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Fucoxanthin: From chemical properties and sources to novel anticancer mechanistic insights and synergistic therapeutic opportunities

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ABSTRACT

Fucoxanthin (FX) is a carotenoid of marine origin primarily distributed in brown seaweeds and has garnered interest for its antioxidative, anti-inflammatory, and anticancer properties. Despite its potential, a comprehensive understanding of its anticancer effects and mechanisms of action remains elusive.

The aim of this review is to present novel insights into the anticancer effects of FX, shedding light on previously unexplored molecular mechanisms and its synergistic potential with established chemotherapeutic agents.

A comprehensive search was conducted employing databases like PubMed/MedLine, Scopus, and Web of Science to aggregate relevant pharmacological experimental studies. The results of the studies showed that FX exhibits anticancer activity against various cancer types, including breast, colorectal, and lung cancer, through multiple pathways: cell cycle arrest, apoptosis induction, and inhibition of angiogenesis. Additionally, FX potentiates the effects of existing chemotherapeutic agents, making it a potential candidate for combination therapies. The evidence suggests that FX possesses considerable anticancer properties, acting through diverse molecular mechanisms; the heterogeneity of study designs and the limited number of clinical trials make it hard to conclude. Further in-depth studies, particularly randomized controlled trials, are essential for validating FX's efficacy and for paving the way for its integration into standard cancer treatment regimens; additional research is needed to explore its pharmacokinetics, safety profile, and potential synergistic effects with existing chemotherapeutics.

Abbreviations: ABCC1, ATP Binding Cassette Subfamily C Member 1; ABCG2, ATP Binding Cassette Subfamily G Member 2; AP-1, Activator protein-1; ATL, Adult T-cell leukemia (lymphoma); Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; CASP3, Caspase-3; CASP8, Caspase-8; CDK4, Cyclin-dependent kinase 4; CDK6, Cyclin-dependent kinase 6; cIAP2, Cellular inhibitor of apoptosis 2; CML, Chronic myelogenous leukemia; Cx32, Connexin 32; Cx43, Connexin 43; CYP3A4, Cytochrome P450 Family 3 Subfamily A Member 4; DEN, Diethylnitrosamine; DNA, Deoxyribonucleic acid; ERCC1, DNA excision repair protein; Fx, FX; FxRF, FX-rich fraction; GADD45alpha, Growth arrest and DNA damage-inducible protein 45 alpha; GST, Glutathione S-transferase; HCC, Hepatocellular carcinoma; HTLV-1, Human T-lymphotropic virus 1; IκBα, Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; JNK-c, Jun N-terminal kinase; MDR, Multidrug resistance; MDRP1, Multi-drug resistance protein 1; NAC, N-acetylcysteine; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PARP, Poly-ADP-ribose polymerase; PXR, Pregnane X receptor; ROS, Reactive oxygen species; TNBC, Triple-negative breast cancer; TP, Thymidine phosphorylase; TRAIL, Tumor necrosis factor-related apoptosis-inducing ligand; XIAP, X-linked inhibitor of apoptosis protein.

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1. Introduction

Cancer diseases are currently one of the greatest threats to modern societies, and their effective treatment is one of the greatest challenges for modern medicine. According to WHO Global Cancer Observatory (GLOBOCAN) in general over 18 million new cases of cancer were diagnosed in 2018 (Mattiuzzi and Lippi, 2019), whereas it was estimated that in 2020 over 10 million people died because of cancer (Sung et al., 2021). Breast (2.26 million cases), lung (2.21 million) and colorectal (1.93 million) are the three most frequently observed types of cancers (Sung et al., 2021). Age-standardized calculations revealed that Australia with a rate of 452.4 new cases per 100,000 people has the highest cancer rate in the world, followed by New Zealand (422.9 per 100,000), Ireland (372.8 per 100,000) and USA (362.2 per 100,000). Interestingly, among the developed countries with the highest cancer incidence rate, there are no Asian countries. It is also worth noting that this percentage is low among Arab countries (Arafa and Farhat, 2022; Bray et al., 2022). Cancer is very often associated with improper lifestyle choices, especially inadequate nutrition and a sedentary lifestyle (Koch, 2019). There are many therapeutic strategies in the treatment of cancer, among which chemotherapy, surgery and radiotherapy are the most important and frequently used (Elshimali et al., 2018; Huang et al., 2021). Surgery is very often used at the initial state to treat localized tumors with adequate accessibility. At later stages targeted treatments, immunotherapy, radiation and chemotherapy are introduced including or after surgical treatment (Farhan, 2023). Chemotherapy using synthetic or semi-synthetic derivatives is still a leading method of cancer treatment. Although chemotherapeutic drugs have many serious side effects, improvements in anticancer drugs led to significant elevation in patients' life quality and length of time without relapse (Nikolaou et al., 2018). In addition to serious side effects that can significantly reduce the quality of life of patients and even kill the patient, a separate issue of concern is the growing resistance of cancers to drugs. Acquired drug resistance is a major cause of cancer treatment failure using chemotherapeutic drugs these days (Farhan, 2023; Nikolaou et al., 2018). There are various mechanisms leading to an increased resistance of cancer cells against chemotherapeutics, including an altered drug target, an upregulated drug efflux, apoptosis and re-pair signalling pathways (Farhan et al., 2023; Vafadar et al., 2019).

Considering the above-mentioned data, the search for new, more effective and safe anti-cancer drugs is still underway, among which natural products occupy an important place. So far, many different natural substances with anti-cancer potential have been studied and described, but only some of them revealed beneficial results in both *in vitro* and *in vivo* studies, and even fewer have entered clinical trials. Additionally, only some natural products have the possibility of combining with synthetic drugs, which allows to reduction the doses of conventionally used drugs, and sometimes overcomes the drug resistance of the tumor tissue. Among the numerous natural products with very promising anti-cancer properties is fucoxanthin (FX), a lipophilic carotenoid compound, found in selected macro- and microalgae species.

This comprehensive review aims to shed light on the latest evidence regarding the structure–activity relationship, molecular mechanisms, signaling pathways, and cellular effects of FX on tumor cells. It addresses the existing gap in understanding the intricate molecular interactions and mechanisms of FX's anticancer properties, offering new perspectives and highlighting its novel interactions with cellular pathways. Moreover, it proposes potential synergies with conventional cancer treatments. Overall, this synthesis provides valuable insights for identifying new candidates in the exploration of adjuvant therapies for cancer treatment and prevention.

2. Review methodology

This comprehensive review was carried out using comprehensive searches across electronic databases such as PubMed/MedLine, Scopus and Web of Science; the search covered a period from January 2000 to August 2023 and a combination of keywords and Medical Subject Headings (MeSH) terms was employed. Keywords included: "Fucoxanthin," "Anticancer," "Molecular Mechanisms," "Apoptosis," "Cell Cycle Arrest," "Angiogenesis," "Marine Carotenoids," "Combination Therapy," "Systematic Review," and "Pharmacokinetics."; corresponding MeSH terms like "Fucoxanthin," "Antineoplastic Agents," "Molecular Mechanisms of Pharmacological Action," "Apoptosis," "Cell Cycle Checkpoints," "Angiogenesis Inhibitors," "Seaweed," "Drug Synergism," "Review Literature as Topic," and "Pharmacokinetics" were also used. Articles considered for inclusion had to meet several criteria: they must be written in English, be peer-reviewed, focus on the anticancer effects of FX, and provide molecular or mechanistic insights into its action. Original research articles, clinical trials, and review articles were all considered acceptable types of articles. Exclusion criteria included articles not written in English, those focusing solely on the antioxidant or anti-inflammatory properties of FX without addressing its anticancer effects, non-peer reviewed articles, articles involving FX analogues or derivatives without studying FX itself, and articles published before the year 2000. The chemical structure has been validated according to PubChem and the taxonomy of the plant has according to the World Flora Online. The results of this survey will be presented in the text below, but also Tables and Figures.

3. Characterizing FX: structure, sources and computational insights into anticancer properties

3.1. Chemical structure of FX

FX (IUPAC systematic name: (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5′,6′-Epoxy-5,3′-dihydroxy-8′-oxo-6,7-didehydro-5,6,5′,6′,7′,8′-hexahydro-β,β-caroten-3-yl acetate) belonging to the group of primary epoxy carotenoids (Imchen and Singh, 2023) is a lipophilic compound with the molecular weight of 658.906 g/mol. It has a complex and therefore, unique molecular structure (Fig. 1), which results in its remarkable pharmacological effects (Mumu et al., 2022). The large chemical reactivity of the epoxide functional group is affected by the presence of two polar bonds along with the inherent ring strain although the heterocycle formation is energetically favorable which is confirmed by the negative enthalpy of the reaction (ΔH) (Kaur and Singh, 2022).

The complete chiral structure was first determined in 1990 using 1D and 2D NMR studies (Englert et al., 1990). One end of the molecule contains an allenic moiety while the other end contains the 5,6-monoepoxide structure. There is in between a long polyene carbon chain with the methyl substituents. In addition, there are carbonyl, hydroxyl and carboxyl moieties in the structure (Gundermann and Büchel, 2014). Due to the presence of the conjugated double bonds in the polyene chains, FX, like all the carotenoids, has geometric isomers depending on the disposition of substituent groups: (*Z*) and (*E*) which differ one from another in the physicochemical properties. The (*Z*) isomers are less stable from the thermodynamic point of view than the (*E*) forms due to a greater steric hindrance between the hydrogen atoms located near the methyl groups (Nakazawa et al., 2009). The isomer of FX occurring the most frequently in nature is the (*E*) isomer (all (*E*)-FX; Fig. 2) which is generally more stable and more active as an antioxidant than the (*Z*) counterparts (Kawee-ai et al., 2013; Nakazawa et al., 2009). As regards the anticancer activity, the isomeric structure determines the antiproliferative and inhibitory effects of FX (Peng et al., 2011), however,

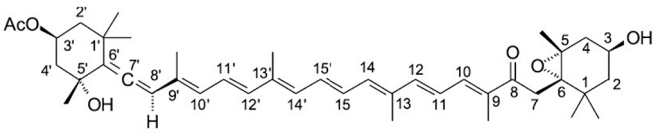

Fig. 1. Chemical structure of FX.

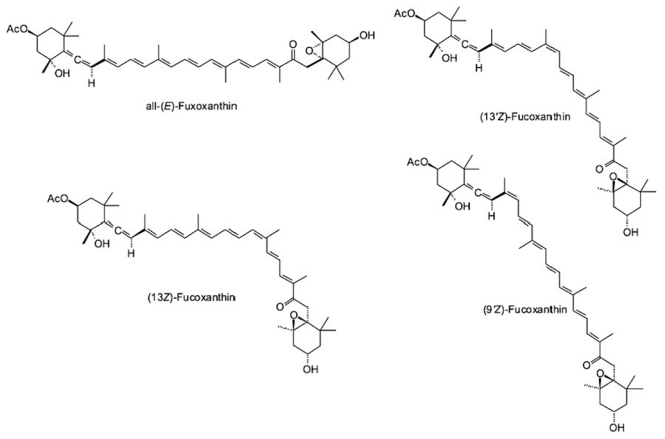

Fig. 2. Chemical structures of FX geometric isomers.

stronger ones were observed in the case of (*Z*)-FX which can result from the hindrances affected by their structure. The mixture of (13′*Z*)- and (13*Z*)-FX (Fig. 2) shows a greater antiproliferative activity than the one investigated for the (9′*Z*)- and all-(*E*) isomeric forms in terms of their activity on cancer cells. It was found that the (13′*Z*)-FX was characterised by the highest inhibitory action related to HL-60 and Caco-2 cells growth, followed by the all-(*E*; 13*Z*), or (9′*Z*) isomers (Fig. 2), which may be due to higher apoptosis-inducing activity (Nakazawa et al., 2009).

3.2. Natural sources of FX

FX, a marine carotenoid, is an orange pigment widely spread in the Protista kingdom. It occurs in chloroplasts of both the macroalgae and microalgae (Guo et al., 2020; Iio et al., 2011; Mumu et al., 2022), however, it was observed that the microalgae produce a higher amount of FX than the macroalgae (Kim et al., 2012). The first extraction of FX was performed in 1950 from three marine seaweeds (Aronoff, 1950). Since then, many scientific reports have pointed out a variety of natural sources from which FX was extracted (Table 1).

3.3. In silico studies of anticancer properties

The computational *in silico* approaches, including molecular dynamics simulation, molecular docking, Absorption-Distribution-Metabolism-Excretion (ADME), or Quantitative Structure-Activity Relationship (QSAR) analyses are commonly employed to investigate a wide spectrum of therapeutic potential of compounds occurring in plant materials. Januar and co-investigators studied FX for its antitumor cytotoxic potential using computational (*in silico*) methods. For this purpose, the p53, the cyclin-dependent kinase 2 (CDK2) as well and tubulin were applied as anticancer receptors. Molecular docking was used for the comparison of three binding sites of tubulin (1SA0-colchicine, 1JFF-paclitaxel and 1Z2B-vinblastine site) with FX and native ligands binding energies in the p53 gene (1RV1), CDK2 (1AQ1). Since the binding energy of FX is quantitatively similar to only the tubulin-colchicine energy level, FX is predicted to form a stable complex with tubulin in the colchicine site. The most logical anticancer mechanism of FX action is to bind tubulin and by this activity cause the depolymerization of microtubules and cell cycle arrest. It was assumed that the oxygenated site of the cyclohexane ring in FX played an important role in the binding mechanism (Januar et al., 2012).

The *in-silico* approach was also used by Garg and co-investigators for the assessment of the possibility of preventing the formation of the mortalin-p53 complex by FX using both the molecular docking and the molecular dynamics simulations (Garg et al., 2019). The investigations demonstrated that FX docked and formed stable interactions in the p53-mortalin in binding site, thus, the complex formation is counteracted.

Table 1
Chosen macro- and microalgae sources of FX.

Species	Family	References
Chaetoceros muelleri	Chaetocerotaceae	(Ishika et al., 2019)
Alaria esculenta	Alariaceae	(Shannon and Abu-Ghannam, 2018)
Ascophyllum nodosum	Fucaceae	(Shannon and Abu-Ghannam, 2018)
Amphora sp.	Catenulaceae	(Ishika et al., 2019)
Colpomenia sinuosa	Scytosiphonaceae	(Noviendri et al., 2021)
Cylindrotheca closterium	Bacillariaceae	(Wang et al., 2018)
Cystoseira barbata	Sargassaceae	(Sellimi et al., 2017)
Cystoseira indica	Sargassaceae	(Fariman et al., 2016)
Cystoseira hakodatensis	Sargassaceae	(Susanto et al., 2016)
Dictyopteris australis	Dictyotaceae	(Ktari et al., 2021)
Dictyota dichotoma	Dictyotaceae	(Ktari et al., 2021)
Dictyota indica	Dictyotaceae	(Noviendri et al., 2021)
Ecklonia kurome	Lessoniaceae	(Susanto et al., 2016)
Fucus vesiculosus	Fucaceae	(Shannon and Abu-Ghannam, 2018)
Himanthalia elongata	Himanthaliaceae	(Shannon and Abu-Ghannam, 2018)
Hincksia mitchellae	Acinetosporaceae	(Chen et al., 2017)
Isochrysis galbana	Isochrysidaceae	(Kim et al., 2012)
Iyengaria stellata	Scytosiphonaceae	(Noviendri et al., 2021)
Laminaria japonica	Laminariaceae	(Chen et al., 2018; Xu et al., 2018)
Lobophora variegata	Dictyotaceae	(Nunes et al., 2019)
Nitzschia laevis	Bacillariaceae	(Lu et al., 2018; Sun et al., 2019)
Nizamuddinia zanardinii	Sargassaceae	(Chesalin et al., 2017)
Odontella aurita	Odontellaceae	(Xia et al., 2013)
Padina gymnospora	Dictyotaceae	(Baliano et al., 2016)
Padina pavonica	Dictyotaceae	(Hegazi et al., 1998)
Padina tenuis	Dictyotaceae	(Karkhane Yousefi et al., 2018)
Padina tetrastromatica	Dictyotaceae	(Sharma and Baskaran, 2021)
Padina australis	Dictyotaceae	(Susanto et al., 2016)
Phaeodactylum tricornutum	Phaeodactylaceae	(Neumann et al., 2019)
Saccharina japonica	Laminariaceae	(Lim et al., 2018)
Sargassum angustifolium	Sargassaceae	(Oliyaei et al., 2021)
Sargassum binderi	Sargassaceae	(Jaswir et al., 2012)
Sargassum duplicatum	Sargassaceae	(Jaswir et al., 2012)
Sargassum fusiforme	Sargassaceae	(Terasaki et al., 2009)
Sargassum horneri	Sargassaceae	(Susanto et al., 2016)
Sargassum muticum	Sargassaceae	(Milledge et al., 2016)
Sargassum plagyophyllum	Sargassaceae	(Jaswir et al., 2013)
Sargassum polycystum	Sargassaceae	(Lim et al., 2018)
Sargassum siliquosum	Sargassaceae	(Lim et al., 2018; Susanto et al., 2016)
Spatoglossum asperum	Dictyotaceae	(Din et al., 2022)
Sphaerotrichia divaricata	Chordariaceae	(Maeda et al., 2018)
Tisochrysis lutea	Isochrysidaceae	(Mohamadnia et al., 2022)
Turbinaria decurrens	Phaeophyceae	(Qurrota'Ayun et al., 2018)
Turbinaria triquetra	Sargassaceae	(Mumu et al., 2022)
Turbinaria ornata	Sargassaceae	(Susanto et al., 2016)
Undaria pinnatifida	Alariaceae	(Yan et al., 1999)
Zonaria tournefortii	Dictyotaceae	(Nunes et al., 2019)

Since FX can induce the abrogation of the mortal in–p53 interactions, it can be treated as a selectively toxic agent for cancer cells (Kaul et al., 2005; Wadhwa et al., 2002). As follows from the studies, FX acts as a competitive inhibitor capable of the p53 nuclear translocation and for the transcriptional activation function or the reactivation in cancer cells (Garg et al., 2019). This compound can become a competitive inhibitor by hindering the interactions of p53 with mortalin. These interactions are unique for the cancer cells particularly because the p53 mutant cell lines are treated as more aggressive than their null counterpart or wild type because of the increased functions inducing the resistance against the stress and the escalation of metastatic capabilities. To verify the cytotoxic potential of FX towards the cancer cells, the *in vitro* analysis was performed on the representative cells with the wild-type p53 (cell lines MCF7 and U2OS), null p53 (cell lines SKOV3 and H1299), and mutant p53 (cell lines A549, DLD-1, and MDA-MB-231). The results confirmed the cytotoxicity effect of FX for these cancer cells, irrespective of their p53 status (Garg et al., 2019).

The estimation of anticancer potential of the compounds found in the alga *K. alvarezii*, including FX and maraniol was also made by Dibha and co-investigators employing computational methods. The interactions between FX and the nuclear factor-kappa B (NF-kB) kinase (NIK) protein in breast cancer were examined using the molecular docking method. In addition, ribociclib, a substance used in the treatment of breast cancer, was used as a control for the study. There were also determined several physicochemical parameters i.e., the lipophilic descriptor $logP_{ow}$ (logarithm of *n*-octanol/water partition coefficient), solubility and molecular weight. As regards the ADME pharmacokinetic parameters, the ability of FX to cross both the blood–brain barrier (BBB) and the gastrointestinal absorption there was not confirmed (Dibha et al., 2022).

Jung et al. used the docking studies of FX against both the monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B) binding sites whose chemical inhibition can play an important role in cancer treatment, and therefore it may constitute valuable therapeutic approach in this regard (Aljanabi et al., 2021). The enzyme-based kinetic results displayed a reversible competitive both MAOs inhibition by FX. Due to the hydrogen binding and the hydrophobic interactions FX accommodates the binding sites of MAO-A and B (Jung et al., 2016). The results showed that FX exhibits significant inhibitory activity against both MAO-A and B (IC_{50} values of 197.41 ± 2.20 and 211.12 ± 1.17 μM, respectively).

4. Metabolites and semi-synthetic derivatives

Two main FX metabolites are formed in the digestive tract of mammals during the process of its metabolism: fucoxanthinol (FXol) and amarouciaxanthin A. FXol (Fig. 3) being a FX hydrolysate is considered to be the primary active metabolite of FX (Komba et al., 2015). The research carried out by Hashimoto et al. showed that 4 h after the oral administration of *Laminaria japonica* (kombu) extract containing 31 mg of FX the maximum concentration of FXol (44.2 nM/L) in the human blood plasma was observed (Hashimoto et al., 2012). It suggested that a larger part of dietary FX is absorbed in the small intestine as FXol (Sugawara et al., 2002). In the *in vitro* study on mice, it was observed that one hour after the administration of 40 nM of FX, the concentration of FXol was found to be 10.4 ± 5.3 nM/L in the plasma samples (Sugawara et al., 2002). FXol found in the egg yolk from the white leghorn laying hens fed with the brown seaweed *F. serratus* demonstrates the total deacylation of FX in the intestinal lumen (Strand et al., 1998).

FXol is further bioconverted into amarouciaxanthin A by dehydrogenation taking place in the liver microsomes, which was confirmed by Hashimoto et al., Asai et al., Tsukui et al., Sangeetha et al., and Airanthi et al. (Airanthi et al., 2011; Asai et al., 2004; Hashimoto et al., 2009; Peng et al., 2011; Sangeetha et al., 2010, 2009; Tsukui et al., 2009). Moreover, it was observed that FXol was further transformed into halocynthiaxanthin in marine animals (Maoka, 2011; Matsuno, 2001). Both metabolites exhibited the induction of apoptosis greater than that of FX in human leukaemia HL-60 cells, MCF-7 breast cancer cells as well as in Caco-2 colon cancer cells (Konishi et al., 2006). It was also confirmed in the *in vitro* studies that FX, FXol, and amarouciaxanthin A are involved in the antiproliferative activity via induction of apoptosis in human prostate cancer PC-3 cells (Asai et al., 2004).

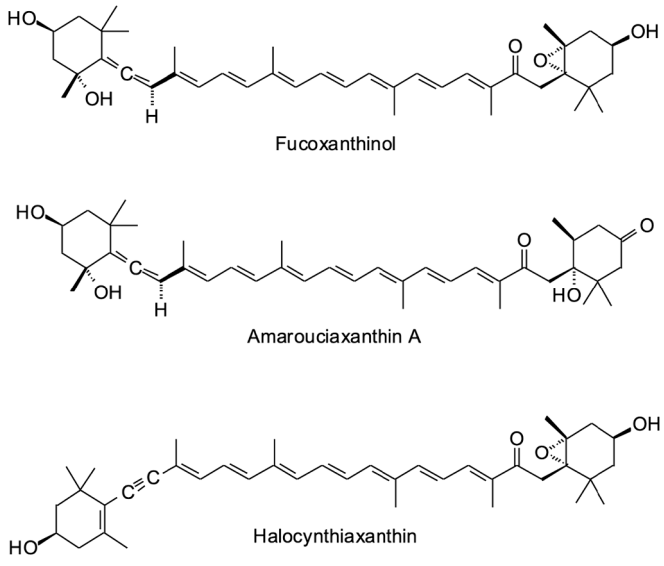

Fig. 3. Chemical structures of FX metabolites, FXol, amarouciaxanthin and halocynthiaxanthin.

Komba et al. synthesized lithocholic acid derivatives of FX to improve its intestinal absorption based on the (Komba et al., 2015). For this purpose, a FX-lithocholic acid complex was designed. Finally, two derivatives, namely lithocholyl-FX and lithocholyl-FX levulinate, were obtained (Fig. 4). To evaluate the efficiency of intestinal absorption, Caco-2 cells were used as a model of the intestinal epithelial cells. No advantageous changes in absorption were observed as a result of the FX chemical modification (Komba et al., 2015).

5. Bioavailability and pharmacokinetics of FX

Bioavailability is involved in the drug moiety circulation in the body space (extent) and time (rate). Although the ADME (absorption, distribution, metabolism and excretion) paradigm has been applied in the drug regulation and clinical practice for decades, the ABCD (administration, bioavailability, clearance and distribution) model proposed by Doogue and Polasek (Doogue and Polasek, 2013) can be used for the determination of pharmacokinetics. Since FX proved to have beneficial properties for human health, including anticancer (Kumar et al., 2013; Lau and Kwan, 2022; Martin, 2015; Muthuirulappan and Francis, 2013) it is treated as a promising phytochemical for diverse pharmacological targets (Mumu et al., 2022). However, its action is limited due to weak stability resulting in a relatively low bioavailability (Zhao et al., 2019, 2014).

As mentioned above, the all-(*E*)-FX form is generally more stable than the (*Z*) counterparts, however, it can be degraded by oxidation, photodegradation as well and by isomerisation. The formation of (*Z*) isomers such as (9*Z*; 13*Z*), or (13*Z*,15*Z*) forms depends on the treatment conditions (Chen and Huang, 1998; Henry et al., 1998; Pesek and Warthesen, 1990). For example, the increase in heating temperature can degrade the (9′*Z*) isomer and simultaneously promote the formation of (13Z) and (13′Z) forms. These energetically unfavourable, non-spontaneous reactions follow simple first-order kinetics, however, the degradation of total and all-(*E*)-FX, contrary to the (*Z*) forms has been shown to satisfy the Arrhenius-type temperature dependence (Zhao et al., 2014). Besides the temperature-stability dependence, the degradation of FX can be due to oxygen, light, and the presence of heavy metals, which causes oxidative stress in the algae during their processing and storage (Pinto et al., 2003; Sun et al., 2018). However, Zhao et al. reported that pH is a more influential factor in FX stability than those mentioned above (Zhao et al., 2019). Many scientific hypotheses were made concerning the limited bioavailability of FX. These findings can be divided into endogenous, originating from humans (intestinal absorption of epoxyxanthophylls from the diet) as well as exogenous, resulting from the features of raw material (dietary fiber present in the algal matrix (Yonekura and Nagao, 2009)) factors.

Due to poor bioavailability of epoxy-xanthophylls from diets into human plasma, many attempts have been carried out to increase the bioavailability of FX. One of the methods for improving the bioavailability of FX is nanoencapsulation. Koo et al. applied two types of nanoparticles incorporated in the *Phaeodactylum tricornutum* extract, i.e., built with alginate and casein as well as the same additionally coated with chitosan. Both types of nanoparticles resulted in the controlled release of FX during the simulated gastrointestinal digestion and fold

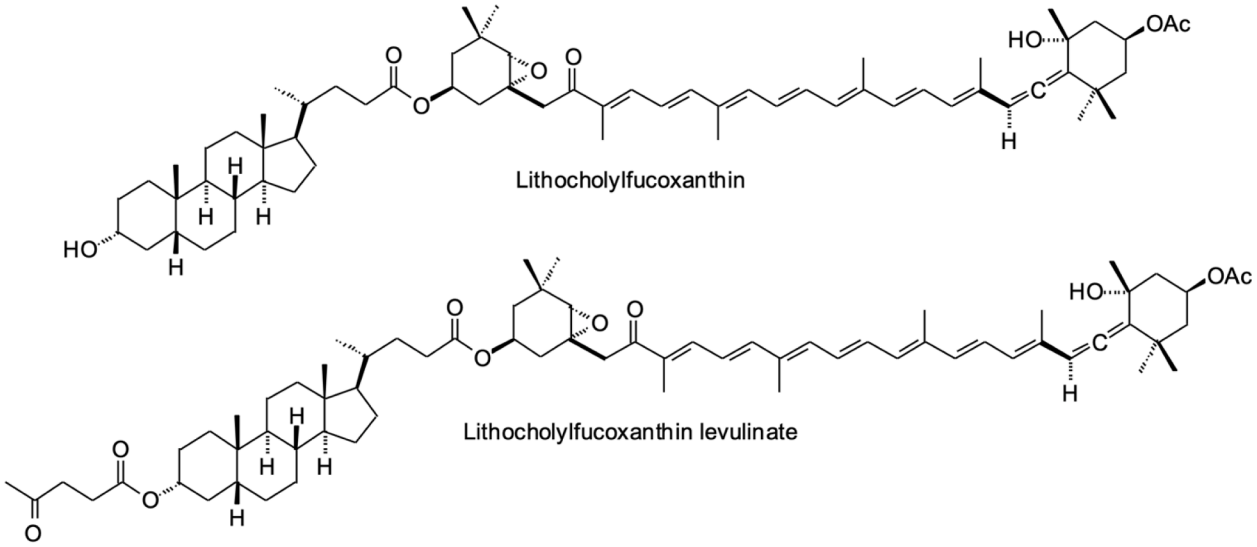

Fig. 4. Chemical structures of the FX derivatives, lithocholyl-FX and lithcholyl-FX levulinate.

improvement of the membrane permeability in the Caco-2/TC7 cells compared to the non-encapsulated extract (Koo et al., 2023, 2016). Several other biopolymers, e.g., chitosan oligosaccharides/PLGA (poly (lacticcoglycolic acid)), zein/casein and zein have also been used to enhance the storage stability and bioavailability of FX. Nevertheless, there is still a need to search for new natural biopolymers as well as for novel preparation methods to be able to use the nanocarotenoids complexes in the food/drug industry (Sridhar et al., 2021). To reduce the endogenous cause of low FX bioavailability, a dietary combination of FX isolated from the raw algae material and an edible oil or lipid has been applied. It was shown that such combinations can increase the intestinal absorption rate of FX in humans (Peng et al., 2011).

6. Anticancer properties of FX: a mechanistic synopsis

6.1. Interplay between FX and various hallmarks of cancer

6.1.1. Sustaining proliferative signaling

In the context of lung cancer, particularly non-small cell lung carcinoma (NSCLC) cell lines like A549 and H1299, FX has been shown to modulate the Phosphoinositide 3-kinase(PI3K)/Protein kinase B (Akt)/NF-κB signaling pathway (Méresse et al., 2020). This modulation leads to a notable down-regulation of proteins that generally promote cell growth, thereby interrupting the cancer cell's ability to sustain proliferative signaling. Additionally, in liver cancer cells, specifically HepG2, FX treatment resulted in reduced expression levels of cyclin D, mRNA, and its protein, which are essential for the progression of cells from the G1 to S phase. The downregulation of cyclin D suggests that FX can arrest the cell cycle at the G1 phase, further curtailing the cell's proliferative potential (Méresse et al., 2020) (see Fig. 5).

6.1.2. Evading growth suppressors

FX exhibits a unique ability to counteract the cancer cell's mechanisms for evading growth suppressors (Ahmed et al., 2022). For instance, in A549 lung cancer cells, it upregulates the expression of p21 and p53, proteins that act as inhibitors of the cell cycle and thus function as tumor suppressors. In prostate cancer, specifically in DU145 cells, FX was found to induce the expression of the Growth Arrest And DNA Damage Inducible Alpha (GADD45A) gene, responsible for growth arrest and DNA-damage response, further bolstering the cell's natural growth suppression mechanisms (Ahmed et al., 2022; Satomi, 2012) (see Fig. 5).

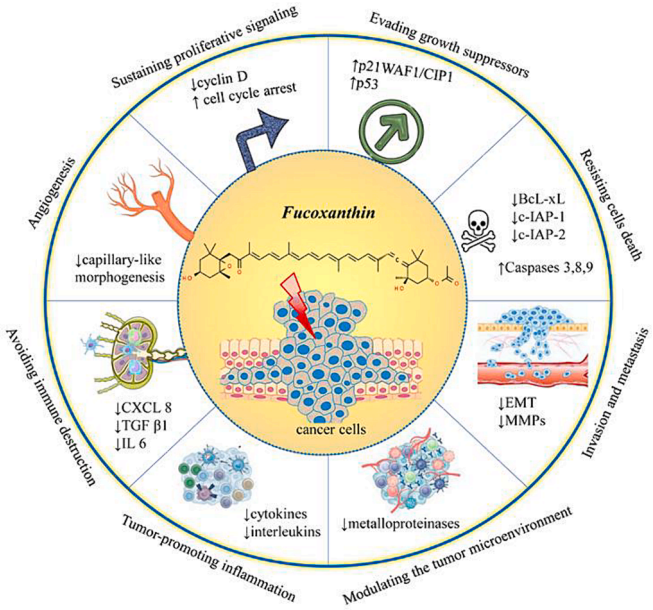

Fig. 5. Modulatory effects of FX on cancer hallmarks. The diagram depicts the anticancer effects of FX on key cancer hallmarks; it highlights FX's role in inhibiting cell proliferation, growth, angiogenesis, and metastasis, while promoting apoptosis and modulating inflammatory responses within the tumor microenvironment. Abbreviations: Bcl-xL: B-cell lymphoma extra-large; CXCL8: C-X-C motif chemokine ligand 8; EMT: Epithelial-mesenchymal transition; c-IAP-1: cellular Inhibitor of apoptosis protein 1; IAP-2: cellular Inhibitor of apoptosis protein 2; IL-6: Interleukin 6; MMPs: Matrix metalloproteinases; TGF β1: Transforming growth factor beta 1; p53 and p21 (WAF1/CIP1): transcription factors 53 and 21; Cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1.

6.1.3. Resisting cell death

Across multiple types of cancer, FX has demonstrated a potent ability to activate pro-apoptotic pathways. In lung cancer cells, it increases the activity of caspase-3 and caspase-8, enzymes crucial for the initiation of apoptosis (Xiao et al., 2020). Similarly, in skin cancer cells, FX upregulates the expression of key proteins involved in apoptosis, such as caspases 3 and 9, while downregulating anti-apoptotic markers like the B-cell lymphoma-extra-large (BcL-xL), and the Cellular Inhibitor of Apoptosis Protein 1 and 2 (c-IAP-1 and c-IAP-2); this dual action

effectively undermines the cancer cell's ability to resist programmed cell death (Rengarajan et al., 2013) (see Fig. 5).

6.1.4. Angiogenesis

Angiogenesis is represented by the formation of new blood vessels and cancer cells often stimulate this process to ensure a sufficient supply of nutrients and oxygen ((Saman et al., 2020; Tu et al., 2023). FX also shows promise in inhibiting angiogenesis, the formation of new blood vessels that tumors need for growth and metastasis; it has shown anti-angiogenic activities in human umbilical vein endothelial cells (HUVECs), significantly inhibiting cell migration and capillary-like morphogenesis, which are critical for vascular development in tumors (Sugawara et al., 2006) (see Fig. 5).

6.1.5. Invasion and metastasis

In both lung and skin cancer models, FX inhibits the epithelial-to-mesenchymal transition (EMT), a crucial step in cancer metastasis (Luan et al., 2022). It also suppresses the expression of matrix metalloproteinases (MMPs), which belong to the enzymes degrading the extracellular matrix and facilitate tissue invasion (Ahmed et al., 2022). In prostate cancer cells, particularly DU145 and LNCaP, FX treatment resulted in reduced expression of MMPs and other proteins typically involved in cellular migration and invasion (Ahmed et al., 2022) (see Fig. 5).

6.1.6. Avoiding immune destruction

FX appears to modulate the immune environment within tumors by decreasing the expression of inflammatory cytokines like the C-X-C motif chemokine ligand 8 (CXCL8), transforming growth factor β1 (TGFβ1), and Interleukin-6 (IL-6) in HUVECs and prostate cancer cells (Calabrone et al., 2023). This could potentially make it more difficult for cancer cells to evade immune detection (see Fig. 5)

6.1.7. Tumor-promoting inflammation

By downregulating proinflammatory markers such as IL-6 and CXCL8 across various cancer types, FX may play a role in attenuating the chronic inflammation that often facilitates tumor growth (Calabrone et al., 2023) (see Fig. 5).

6.1.8. Modulating the tumor microenvironment

In lung cancer cells, FX has been shown to impact tissue inhibitors of metalloproteinases (TIMPs), which are known to play a significant role in modulating the extracellular matrix and, consequently, the tumor microenvironment. The effects could potentially limit the invasive ability of tumor (Ahmed et al., 2022) (see Fig. 5).

6.2. Anticancer effects of FX on different type of cancers

6.2.1. Lung cancer

Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two major types of lung cancer. NSCLC comprises about 85 % of lung cancer cases, and lung adenocarcinoma (LUAD) is the main pathological type of NSCLC (Travis et al., 2015). The anticancer effect of FX was investigated *in vitro* mainly using human non-small cell lung carcinoma cell lines: A549 and H1299 as well as PC9, H460, SPC-1A, and NCI-H522 (Luan et al., 2022; Mei et al., 2017; Ming et al., 2021; Nurcahyanti et al., 2021; Wang et al., 2014b) (see Table 2). In addition, one study was performed on small-cell lung carcinoma cell line H446 (Ming et al., 2021). The IC_{50} values of FX for tested cell lines were <100 μM and depended on the time of treatment – the longer the time of FX treatment, the lover the viability of cancer cells. Following 72 h treatment the IC_{50} values were usually below 20 μM (Ming et al., 2021).

Among the mechanisms of action of FX, several properties are distinguished (Table 2). The compound was shown to induce cell cycle arrest by modified expression of proteins regulating cell cycle: upregulation of p21, p53 in A549 and p21 in H1299 (Mei et al., 2017).

Table 2
Effects of FX on lung tumor cells.

Type of lung cancer cells	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	Reference
A549 human non-small cell lung cancer	18 μM (48 h)	↑ROS	↑Cytotoxicity	(Nurcahyanti et al., 2021)
	10–30 μM (48 h)	↓ETM-related proteins ↓TIMP ↓PI3K/Akt/NF-κB	↓Cancer cells invasion ↓Migration	(Ming et al., 2021)
	52 μM (24 h) 29 μM (48 h) 14 μM (72 h)	↑p21, ↑p53, ↑PUMA, ↑Fas; ↓Bcl-2 ↑Caspases 3, 8	↑Cytotoxicity ↑Cell cycle arrest ↑Apoptosis	(Mei et al., 2017)
	25 μM (48 h)	↑Bax, ↓caspase 3, ↓ PARP; ↓Bcl-2, ↓Caspase 3, ↓PARP	↑Cytotoxicity ↑Apoptosis	(Luan et al., 2022)
		↓Wnt/β-catenin	↓TGF-β1-induced invasion ↓Migration	(Luan et al., 2022)
	45 μM (48 h) 26 μM (72 h)		↑Cytotoxicity	(Wang et al., 2014b)
	30 μM (24 h) 23 μM (48 h) 18 μM (72 h)	↓PI3K/Akt	↑Cytotoxicity ↑Cell cycle arrest in G0/G1 phase ↑Apoptosis	(Fang et al., 2022)
	5, 10, 20 μM (24 h)	↓Vimentin, ↓N-cadherin, ↑E-cadherin	↓Cancer cells invasion ↓Migration	
H1299 human non-small cell lung cancer	10–30 μM (48 h)	↓ETM-related proteins ↑ TIMP-2; ↓PI3K/Akt/NF-κB	↓Cancer cells invasion ↓Migration	(Ming et al., 2021)
	81 μM (24 h) 56 μM (48 h) 23 μM (72 h)	↑p21, ↑PUMA, ↑Fas; ↓Bcl-2 ↑caspases 3, 8	↑Cytotoxicity ↑Cell cycle arrest ↑Apoptosis	(Mei et al., 2017)
	50 μM (48 h)	↑Bax, ↓caspase 3, ↓PARP ↓Bcl-2	↓TGF-β1-induced invasion	(Luan et al., 2022)
	50 μM	↓Wnt/β-catenin	↓Migration	(Luan et al., 2022)
	41 μM (24 h) 30 μM (48 h) 22 μM (72 h)	↓PI3K/Akt	↑Cytotoxicity	(Fang et al., 2022)
	5, 10, 20 μM (24 h)	↓Vimentin, ↓N-cadherin ↑E-cadherin	↓Cancer cell migration ↓Invasion	
H460 human non-small cell lung cancer	69 μM (24 h) 41 μM (48 h) 17 μM (72 h)	↑Cell Cycle Arrest G0/G1 phase ↑p21, ↑ p53 ↑PUMA, ↑Fas ↓Bcl-2	↑Cytotoxicity ↓Proliferation ↑Apoptosis	(Mei et al., 2017)
SPC-A1 human non-small cell lung cancer	60 μM (24 h) 38 μM (48 h) 15 μM (72 h)	↑Caspases-3,-8		(Mei et al., 2017)

(continued on next page)

Table 2 (*continued*)

Type of lung cancer cells	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	Reference
NCI-H522 human non-small cell lung cancer	46 μM (48 h) 24 μM (72 h)			(Wang et al., 2014b)
PC9 Gefitinib resistant human non-small cell lung cancer	46 μM (48 h) 24 μM (72 h)			(Ming et al., 2021)
H446 small cell lung cancer	5–15 μM (48 h)	↓ETM ↓TIMP-2	↓Cancer cells invasion ↓Migration	(Ming et al., 2021)

Abbreviations: ETM: Epithelial-to-Mesenchymal Transition; TIMP: Tissue Inhibitor of Metalloproteinases; PI3K/Akt/NF-κB: Phosphatidylinositol 3-Kinase/ Protein Kinase B/Nuclear Factor-kappa B; p21; protein p 21; p53: Protein 53; PUMA: p53 Upregulated Modulator of Apoptosis; Fas: Fas Receptor; Bcl-2: B-cell lymphoma 2; caspases: Cysteine-dependent Aspartate-specific Proteases; Bax: BCL2 Associated X Protein; PARP: Poly (ADP-ribose) polymerase; ROS: Reactive Oxygen Species; Wnt/β-catenin: Wingless/Integration1/Beta-catenin; Akt: Protein Kinase B; N-cadherin: Neural Cadherin; E-cadherin: Epithelial Cadherin.

Proapoptic properties were also shown for FX. The apoptosis in A549 and H1299, p53 Upregulated Modulator of Apoptosis (PUMA) and Fas receptor were upregulated, and B-cell lymphoma 2 (Bcl-2) was downregulated following the treatment of FX. Also, the activity of caspases-3 and −8 was increased upon FX administration in a dose-dependent manner (Mei et al., 2017). Moreover, the levels of BCL2 Associated X Protein (Bax), cleaved caspase 3, and cleaved Poly (ADP-ribose) polymerase (PARP) were increased while those of Bcl-2, caspase 3, and PARP decreased in A549 and H1299 cell lines (Luan et al., 2022). FX significantly inhibited lung cancer cell migration in a wound healing assay *in vitro* at 10 and 30 μM in A549 and H1299 and at 5 and 15 μM in the H446 cell line. FX inhibited migration and invasion in a transwell migration assay at 20 μM in A549 and H1299 cells and 10 μM in H446 cells. These results suggest that cell small-cell lung carcinoma cells are more sensitive to FX treatment than non-small cell lung carcionoma cells (Ming et al., 2021). Inhibition of the epithelial-to-mesenchymal transition (EMT), mechanisms enhancing metastasis (Liao and Yang, 2017), FX treatment inhibited the expression of EMT-related proteins in A549, H1299, and H446 cell lines, including Snail, Twist, MMP-2, Fibronectin, and N-cadherin (Ming et al., 2021). FX (25 or 50 μM) also effectively reversed cell invasion, migration, and TGF-β1-induced EMT. Compared with the TGF-β1-treated group, FX-treated cells showed increased E-cadherin expression, whereas the expression of vimentin was reduced. FX also significantly reduced migration distances and the number of invasive cells. It potentially targets the Wingless/Integration1/Beta-catenin (Wnt/β-catenin) pathway in A549 and H1299 cell lines (Luan et al., 2022). In cells treated with FX (5, 10, 20 μM), the expression levels of Vimentin and N-cadherin were significantly decreased, while E-cadherin levels were significantly increased (Fang et al., 2022).

An increased expression of tissue inhibitor of metalloprotease-2 (TIMP-2) in A549, H1299, and H446 cell lines (Ming et al., 2021) was observed after the treatment with FX. The tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of matrix metalloproteinases (MMPs). The MMPs degrade the components of the extracellular matrix and play an important role in the invasion of tumor cells. Their proteolytic activities are inhibited by TIMPs. An increasing number of studies have shown that the high invasive ability of tumor cells is closely related to the downregulation of TIMPs ((Salimi Sartakhti et al., 2017). Also, the ability to inhibit the PI3K/Akt/NF-κB signaling pathway was verified for FX. The activation of this pathway upregulated the expression of EMT-related protein (Akca et al., 2011) expressions of PI3K, phosphorylated Akt (p-Akt), and NF-κB, p65 that were significantly decreased in FX-treated A549, H1299, and H446 cell lines (Ming et al., 2021). The levels of p-Akt and PI3K were also decreased in A549 and H1299 cell lines following 24 h treatment with 5, 10 and 20 μM FX (Fang et al., 2022).

6.2.2. Skin cancer

At lower concentrations (5, 10 and 30 μM) FX showed no cytotoxicity towards B16F10 cells but significantly suppressed their metastatic and invasive potential. FX suppressed the expression and secretion of MMP-9 which plays a critical role in tumor migration and invasion, and also suppressed the invasion of highly meta-static B16-F10 murine melanoma cells as shown using the transwell invasion assay. In addition, FX diminished the expressions of the cell surface glycoprotein CD44 and CXC chemokine receptor-4 (CXCR4), involved in invasion, migration and cancer-endothelial cell adhesion. FX markedly decreased cell migration and inhibited the formation of actin fibers. Moreover, the adhesion of B16-F10 melanoma cells to the endothelial cells was significantly inhibited in the presence of FX (Chung et al., 2013).

The anticancer activity of FX towards skin cancer was analyzed on melanoma cell lines (Table 3). Exposure of mouse melanoma cell line B16F10 to high concentrations of FX (50, 100 and 200 μM) was shown to cause cell cycle arrest in the G0/G1 phase by modulating the expression of the checkpoint proteins. The expression of retinoblastoma protein (pRb) decreased and the levels of transcription factors p15INK4B and p27Kip1 significantly increased following FX treatment. The presence of FX promoted a concentration-dependent reduction in cyclins D1 and D2 and CDK4 levels. FX induced a pro-apoptotic effect in melanoma cells by upregulating the levels of key pro-apoptotic proteins such as caspases 3 and 9. Additionally, anti-apoptotic markers, including BcL-xL, c-IAP-1, c-IAP-2 and the X-linked inhibitor of apoptosis (XIAP), were downregulated following FX exposure, confirming its apoptosis-mediated cytotoxic activity (Kim et al., 2013).

The cytotoxic concentrations of FX towards human melanoma cell lines were significantly lower than for the mouse melanoma as the IC_{50}

Table 3
FX effects and potential mechanisms on skin cancer.

Model	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	Reference
B16F10 metastatic murine melanoma	5, 10, 30, 100 μM (24 h)	↓ MMP-9 ↓CD44 ↓CXCR4	↓Cellular viability ↓Metastasis ↓Invasion ↓Migration	(Chung et al., 2013)
	50, 100, 200 μM (24 h)	Dose-dependent decrease in cyclins D1, D2 ↓CDK4 level ↑Caspase-3, ↑caspase-9, ↑PARP;↓ Bcl-xL	↓Cell viability ↑Cell cycle arrest in the G0/G1 phase ↑Apoptosis	(Kim et al., 2013)
Malme-3M human melanoma	28 μM (48 h) 17 μM (72 h)	↓Cancer cell viability	↑Cytotoxicity	(Wang et al., 2014b)
A2058 human metastatic melanoma cells	14.5 μM (72 h)			(Gonçalves de Oliveira-Júnior et al., 2020)
SK-MEL-28 human melanoma	114 μM (48 h)			(Imbs et al., 2013)

Symbols and abbreviations: ↓: Decrease or downregulation; ↑: Increase or upregulation; MMP-9: Matrix Metallopeptidase 9; CD44: Cluster of Differentiation 44; CXCR4: C-X-C Chemokine Receptor Type 4; CDK4: Cyclin-Dependent Kinase 4; PARP: Poly (ADP-ribose) Polymerase; Bcl-xL: B-cell lymphoma-extra-large.

values for human melanoma Malme-3 M and A2058 cell lines following 72 h treatment were 17.33 and 14.57 μM, respectively (Gonçalves de Oliveira-Júnior et al., 2020; Wang et al., 2014b). The human SK-MEL-28 melanoma cell line was less sensitive to FX treatment - the IC_{50} value following 48 h exposure was 114 μM (Imbs et al., 2013).

The anti-cancer properties of FX concerning skin cancer might also implicate prevention of the ultraviolet (UV)-induced damages, known as the leading cause of all skin cancers (D'Orazio et al., 2013). Carotenoids are known as promising photoprotective agents to be used for both oral and topical applications (Genç et al., 2020; Sies and Stahl, 2004). One of the important features of FX in the prevention of skin cancer is its ability to quench singlet oxygen and scavenge free radicals formed following UV exposure, leading to DNA damage and carcinogenesis (Sachindra et al., 2007). In addition, contrary to most other carotenoids, FX quenches free radicals in anoxic conditions, which is a very rare ability (D'Orazio et al., 2012). *In vitro* studies on FX in the prevention of UV-mediated carcinogenesis have been performed using keratinocyte and fibroblast cell lines. A 5 μM treatment of HaCaT keratinocytes slightly decreased UV-mediated cell death and cell-cycle alterations. FX in combination with rosmarinic acid was also able to suppress the formation of inflammasome following UV exposure (Rodríguez-Luna et al., 2019). Also, in HaCaT cells, pretreatment with 10–50 μM FX decreased the release of IL-6 ((Rodríguez-Luna et al., 2018), which is an early symptom of skin aging and carcinomas, and prevented the increase in the reactive oxygen species (ROS) levels induced in response to the UVA and UVB exposure ((Rodríguez-Luna et al., 2018; Tavares et al., 2020). Moreover, 10 μM FX inhibited keratinocyte proliferation in response to UVB radiation, a mechanism also involved in the development of skin cancers (Rodríguez-Luna et al., 2018). In human fibroblasts, FX showed an intense protective activity against UVB irradiation by counteracting UVB-induced ROS production and partially preventing UVB-mediated cell death. Cell survival rate was increased following FX pre-treatment, reaching around 81.47 % at 100 μM, and the inhibitory effect of cell damage exhibited dose-dependent behavior. At concentrations of 50 μM and 250 μM, FX suppressed UVB-mediated DNA damage, probably due to its antioxidant activity, leading to its ability to prevent the formation of neoplastic lesions ((Heo and Jeon, 2009). Chemo-preventive effect of FX in skin cancer can be obtained by topical applications as this compound hardly reaches an effective concentration in the skin upon oral administration (Hashimoto et al., 2009). Using 3D reconstructed human skin *in vitro* models, Tavares et al. showed that FX (0.5 %, w/v) incorporated in sunscreen lotion efficiently synergized the effect of two common sun-screen compounds - avobenzone and ethylhexylmethoxycinnamate, through UVR absorption. FX showed an acceptable degree of photodegradation that was accompanied by a 72 % enhancement in UVA and UVB absorption and showed no phototoxicity (Tavares et al., 2020). Preventive role of FX in the development of skin cancer might also involve epigenetic modifications leading to the activation of the nuclear factor erythroid-2-related factor-2 (Nfr2) pathway and subsequent inhibition of cell *trans*-formation, as shown using mouse epidermal cell line JB6 P + activated with TPA (12-o-tetradecanoyl phorbol 13-acetate) (Wang et al., 2022; Yang et al., 2018).

6.2.3. Prostate cancer

The effect of FX on prostate cancer has been extensively studied using various cellular models, e.g., androgen-sensitive LNCaP and R22v1 cell lines ((Calabrone et al., 2023; Kotake-Nara et al., 2001; Satomi, 2012) and DU145 and PC3 androgen-insensitive cells (Calabrone et al., 2023; Kang et al., 2018; Kotake-Nara et al., 2005a; Li et al., 2021; Satomi and Nishino, 2009; Yoshiko and Hoyoku, 2007). Available scientific data are gathered in Table 4.

The potential mechanism of FX action includes the cell cycle arrest. FX tested in DU145 cells induced cell cycle arrest at the G1 phase and increased the expression of GADD45A (Growth arrest and DNA-damage-inducible, alpha), a cell cycle-related gene (Yoshiko and Hoyoku, 2007). The inhibition of Stress-Activated Protein Kinase/c-Jun N-terminal

Table 4
FX mechanisms on prostate cancer.

Model	IC_{50}/ time effect	Mechanisms/ Signaling pathways	Results	References
R22v1 androgen-sensitive human prostate carcinoma epithelial cell line	20 and 100 μg/mL (48 h and 72 h)	↑Apoptosis	↓Cancer cell viability	(Calabrone et al., 2023)
LNCaP androgen-sensitive human prostate adenocarcinoma derived from lymph node metastasis	5, 10 and 50 μg/mL (48 h and 72 h)			
	10 and 20 μM			(Kotake-Nara et al., 2001)
	2.5 and 5 μM (24 h)	↓SAPK/JNK ↑GADD45A	↓Cancer cell viability ↑Cell cycle arrest	(Satomi, 2012)
DU145 androgen-insensitive human prostate adenocarcinoma derived from brain metastasis	10 and 20 μM	↑Apoptosis	↓Cancer cell viability	(Kotake-Nara et al., 2001)
	3 μM (7 2 h)	↑GADD45A	↑Cell cycle arrest at G1	(Yoshiko and Hoyoku, 2007)
	5.2 μM	↑SAPK/JNK, ↑GADD45A		(Satomi and Nishino, 2009)
	10 and 20 μM	↑Apoptosis	↓DHT production	(Kang et al., 2018)
	5, 10 and 50 μg/mL (48 h and 72 h)		↓Cell viability	(Calabrone et al., 2023)
	20 μg/mL		↓Expression of angiogenesis ↓Inflammation-related proteins	
PC-3 androgen-insensitive human prostate adenocarcinoma derived from bone metastasis	20 μM		↓Cancer cell viability	(Kotake-Nara et al., 2005a)
	10 and 20 μM			(Kotake-Nara et al., 2001)
	10, 50, 100 and 200 μM (24 h and 48 h)	↑Apoptosis, ↑Mitochondrial dysfunction, ↓Antioxidant capacity		(Li et al., 2021)

Abbreviations: SAPK/JNK: Stress-Activated Protein Kinase/c-Jun N-terminal Kinase;GADD45A: Growth Arrest -and DNA Damage Inducible Alpha DHT: dihydrotestosterone.

Kinase (SAPK/JNK) pathway suppressed the induction of GADD45A expression and G1 arrest by FX in DU145 cells (Satomi and Nishino, 2009). In LNCaP cells FX induced activation of the SAPK/JNK pathway followed by upregulation of GADD45A expression (Satomi, 2012). GADD45 is known to be involved in G1 and G2/M arrest (Wang et al., 1999). Also, FX treatment of PC-3, DU145 and LNCaP cells caused DNA fragmentation and induced changes in cellular morphology characteristic for apoptosis (Kotake-Nara et al., 2001). PC-3 cells treatment with FX decreased the protein expressions of Bcl-2 and increased caspase 9 and 3/7 activation in a dose-dependent manner. Kotake-Nara et al. detected decreased expression of Bax, whereas Li et al. measured increased expression of Bax in FX-treated PC-3 cells (Kotake-Nara et al., 2005a; Li et al., 2021). Other studies demonstrated that FX treatment of PC-3 cells significantly decreased mitochondrial fragmentation and membrane potential, and increased superoxide levels in the mitochondria in a dose-dependent manner (Li et al., 2021). FX was also shown to exhibit antiangiogenic potential. The treatment of HUVEC with 20 μg/mL FX reduced cellular migration in both Matrigel and scratch assays; at 50, 100, and 200 μg/mL FX reduced capillary-like morphogenesis of HUVECs *in vitro*; FX treatment at concentrations higher than 5 μg/mL was also cytotoxic for HUVEC following 48 h and 72 h; treatment of DU145 cells plated on Matrigel inhibited interconnected structures, similar to capillaries, which were defined as vascular mimicry; in HUVECs, FX strongly downregulated angiopoietin 2, angiogenin, and CXCL5 (C-X-C Motif Chemokine Ligand 5), while the expression of PLGF (Placental Growth Factor), MMP1 (Metalloproteinase-1), IL6, TIMP1, TIMP2, and PDGF-BB (Platelet derived growth factor) was significantly downregulated. Also, in DU145 cells, the expression of angiogenin, angiopoietin 2, CXCL5, TIMP1, TIMP2, GRO (Growth-regulated oncogene), PDGF-BB, and IL1β was similarly suppressed (Calabrone et al., 2023).

The anticancer potential of the carotenoid was stimulated by its demonstrated anti-inflammatory activity. Inflammatory response induced in HUVECs with TNFα (10 ng/mL) and treated with 20 μg/mL FX showed a statistically significant decrease in the expression of various proinflammatory markers, including IL-6, TGF-β1, TGF-β2, CXCL8, VEGF, TIMP-1, TIMP-2, MMP-9, VCAN (Versican), and STAT3 (Signal transducer and activator of transcription 3) genes. Also, the DU145 cells treatment with 20 μg/mL FX showed decreased expression of IL-10, IL-6 TGF-β1, IL-10, CXCR4 (C-X-C chemokine receptor type 4), MMP-9 and TIMP-1 genes (Calabrone et al., 2023). FX was also reported to inhibit dihydrotestosterone (DHT) production in cultured DU145 cells at 10 and 20 μM without influencing their viability (Kang et al., 2018). DHT, an active metabolite of testosterone (testosterone is converted to DHT by 5α-reductase) is involved in several diseases in older males, including benign prostatic hyperplasia and androgenic alopecia. 5α-Reductase inhibitors, such as finasteride and dutasteride, are potently able to decrease serum and prostatic DHT levels and are used to treat male patients with these diseases (Azzouni and Mohler, 2012).

6.2.4. Cervical cancer

The anticancer properties of FX in cervical cancer were related to its apoptosis-inducing activity. The compound targeted the PI3K/Akt pathway. Decreasing the levels of phosphorylated Akt following FX treatment in HeLa, SiHa and CaSki cell lines resulted in the downregulation of Bcl-2 protein levels and upregulation of Bax and cleaved caspase-3 (Jin et al., 2018; Ye et al., 2014, 2017). In addition, the treatment of HeLa cells with FX induced the expression of two proteins involved in autophagy - LC3 II (the autophagosome marker) and Beclin 1 (the initiation factor for autophagosome formation) in a dose-dependent manner. Also, the phosphorylation of Akt and its downstream proteins p70S6K, p53, and mTOR (mechanistic target of rapamycin) notably elevated the expression of PTEN (Phosphatase and Tensin homolog) (Hou et al., 2013) was described under the influence of FX. Autophagy, a signal transduction pathway affecting the G1 phase progression during G1 arrest, can repair cell damage and inhibit cell death (Platini et al., 2010). Available data related to the treatment of cervical cancer using FX in *in vitro* model are summarized in Table 5.

6.2.5. Brain cancer

Mechanisms of FX action in glioma cell lines include apoptosis, the inhibition of cell migration and invasion, the antiangiogenic potential and the cytotoxic properties. Morphological changes together with decreased ATP levels and mitochondrial membrane potential were found in GBM1 primary glioblastoma cell lines treated with FX ((Lopes et al., 2020). Interestingly, FX was not cytotoxic to normal neurons exposed to 25 μM and 50 μM FX for 72 h (Liu et al., 2016). The observed reduced migration and invasion in GBM1 primary glioblastoma cells treated with FX (40–100 μM) using scratch assay, Transwell assay, and Matrigel invasion assay (Lopes et al., 2020) was significant. Similar effects were observed in U87 and U251 human glioma cell lines following the treatment with FX. The effect was mediated by the inhibition of the p38 signaling pathway downregulation of MMP-9 and MMP-2 expression (Liu et al., 2016). The compound inhibited tube formation of HUVECs (Lopes et al., 2020) in a conditioned medium of FX-treated GBM1 cells (100 μM) demonstrating the antiangiogenic potential of the compound.

Regarding neuroblastoma, FX has been demonstrated to be cytotoxic to human SK-N-SH neuroblastoma cells, although the mechanism of this

Table 5
FX effects on cervical cancer cell lines.

In vitro model	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	References
HeLa human cervical carcinoma	10 μM (24 h)	↓PI3K/Akt, ↓Bcl-2, ↑Bax, ↑Caspase-3, ↑NF-κB	↓Cancer cells viability	(Ye et al., 2014)
	55 μM (48 h)	↑p21, ↓cyclin D1, ↓CDK2	↑Cytotoxicity ↑Go/G1 cell cycle arrest	(Hou et al., 2013)
	10, 20, 40 μM (24 h)	↑LC3 II, ↑Beclin 1 ↓Akt, ↓p53, ↓p70S6K, ↓mTOR, ↑PTEN	↑Autophagy	
	1445 μM (24 h)	↓HIST1H3D	↑Cytotoxicity ↑Cell cycle arrest at G0/G1 phase, ↑Apoptosis, ↓Cancer cell colony formation	(Ye et al., 2020) (Ye et al., 2020)
SiHa human cervix squamous carcinoma	1641 μM (24 h)	↓HIST1H3D		
	0.5 μM (48 h)	↓PI3K/Akt, ↑ Bax, ↑caspase-3, ↓ Bcl-2	↑Apoptosis	(Jin et al., 2018)
	37 μM (48 h) 19 μM (72 h)	↓Cancer cell viability	↑Cytotoxicity	(Wang et al., 2014b)
	10 μM (24 h)	↓PI3K/Akt	↑Cytotoxicity ↑Sensitivity of cancer cells to FX	(Ye et al., 2017)
CaSki human cervical carcinoma	10 μM (24 h)			

Abbreviations and symbols: ↑increase, ↓decrease, PI3K/Akt: Phosphoinositide 3-kinases/Protein kinase B, Bcl-2: B-cell lymphoma 2, Bax: Bcl-2-associated X protein, caspase-3: Cysteine-aspartic proteases 3, NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells, p21: Cyclin-dependent kinase inhibitor 1 or CDKN1A, cyclin D1: Regulatory subunit of cyclin-dependent kinases, CDK2: Cyclin-Dependent Kinase 2, LC3 II: Microtubule-associated proteins 1A/1B light chain 3B, lapidated form Beclin 1: Autophagy related protein, Akt: Protein kinase B, p53: Tumor protein p53, p70S6K: Ribosomal Protein S6 Kinase B1, mTOR: Mechanistic Target of Rapamycin, PTEN: Phosphatase and Tensin homolog, HIST1H3D: Histone Cluster 1 H3 Family Member D.

cytotoxicity was not explored (Wang et al., 2014b). Studies on the human neuroblastoma cell line GOTO showed that FX treatment significantly slowed its growth rate, with this anti-proliferative effect linked to the downregulation of N-myc oncogene expression (Okuzumi et al., 1990). Notably, halocynthiaxanthin, a natural metabolite of FX, exhibited greater cytotoxicity towards GOTO cells compared to FX, with IC_{50} values of 7.5 μg/mL for FX and 5 μg/mL for halocynthiaxanthin, respectively (Nishino et al., 1992). Studies on human glioblastoma revealed that FX induced toxicity and apoptosis, through increased DNA damage, cell cycle arrest and reduced DNA replication (Pruteanu et al., 2020). Table 6 summarizes available data on FX treatment for brain cancer *in vitro* models.

Table 6
FX effects on brain cancer cell lines.

In vitro model	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	References
C6 rat glioma A172 human glioblastoma GBM1 human primary glioblastoma	100 μM (24 h) 100 μM (24 h) 100 μM (24 h)	↓MMP-2 ↓MMP-9	↓Viability ↑Apoptosis ↓Invasion, ↓Migration ↓Mitochondrial membrane potential	(Lopes et al., 2020)
U87MG human glioblastoma	20 μM (72 h)	↑DNA damage, ↓DNA replication ↑Cell cycle arrest	↑Cytotoxicity ↑Apoptosis	(Pruteanu et al., 2020)
U87 human glioma	25–100 μM (24 h and 48 h) 25 and 50 μM (24 h and 48 h)	↓Akt/mTOR pathway; ↑Bax, ↓Bcl-2,↑ PARP, ↑Caspases 9, 3 ↓ MMP-9, MMP-2 and uPa protein levels; ↓p38	↓Viability ↑Apoptosis ↓Depolarization of mitochondrial potential ↓Migration ↓Invasion	(Liu et al., 2016)
U251 human glioma	25–100 μM (24 h and 48 h) 25 and 50 μM (24 h and 48 h)	↓ Akt/mTOR pathway; ↑ Bax, ↓Bcl-2; ↑PARP, ↑caspase-9, −3 ↓MMP-9, ↓MMP-2, ↓p38	↓Viability ↑Apoptosis ↑Depolarization of mitochondrial Potential ↓Migration ↓Invasion	
SK-N-SH human neuroblastoma	53 μM (48 h) 31 μM (72 h)	↓Cancer cell viability	↑Cytotoxicity	(Wang et al., 2014b)
GOTO human neuroblastoma	10 and 20 μg/mL (3–7 days) 10 μg/mL (4 h, 8 h and 24 h) 10 μg/mL (24 h)	↓N-myc mRNA expression	↓Cancer cells growth ↑Cell cycle arrest at G0/G1 phase ↑Cytotoxicity	(Okuzumi et al., 1990) (Nishino et al., 1992)

Abbreviations and symbols: Akt: Protein kinase B, Bax: Bcl-2-associated X protein, Bcl-2: B-cell lymphoma 2, Caspases: Cysteine-aspartic proteases, DNA: Deoxyribonucleic Acid, MMP-2: Matrix Metallopeptidase 2, MMP-9: Matrix Metallopeptidase 9, PARP: Poly (ADP-ribose) polymerase, mTOR: Mammalian Target of Rapamycin, p38: Mitogen-activated protein kinase, uPa: Urokinase-type plasminogen activator, ↑: Increase, ↓: Decrease.

6.2.6. *Leukemia*

Adult T-cell leukaemia (lymphoma) (ATL) is a rare but fast-growing T-cell lymphoproliferative neoplasm induced by human T-lymphotropic virus 1 (HTLV-1) (Mehta-Shah et al., 2017). Cytotoxicity of FX was also evaluated in two K562 and TK6 human leukaemia cell lines *in vitro*. FX at 10 μM increased by 30 % the cytotoxicity in K562 cells compared with the control. In turn, in TK6 cells, FX did not cause any significant cytotoxic effect. Interestingly the antiproliferative effect of this active agent was more evident than the cytotoxic one. It has been demonstrated that FX in the same concentration decreased cancer cell proliferation with an inhibition of 51 % and 56 % for TK6 and K562 cells, respectively. All these effects were confirmed using phase contrast microscopy. Despite the antiproliferative effect caused by FX, cell death or induction of DNA damage were not observed; therefore, it requires further research (Almeida et al., 2018).

Another study confirmed that FX can induce apoptosis via a ROS-mediated Bcl-xL pathway in human leukaemia HL-60 cells. It has been demonstrated that ROS were generated during apoptosis and cytotoxicity induced by FX, and a ROS sweeper, *N*-acetylcysteine (NAC), suppressed all these effects. Additionally, the cleavage of PARP and caspases -3 or -7 as well as a decrease in Bcl-xL level was caused by FX. Interestingly, NAC pre-treatment significantly decreased PARP and caspase-3 or -7 cleavage, and the reduction of Bcl-xL level (Kim et al., 2010). Slightly different results were obtained by another research group. They demonstrated that apoptosis induced by FX was connected with a loss of mitochondrial membrane potential, but not with an increase in ROS level in HL-60 leukemia cells. They confirmed that FX induced cleavage of poly (ADP-ribose) polymerase and pro-caspase-3; however, it has happened without any effect on Bax, Bcl-2 or Bcl-xL protein levels. The authors conclude that FX-mediated apoptosis can be induced only through caspase-3 activation or mitochondrial membrane permeabilization (Kotake-Nara et al., 2005b). It has been demonstrated that FX inhibits the viability of both ATL cells as well as HTLV-1-infected T-cell lines. Interestingly, normal peripheral blood mononuclear cells and uninfected cell lines were not sensitive to FX. Additionally, FX induces apoptosis through increased Bcl-2, survivin, c-IAP 2 and XIAP expression. Induction of apoptosis was also connected with caspase-3, −8, and −9 activation, and suppression of JunD expression and phosphorylation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) causing the inactivation of NF-κB and activator protein-1 (AP-1). It has been detected that FX also induces cell cycle arrest in the G1 phase by a decrease of CDK4, CDK6, cyclin D1 and cyclin D2 as well as an increase in GADD45A expression. All these data suggest that FX could be a potential therapeutic agent for ATL patient treatment (Ishikawa et al., 2008) (See Table 7).

6.2.7. *Digestive cancer*

6.2.7.1. Gastric cancer. Mechanisms of FX action in gastric cancer cells include the targeting of the JAK/STAT signaling pathway, leading to the decreased expression of Cyclin B1, survivin, Mcl-1, and STAT3 in MGC-803, SGC-7901 and BGC-823 human gastric cancer cell lines ((Yu et al., 2018, 2011). It is worth noting that the Janus group of tyrosine kinases (JAK) and also the family of STAT, the signal transducer and activator of transcription, are critical factors of different transduction pathways involved in the process of proliferation, survival, apoptosis and differentiation of gastric cancer cells. Among them, STAT3 is particularly associated with tumorigenesis. Thus, the activation of STAT3 induced by FX is involved in tumorigenesis thanks to its ability to induce proliferation and inhibit apoptosis (Levy and Darnell, 2002). Other studies confirmed an FX-dependent autophagy in human gastric SGC-7901

Table 7
Presents available data related to the treatment of leukemia using fx *in vitro* model using various cell lines.

Model	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	References
K562 human CML	10 μM (24 h)	↑ Bcl-2 ↑Caspase3	↑Cytotoxicity ↓Proliferation	(Almeida et al., 2018)
TK6 human CML	10 μM (24 h)		↓Proliferation	
HL-60 human promyelocytic leukemia	12.1 μM (7 2 h)	↑ROS, ↑Caspases3, 7, ↑PARP, ↓Bcl-xL	↑Cytotoxicity ↑Apoptosis	(Kim et al., 2010)
HL-60 human promyelocytic leukemia	10 μM (24 h)	↓Mitochondrial membrane potential ↑Caspase-3↑ poly (ADP-ribose) polymerase	↑Apoptosis	(Kotake-Nara et al., 2005b)
Adult T-cell leukemia (ATL) cells obtained from patients, infected with human T-cell leukemia virus type 1 (HTLV-1)	10 μM (24 h)	↑Cell cycle arrest at ↑G1 phase ↓Cyclin D1, ↓Cyclin D2, ↓CDK4, ↓CDK6, GADD45alpha, ↑Apoptosis ↑ Bcl-2, ↑XIAP, ↑cIAP2,↑Survinin, ↑Caspase-3, 8, 9	↑Cell cycle arrest ↑Apoptosis	(Ishikawa et al., 2008)

Abbreviations and symbols: ↑increase; ↓decrease; Bcl-2: B-cell lymphoma 2; caspase: Cysteine-dependent Aspartate-specific Proteases; Bax: BCL2 Associated X Protein; ROS: Reactive Oxygen Species; PARP: Poly (ADP-ribose) polymerase; Bcl-xL: B-cell lymphoma-extra-large, GADD45alpha: Growth Arrest and DNA Damage-inducible 45 alpha; XIAP: X-linked Inhibitor of Apoptosis Protein; cIAP2: Cellular Inhibitor of Apoptosis Proteinclin-Dependent Kinase 4; CDK6: Cyclin-Dependent Kinase 6.

cancer cells that was observed by electron transmission microscopy studies and was characterized by detected over-expression of beclin-1 (Zhu et al., 2018). Table 8 summarizes anticancer effects of FX on gastric cancer cell lines *in vitro*.

Abbreviations and symbols:↑increase; ↓decrease:. AK: Janus Kinase; STAT: Signal Transducer and Activator of Transcription; Cyclin B1: A specific type of cyclin protein that plays a role in cell cycle progression; Mcl-1: Myeloid Cell Leukemia 1; Beclin-1: A protein involved in autophagy and cellular homeostasis; LC3: Microtubule-associated protein 1A/1B-light chain 3; p-STAT3: Phosphorylated Signal Transducer and Activator of Transcription 3, JAK: Janus Kinase, STAT: Signal Transducer and Activator of Transcription; Cyclin B1: A specific type of cyclin protein that plays a role in cell cycle progression; Mcl-1: Myeloid Cell Leukemia 1; Beclin-1: A protein involved in autophagy and cellular homeostasis; LC3: Mi-crotubule-associated protein 1A/1B-light chain 3; p-STAT3: Phosphorylated Signal Transducer and Activator of Transcription 3.

6.2.7.2. Colon cancer. FX dose-dependently inhibited WiDr, Lovo and HCT-116 human colon cancer cell viability, proliferation, and apoptosis as well as caused cell cycle arrest at G0/G1 phase in the concentration range from 20 to 100 μM (Wang et al., 2014; Das et al., 2005; Tamura et al., 2019). Despite numerous data on the cytotoxic activity of FX on human colon cancer cell lines, the mechanisms of FX action has not been extensively investigated. Ravi (Ravi et al., 2018), Sui (Sui et al., 2021), Lopes-Costa (Lopes-Costa et al., 2017) and Hosokawa (Hosokawa et al., 2004) showed that the treatment with FX induced DNA fragmentation and decreased Bcl-2 protein expression, indicating the activation of apoptotic pathways, in Caco-2, HT-29, HTC116 and DLD-1 human colon cancer cell lines. Ravi (Ravi et al., 2018) and Sui (Sui et al., 2021) showed increased ROS levels in the cells treated with FX-loaded nanogels and nanoparticles, suggesting that the apoptosis is induced by intracellular oxidative stress. FX might be implicated in the prevention of colon cancer due of its significant beta-glucuronidase inhibitory activity. Intestinal bacterial beta-glucuronidases were able to retoxify the compounds previously detoxified by liver glucuronidation; they were also shown to start the metastatic and invasive potential of colon cancer cells ((Maruti et al., 2010). In the study by Kawee-Ai and Kim (Kawee-Ai and Kim, 2014), FX showed the ability to inhibit beta-glucuronidase in a concentration-dependent manner (IC_{50} of 2.32 mM). FX was also found to diminish the beta-glucuronidase activity of DLD-1 cells (at 20–50 μM). Table 9 summarizes available data of anticancer activity of FX on colon cancer cell lines *in vitro*.

6.2.8. Liver cancer

Hepatocellular carcinoma (HCC) is an important cause of morbidity and mortality worldwide. This type of neoplasm is particularly common due to insufficient early detection programs and lack of effective form of treatment, especially for patients with advanced or moderately advanced disease (Parikh and Pillai, 2021). The main risk factor for HCC is cirrhosis, which is the primary indication for the screening test (Hartke et al., 2017). The influence of FX on liver cancer cell lines was examined *in vitro*. It was found that FX reduces the viability of HepG2 cells and arrests the cell cycle in the G0/G1 phase. Western blotting analysis confirmed that cyclin D expression was reduced by FX treatment. Moreover, RT-PCR analysis demonstrated that this natural agent is also able to reduce cyclin D mRNA levels; therefore, both transcription repression and protein degradation may be responsible for the reduction of cyclin D levels in HepG2 cells exposed to FX (Das et al., 2008). *In vitro* cytotoxicity against HepG2 cell line was also reported by Wang et al. (Wang et al., 2014), and Ayyad et al. (Ayyad et al., 2011). Wang and co-

Table 8
FX effects on gastric cancer cell lines *in vitro*.

In vitro model	IC_{50}/time effect	Mechanisms/Signalling pathways	Results	References
MGC-803 human gastric adenocarcinoma	50 μM, 75 μM (24 h, 48 h)	Targeting JA.STAT signaling pathway ↓CyclinB1, ↓survivin, ↓STAT3	↑Cell cycle arrest at G2/M phase ↑Apoptosis	(Yu et al., 2011)
SGC-7901 human gastric adenocarcinoma, medium differentiated, derived from metastatic lymph node around the stomach	50 or 75 μM (24 h)	Targeting the JAK/STAT signaling pathway ↓Mcl-1 ↓STAT3 ↓p-STAT3	↓Proliferation ↑Apoptosis ↑Cell cycle arrest at S phase	(Yu et al., 2018)
BGC-823 human gastric adenocarcinoma, poorly differentiated, derived from primary tumor			↓Proliferation, ↑Apoptosis ↑Cell cycle arrest at G2/M phase	
SGC-7901 human gastric adenocarcinoma	12.5–100 μM (12, 24, 48 h)	↑Beclin-1, ↑LC3, ↑Caspase-3, ↓Bcl-2	↓Cellular viability ↑Apoptosis ↑Autophagy	(Zhu et al., 2018)

Table 9
FX effects on colon cancer cell lines *in vitro*.

In vitro model	IC_{50}/time effect	Mechanisms/Signalling pathways	Results	References
WiDr human colon adenocarcinoma	42 μM (48 h) 25 μM (72 h)	↓Cancer cell viability	↑Cytotoxicity	(Wang et al., 2014b)
Lovo, human colon adenocarcinoma	39 μM (48 h) 22 μM (72 h)			
WiDr human colon adenocarcinoma	25–100 μM (24, 48, 72 and 96 h)	↑p21	↓cell proliferation, ↑Cell cycle arrest at G0/G1 phase	(Das et al., 2005)
HCT116 human colon cancer	25 μM (24 h)		↑Cell cycle arrest at G0/G1 phase	
	25 μM (24, 36 and 48 h)	↑ROS ↑Chromatin condensation ↑DNA degradation	↑Apoptosis ↑Cell cycle arrest at G0/G1 phase	(Tamura et al., 2019)
Caco-2 human colorectal adenocarcinoma	10 μM FX incorporated in nanogels	↓Bcl-2, ↑mitochondrial membrane potential ↑Caspase-3	↓Viability ↑Apoptosis	(Ravi et al., 2018)
	20 μM FX Polyvinylpyrrolidone Nanoparticles	↑DNA fragmentation ↓PPARγ	↓Viability of cancer cells	(Sui et al., 2021)
HT29 human colorectal adenocarcinoma	10 μM, 50 μM, 100 μM (48 h)	↓Bcl-2		(Lopes-Costa et al., 2017)
HCT116	50 μM and 100 μM (48 h)			
Caco-2 human colorectal adenocarcinoma	15.2 μM (72 h)			(Hosokawa et al., 2004)
HT29 human colorectal adenocarcinoma	15.2 μM (72 h)			
DLD-1 human colorectal adenocarcinoma	15.2 μM (72 h)			
DLD-1 human colorectal adenocarcinoma	20–50 μM FX	↓Beta-glucuronidase activity	↓Invasion ↓metastasis	(Kawee-Ai and Kim, 2014)

workers calculated the IC50 values of FX following 48 h and 72 h treatment (59 μM and 26 μM, respectively). Ayyad reported that the cytotoxic effect of FX was related to DNA damage (Ayyad et al., 2011). Yoshiko and Hoyoku did not observe reduction in cellular viability following FX treatment (20 μg/mL, 96 h) but showed cell cycle arrest at G1 phase and increased expression of GADD45A and GDD45B genes in response to FX. In the other experiments, the effect of the *C. calcitrans* extract (CME) and its FX-rich fraction (FxRF) on the inhibition of the growth of HepG2 cells was assessed. The effectiveness of the total extract and FxRF was checked by cytotoxicity tests, and morphological and cell cycle analysis. The results indicated that both the *C. calcitrans* extract and the FxRF fraction induced a dose- and time-dependent cytotoxic effect on HepG2 cells. However, the antiproliferative effect was more evident in FxRF compared to CME. Interestingly, FxRF induced sub-G0/G1 DNA accumulation while CME caused cell cycle arrest in the G2/M phase (Foo et al., 2019). Further data showed that FX can affect cell cycle arrest and enhance intercellular communication in SK-Hep-1 hepatoma cells. Moreover, FX significantly and dose-dependently inhibited the proliferation of SK-Hep-1 cells after 24 h of incubation. In addition, FX caused the induction of apoptosis in SK-Hep-1 cells and the arrest of the cell cycle in the G0/G1 phase, as evidenced by the growth of % sub-G1 cells, as well as the induction of DNA strand breaks (Liu et al., 2009).

In addition to *in vitro* studies, the protective role of FX in diethylnitrosamine-induced hepatocarcinogenesis was described experimentally in adult rats (Jin et al., 2019). In the study, the levels of lipoproteins, liver enzymes and oxidative stressors were compared in different groups in both tissues and blood. The experimental rats were divided into four groups. The first control group was administered isotonic saline intraperitoneally, the second group received 0.01 % diethylnitrosamine (DEN) via drinking water to induce hepatocellular carcinoma, the third group was orally treated with concomitant 0.01 % DEN and FX and finally, the fourth one was orally administered FX alone for 15 weeks. The results of the study clearly showed a decrease in the body weight of the animals, a decrease in the level of albumin and an increase in the level of all liver enzymes, bilirubin and stress markers in DEN-induced rats. However, concomitant FX supplementation restored these parameters to normal levels. Self-administration of FX alone showed no changes. Based on these results, it can be concluded that FX can be used as a chemotherapeutic agent against liver cancer (Jin et al., 2019). Data related to the application of FX in studies using liver cancer cell lines are presented in Table 10.

6.2.9. Other types of cancers

Anticancer properties of FX has been also investigated using other *in vitro* models, indicating its bread spectrum of anti-cancer potential. Rokkau et al. showed significant cytotoxic effect of FX (IC_{50} = 10 μM) towards human and mouse osteosarcoma cells, associated with the cell cycle arrest at G1 phase and downregulated expression of CDK4, CDK6, cyclin E, survivin, XIAP, Bcl-2 and Bcl-xL. Increased apoptosis in FX-treated cells was also connected with increased activation of caspases 3, 8 and 9. FX was also effective against human endometrial carcinoma cell line HEC-1A. 25 h treatment with 7.5 μM or 10 μM FX increased apoptosis, as confirmed by elevated Bax and caspase-3 and decreased Bcl-2 levels. Increased ROS, COX-2, TNFα, IL-6 and NF-κB levels suggested that the prooxidant and pro-inflammatory mechanisms were involved in FX action on this type of cancer cells (Qu et al., 2022). Zhang et al. (Zhang et al., 2008) confirmed also anti-cancer potential of FX against bladder cancer cell lines. Similarly to previously described *in vitro* models, observed cytotoxic effects was related to increased DNA fragmentation, activation of caspase-3 (Zhang et al., 2008), upregulation of p21 and decreased activity of CDK2, CDK4, cyclin D1 and cyclin E (Wang et al. 2004). Interestingly, Long et al. showed that the cytotoxic effect of FX on human nasopharyngeal carcinoma results from the activation of autophagy and autophagy-related proteins, such as LC3 (microtubule-associated protein 1A/1B-light chain 3), ATG4B

Table 10
FX effects on liver cancer cell lines *in vitro*.

In vitro model	IC_{50}/time effect	Mechanisms/Signalling pathways	Results	References
HepG2 human hepatocellular carcinoma	25 μM (24 h)	↓Cyclin D	↓Viability ↑Cell cycle arrest ↑G0/G1 phase	(Das et al., 2008)
	59 μM (48 h) 26 μM (72 h)	↓Cancer cell viability	↑Cytotoxicity	(Wang et al., 2014b)
	20 μg/mL (96 h)	↑DNA damage	↑Cytotoxicity	(Ayyad et al., 2011)
	3 μM (72 h)	↑GADD45A, ↑GADD45B genes	↑Cell cycle arrest ↑G1 phase no apoptosis	(Yoshiko and Hoyoku, 2007)
	FX rich fraction of *Chaetoceros calcitrans* extract) 80 μg/mL (24 h) 40 μg/mL (48 h) 19 μg/mL (72 h)	↓Genes involved in cell signaling (Akt1, ERK1/2, JNK) ↓ SOD1, ↓SOD2, ↓CAT	↑Cytotoxicity ↑Apoptosis ↓Proliferation	(Foo et al., 2019)
SK-Hep-1 human hepatoma	1–20 μM (24 h)	↑DNA strand breaks ↑Gap junctional intercellular communication ↑Cx43, ↑Cx32 mRNA, ↑protein expression ↑Intracellular Ca2 + levels	↓Proliferation ↑Cell cycle arrest ↑G0/G1 phase ↑Apoptosis	(Liu et al., 2009)

Abbreviations and symbols: ↑increase; ↓decrease; AKT: serine/threonine-specific protein kinases involved in multiple cellular processes; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; SOD: Superoxide Dismutase; CAT: catalase; GADD45A and GADD45B: genes encoding Growth Arrest And DNA Damage-Inducible Protein GADD45 Alpha and Beta.

(autophagy related 4B cysteine peptidase) and ATG7 (autophagy related 7 protein). (Long et al., 2020) Available data on FX effects on other cell lines *in vitro* models are presented in Table 11. A comprehensive summary of the cellular and molecular anticancer mechanisms of FX is provided in Fig. 6.

7. Synergistic anticancer effects of FX and traditional chemotherapy

Currently, combined forms of therapy with two or more active agents are considered promising anti-cancer tools to achieve better therapeutic responses and reduce therapy-related side effects. Cisplatin is an antineoplastic drug that has been widely used in the therapy of various types of cancers, e.g., triple-negative breast cancer (TNBC), ovarian, testicular, bladder, lung or hepatoma; the mechanism of action of cisplatin is the ability to crosslink with the purine bases and interfere with deoxyribonucleic acid (DNA) repair, causing DNA damage and subsequently inducing apoptosis in cancer cells (Aldossary, 2019; Dasari and Tchounwou, 2014). Unfortunately, cisplatin use is associated with various serious toxic side effects including nausea, hepatotoxicity, nephrotoxicity, neurotoxicity, or cardiotoxicity (Aldossary, 2019; Dasari and Tchounwou, 2014). Therefore, the combined treatment with anticancer drugs and nutritional factors seems to be a promising therapeutic strategy for improving the safety and efficacy of cisplatin chemotherapy.

The antiproliferative effect of FX and cisplatin was described in HepG2 human hepatoma cancer cells. The FX pretreatment at a concentration of 1–10 μM followed by cisplatin at 10 μM for 24 h significantly decreased cancer cell proliferation in comparison with cisplatin used alone. In the study cisplatin induced mRNA expression of thymidine phosphorylase (TP) and excision repair cross complementation 1 (ERCC1) via phosphorylation of p38, ERK (Extracellular signal-regulated kinase) and PI3K/Akt signaling pathways. Interestingly, FX significantly attenuated cisplatin-induced TP and ERCC1 gene expression, causing the improvement of cisplatin cytotoxicity. Moreover, FX increased cisplatin-induced NFκB expression and enhanced the NFκB-regulated Bax/Bcl-2 mRNA ratio. In summary, the obtained results suggest that a combination of FX and cisplatin may be a novel therapeutic strategy against human hepatoma (Liu et al., 2013).

The anti-cancer potential of FX in combination with cisplatin was also investigated in human A549 lung cancer and HeLa cervical cancer cell lines. The drug combination method developed by Chou and Talalay theorem was used. In the study selective sensitization of cancer cells to cisplatin after FX treatment was demonstrated in both analyzed cell lines (Nurcahyanti et al., 2021).

Multidrug resistance (MDR) reversal by FX as well as the ability of FX to increase the doxorubicin cytotoxicity in the Adriamycin-resistant MCF-7/ADR breast, SKOV-3/ADR ovarian, HepG-2/ADR hepatic cell lines variants were investigated (Eid et al., 2020). Doxorubicin (Adriamycin) is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces* strains and widely used in cancer therapy. Doxorubicin inhibits DNA topoisomerase II and induces DNA damage in cancer cells (Kciuk et al., 2023). It has been demonstrated that FX at 20 μM synergistically increased the cytotoxic effect of doxorubicin and significantly reduced the doses of doxorubicin in MCF-7/ADR breast, SKOV-3/ADR ovarian and HepG-2/ADR doxorubicin-resistant cells. The combination of the FX and doxorubicin decreased multi-drug resistance protein 1 (MDRP1, ABCB1), ATP Binding Cassette Subfamily C Member 1 (ABCC1) and ATP Binding Cassette Subfamily G Member 2 (ABCG2) expression as well as increased the levels and activity of caspase-3, caspase-8 and p53. Additionally, the FX/doxorubicin combination decreased the levels and activity of glutathione *S*-transferase (GST), Cytochrome P450 Family 3 Subfamily A Member 4 (CYP3A4) and Pregnane X receptor (PXR) proteins in ADR-resistant cancer cells, and induced apoptosis compared with untreated control. All these data suggest that FX and doxorubicin applied together can overcome multidrug resistance in analyzed cancer cells (Eid et al., 2020).

The cytotoxic effect of FX individually or in combination with doxorubicin as well as imatinib was also determined in K562 and TK6 human leukaemia cell lines (Almeida et al., 2018). Imatinib is the tyrosine kinase inhibitor successfully used in the therapy of chronic myelogenous leukaemia (CML) (Iqbal and Iqbal, 2014; Tolomeo et al., 2009). In the study Imatinib induced a cytotoxic effect in TK6 cells and inhibited the proliferation of K562 leukaemia cells; in turn, doxorubicin decreased cell viability and proliferation in both analyzed cell lines. FX used individually increased cytotoxicity in K562 cells and decreased the proliferation of K562 and TK6 leukaemia cells. Interestingly, FX in combination with imatinib or doxorubicin inhibited the proliferation of both tested cell lines, however, no statistically significant differences were observed about FX applied alone. The study confirmed the cytotoxic effect of FX in both leukaemia cell lines, however; further studies

Table 11
FX effects on other cancer cell lines.

In vitro model	IC_{50}/ time effect	Mechanisms/ Signalling pathways	Results	References
Saos-2 human osteosarcoma	20 μM (24 h) 10 μM (24 h)	Cell cycle arrest at G1 ↓CDK4, ↓CDK6, ↓cyclin E,	↑cytotoxicity ↓viability of cancer cells	(Rokkaku et al., 2013)
MNNG human osteosarcoma	10 μM (24 h)	↑Apoptosis ↓Survivin,↓ XIAP, ↓Bcl-2,		
143B human osteosarcoma	10 μM (24 h)	↓Bcl-xL; ↑caspases-3, −8 and −9		
LM8 mouse osteosarcoma	10 μM (24 h)			
HEC-1A human endometrial adenocarcinoma	7.5 μM (24 h)		cytotoxicity	(Qu et al., 2022)
	7.5 and 10 μM (24 h)	↑ROS, ↑Apoptosis; ↓Mitochondrial membrane potential, ↓PI3K/Akt/ mTOR, ↑TNF-α, ↑NF-κB, ↑COX-2, ↑ IL-6,↑Cyclin D1, ↓ Bcl-2, ↑Bax, ↑Caspase-3		
T24 human blader cancer	5 and 10 μM (72 h)	↑p21, ↓CDK-2, ↓CDK-4, ↓Cyclin D1 ↓Cyclin E	↓ proliferation ↑Cell cycle arrest ↑ G0/G1 phase	(Wang et al., 2014b)
	20 and 40 μM (72 h)	Abrogation of mortalin-p53 complex and the reactivation of nuclear mutant-type p53	↑Apoptosis	
EJ-1 human bladder cancer	6.25 μM–22.5 μM (72 h)	↑Caspase-3 ↑DNA fragmentation	↓Viability ↑Apoptosis	(Zhang et al., 2008)
C666-1 human nasopharyngeal carcinoma	25 μM (24 h)	↑Autophagy-related proteins ↑LC3, ↑ATG4B, ↑ATG7	↓Viability ↑Apoptosis ↑Autophagy	(Long et al., 2020)

Abbreviations and symbols: CDK: cyclin-dependent kinase; XIAP: X-linked inhibitor of apoptosis protein; Bcl-2: B-cell lymphoma 2 protein family; Bcl-xL: B-cell lymphoma-extra-large protein; ROS: Reactive oxygen species; PI3K: Phosphoinositide 3-kinase; Akt: serine/threonine-specific protein kinases; mTOR: mammalian target of rapamycin kinase; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; COX: cyclooxygenase; IL: interleukin; Bcl-2: B-cell lymphoma 2; Bax: Bcl-22 Associated X Protein; p21: protein regulating cell proliferation by inhibiting the cell cycle through the cyclin kinase pathway;p53: regulatory protein that is often mutated in human cancers; LC3: Microtubule-associated protein 1A/1B-light chain 3; ATG4B: Autophagy Related 4B Cysteine Peptidase; ATG7: Autophagy Related 7 protein.

are needed to investigate the molecular targets and signal transduction pathways involved in that effect (Almeida et al., 2018). The cytotoxic effect of FX in combination with another promising cancer therapeutic, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), was analyzed in *in vitro* and *in vivo* models (Ye et al., 2017). TRAIL is a member of the tumor necrosis factor (TNF) ligand family that selectively induces apoptosis in cancer cells both *in vitro* and *in vivo*; however, it does not affect most normal cells. Several chemotherapeutic agents have been shown to sensitize tumor cells to TRAIL-mediated apoptosis. A combination of TRAIL with other therapeutic agents, including natural products seems to promise a therapeutic strategy in cancer treatment (Wu et al., 2004). Two cervical cancer cell lines, CaSki TRAIL-sensitive and SiHa TRAIL-resistant, were used in the study. As might be expected the cytotoxic experiments showed that CaSki cells have a much lower IC_{50} for TRAIL than the SiHa cell line. Interestingly, CaSki TRAIL-sensitive cells demonstrated resistance to FX compared to SiHa cells, suggesting a reverse relation between FX and TRAIL in cervical cancer cells. Additionally, FX treatment induced apoptosis in both analyzed cell lines. Next, CaSki and SiHa cells were treated with TRAIL and FX alone, or in the combination of both substances to determine the type of pharmacological interaction between agents. The obtained results showed a significantly increased cytotoxic effect of both drugs used in the combination. To investigate the role of TRAIL and FX in tumor growth, an ectopic implantation model in nude mice was used. In the experiments, CaSKi cells were subcutaneously injected into mice, and then the mice were divided into three groups when the tumor size reached about 5 mm. The first group was treated with TRAIL, the second with FX, and the third group with a combination of both drugs. Both TRAIL and FX used alone suppressed tumor growth compared with the control. Interestingly, the injection of TRAIL in combination with FX showed a greater effect in inhibiting tumor growth than drugs applied individually. The results of the study also revealed that TRAIL-resistant cervical cancer cells overexpress the PI3K/Akt signaling pathway, which may be an effective target for FX. The combination of TRAIL and FX suppresses the PI3K/Akt signaling pathway. The results of the study suggest that the combination of FX and TRAIL may be an effective method in the treatment of cervical cancer (Ye et al., 2017).

Table 12 summarizes available data suggesting that FX can be effectively combined with classical chemotherapeutic drugs, line cisplatin or doxorubicin, in the treatment of various cancers. However, majority of the conducted studies were performed on cancer cell lines and only few experiments were carried out *in vivo* and *in vivo*. Therefore, more studies are needed, especially in *in vivo* and clinical models, to fully elucidate practical potential of FX in the oncological treatment.

8. Toxicity and safety

As described above FX bears a variety of pharmacological applications; the performed studies confirm its therapeutical potential together with high safety. This component of brown seaweed algae belongs to foods, which explains its low toxicity. In the search for toxic effects exhibited by the compound the researchers achieved very high doses. In the studies of Ishikawa et al., the oral doses of 200 mg/kg b.w. FX administered daily in rats did not show any side effects (Ishikawa et al., 2008). In addition, no mortality or adverse effects were observed for higher doses of the compound as well. Beppu and collaborators administered to mice 1 and 2 g/kg b.w. as a single dose, and later additional doses of 0.5 and 1 g/kg for the following 30 days (Beppu et al., 2009b). The performed histological tests revealed no changes in the tissues, however, the authors noted an increased level of total cholesterol in plasma. In another study the authors confirmed no mutagenicity of the compound in the same doses in the assays performed on *Escherichia coli* WP2uvrA/pKM101 and *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537, with or without metabolic activation (Beppu et al., 2009a). Also, no genotoxicity at a dose lower than 2 g/plate, no mutagenicity (<5 g/plate), no abnormalities in the internal organs (>200 mg/kg/day) and no mortality until 2 g/kg b.w. were reported by Peng and colleagues (Peng et al., 2011).

Most of the described anti-cancer *in vitro* studies did not include a non-cancer cell line control which raise a question of FX safety and selectivity towards particular type of cancer cells. However, from other studies, it is known that FX causes no side effects following application at 0.5 % *w/v* on human skin and 20–2000 mg/kg body weight following oral application in rodents (Spagolla Napoleão Tavares et al., 2020). Toxicity studies that were performed on 4-week-old rats, with oral

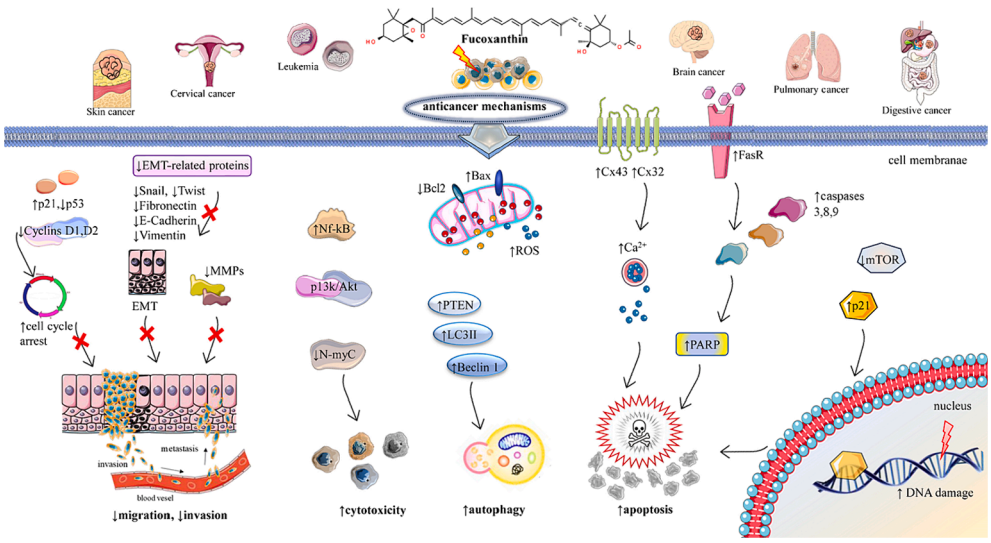

Fig. 6. Mechanisms of FX's anticancer activity across different cancer types. The figure illustrates the diverse anticancer mechanisms exerted by FX across multiple cancer types; it modulates several pathways contributing to its anticancer properties; induces cell cycle arrest, hinders epithelial-mesenchymal transition (EMT), inhibits migration and invasion processes, it promotes the generation of reactive oxygen species (ROS), autophagy, and cytotoxicity while inhibiting cell survival and growth pathways and also encourages DNA damage and apoptosis, further solidifying its role as a potent anticancer agent; together, these mechanisms elucidate FX's broad-spectrum anticancer potential. EMT: Epithelial-Mesenchymal Transition; ROS: Reactive Oxygen Species; MMPs: Matrix Metalloproteinases; NF-kB: Nuclear Factor Kap-pa-light-chain-enhancer of Activated B cells. PI3K/Akt: Phosphatidylinositol-3-Kinase/Protein Kinase B; mTOR: Mammalian Target Of Ra-pamycin; Bcl2: B-cell Lymphoma 2; Bax: Bcl2 Associated X protein; Cx43 and Cx32: Connexins 43 and 32; Ca2+: Calcium ion; PTEN: Phosphatase and Tensin Homolog; LC3II: Microtubule-associated protein 1A/1B-light chain 3 II; PARP: Poly ADP Ribose Polymerase; FasR: Fas Receptor.

Table 12
Anti-cancer activity of FX in combination with other chemotherapeutic agents.

In vitro/ in vivo	Type of cancer	Model	Combined therapy	Mechanism of action	References
In vitro	Hepatoma	HepG2 cells	Cisplatin	FX pretreatment followed by cisplatin significantly decreased cell proliferation in comparison with cisplatin used alone FX attenuated cisplatin-induced TP and ERCC1 gene expression via p38, ERK and PI3K/Akt signaling pathways, causing the improvement of cisplatin cytotoxicity FX increased cisplatin-induced NF-κB expression - ↑Bax/Bcl-2 mRNA ratio	(Liu et al., 2013)
	Lung and cervical	A549 HeLa cells		Selective sensitization of cancer cells to cisplatin after FX treatment	(Nurcahyanti et al., 2021)
	Breast, ovarian, hepatoma	MCF-7/ADR, SKOV-3/ADR HepG-2/ADR cells	Doxorubicin (adriamycin)	FX synergistically increased the cytotoxic effect of doxorubicin and significantly reduced the doses of doxorubicin FX + doxorubicin decreased MDRP1 (ABCB1), ABCC1 and ABCG2 expression as well as increased the levels and activity of CASP3, CASP8 and p53 FX/doxorubicin combination decreased the levels and activity of GST, CYP3A4 and PXR proteins in ADR-resistant cancer cells, and induced apoptosis compared with untreated control	(Eid et al., 2020)
	Leukemia	K562 and TK6 cells	Doxorubicin and imatinib	FX in combination with imatinib or doxorubicin inhibited the proliferation of leukemia cell lines	(Almeida et al., 2018)
In vitro *In vivo*	Cervical	CaSki TRAIL-sensitive and SiHa TRAIL-resistant cells; CaSKi cells subcutaneously injected into mice.	TRAIL	↑Cytotoxic effect after TRAIL/FX combined versus individual treatment in CaSKi cells a combination of TRAIL and FX suppresses the PI3K/Akt signaling pathway *in vitro* injection of TRAIL in combination with FX showed a greater effect in inhibiting tumor growth than drugs applied individually in mice	(Ye et al., 2017)

Abbreviations: ABCC1: ATP Binding Cassette Subfamily C Member 1, ABCG2: ATP Binding Cassette Subfamily G Member 2, ADR: adriamycin-resistant, Bax/Bcl-2: Bcl-2-associated X protein/B-cell lymphoma 2, CASP3: caspase-3, CASP8: caspase-8, ERCC1: excision repair cross complementation 1, ERK: Extracellular signal-regulated kinase, CYP3A4: Cytochrome P450 Family 3 Subfamily A Member 4, GST: glutathione S-transferase, MDRP1 (ABCB1): multi-drug resistance protein 1, NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells, PI3K/Akt: Phosphatidylinositol-3-Kinase/Protein Kinase B; PXR: Pregnane X receptor, TP: thymidine phosphorylase, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

dosing of FX, did not show any adverse effects after daily administration of drugs. Moreover, *in vivo* studies with FXol did not reveal any harmful adverse effects (Ishikawa et al., 2008).

9. Clinical limitations and pitfalls for the therapeutic application of FX in oncology

Currently FX has shown promise as an anticancer agent in various pre-clinical models, but several key limitations and pitfalls must be

acknowledged for its potential clinical application. Firstly, most of the existing research has been limited to *in vitro* and *in vivo*, with very few human trials conducted to date; the applicability of these findings to human health remains uncertain. Secondly, the bioavailability of FX in humans is not fully understood; although preclinical studies have demonstrated promising absorption rates, the metabolic processes in humans may differ, potentially impacting its effectiveness as an anticancer agent. Thirdly, the potential for drug-drug interactions with FX remains underexplored; many cancer patients are on multi-drug regimens, making it crucial to understand how FX could interact with other medications to ensure both safety and efficacy. Another limitation is the lack of standardized dosing regimens for FX; it remains unclear whether the compound is most effective when administered alone or in combination with other anticancer agents. Lastly, while FX has been found to target multiple signaling pathways involved in cancer development, the specificity of these effects in human tissues has yet to be established and there may be a risk of off-target effects, complicating its transition from experimental settings to clinical applications. Despite the promising anticancer effects of FX in preclinical pharmacological studies, there is a need for extensive research to address these clinical limitations and pitfalls before it can be reliably considered for therapeutic applications.

10. Conclusions and future perspectives

FX a carotenoid pigment, is primarily found in various species of brown algae and has piqued the interest of scientists due to its potent anticancer properties, as evidenced by numerous scientific studies. These anticancer effects are believed to stem from fucoxanthin's broad range of biological activities, such as cytotoxicity, pro-apoptotic actions, enhancement of autophagy, and anti-invasive, anti-migratory, and anti-angiogenic effects. Remarkably, fucoxanthin shows an additive or synergistic effect when used alongside conventional chemotherapy drugs like cisplatin and doxorubicin, enhancing their effectiveness while reducing their harmful side effects. FX's mechanism of action is closely linked to its interaction with key cellular proteins, including CDK2, tubulin, and mortalin. These interactions are crucial for fucoxanthin's ability to inhibit cancer cell proliferation and induce apoptosis. By binding to CDK2, fucoxanthin disrupts cell cycle regulation, leading to cell cycle arrest. Its interaction with tubulin impacts the cellular cytoskeleton, essential for cell division and migration, while its affinity for mortalin disrupts cancer cells' stress response mechanisms, promoting cell death. These molecular interactions emphasize the complexity of fucoxanthin's anticancer activity and highlight the need for further research to fully harness its therapeutic potential. Structurally, fucoxanthin exists in two isoforms, (*Z*) and (*E*), differentiated by the orientation of a double bond in their molecular structure. The (*E*) isoform is more common and stable but has a reduced anticancer activity. Future research should focus on identifying abundant sources of the (*Z*) isoform for commercial use. Establishing dedicated cultivation areas for brown algae could enable sustainable fucoxanthin extraction without overexploiting natural resources. Additionally, developing efficient and scalable methods for compound recovery is essential. Future research should prioritize animal and human trials to further explore the compound's efficacy and safety (Supplementary file 1). Given fucoxanthin's relatively poor solubility, efforts to enhance its bioavailability, such as through nanocapsulation or the semi-synthesis of more soluble derivatives, are crucial. In summary, the existing body of research supports the need for further investigation into FX, particularly given its favourable safety profile, which is promising for its potential integration into human cancer treatment strategies. Exploring fucoxanthin's interactions with essential cellular proteins and their implications for cancer therapy could open new avenues for developing effective anticancer treatments.

Author contributions

Conceptualization and design were performed by J.S.-R., D.C.; investigation, data curation, and writing were performed by W.K., W.K.-K., A.W., E.O., K.S., K.G.-B.; review and editing were performed by W.K., D.C., W.N.S., I.D., J.S.-R.; validation, supervision W.K., D.C., W.N.S., I. D., J.S.-R.; All the authors read and approved the final manuscript; have read and agreed to the published version of the manuscript.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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