Bioactive Compounds from Seaweed with Anti-Leukemic Activity: A Mini-Review on Carotenoids and Phlorotannins

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Abstract: Chronic Myeloid Leukemia (CML) represents 15-20% of all new cases of leukemia and is characterized by an uncontrolled proliferation of abnormal myeloid cells. Currently, the first-line of treatment involves Tyrosine Kinase Inhibitors (TKIs), which specifically inhibits the activity of the fusion protein BCR-ABL. However, resistance, mainly due to mutations, can occur. In the attempt to find more effective and less toxic therapies, several approaches are taken into consideration such as search of new anti-leukemic drugs and “combination chemotherapy” where different drugs that act by different mechanisms are used. Here, we reviewed the molecular mechanisms of CML, the main mechanisms of drug resistance and current strategies to enhance the therapeutic effect of TKIs in CML. Despite major advances in CML treatment, new, more potent anticancer drugs and with fewer side effects are needed. Marine organisms, and particularly seaweed, have a high diversity of bioactive compounds with some of them having anticancer activity in several in vitro and in vivo models. The state-of-art suggests that their use during cancer treatment may improve the outcome. We reviewed here the yet few data supporting anti-leukemic activity of some carotenoids and phlorotannins in some leukemia models. Also, strategies to overcome drug resistance are discussed, particularly the combination of conventional drugs with natural compounds.

Keywords: Carotenoids, imatinib, leukemia, seaweed, phlorotannins, Acute Myeloid Leukemia (AML).

1. INTRODUCTION

Leukemia is considered as a group of cancers that affects the blood-forming cells present in bone marrow and the hematopoietic process [1]. According to the GLOBOCAN project 2012, leukemia has a worldwide incidence of 351,965 cases which correspond to 2.5% of all cancers, and a mortality of 255,471 cases corresponding to 3.4% of all cancer deaths [2]. This disease can affect the lymphoid or the myeloid stem cells, both resulting in the production of many white blood cells that are abnormal and do not mature normally [3]. Referring to morphology, genetics, and clinical features, leukemia could be classified into four main groups [4]: Acute Lymphoid Leukemia (ALL), Chronic Lymphoid Leukemia (CLL), Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML).

CML appears due to a translocation of genetic information which generates a constitutively active BCR-ABL oncoprotein [5]. Currently, the standard first-line of treatment is the Tyrosine Kinase Inhibitors (TKIs) such as imatinib that inhibits the catalytic activity of the BCR-ABL oncoprotein. However, this treatment is not curative since most of the patients have residual disease [6, 7]. Despite alternative options, such as switching to other TKI drugs or making stem cell transplants, there is a huge need for novel drugs and treatment strategies aiming at cure [8].
Marine organisms have a huge chemical and biological diversity, being a rich source of compounds with a wide range of bioactivities, namely of medical interest [9]. For at least 2000 years, seaweed has been used for diverse purposes, such as for human and animal nutrition, or as ingredient in traditional medicine, among others [10]. Seaweeds have a high diversity of phytochemicals in their composition, such as carotenoids and phlorotannins that present several bioactivities, namely anticancer action against numerous cancer cell lines [11, 12]. In this mini-review, we briefly describe the main molecular mechanisms in CML and what is known about the anticancer effects against CML of some of the seaweed compounds, mainly carotenoids and phlorotannins, either alone or in combination with currently leukemia treatments.

2. CHRONIC MYELOID LEUKEMIA

CML starts in the blood-forming cells of the bone marrow and is characterized by uncontrolled proliferation of neoplastic hematopoietic precursor cells and weakened production of the normal hematopoiesis, causing several abnormalities in the blood, such as neutropenia, anemia and thrombocytopenia [13]. The incidence of CML increases with age, with the median age of CML diagnosis being 64 years. The course of CML invariably includes: (i) a Chronic Phase (CP), lasting a median of 4-5 years, normally asymptomatic, presenting a myeloid hyperplasia in the bone marrow and peripheral blood with less than 20% of undifferentiated myeloblasts; (ii) an intermediate stage, called the accelerated phase (AP), with few symptoms and an increase of primitive (undifferentiated) cells in the peripheral blood and bone marrow; (iii) and lastly the blast crisis (BC), with extremely poor prognosis, has a rapid progression and the number of undifferentiated myeloblasts is higher than 20% [1].

2.1. Mechanisms Underlying Chronic Myeloid Leukemia

CML is a hematopoietic stem cell disease in which there is a chromosome translocation between chromosomes 9 and 22 during cell division, resulting in a shorter chromosome 22 called Philadelphia chromosome. This translocation t(9;22) (q34;11) of DNA means that part of chromosome 22 Breakpoint Cluster Region (BCR) gene at band q11 fuses with chromosome 9 Abelson murine leukemia viral oncogene homolog 1 (ABL) gene at band q34, leading to the formation of a new oncogene BCR-ABL [5]. ABL is a proto-oncogene that encodes for a protein tyrosine kinase found mainly in the nucleus, which regulates the cell cycle, differentiation, migration, invasion, genomic instability and the response to genotoxic stress [14]. The BCR gene encodes for a serine/threonine kinase that can act as a GTPase-activating protein for members of the Rho family of guanine nucleotide exchange factors and can also phosphorylate histones and caspase [15].

Fig. (1). Mechanisms of drug resistance induced by BCR-ABL. In a normal cell pathways (represented as black lines) DNA repair mechanisms are activated and DNA damage can be completely repaired - the cell will survive. In the case of inefficient repair, and if the cell cycle checkpoints are not strong enough to allow more time for repair, death signals are activated by the induction of apoptosis. In a BCR-ABL expressed cell pathways (represented as gray lines), the global levels of DNA repair mechanisms are increased. This also affects the checkpoints, providing more time for repair, however, the efficiency is compromised. In addition, in case of some unrepair or misrepair lesion escape, the apoptotic signals are inhibited by the regulation of Bcl-2 protein family. Overall, this process leads to an accumulation of DNA damage, increasing the genomic instability. Adapted from [20].
BCR-ABL encodes a new protein, p210<sup>BCR-ABL</sup>, which shows deregulated tyrosine kinase activity. The critical functional changes found with the expression of this new protein are: ABL protein becomes constitutively active as a protein tyrosine kinase enzyme; attenuation of DNA protein-binding activity of ABL; and the improvement of ABL binding to the cytoskeletal actin microfilaments [16]. BCR-ABL modulates different signaling pathways, such as PI3K/AKT/mTOR, JAK-STAT, Wnt/β-catenin and autophagy. This brings benefits in terms of cell survival, proliferation, differentiation, and migration, allowing a deregulated cell proliferation, protection against cell death in the absence of external factors, and promotion of invasion and metastasis [17]. Modulation of survival pathways by BCR-ABL activation (Fig. 1) has also been related to resistance to genotoxic therapeutics since BCR-ABL-positive cells can repair DNA damage more quickly through the upregulation of RAD51 (a protein of homolog recombination repair system), decreasing its degradation and activating it through post-translational modification. The expression of RAD51 in the cells seems to be positively correlated with the appearance of a resistance phenotype [18]. The capacity to repair DNA is not only influenced by the efficiency of the repair mechanisms but also by the time that the cell has to carry out the mechanism. BCR-ABL-positive cells can activate DNA damage-dependent cell cycle checkpoints faster by displaying a pronounced G2/M delay [19]. Another mechanism involved in drug resistance is the modulation of Bcl-2 family members, namely the upregulation of anti-apoptotic proteins (e.g. Bcl-xl and Bcl-2) [20].

Animal models confirmed that BCR-ABL plays a crucial role in the pathogenesis of CML, increasing cell proliferation, blocking apoptosis and stromal interactions of the cell [21, 22]. Hence, the development of targeted therapy with a specific action on tyrosine kinase was a breakthrough in the treatment of myeloid leukemia [1].

2.2. Treatment of Chronic Myeloid Leukemia Patients with TKIs

TKIs are the most common drugs used in CML therapy. They bind competitively to the adenosine triphosphate (ATP) binding site of the BCR-ABL protein (Fig. 2). Consequently, TKIs inhibit the phosphorylation of proteins related to BCR-ABL signals transduction [1, 23]. The outcome is the induction of apoptosis in BCR-ABL-positive cells without affecting normal cells [24]. In the early 1990s, were developed the first TKI - Imatinib mesylate (also known as STI-571 or Gleevec). In 2001, imatinib was approved by Food and Drug Administration (FDA) for the treatment of CML in chronic phase and is known as the first generation of TKI [25]. Imatinib results in the inhibition of proliferation, restoration of the cell cycle, apoptosis induction and reversal of genetic instability in BCR-ABL dependent cell. Imatinib proved to be an effective treatment since patients showed 85% of overall survival [1].

Despite the specific mechanisms of action, around 33% of patients started to fail to achieve a Complete Cytogenetic Response (CCyR), either because of toxicity or (mostly) due to the appearance of resistant phenotype over time [16, 26]. The resistance to imatinib may be due to BCR-ABL-dependent or independent mechanisms. The BCR-ABL-dependent ones include, most frequently, point mutations in the ABL Tyrosine Kinase Domain (TKD) and gene amplification of ABL [26]. An important mutation results in the formation of amino acid substitutions in imatinib binding sites, frequently called “gatekeeper” mutations (e.g. T315I) [1]. The BCR-ABL-independent mechanisms of resistance involve: (1) decrease in drug uptake by the expression of the human Organic Cation Transporter 1 (hOCT1) [27]; (2) increase in drug efflux by overexpression of P-glycoprotein (P-gp) efflux pumps [23]; (3) increase of plasma protein α1 acid glycoprotein (AGP), which binds to imatinib preventing the ABL kinase inhibition [28] and (4) increase of prostaglandin-endoperoxide synthase 1 (or cyclooxygenase 1), which plays a significant role in imatinib metabolism, viz. because participation of the enzyme in membrane transport and drug metabolism and resistance [29].

The second-generation of TKIs were developed in response to the emergence of imatinib resistance and intolerance. Those TKIs included nilotinib (Tasigna®), dasatinib (Sprycel®) and bosutinib (Bosulif®). These drugs achieved positive molecular responses in imatinib-resistant patients, yet each drug induced its own mutations, and none could inhibit BCR-ABL T315I [1, 7, 16, 30]. In 2012, it was approved the first TKI of third generation - ponatinib (Iclusig®). This is the only TKI that presents activity against the T315I mutation [1, 31].

2.2.1. Current Strategies to Enhance the Therapeutic Effects of TKIs

The development of resistance in leukemia cells and the potential toxicity have been great limitations for TKIs application [32]. In 2013, FDA issued a Drug Safety Communication that warned for “an increasing frequency of reports of serious and life-threatening blood clots and severe narrowing of blood vessels (arteries and veins) of patients taking the leukemia chemotherapy drug Iclusig (ponatinib).” Full approval and label by FDA for Ponatinib in CML and ALL were granted only in 2016 [33]. The future of CML therapy has passed to discover new potent agents that could act even in cases of imatinib-resistance and/or by combinatorial chemotherapies (Fig. 3). Tolomeo et al. showed that 3'hydroxypterostilbene, a natural pterostilbene analogue, showed anticancer activity through a pro-apoptotic effect in resistant lymphoma and leukemia cell lines without cytotoxicity in normal hematopoietic stem cells [34]. Zhang et al. verified that beta-phenylethyl isothiocyanate (PEITC), a natural compound found in edible vegetables, is a promising compound, because it is capable of inducing cell death in imatinib-resistant CML cells through a ROS-mediated mechanism [35]. More recently, Eucker et al. showed that using mTOR inhibitor RAD001 in imatinib-resistant leukemia cells, it is possible to decrease cell proliferation by inducing cell cycle arrest and apoptosis [36].

Combination chemotherapy is based on joining two or more therapeutic drugs, either established ones and/or new agents, ideally with different modes of action and non-overlapping toxicities, and is a well-proven good strategy to overcome chemoresistance in several types of cancer [37, 38].
Fig. (2). ABL through binding of TKIs (of 1st and 2nd generation) that inhibit the phosphorylation of BCR-ABL targets. C) Inactivation of the kinase domain of BCR-ABL mutant T315I by ponatinib (TKIs of 3rd generation). The mutation T315I prevents the binding of TKIs to the Kinase domain and only ponatinib has a chemical structure able to bind BCR-ABL. P – Phosphate.

Fig. (3). Strategies to enhance the therapeutic effects of TKIs. Solid lines represent the effect of BCR-ABL, whereas dashed lines represent the effect of TKIs and the possible strategies.
Nimmanapalli et al. showed that imatinib, when combined with suberoylanilide hydroxamic acid (SAHA), known as an inhibitor of histone deacetylases, can enhance the cytotoxicity effects in leukemia cells (K562 cell line) by up-regulation of p21 and p27 and down-regulation of BCR-ABL levels with the induction of apoptosis in BCR-ABL-positive cells [39]. A decrease in phospho-AKT and Bcl-xL levels was also observed. Bu et al. reported that the co-treatment with SAHA and S116838 (a small molecule multi-targeted tyrosine kinase inhibitor) reduced cell viability and induced cell death by down-regulation of Mcl-1 and XIAP and promoted Bim expression and mitochondrial damage [40]. After experiments in two CML murine models, Hu et al. concluded that low doses of imatinib combined with proteasome inhibitor might help CML treatment by the inhibition of short-term cell growth and long-term clonogenic activity and induction of apoptosis in BCR-ABL positive cells [32]. The results showed inhibition of Bcl-2, increase of cytochrome c and activation of caspases, along with the inhibition of proteasomal degradation of protein phosphatase 2A (PP2A). Lin et al. demonstrated that by down-regulating RanGTPase and activating protein 1 (RanGAP1), it is also possible to improve imatinib efficiency, because RanGAP1 can mediate BCR-ABL nuclear entrapment to activate the p73-dependent apoptosis pathway [41]. Also, platinum-based anticancer agents, such as cisplatin and oxalipatin, exercised a synergistic effect when combined with imatinib in BCR-ABL-positive cells, by the inhibition of BCR-ABL and its downstream ERK phosphorylation [42]. Zhang et al. suggested the synergistic effects of imatinib with Grb SH2 domain binding antagonists by cell cycle arrest, induction of apoptosis, reduced protein tyrosyl phosphorylation and Grb2 recruitment [43]. Several other drugs, e.g. arabinoside, a synthetic pyrimidine nucleoside derived from spongouridin isolated from a Caribbean sponge; daunorubicin, an anthracycline drug derived from Streptomyces peucetius; and homoharringtonine, a natural alkaloid obtained from Cephalotaxus fortunei) when in combination with imatinib, also improve the therapeutic effect in imatinib-resistant CML cells [44]. The synergistic effect was also observed with other TKIs. Crawford et al. showed the synergism of carfilzomib with imatinib and nilotinib, by the reduction of cell proliferation and induction of apoptosis in various CML models [45]. Equally, ruxolitinib, a JAK2 inhibitor, combined with nilotinib can eradicate CML disease by reduced JAK2/STAT5 pathway [46]. Also, the combination of Bcl-2 inhibitors with BCR-ABL tyrosine kinase inhibitors, mainly nilotinib, could improve cure rates of CP and BC of CML in mice [8].

Besides the encouraging data obtained with the current therapies, new anti-leukemic compounds are needed and marine organisms, due to the extremely high bio and chemical diversity, have emerged as a hopeful source of bioactive compounds.

3. MARINE ORGANISMS AS A SOURCE OF BIOACTIVE COMPOUNDS

The interest in natural products for oncology therapy is not new and nowadays, almost 60% of the existing anticancer drugs are derived from natural origin [47]. Marine algae can be divided into four main groups, per the type of pigments, morphology, anatomy and reproductive structures: Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae) and Cyanophyceae (blue-green algae) [48]. Seaweeds are multicellular eukaryotic and macroscopic organisms living in salty water and mostly belong to the green, brown and red algae. Seaweeds are a rich source of polysaccharides, polyunsaturated fatty acids, minerals, protein, vitamins, and low-fat carbohydrates [49]. Since ancient times, seaweeds have been integrated into the human diet, particularly in Asian cuisine, and nowadays the consumption of this natural product around the world is increasing [48]. Many other “outside the kitchen” applications have been emerging, aiming at a wide range of industrial and biotechnological usages, and evoking particular challenges, namely in the industrial production of such diverse and coveted seaweed bioactive compounds [50, 51].

In the context of their bioactives, it is worth stressing that seaweeds are continuously exposed to high levels of light and oxygen that normally contribute to the formation of free radicals and other oxidative reagents. However, the absence of photodynamic damage in those algae could be due to the presence of bioactive components with the ability to protect themselves [52]. In detail, the bioactives produced by a variety of seaweed species provide several properties of great interest in biomedicine, such as antibacterial, antiviral, immunosuppressant, antioxidant and antitumor [53, 54]. Hence, we will highlight the findings that showed the benefits of bioactive compounds, such as carotenoids and phlorotannins, present in seaweed, alone or in combination with anticancer drugs to treat leukemia especially CML.

3.1. Carotenoids

Seaweed pigments can be divided into three main groups: chlorophylls, carotenoids, and phycobiliproteins. Carotenoids, organic pigments found in chloroplasts and chromoplasts, exhibit a purple, red, orange and yellow color — actually, carotenoids are a class of tetraterpene pigments found in bacteria, fungi, algae, as well as in higher plants and animals [55]. Nowadays, more than 700 carotenoids have been found in nature [56-58]. The intake of dietary carotenoids has been correlated with a lower incidence of cardiovascular and neurodegenerative diseases, cataract formation and cancer [59, 60]. Diverse biological functions such as provitamin A activity, antioxidant, antiproliferative and pro-apoptotic activity and enhancement of the immune system have been attributed to carotenoids [61-63]. The antioxidant activity of carotenoids is related to their structure; the number of double bonds and the presence of functional groups influence their ability to interact with different radicals [64].

Seaweeds are rich in carotenoids, such as, fucoxanthin, astaxanthin, siphonoxanthin, violaxanthin, neoxanthin, β-carotene, capsanthin, lutein, among others (Fig. 4), which are differently distributed according to the classes of algae (to review this topic see the reference [58]). Fucoxanthin is one of the most abundant carotenoid found in nature, particularly in marine environments. This xanthophyll represents more than 10% of the total carotenoid production. Fucoxanthin is...
widely distributed in brown algae (Phaeophyceae) and diatoms (Bacillariophyta) and has an unusual chemical structure that includes an allenic bond and a 5,6-monoepoxide moiety [65]. Such structure caused unique functionalities, e.g., a potent antioxidant action [66].

Fucoxanthin has preventive effects against several types of cancer, including leukemia, due to its mechanisms of action, which includes antiproliferation, cell cycle arrest, apoptosis induction, and suppression of angiogenesis [65, 67, 68]. Furthermore, the anticancer effects of fucoxanthin seem to be in some cases specific for cancer cells, without cytotoxic effects against the normal cell lines [62]. However, in leukemia, the effects of fucoxanthin are poorly understood. Here, we will summarize the current knowledge of the effects of fucoxanthin and other seaweed carotenoids against CML.

### 3.1.1. Anti-Leukemic Activity of Carotenoids

The anticancer activity of carotenoids was demonstrated in different types of cancer cell lines [65], including leukemia (Table 1). Recently, we showed that fucoxanthin has cytotoxic and antiproliferative effects in K562 and TK6 malignant cells, without the induction of DNA damage and apoptosis [69]. Fucoxanthin and fucoxanthinol (its deacetylated metabolite) also decrease the viability of HTLV-1 infected T-cell lines and primary ATL (adult T cell leukemia) cells, without evidencing cytotoxicity in normal peripheral Blood Mononuclear Cells (PBMCs) [62]. The cytotoxic effect of fucoxanthin and fucoxanthinol is mainly due to the induction of G1 cell cycle arrest and apoptosis, and were greater than the cytotoxicity of other carotenoids (β-carotene and astaxanthin). The cell cycle arrest was associated with the down-regulation of cyclin D1, cyclin D2, CDK4 and CDK6 expression and up-regulation of GADD45α, which inhibit the entry of cells into S phase [62]. Later, authors also showed the induction of apoptosis with the activation of caspase-3, -8 and -9, and down-regulation of anti-apoptotic proteins expression, such as XIAP, cIAP2, Bcl-2 and survivin. In CML blast phase and imatinib resistant cells, survivin, a member of the inhibitor of apoptosis (IAP) family proteins, is overexpressed. Down-regulation of survivin expression induces cell cycle arrest and cell death and has been suggested as a potential therapeutic strategy in patients with CML [70]. NF-κB pathway is involved in the regulation of proliferation, apoptosis and cell differentiation, contributing for cancer pathogenesis, namely in CML, where that pathway is aberrantly activated at the CML blast phase [71, 72]. Inhibition of NF-κB pathway has been reported as a promising therapeutic strategy [72]. Fucoxanthin and fucoxanthinol inhibit the NF-κB pathway by suppressing IkBα phosphorylation [62]. Konishi et al. demonstrated that fucoxanthinol exhibits antiproliferative effects in HL-60 cells by the induction of apoptosis with an increase of DNA fragmentation [73]. Nakazawa et al. observed that fucoxanthin showed antiproliferative and pro-apoptotic effects in HL-60 leukemia

![Fig. (4). Chemical structure of some carotenoids.](image-url)
Table 1. Anti-leukemic activity of carotenoids and phlorotannins and its mechanism of action.

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<th>Natural Compounds</th>
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<td>HTLV-1 infected T-cell lines and primary ATL cells</td>
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<td>Fucoxanthinol</td>
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<td>HL-60 cells and HUVECs</td>
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cells with the down-regulation of anti-apoptotic Bcl-2 proteins [74, 75]. Cleavage of caspase-3 and -7, and poly (ADP-ribose) polymerase (PARP) and decrease of Bcl-xL levels caused by the generation of ROS, and cell cycle arrest in G1 stage were observed in leukemic cells treated with fucoxanthin [76]. However, Kotake-Nara et al. demonstrated that apoptotic activity of fucoxanthin in promyelocytic cell lines was associated with the loss of mitochondrial membrane potential and cleavage of procaspase-3 and PARP, without any effect on ROS level and protein level of Bcl-2, Bcl-xL and Bax [77]. Fucoxanthin also inhibits JAK/STAT and PI3K/AKT pathways that are involved in CML pathogenesis, however, the effect of fucoxanthin in these pathways in CML still unknown [65]. Other carotenoids present in seaweeds, such as siphonaxanthin, neoaxanthin, violaxanthin, and astaxanthin, decreased cell viability in HL-60 cells. In the case of siphonaxanthin, the cytotoxic effect was higher than fucoxanthin, inducing cell cycle arrest, by up-regulation of GADD45α, and apoptosis, by caspase-3 activation with a decrease of Bcl-2 expression. Siphonaxanthin also sensitizes HL-60 cells to TRAIL-induced apoptosis, by an increase of the expression of Death Receptor (DR5) [78]. Besides the antiproliferative and pro-apoptotic effects, fucoxanthin and siphonaxanthin also showed anti-angiogenic effects - mediated by down-regulation of signal transduction by Fibroblast Growth Factor 2 (FGF-2) and its receptor (FGFR-1) - in Human Umbilical Vein Endothelial Cells (HUVECs) [79, 80]. Activation of telomerase has been associated with CML pathogenesis and its inhibition could lead to CML stem cell elimination [81, 82]. Some carotenoids such as lycopene, when in encapsulated form, significantly decrease cell proliferation and induce apoptosis, by inhibiting telomerase activity in K562 leukemia cells [83]. Also, β-ionone induce apoptosis in K562 cells by the inhibition of telomerase activity [84]. β-cryptoxanthin-loaded nanoliposomes have strong apoptosis induction in K562 cells, [85]. Zhang et al. verified in K562 cells that carotenoids such as β-carotene, astaxanthin, capsanthin, and bixin inhibit cell viability and cell proliferation, with an increase of cells in G0/G1 phase, and induce apoptosis [86]. These effects were accomplished with the up-regulation of PPARγ, p21 and Nrf2 and down-regulation of cyclin D1 expression. According to the authors, the antiproliferative effects of these carotenoids in K562 cells seem to be at least partly due to the effects on PPARγ and Keap1-Nrf2/EpRE/ARE signaling pathways [86]. PPARγ is a transcription factor involved in the regulation of cell differentiation, proliferation and apoptosis and its agonists have been reported as having anticancer activity, inhibiting cell proliferation and inducing apoptosis in CML [87, 88]. Up-regulation of p21, a cell cycle inhibitor, has been induced in different cell models by several carotenoids such as fucoxanthin, β-cryptoxanthin and β-carotene [89-91]. Cyclin D1, which is involved in the regulation of cell proliferation in G1 phase, is overexpressed in CML, and its inhibition promotes cell cycle arrest [92]. In HL-60 cells, bixin induces cytotoxicity without genotoxic, mutagenic and apoptosis effect [93]. Palozza et al., Sacha et al. and Upadhyaya et al. also found pro-apoptotic effects of β-carotene in HL-60 leukemia cells [94-96]. The data suggest that the antiproliferative and pro-apoptotic effects of β-carotene are related with an increase of ROS and its regulation by NF-kB, which raised gene expression of c-myc [94]. At a concentration achievable in human serum after supplementation, β-carotene induced apoptosis by up-regulation of Bax expression in U-937 leukemia cell line [95].

3.2. Phlorotannins

Phlorotannins are tannin derivatives that appear in the form of polyphenols formed by polymerization of phloroglucinol units (Fig. 5). Regarding the link between phloroglucinol units and the number of hydroxyl groups, phlorotannins can be classified into two subgroups: phloretins, fuhalols, fucols, fucophloretins, eckols and carmalols [103, 104]. Some of the most known phlorotannins are phloroglucinol, eckol, dieckol, 8,8’-biekol, 6,6’-biekol, dioxyindolhydroeckol, phlorofucofuroeckol-A, among others (for more detail about chemical structure read the review [105]). They are highly hydrophilic, display a wide range of molecular sizes, and are present mainly in brown algae in which they are crucial for photoprotection [49, 105]. Phlorotannins have been clarified to exhibit several biological activities, such as anti-diabetic, antioxidant, anti-inflammatory, anti-bacterial, anti-allergic and anticancer [106, 107].

In leukemia, the effects of phloroglucinol are poorly understood. As such, it will be summarized below the meagre current knowledge regarding the anticancer effects of this compound against leukemic cells, as well as the evidence supporting cytoprotective effects in normal cells.

3.2.1. Anti-Leukemic Activity of Phlorotannins

Phlorotannins, especially phloroglucinol and eckol, have been shown to have cytoprotective effects in normal cells derived from the toxicity found in cancer treatments, proving to be a good candidate to improve the quality of these treatments [105, 108-110]. Both phloroglucinol and eckol showed radioprotective and antioxidant effects, in vitro and in vivo, protecting intestinal stem cells against damage caused by gamma-irradiation [110]. The protective effect of phloroglucinol, in mice against intestinal damage caused by ionizing radiation, was accomplished by the down-regulation of pro-apoptotic proteins such as p53, Bax and Bak and up-regulation of anti-apoptotic proteins such as Bcl-2 and Bcl-X<sub>L</sub> [108]. In vitro, phloroglucinol, decreased the level of ROS, lipid peroxidation, DNA damage and protein oxidation and restored the level of reduced glutathione. The protective effect of phloroglucinol against radiation-induced apoptosis was accompanied by up-regulation of bcI-2 expression that prevents the loss of mitochondrial membrane potential and subsequently decreases the levels of caspase-9 and -3. Phloroglucinol also inhibited JNK activation, SEK1 phosphorylation and AP-1 activity suppressing radiation-induced apoptosis through SEK1-JNK-AP-1 pathway. In this way, phloroglucinol efficiently protected cells against radiation and extended the survival of mice exposed to lethal doses of radiation [109]. Phloroglucinol, eckol and other phlorotannins showed the ability to inhibit ROS generation [111, 112].
Phlorotannins showed anticancer activity in several cell models, such as colon cancer cells, hepatocellular carcinoma cells, and ovarian cancer cells [104, 113-115]. However, anticancer activity of phlorotannins on leukemia cell lines remains poorly explored. One extract of phlorotannins from *Laminaria japonica* showed cytotoxic activity in murine leukemic cells (P388) with apoptosis induction [97]. According to Liu *et al.*, hyperforin, a phloroglucinol-type element isolated from the plant *Hypericum perforatum*, decreased cell viability by inducing cell cycle arrest in G1 phase and apoptosis in K562 cells, with little cytotoxic effect in HU-VECs [98]. Further, hyperforin induced mitochondrial dysfunction through regulation of Bcl-2 family proteins (downregulation of antiapoptotic and up-regulation of proapoptotic proteins). The authors observed the activation of caspase -3, -8 and -9 cascade, and subsequently cleavage of PARP (a nuclear enzyme involved in DNA repair), as well as up-regulation of p53 and p27kip1 in K562 cells treated with hyperforin [98]. The antiproliferative and apoptotic effects of hyperforin were reported in other leukemia cells U937 increasing the activity of caspase-9 and -3 [116]. The apoptotic effect of hyperforin seems to be related to inhibition of AKT1 pathways in leukemic cells [99]. Quiney *et al.* reported that leukemic cells isolated from patients with B-cell Chronic Lymphocytic Leukemia (B-CLL), treated *ex vivo* with hyperforin, showed increased apoptosis by disruption of the mitochondrial transmembrane potential, activation of caspase-3, and cleavage of the PARP [100]. Furthermore, downregulation of Bcl-2 proteins, Mcl-1 (antiapoptotic proteins) and Nitric Oxide (NO) synthase of type 2 were also observed. In the same study, downregulation of the p27kip1, a cell cycle inhibitor, was detected in contrast with the results from Liu *et al.* [98, 100]. Zaher *et al.* showed that the mitochondrial pathway of caspase-dependent apoptosis triggered by hyperforin was induced by up-regulation of Noxa (protein of Bcl-2 family), suggestively via post-translational regulation because Noxa mRNA levels were unchanged [117]. Hyperforin also showed anti-angiogenic activity in B-CLL cells by inhibition of Matrix Metalloproteinase-9 (MMP-9) and of vascular endothelial growth factor (VEGF) production [101]. The acylphloroglucinols hyperforin and myrtucommulone A induced apoptosis in HL-60 cells, by the activation of intrinsic apoptotic pathway with the loss of the mitochondrial membrane potential and release of cytochrome c [102].

*Fig. (5).* Chemical structure of some phlorotannins.
3.3. Potentiation of TKIs by Natural Compounds

The combination of natural compounds with conventional anticancer drugs may improve the cancer treatment by changing the biological disposition to minimize toxicity, maximizing efficacy and possibly reducing the dose of therapy [13, 118]. Regarding the combination of TKIs with either carotenoids or phlorotannins derived from marine organisms, to the best of our knowledge, no data are available until now, except for one recent paper that reported that fucoshandin in combination with imatinib increased antiproliferative effects of the anticancer drug in K562 and TK6 cells [69]. However, the antiproliferative effect observed was mainly due to the action of fucoshandin alone [69]. The anticancer activities of isolated compounds reveal promisor effects of carotenoids and phlorotannins in future combinations with TKIs. For example, in CLL and CML cells, hyperforin decreased the activity of P-glycoprotein (P-gp or MDR1) and Breast Cancer Resistance Protein (BCRP), two drug transporters overexpressed in leukemic cells [119]. Carotenoids such as fucoshandin and canthaxanthin also down-regulate MDR1 expression increasing the cytotoxic activity of anticancer drugs [120, 121]. Accordingly, inhibition of these drug transporters is an alternative to overcome TKI drug-resistance [122]. Some natural compounds seem to potentiate the anticancer effect of TKIs against CML cells, as is the case of the natural compounds mentioned below. Lin et al. showed that sulforaphane, an organosulfur compound, enhances the anticancer effect of imatinib over resistant leukemia stem cells, inducing apoptosis by up-regulation of caspase 3, PARP, and Bax, and down-regulation of Bel-2 expression. The combination also reduced BCR-ABL, β-catenin, and MDR-1 protein expression [123]. Likewise, α-Bisabolol, a natural sesquiterpene alcohol, induced apoptosis through an increase of ROS, disruption of mitochondrial potential and plasma membrane integrity, while potentiating the anticancer effect of imatinib (and nilotinib) in TKI-resistant leukemia cells [124]. Ponatinib is a third-generation TKI that is effective against resistant CML cells (with T315I mutation) but induces severe side effects, such as cardiac toxicity and several vascular problems. Forskolin, a diterpene produced in the roots of the Indian plant Coleus forskohlii, synergistically enhanced the anticancer activity of ponatinib in drug-resistant CML cells [125]. Facing the state-of-the-art, there is evidence of promising effects of some natural compounds in combination with TKIs. In view of the anti-leukemic activity of some carotenoids and phlorotannins and the scarce data about their effects when combined with TKIs, such combinations should be worth exploring by replication and expanding the diversity of bioassays and tested conditions.

CONCLUSION

The continuous increase of cancer incidence and prevalence over the world, and in particular of hematologic malignancies, a broader repertoire of therapeutic solutions than those currently available is needed. The introduction of TKIs has transformed the outcome of CML treatment by significantly improving long-term survival; however, their therapeutic efficacy is compromised since they rarely eliminate completely CML stem cells, even in patients with complete molecular responses. Furthermore, long-term treatment with TKIs comes with several health problems and high financial costs. Thus, the facts highlight the need to search for more efficient agents with the ability to mitigate side effects. Marine organisms, such as seaweed, have been consistently reported as relevant sources of new bioactive compounds with therapeutic interest in oncology. This review first summarizes the main molecular mechanisms involved in CML and current therapies. Then it discusses the anti-leukemic activity of some carotenoids and phlorotannins present in seaweed. Several carotenoids showed anti-leukemic effects, mainly by inhibition of cell proliferation, cell cycle arrest, induction of apoptosis, and suppression of angiogenesis, show some selective toxicity against cancer cells. The potential anti-leukemic effect of phlorotannins is less explored until now, but antiproliferative, pro-apoptotic and anti-angiogenic effects have been reported in some leukemic cell lines. While several studies provide evidence of anti-leukemic activity of carotenoids and phlorotannins, the knowledge about the mechanisms involved is still limited and further research is needed to evaluate in depth the potential of these natural compounds as adjuvants in leukemia treatments. The use of additional in vitro and in vivo models should help in such efforts. Moreover, the interaction of natural compounds with anti-leukemic drugs is a very exciting topic since some natural compounds showed the ability to potentiate the anti-leukemic activity of some TKIs, strongly suggesting that combination chemotherapy is more successful than single drug therapies. However, the effect of marine carotenoids and phlorotannins in combination with TKIs are until now unknown and in our point of view, do deserve investigation efforts.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABL</td>
<td>Abelson 1</td>
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<tr>
<td>AP</td>
<td>Accelerated Phase</td>
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<tr>
<td>AGP</td>
<td>Acid Glycoprotein</td>
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<tr>
<td>AP-1</td>
<td>Activator Protein-1</td>
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<tr>
<td>ALL</td>
<td>Acute Lymphoid Leukemia</td>
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<tr>
<td>AML</td>
<td>Acute Myeloid Leukemia</td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>ATL</td>
<td>Adult T Cell Leukemia</td>
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<tr>
<td>B-CLL</td>
<td>B-Cell Chronic Lymphocytic Leukemia</td>
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<tr>
<td>BC</td>
<td>Blast Crisis</td>
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<tr>
<td>BCR</td>
<td>Breakpoint Cluster Region</td>
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<tr>
<td>BCRP</td>
<td>Breast Cancer Resistance Protein</td>
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<tr>
<td>JNK</td>
<td>C-Jun NH2-Terminal Kinase</td>
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<td>CLL</td>
<td>Chronic Lymphoid Leukemia</td>
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CP = Chronic Phase
CML = Chronic Myeloid Leukemia
CCyR = Complete Cytogenetic Response
DR5 = Death Receptor
FGF = Fibroblast Growth Factor
FGFR = Fibroblast Growth Factor Receptor
hOCT1 = Human Organic Cation Transporter 1
HUVECs = Human Umbilical Vein Endothelial Cells
MMP-9 = Matrix Metalloproteinase-9
MMP = Mitochondrial Membrane Potential
MKK4 = Mitogen-Activated Protein Kinase Kinase-4
MDR = Multidrug Resistance
NO = Nitric Oxide
P-gp = P-glycoprotein
PBMCs = Peripheral Blood Mononuclear Cells
PP2A = Protein Phosphatase 2A
PARP = Poly ADP-Ribose Polymerase
RanGAP1 = RanGTPase Activating Protein 1
ROS = Reactive Oxygen Species
SAHA = Suberoylanilide Hydroxamic Acid
TKD = Tyrosine Kinase Domain
TKI = Tyrosine Kinase Inhibitor
VEGF = Vascular Endothelial Growth Factor

CONSENT FOR PUBLICATION
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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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REFERENCES


