has to be sought to offset high carbon expenses. Crude glycerol obtained during biodiesel processing can offer such a tool. While the demand for biodiesel continues to increase, crude glycerol is flooding the market (106). The prices of synthetic glycerol decreased from $0.25/lb in 2004 to $0.025-0.05/lb in 2006 (66). The low demand and increased supply of crude glycerol have driven biodiesel manufacturers to search for ways to dispose of this by-product (107).

4. Tamoxifen

Tamoxifen is one of the well-recognised and most recommended specific oestrogen receptor (ER) modulators (108). The FDA affirmed its use for the treatment of women and men with early-stage breast cancer or malignant growth after a medical procedure or to diminish the danger of disease recurrence (109). Tamoxifen has been used to decrease the chance of breast malignancies in women who have not been diagnosed; however, they are at higher-than-normal risk for these malignancies. In women with ER-positive early breast cancer, the risk of recurrence reduced to 50% after 5 years of treatment with tamoxifen [Early Breast Cancer Trialists' Collaborative Group (2015)]. The long-term use of tamoxifen is related to extreme reactions, for example, nausea, and can cause aneuploidy and endometrial and hepatic diseases. The critical characteristics of tamoxifen are revealed in Table III.

Tamoxifen (ICI 46, 474) is a triphenylethylene derivative and exists in *trans*-isomeric form. This tetra-substituted olefin is a nonsteroidal antihormonal drug used for the treatment of breast cancer. It is administered orally and available in the form of citrate salt with the common marketed name Nolvadex (110-114). The citrate salt of tamoxifen exists in the form of a fine white crystalline powder, which is readily soluble in organic solvents, for example acetone, methanol and ethanol, and is partially soluble in water. The stability of tamoxifen citrate depends on its exposure to light and moisture. If properly stored, it can be stable for 5 years. However, humid conditions are unsuitable for this compound, and it becomes hygroscopic under conditions of high humidity. It has been applied worldwide for >40 years and is the most common anti-oestrogen drug used in chemotherapy. It is usually prescribed for the treatment of oestrogen-positive breast cancer. Tamoxifen binds competitively to the ER of cancer cells to

Table III. Tamoxifen characteristics

Name	Tamoxifen
Molar mass	371.515 g/mol
Formula	$C_{26}H_{29}NO$
Other names	TMX; ICI-46474
Trade name	Nolvadex, Genox, Tamifen, and others
Elimination half-life	5-7 days
Bioavailability	~100%

form a nuclear complex, which results in oestrogen inhibition and decreases DNA synthesis. The pharmacological activity of this drug requires metabolic activation. The 2D6 cytochrome P450 converts the pharmacologically inactive tamoxifen into endoxifen (100). Tamoxifen has been found to decrease the rate of breast cancer with its long-term use. It is amongst the highly recommended breast cancer drugs due to its fewer side effects than other treatments. It has been the primary choice for the therapy of postmenopausal, node-positive, oestrogen/progesterone receptor-positive and postmenopausal, node-negative, oestrogen/progesterone receptor-positive women since the '90s. It is one of the common drugs recommended after breast cancer surgery and radiation therapy and for the treatment of women who are at risk of developing invasive carcinoma (115).

Nausea, vaginal dryness, irregular periods, hot flashes and decreased urine amount are amongst the common side effects of tamoxifen (92,116). Another important side effect of tamoxifen is its agonistic effect on all tissues, namely, bones, ovaries, heart and liver, including breast tissues. It participates in forming excess reactive oxygen species within the mitochondria, leading to cell apoptosis (94). In a few cases, the long-term use of tamoxifen also involves the blistering, peeling and loosening of the skin, cataracts, anxiety, chest pain, cough, confusion, pelvic pain, pain and swelling of arms and legs and diarrhoea.

5. Immunological mechanisms of action

As aforementioned, tamoxifen has differential effects on various tissues. Two types of ER exist, namely, ER-α and ER-β (117). Tamoxifen exhibits differential expression with both of these ligand receptors (81). For decades, the antiproliferative action of tamoxifen was considered to be due to its agonistic effect on ER-α. However, in 1996, tamoxifen was reported to have an antagonistic effect on ER-β (118,119). The expression of tamoxifen on ER-α either leads to the inhibition of cell growth or death of cancer cells. The anti-oestrogenic mechanism of tamoxifen on ER-α can induce apoptosis *in vivo* and *in vitro*. However, the *in vitro* mechanism of tamoxifen was found to be concentration-dependent; at low concentrations (nM), tamoxifen can only inhibit the growth of cells, whereas at high concentrations (μM), it leads to cell death (120).

6. Metabolism of tamoxifen

N-desmethyl tamoxifen and 4-hydroxy-*N*-desmethyl tamoxifen are the primary metabolites found after the oral administration of tamoxifen. The enzyme responsible for the metabolism of tamoxifen into 4-hydroxy tamoxifen is cytochrome P450 2D6, which undergoes further metabolism to afford endoxifen. These active metabolites account for tamoxifen's antiproliferative and ER binding ability, and their decreased production will affect the efficacy of the drug because they have the potential to induce apoptotic cell death in MCF-7 (ER-positive), MDA MB 231 (ER-negative) and BT-20 (ER-negative) cell lines (121). Therefore, induced apoptosis might be the primary mechanism of the antitumour activity of tamoxifen. Nevertheless, several attempts have been made to study the non-ER-mediated mechanism of tamoxifen at the cellular and molecular levels due to its efficacy in ER-negative cancers. It has also been used to treat other malignancies, including hepatocellular, ovarian, renal and pancreatic cancers, other than breast carcinoma (122). Therefore, the antitumour effect of tamoxifen is due to a combination of ER-mediated (genomic) and non-ER-mediated (non-genomic) mechanisms. Various signalling proteins are involved in nongenomic pathways, including TGF-β, calmodulin, MAP kinases and protein kinase C (123-125). The statistics of tamoxifen are revealed in Table IV.

7. Other related drugs

Other antioestrogen drugs are used as the second line of breast cancer treatment. Several studies have been conducted to evaluate the comparative effects of tamoxifen and other antioestrogen drugs, for example megestrol acetate. In a survey, Nie *et al* concluded that tamoxifen and megestrol acetate could induce responses as initial treatments. They also studied the combined effects of both drugs however they did not obtain encouraging results (126). However, tamoxifen is still preferable because it has fewer side effects than megestrol acetate. Letrozole, an aromatase inhibitor, has been used to treat metastatic breast cancer in postmenopausal women who are steroid hormone receptor-positive. Compared with tamoxifen, letrozole effectively reduced the risk of periodic disease development in postmenopausal women with endocrine-responsive breast cancer (127).

Tamoxifen is one of the most common drugs used as a first-line breast cancer treatment around the globe. Although the pharmacokinetics and pharmacodynamics parameters of tamoxifen have been widely studied, its paragenetic factor still remains to be explored. *In vivo* studies are performed to evaluate and screen the effects of new drug molecules for the treatment of any disease. Various studies have been conducted to assess the *in vivo* effects of tamoxifen and reported different results. The over-dosage of tamoxifen has not demonstrated any acute toxicity. However, tamoxifen has been found to induce retinopathy in a dose-dependent manner (128). Data on toxicity in children are unavailable. Tamoxifen has exhibited osteogenic agonist effects in some animal species at high dosages (129). The effect of tamoxifen and microalgal extracts on normal and breast cancer cells is depicted in Fig. 3. As aforementioned, tamoxifen is used as a first-line breast cancer treatment.

Nonetheless, an important side effect of tamoxifen is damage to normal breast cells. Microalgal extracts may affect both cell lines; however, not normal cells. The present

Table IV. Statistics of Tamoxifen.

First author/s, year	Disease	Treatment	Type of cells	Significance	(Refs.)
Shen *et al*, 2010	Lung cancer	(Tamoxifen + Gefitinib) 106 mol/l for 72 h	A549 and H1650	Significant	(130)
Torti *et al*, 1984	Prostate cancer	Tamoxifen Citrate, Oral (10-50 mg/twice day)	*In vivo*	Non-significant	(131)
Hoelting *et al*, 1995	Follicular and papillary thyroid cancer	1.5 μM	FTC133, FTC236, and FTC238	Significant	(132)
Chen and Thompson, 2003	Breast cancer	10 μM	MDA-MB-435 and MDA-MB-231	Significant	(133)
He *et al*, 2015	Glioblastoma	15 μM	A172, U251, BT325 and U87	Significant	(134)

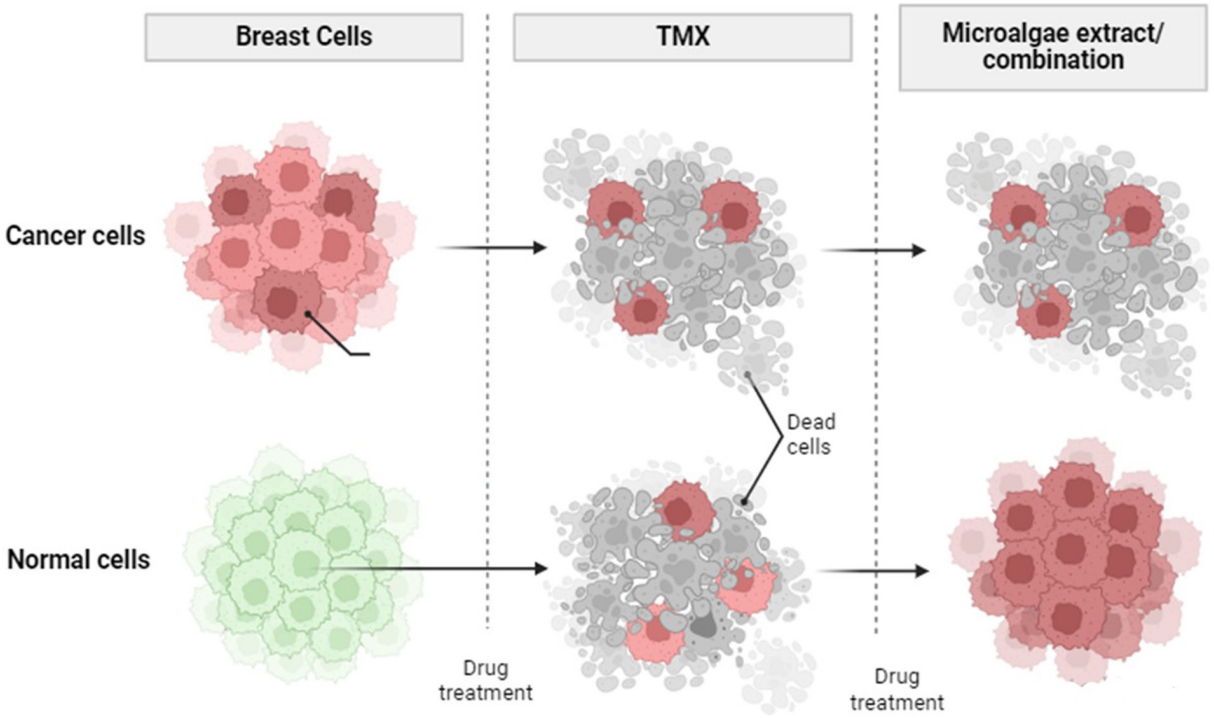

Figure 3. Different breast cancer treatments. The potential effect of microalgal extracts on breast cancer and normal cells and the effect of chemotherapy or hormonal treatments, which eliminate all cells, including normal breast cancer cells, is demonstrated. Created with BioRender.com. TMX, tamoxifen.

review highlights that the co-application of tamoxifen with microalgal extracts may lead to the targeted apoptosis of breast cancer cells and have minimal cytotoxic effects on normal cells.

8. Perspectives

The prospective product areas of algae include nutrient-rich food, bioenergy, bioactive medicine, novel enzymes, special chemicals, bio-fertilisers and bioremediation. They do not depend on one factor and are instead multifactorial with multiple target pathways. Therefore, further studies must be conducted to enhance the positive effects of already available drugs. Given that natural products have always been a great source of interest, the co-application of active algal extracts with tamoxifen can enhance activity against cancer cells or might be a helpful treatment for minimising the side effects of tamoxifen in other ways. Microalgal extracts may affect both cell lines; however, not normal cells. The present review highlights that the co-application of tamoxifen with microalgal extracts may lead to the targeted apoptosis of breast cancer cells and have minimal cytotoxic effects on normal cells. More research interest is necessary to clarify their ideal dosages, mechanisms of action and potential adverse effects.

9. Future directions

Tamoxifen is one of the most common drugs used in the first-line treatment of breast cancer worldwide. Although

its pharmacokinetics and pharmacodynamics parameters have been widely studied, its paragenetic factor has yet to be explored. Previous studies have determined that microalgal extracts, especially those with astaxanthin, have an essential role in anticancerous effects. However, to date, no studies have been conducted to investigate the modulatory action of microalgal extracts targeting specific mechanisms related to inflammation or immunological pathways. Previous studies have demonstrated that the cell cycle and treatment progression of cancer are unclear. They do not depend on one factor and are instead multifactorial with multiple target pathways. Therefore, further studies must be conducted to enhance the positive effects of already available drugs. Given that natural products have always been a great source of interest, the co-application of active algal extracts with tamoxifen can enhance activity against cancer cells or might be a helpful treatment for minimising the side effects of tamoxifen in other ways.

10. Conclusion

The regulation of breast cancer immunotherapy will benefit immensely from the use of microalgal extracts and tamoxifen. Both substances have the potential to strengthen the immune system's response to breast cancer cells, thereby improving therapy outcomes in affected patients. Tamoxifen's well-established involvement in the treatment of breast cancer and astaxanthin's antioxidant qualities imply a synergistic relationship that could enhance the efficacy of immunotherapy. However, before considering the inclusion of these two essential compounds in clinical practice for improving breast cancer immunotherapy, additional research is necessary to clarify their ideal dosages, mechanisms of action and potential adverse effects.

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Availability of data and materials

The data generated in the present study may be found in PubMed and Google Scholar at the following URL: https://scholar.google.com/, https://pubmed.ncbi.nlm.nih.gov/.

Authors' contributions

OMAS and TADAATD conceptualized the study. OMAS and HAA wrote the original draft. OMA, HAA, IAMT and TADAATD reviewed and edited the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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