Cytotoxicity of *Cichorium intybus* L. metabolites (Review)

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Abstract. *Cichorium intybus* L. (Chicory) is a widely distributed, edible, perennial, herbaceous member of the Asteraceae family. Besides its use in modern Chinese herbal medicine, its ethnomedicinal use is evident in the text from ancient Greece, Egypt and China. It is also used as a food and coffee substitute, which is mainly responsible for its extensive domestication. In recent decades, cytotoxic studies of *C. intybus* extracts have shown its antitumor potential. These studies also identified metabolite constituents including guaianolides, 6-methoxyflavone, eudesmanolides, germacranolides, polyacetylene, sterol, anthocyanin, delphinidin, 3,4-dihydroxyphenethyl and other novel compounds. Many of these phytometabolites have shown positive cytotoxic activities *in vitro*, and antitumor action *in vivo* and in clinical trials, demonstrating the potential of *C. intybus* metabolites as antitumor drugs. Structural activity relationship studies have further confirmed these bioactivities. In this review, we focused on the phytochemicals of *C. intybus* with reported cytotoxicity and potential antitumor properties. We also discuss their specificity towards tumor cells, structural activity relationship, the involved signaling pathways and molecular mechanism, with the expectation of the future development of efficient and targeted antitumor therapeutic strategies.

Contents

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1. Introduction

Cichorium intybus L. is a perennial herbaceous plant and is one of the six members of the genus *Cichorium* under family Asteraceae. Although it is a native weed from Europe, *C. intybus* has been naturalized in different parts of the world including Africa, temperate and tropical Asia, Europe, Australia, North and South America (1,2). Its leaf is commonly used as a vegetable and powdered root as a coffee substitute; the whole plant is also used for animal forage (3). *C. intybus* L. has been used as a common medicinal herb worldwide in different civilizations. Ancient Egyptians (3) and Romans (mentioned by 'Horace' in 'Odes') used *C. intybus* as a medicinal plant. However, local nomenclature has vast differences, and so do the medicinal uses. Ethnomedicinal use of *C. intybus* covers diarrhea, liver diseases, prostate and reproductive organ disorder, pulmonary disease, cough, malaria and cancer (1). The recent use of *C. intybus* as an alternate for coffee has led to its commercial cultivation. *C. intybus* is also currently cultivated as a major source of inulin, a dietary fiber fructan (4).

As reviewed in the following sections, several studies have shown that phytochemicals may be a reliable source of compounds with therapeutic benefits. For example, many drugs derived from phytochemicals and their derivatives have shown promise and utility in tumor treatment. *C. intybus* contains diverse types of phytochemicals such as guaianolides, 6-methoxyflavone, eudesmanolides, germacranolides, polyacetylene, sterol, anthocyanin, delphinidin, 3,4-dihydroxyphenethyl and other novel compounds in different quantities. Fractionated and purified extracts of *C. intybus* have been the subject of many studies. Many researchers have reported the therapeutic properties of *C. intybus* compounds, on *in vitro* as well as *in vivo* models of tumors.

C. intybus phytochemicals have shown tissue- and tumor type-specific antitumor activity, which indicates against indiscriminate cytotoxicity and instead suggests the presence of a well-regulated mechanism. In this review, we summarize *C. intybus* derived phytochemicals with reported antitumor properties from traditional and pharmacological trials. We also review their cell specificities and antitumor mechanisms.

2. Literature search method

We used PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (https://scholar.google.com), and Baidu Xueshu (http://xueshu.baidu.com) scientific article search engines as well as PubChem (https://pubchem.ncbi.nlm.nih.gov/), and U.S. National Library of Medicine (https://clinicaltrials.gov/) for the preliminary search. We searched for articles published between 1975 to 2018. Keywords such as chicory, *Cichorium intybus*, phytochemicals, phytometabolites, anticancer, antitumor, cytotoxic, and clinical trial were used individually or in combination to search for antitumor phytometabolites of *C. intybus*. Articles for each metabolite and its activity were searched individually within the same time frame. Chemical structures of metabolites were sourced primarily from research articles and using ChemDraw search and then confirmed from PubChem and Chem Spider databases. Chemical structures were drawn with ChemDraw according to journal guidelines. Illustrations were drawn in combination with ChemDraw and Adobe Illustrator.

3. Antitumor activity of the phytochemicals

The medicinal properties of *C. intybus* L. led to many successful investigations to identify its phytochemical constituents. *C. intybus* is used in a variety of illnesses, and the phytochemicals derived from it are also diverse. Overall, our analysis revealed 87 reported phytochemicals, of which Carazzone *et al* (5) identified 63; Street *et al* (3) reported 84; and Malik *et al* (6) found 78. Several other studies also reported some of these phytochemicals. Most of these compounds show bioactivity, and some have multiple pharmacological properties. However, in the present review, we focus on the phytochemicals with reported antitumor properties, as listed in Table I.

Reactive oxygen species (ROS) are highly reactive radicals, ions or molecules with a single unpaired electron in their outermost shell. Recent studies have shown that ROS contribute to several diseases and disorders (6) including chronic inflammation and a wide variety of different cancers (7). ROS are categorized into two broad types: Free oxygen radicals such as superoxide, hydroxyl radical, nitric oxide, organic radicals, peroxyl radicals, alkoxyl radicals, thiyl radicals, disulfides, sulfonyl radicals, and thiyl peroxyl radicals; non-radical ROS such as hydrogen peroxide, singlet oxygen, ozone/trioxygen, organic hydroperoxides, hypochlorite, peroxynitrite, nitrosoperoxycarbonate anion, nitrocarbonate anion, dinitrogen dioxide, nitronium, and highly reactive lipid- or carbohydrate-derived carbonyl compounds (8). ROS are produced as an inevitable byproduct of cellular processes such as mitochondrial oxidative phosphorylation (8) and play vital roles in the stimulation of cell signaling pathways in response to intracellular and extracellular changes (7). In almost all cancers, the ROS concentration is elevated. However, to survive, cancer cells produce antioxidant proteins to detoxify ROS (8,9). In cancer cells, the ROS elevation can be caused by mitochondrial dysfunction, peroxisome activity, oncogene activity, increased metabolism, increased cellular receptor signaling, increased activity of oxidases, cyclooxygenases, lipoxygenases, and thymidine phosphorylase, as well as through crosstalk with infiltrating immune cells (8,10). The difference between ROS and antioxidant levels creates oxidative stress. Free radicals produced by oxidative stress alter macromolecules such as DNA, proteins, and lipids, and thus play a significant role in inducing carcinogenesis (7,10).

These altered macromolecules, unable to perform their function, can hinder cell growth and even cause death. However, a dysfunctional cell divisional mechanism causes uncontrolled cell division. For example, ROS can upregulate cyclin mRNA levels including cyclin B2, cyclin D3, cyclin E1, and cyclin E2; resulting in fast transition from the G1 to S phase of the cell cycle (8). The uncontrolled cell growth results in a cell mass called a tumor. Afterward, these tumors, unable to support their growth, create new blood vessels around them, a process known as angiogenesis. These blood vessels facilitate growth of the tumors to a point after which cancerous cells start to detach from the malignant tumor and migrate through blood vessels to other tissues of the body known as metastasis (11). ROS can promote tumor cell metastasis by decreasing extracellular matrix anchorage or increasing vascular permeability (8). These transformed cell-containing organs are unable to function properly leading to organ failure and death (11). To be considered as an antitumor drug, the therapeutic agent should have the following qualities of counteracting tumorigenesis: i) counteract ROS; ii) counteract the oxidative stress caused by ROS; iii) prevent angiogenesis; iv) prevent the metastasis of cancerous cells; and v) selective cytotoxicity towards cancerous cells to induce apoptosis.

To be considered as a therapeutic agent, the compound must also show fewer side effects compared with existing antitumor drugs. The present review also describes the possibility of using *C. intybus* phytochemicals and their derivatives as potent tissue-specific antitumor agents.

In vitro studies have revealed the antitumor activities of whole and fractionated extracts of *C. intybus* and its parts with different solvents. A 100 μg/ml hydroalcoholic extract of *C. intybus* leaf was found to be significantly effective against a prostate cancer cell line (LNCaP; percent of inhibition: 3.67±0.12) and a root extract was found to be significantly effective against breast cancer cells (MCF-7), amelanotic melanoma cells (C32) and renal adenocarcinoma cells (ACHN) (percent of inhibition: 12.65±0.26, 30.78±0.75, 14.93±0.29, respectively) (12). A whole plant extract fed to a mouse carcinoma model (dimethyl hydrazine-induced) showed lower expression of natural interferon α (INF-α), and B-cell lymphoma 2 (Bcl-2) and higher expression of interleukin (IL-12 and IL-4), confirming the antitumor property (13). A methanol extract of root from *C. endivia*, a related plant, was found to inhibit the growth of breast cancer cells (MCF-7; IC_{50}: 401 μg/ml) *in vitro* (14), and a whole plant water extract inhibited tumor growth in a colorectal cancer mouse model *in situ* (200 mg/kg body weight) (15). The antitumor activity of a whole ethanolic extract of *C. intybus* root was demonstrated in an Ehrlich ascites carcinoma (EAC) mouse model, resulting in a 70% increase in lifespan with 500 mg/kg/day treatment (16). A comparative study between different plants showed that *C. intybus* seed water extract moderately reduced the development and colony formation in PC-3 prostate cancer cells (2-30%), T47D breast carcinoma cells (2-21%), and RKO

Table I. *Cichorium intybus* L.-derived phytochemicals that exert antitumor properties.

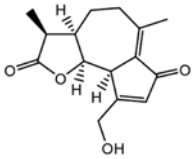
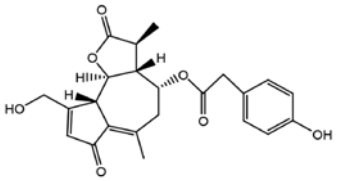
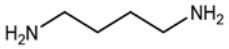
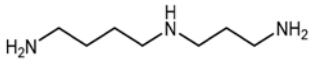
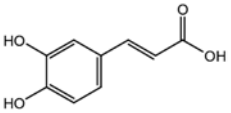
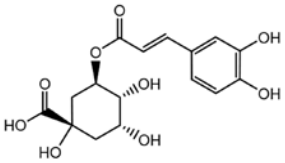
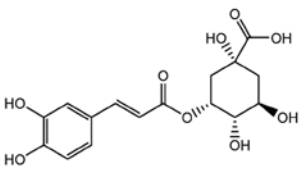
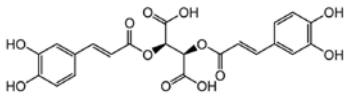
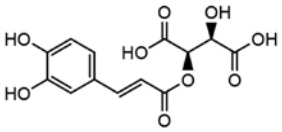
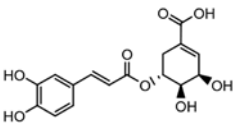
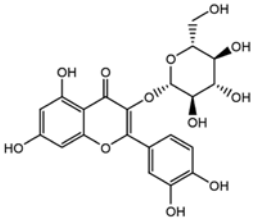
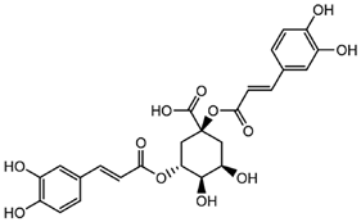
Phytochemicals	Chemical structure	Cell lines	(Refs.)
Jacquilenin		Lung cancer WI38, VA13, A549, HepG2 cells	(34)
11,13-Dihydrolactucopicrin		Nasopharyngeal cancer KB cell, liver cancer Bel 7402 cell	(35)
Putrescine		Breast cancer MDA-MB-231 and T-47D cells	(43-45)
Spermidine		Bone cancer U-2 OS cell, cervical cancer HeLa cell, skin cancer Malme-3M cell, prostate cancer PC-3 and 293 cells	(41,42)
Caffeic acid		Mammary duct carcinoma T-47D cell, promyelocytic leukemia HL-60 cell, liver carcinoma Hep-3B cell	(54-56,58)
Chlorogenic acid		Hepatoblastoma Hep-G2/2.2.15 cell Mouse preadipocyte: 3T3-L1 cell Rat insulinoma INS-1E cell	(61) (62,63) (64)
5-Caffeoylquinic acid		Lung cancer (non-small cell): H1299 cells	(66)
Chicoric acid		Mouse preadipocyte 3T3-L1 cells Cervical cancer HeLa cell line, breast cancer MCF-7 cells	(71) (72)
Trans-caftaric acid		Liver cancer HepG2 cells, cervical cancer HeLa cells	(67)
5-Caffeoylshikimic acid		Rat skeletal myoblasts L6 cells	(68)
Quercetin 3-O-β-D-glucoside		Liver cancer HepG2 cells, colon cancer Caco-2 and 293 cells Gastric BGC-82 cells	(76) (75)
1,3-Dicaffeoylquinic acid		Colon cancer DLD-1 cells	(53,147)

Table I. Continued.

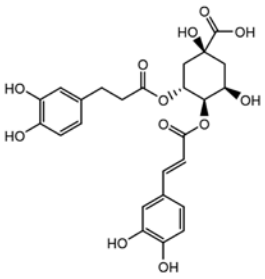
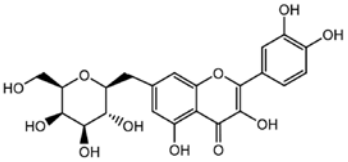
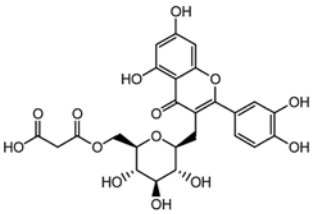
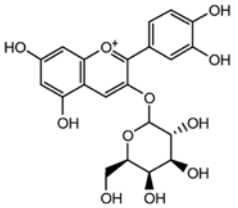
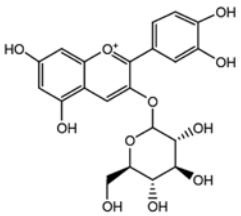
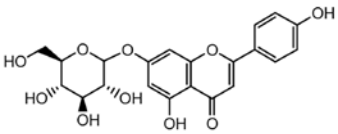
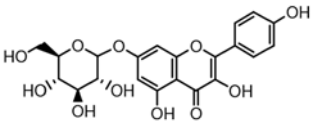
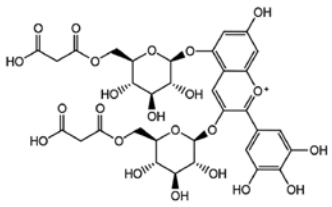
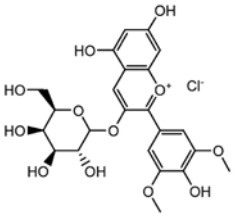
Phytochemicals	Chemical structure	Cell lines	(Refs.)
3,4-Dicaffeoylquinic acid		Stomach cancer: Kato III cells, colon cancer DLD-1 cells, promyelocytic leukemia HL-60 cells	(53)
Quercetin-7-O-galactoside		Liver cancer HepG2 cells Mouse neuroblastoma N2a cells	(73) (79)
Quercetin-3-O-(6"-O-malonyl)-glucoside		Not determined	
Cyanidin-3-O-galactoside		Vulva carcinoma A431 cells	(84)
Cyanidin-3-O-glucoside		Lung carcinoma LLC cells Pancreatic β-cell MIN6 Breast cancer HS578T and MDA-MB-453 cells	(81) (82,83) (83)
Apigenin-7-O-glucoside		Colon cancer HCT116 cells Prostate cancer PC-3 cells	(85) (86)
Kaempferol-7-O-glucoside		Cervical cancer HeLa cells	(87)
Delphinidin 3,5-di-O-(6-O-malonyl-β-D-glucoside)		Breast epithelial MCF10A cells	(90)
Malvidin-3-O-glucoside		Breast cancer MCF-7 cells Vulva carcinoma A431 cells	(106) (84)

Table I. Continued.

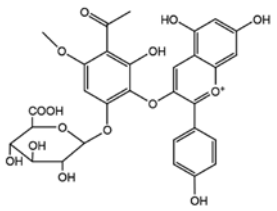
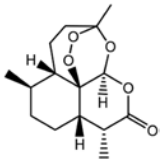
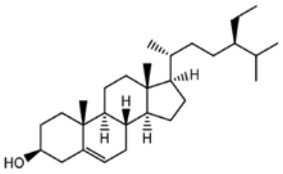
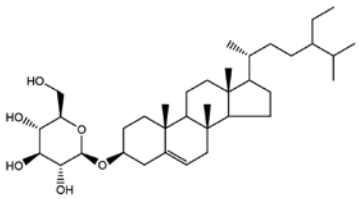
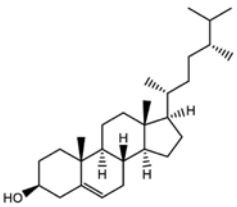
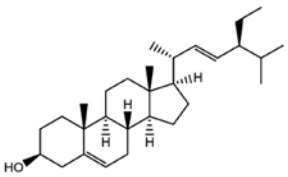
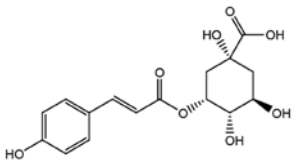
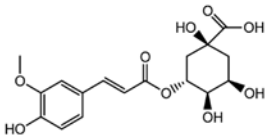
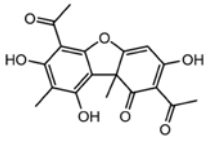
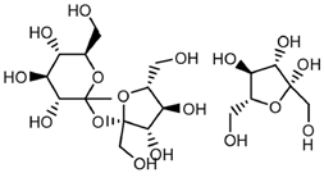
Phytochemicals	Chemical structure	Cell lines	(Refs.)
Pelargonidin-3-O-monoglucuronide		Human monocytic leukemia THP-1 cells	(107)
Artesin/Artemisinin		Colon cancer HCT116 and SW480 cells, leukemia HL-60 cells, breast cancer MCF-7 cells, melanoma KM, MJT3 cells, lung cancer (non-small cell) NSCLC cells, pancreas PANC-1 and MIAPaCa cells, glioma cancer U87MG and A172 cells	(113-115)
β-sitosterol		Cervical cancer HeLa cells	(99)
		Intrahepatic cholangiocarcinoma KKU-M213 and RMCCA-1 cells, immortalized normal cholangiocytes MMNK-1	(93)
		Breast cancer MCF-7 cells	(100,101)
β-sitosterol-3-O-glucoside		Breast cancer MCF-7 cells	(91)
		Leukemia HL-60 cells, liver cancer Hep G2 cells	(103)
Campesterol		Liver cancer HepG2 cells, breast cancer MCF-7 cells	(91)
Stigmasterol		Intrahepatic cholangiocarcinoma KKU-M213 and RMCCA-1 cells, immortalized normal cholangiocytes MMNK-1 cells	(93)
		Breast cancer MCF-7 cells, cervical carcinoma Ca Ski cells, colon cancer HCT-116 cells	(94)
		Chronic myelogenous leukemia K-562 cells	(95)
3-O-p-Coumaroyl quinic acid		Prostate cancer PC-3 cells, mouse preadipocyte 3T3L1 cells	(116)
4-O-feruloylquinic acid		Colon cancer HT-29 cells	(69)
Usnic acid		Breast cancer MCF-7 and MDA-MB-231 cells, lung cancer H1299 cells, prostate cancer LNCaP cells	(117,118)

Table I. Continued.

Phytochemicals	Chemical structure	Cell lines	(Refs.)
Inulin		Transplantable liver tumor TLT cells, mouse mammary carcinoma EMT6 cells	(121-123)

colon cancer cells (6-26%) *in vitro* (17). A methanolic extract of *C. intybus* decreased the viability of breast cancer cells (SKBR3) in a time-dependent manner with an IC_{50} of 800, 400 and 300 μg/ml at 24, 48, and 72 h treatment, respectively (18). The n-hexane extract of the aerial part demonstrated significant antiproliferative (70% at 100 μg/ml) as well as cytotoxic activity (50.3% at 100 μg/ml) against lymphocytic leukemia Jurkat cells (19). In another study, comparing the aerial part's methanolic extract of different plants on five cancer cell lines, *C. intybus* efficiently inhibited Jurkat cell growth (IC_{50} of 138 μg/ml), and moderately inhibited bladder carcinoma cell (Fen), and cervical epithelioid carcinoma cell (HeLa) growth (25% decrease at 200 μg/ml), but had no inhibitory effect on myelogenous leukemia cells (K562) (20). Here we describe *C. intybus*-derived phytochemicals with the reported antitumor, anticancer or antiproliferative properties.

Guaianolides. Sixteen guaianolides have been identified in *C. intybus* L. (Fig. 1A) (21-33) of which only two, 13-dihydro-8-deoxylactucin (jacquilenin) and 11,13-dihydrolactucopicrin, have chemotherapeutic properties. Both were isolated from a fractionated ethanol extract of leaves through a combination of column, thin layer chromatography, and HPLC (23). Leclercq (21) and Van Beek *et al* (27) also purified 11,13-dihydrolactucopicrin from *C. intybus* root methanolic extract. Jacquilenin showed inhibitory activity on the induction of ICAM-1 induced by IL-1α and TNF-α in alveolar basal epithelial adenocarcinoma cells (A549; IC_{50} values of 16.1 and 20.1 μM for IL-1α and TNF-α, respectively) and cytotoxicity against human lung fibroblast cells (WI38 and VA13; IC_{50} values of 2.7 and 8.5 μM, respectively) and hepatocellular carcinoma cells (HepG2; IC_{50} of 25 μM) *in vitro* (34). Isolated 11,13-dihydrolactucopicrin from *Mulgedium tatarica* by Ren *et al* (35) demonstrated antitumor activity in human nasopharyngeal cancer cells (KB; IC_{50} of 22 μM) and human liver cancer cells (Bel 7402; IC_{50} of 30 μM).

Structural activity relationship (SAR) studies by Ren *et al* (35) revealed that the position 8 ester group (γ-butyrolactone) and the methylene group at exocyclic position 11 (α) (Fig. 1A) play a major role in antitumor activities of lactucin-like guaianolides (35). α-methylene-γ-lactone, the '-enone' or unsaturated carbonyl ($O=C-C=CH_2$) system was found to increase the toxicity towards tumor cells (36). Both reported chemoprotective guaianolides in *C. intybus* (Jacquilenin and 11,13-dihydrolactucopicrin) share these features, confirming the structural basis of the activity. Further studies have shown that this structural motif works as a monofunctional alkylate and selectively deactivates the p65 dimer of NF-κB preventing NF-κB transcription in treated cells (37,38). This targeting of specific signaling pathways is also observed with other metabolites.

Polyacetylenes. *C. intybus* contains five polyacetylenes (Fig. 1B) (21,23,26,31,39,40). Putrescine and spermidine extracted from leaves by Krebsky *et al* (39) show potency as antitumor agents (41-45). Putrescine is the precursor of spermidine, and both are ubiquitous constituents of eukaryotic cells (41). Their cellular concentration is associated with the effectiveness of many anticancer therapeutics. The *in vitro* antitumor activity of putrescine has been demonstrated in breast cancer cell line MDA-MB-231 (IC_{50} of 110 μg/ml) (43,45), and it showed antiproliferative effect on T-47D breast cancer cells (0.1 mM) (44). Inhibition of proliferation of human alveolar basal epithelial adenocarcinoma cells (A549; IC_{50} of 4.54 μg/ml), prostate adenocarcinoma cells (LNCaP; IC_{50} of 1.1 μg/ml), breast epithelial cancer cells (T47D; IC_{50} of 0.97 μg/ml), bone osteosarcoma cells (U2-OS, IC_{50} of 4.47 μg/ml), HeLa cells, skin fibroblast cells (Malme-3M), prostate cancer cells (PC-3) and embryonic kidney cells (HEK-293) was demonstrated by Cheng *et al* (42). Putrescine and spermidine were found to be able to cross the cell membrane by a single unique channel or by separate ones (41). The higher lipophilicity of polyacetylene compounds facilitates plasma membrane penetration but often lowers their bioavailability *in vivo* (36). A comparison between different plant-derived polyacetylenes by Kinjo *et al* (46) suggested an inverse relationship of the presence of bulky side chains (hydroxy, methoxy, amino or other groups) with activity and effectiveness. The authors also found that *C. intybus* polyacetylenes were more effective against MK-1 cells than HeLa and B16F10 cells, and their antiproliferative effects were more profound than other chemoprotective characteristics (46). In estrogen-responsive breast cancer cells (ZR-75-1), both putrescine and spermidine were internalized via the same or similar transporter and both V_{max} was rapidly upregulated by estrogens and insulin (47). The presence of positively charged primary and secondary amino groups (at physiologic pH) and hydrophobic methylene bridging groups indicate their capability of acting as ligands at multiple locations of DNA, RNA, proteins, phospholipids and nucleotide triphosphate. Few of these connections are electrostatic and replaced easily by inorganic cations. The rest are specific to the extent of the aliphatic carbon chain (Fig. 1B) (48,49). Although polyamines are critical for cell proliferation, their high concentration can lead to interruption in transcriptions and protein-protein interactions, and thus inhibition of tumor growth.

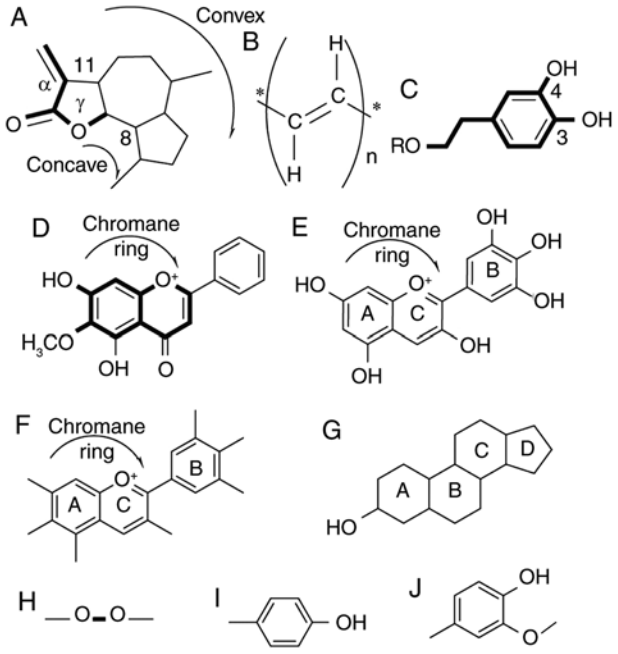


Figure 1. Chemical groups and structural motifs of *C. intybus*-derived phytochemicals. Figures show the primary structural features of (A) guaianolides, (B) polyacetylene, (C) 3,4-dihydroxyphenethyl, (D) 6-methoxyflavone, (E) delphinidin, (F) anthocyanin, (G) phytosterol/sterol, (H) peroxide, (I) phenol and (J) 4-hydroxy-3-methoxyphenyl.

3,4-Dihydroxyphenethyl. Phytometabolites containing a 3,4-dihydroxyphenethyl group (Fig. 1C) are the most frequent type of metabolites found in *C. intybus*. Furthermore, 19 of the 28 phytometabolites (5,24,50-52) were found to exhibit antitumor properties. Major members of this group include hydroxycinnamic acids, quercetins, kaempferols, and cyanidins, among others.

Among the hydroxycinnamic acids, chlorogenic acid and caffeic acid have both been extracted from *C. intybus* hairy root (*Agrobacterium rhizogenes*-induced) culture (24) and leaves [MeOH-HCOOH (99:1, v/v) extract] (5,24,51). Caffeic acid is highly effective against human promyelocytic leukemia cells (HL-60; 90% inhibition at 10 μM) (53,54), mammary duct carcinoma cells (T-47D; IC_{50} of $2.17x10^{-9}$ M) (55) and liver carcinoma cells (Hep3B) (56). It also demonstrated high antioxidant as well as anti-inflammatory activity (57). Caffeic acid was found to be moderately effective against epidermal DNA synthesis, epidermal ornithine decarboxylase activity, and skin tumors induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; IC_{50} of 72.3 μM) (58). Chlorogenic acid showed *in vivo* inhibitory effects on 8-hydroxydeoxyguanosine (8-OH-dG) formation induced by lipid peroxides in 4-nitroquinoline-1-oxide (an oxygen radical-forming carcinogen)-treated animal tongue, but not the endogenous 8-OH-dG (59). Chlorogenic acid was found to protect against environmental carcinogen-induced carcinogenesis (60). An *in vitro* study also demonstrated the antiviral potency of chlorogenic acid in human hepatoblastoma cells (Hep-G2.2.15; IC_{50} >1,000 μM) (61), antitumor potency against mouse preadipocyte cells (3T3-L1; IC_{50} of 72.3 μM) (62,63), and increased insulin secretion in rat insulinoma cells (INS-1E) (64). Caffeic acid contains both phenolic (Fig. 1I) and acrylic functional groups. The amount of absorption in the small intestine (most of the caffeic acid and one-third of the chlorogenic acid) indicates that most chlorogenic acid reaches the colon and only a fraction enters the blood circulation (65).

5-Caffeoylquinic acid, 5-caffeoylshikimic acid, di-caffeoyl tartaric acid (also called chicoric acid), *trans*-caftaric acid and 4-O-feruloylquinic acid have been purified from *C. intybus* leave MeOH-HCOOH (99:1, v/v) extract (5). A previous study showed that 5-caffeoylquinic acid inhibited non-small cell lung cancer cell (H1299) invasion (66). *Trans*-caftaric acid has antioxidant properties and protects against DNA damage caused by ROS. A *trans*-caftaric acid rich extract was found to demonstrate cytotoxicity in HepG2 (liver cancer cells; IC_{50} of 50±12 μg/ml) and HeLa cells (IC_{50} of 32±16 μg/ml) (67). 5-Caffeoylshikimic acid displayed antioxidant and antitumor properties on rat skeletal myoblast (L6; IC_{50} of 90 μg/ml) cells (68). A 4-O-feruloylquinic acid rich extract from *Oplopanax horridus* (Sm.) Miq. exhibited anti-proliferative effects against human colon adenocarcinoma cells (HT-29; 56.5% inhibition with a 0.2 mg/ml extract) (69). Chicoric acid, in synergy with luteolin was found to act as an anti-oxidant and anti-inflammatory agent in mouse macrophage cells (RAW 264.7; luteolin:chicoric acid =1:1, 1:2, 1:4 where IC_{50} of luteolin was 11.6, 9.8 and 9.8 μM, respectively) (70). Chicoric acid caused the apoptosis of mouse 3T3-L1 preadipocytes (71) and displayed antiproliferative activity in MCF-7 cells but promoted the proliferation of the HeLa cell line (72).

Among the eight quercetins (a polyphenolic flavonoid compound) found in *C. intybus*, six were found to inhibit the growth of several malignant tumors (73). Both leaf and flower extracts of *C. intybus* contain quercetin 3-O-β-D-glucoside (52,74), which promoted the apoptosis of human gastric carcinoma cells (BGC-823; 12.1±0.03% inhibition at 100 μM) (75), HepG2 (IC_{50} of 150 μg/ml), Caco-2 (IC_{50} of 79 μg/ml), HEK-293 (IC_{50} of 186μg/ml)cells(76).Quercetin-7-O-galactoside,quercetin-3-O-(6"-O-malonyl)-glucoside, quercetin-7-O-glucoside, quercetin-7-O-glucuronide, quercetin-7-O-(6"-O-acetyl)-glucoside, quercetin-7-O-p-coumaroylglucoside, and quercetin-3-O-glucuronide-7-O-(6"-O-malonyl)-glucoside have been extracted from the leaf (5). Most of the compounds containing 3,4-dihydroxyphenethyl are potent antioxidant and specific inhibitors of NF-κB and Akt. The functionality of quercetin depends largely on the positioning of glycosylation and derivatization of a sugar molecule (77). With the different glycation sites, glucoside derivatives of quercetin show *ex-vivo* 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging capacity at a varying degree (77). Quercetin isomers with a pentyl group in 7 positions were found to significantly inhibit CT26 cell proliferation (78). Quercetin hydrate demonstrated cytotoxicity against a human liver cancer cell line (HepG2) (73) and demonstrated antitumor activity against N2a, a mouse neuroblastoma cell line whereas C3 quercetin showed excellent antioxidant property in *ex-vivo* trials (77,79).

Leaf extract from *C. intybus* also yields phytochemicals such as 1,3-dicaffeoylquinic acid (5), 3,4-dicaffeoylquinic acid (5), cyanidin-3-O-galactoside (5) and cyanidin-3-O-glucoside (50). 1,3-Dicaffeoylquinic acid was found to exhibit antioxidant properties and inhibit oxidative damage created

by $FeSO_4$ and AAPH [2,20-azobis(2-amidinopropane) dihydrochloride] and scavenge ROS. 1,3-Dicaffeoylquinic acid is required in low concentrations than other known antioxidants (80). 3,4-Dicaffeoylquinic acid inhibited the growth of Kato III (human stomach cancer; 20-40% inhibition at 100-500 μM), DLD-1 (20-40% inhibition at 1,000 μM) (53) and HL-60 (40-90% inhibition at 1,000 μM) cells *in vitro* (53). Cyanidin-3-O-glucoside inhibited tumor cell growth, induced apoptosis *in vitro*, and suppressed tumor growth *in vivo* (81). It protected MIN6 (pancreatic β-cells; cell viability 86.6% at 200 μg/ml) cells against apoptosis induced by oxidative stress (82), and showed dose-dependent growth inhibition on tumors derived from HS578T, LLC (Lewis lung carcinoma) (81) and MDA-MB-453 (human breast cancer) cells in a xenografted animal model (83). Cyanidin-3-O-galactoside inhibited the development of the A431 cell line (human vulva carcinoma; IC_{50} >100 μM) (84).

6-Methoxyflavone. The second largest group of phytochemicals is the 6-methoxyflavone (also known as 6-hydroxyflavone) group (Fig. 1D), with 18 members (5,52). Six have shown antitumor properties. Among them, apigenin-7-O-glucoside, kaempferol-7-O-glucoside, isorhamnetin-7-O-glucoside, and isorhamnetin-7-O-glucuronide have been isolated from leaf extract (5). Apigenin-7-O-glucoside was found to be cytotoxic against colon cancer cells (HCT116) (85), androgen-refractory PC-3 cells, and other cancer cell lines (86). Interestingly, there are 11 known isomers of kaempferols in *C. intybus*, but only kaempferol-7-O-glucoside was found to induce G2/M mitotic phase arrest and cell death in a p53-independent manner in HeLa cells (87). Among the three derivatives of isorhamnetin (a 6-O-methylated flavanol) (5), only C7 glucoside and glucuronide isomers were demonstrated to exert antitumor properties (88,89). In lipopolysaccharide-challenged mouse Abelson murine leukemia virus-transformed macrophage cells (RAW264.7), isorhamnetin-3-O-glucuronide increased heme-oxygenase-1 but suppressed p38 and c-Jun N-terminal kinase (JNK) activation (89). This indicates that derivatives of isorhamnetin from *C. intybus* can be modified to generate chemoprotective agents. Among four delphinidins isolated by Nørbaek *et al* (52) from *C. intybus* flower extract, only delphinidin 3,5-di-O-(6-O-malonyl-β-D-glucoside) exerted an antitumor effect, and the mono sugar substitute was effective against breast epithelial cells (MCF10A) (90).

Another study showed that 6-methoxyflavone (Fig. 1D) was associated with HeLa cell growth inhibition (46). However, the precise mechanism is not yet known.

Phytosterol. *C. intybus* contains four phytosterols (Fig. 1G), all with chemoprotective properties. Campesterol and stigmasterol are two of the major phytosterols found in the leaves (39). Campesterol shows antitumor and antiproliferative activity against HepG2 and MCF-7 cells (91). Stigmasterol was found to exhibit antitumor activity against EAC in swiss albino mice (92). The cytotoxicity of stigmasterol was demonstrated by Kangsamaksin *et al* (93) in KKU-M2123, RMCCA-1 and MMNK-1 cell lines; by Syed Abdul Rahman *et al* (94) in MCF-7, CaSki (cervical carcinoma), and HCT-116 cell lines; and by Dutra *et al* (95) in a chronic myelogenous leukemia cell line (K-562). *In vivo*, stigmasterol showed antitumor efficacy against a 1,3-dimethylbutylamine-induced skin carcinoma mouse model (96) and antioxidant activity against EAC in swiss albino mice (92). β-sitosterol is the most abundant phytosterol, present in leaves (39), roots (97), and total areal part extract (26). β-sitosterol increased the activity of antioxidant enzymes, glutathione peroxidase, and superoxide dismutase in cultured macrophage cells with phorbol 12-myristate 13-acetate-induced oxidative stress. This indicates that phytosterols can protect cells from ROS induced damage (98). In *in vitro* experiments, β-sitosterol was found to be cytotoxic to HeLa (99), intrahepatic cholangiocarcinoma (KKU-M213, RMCCA-1) (93), immortalized normal cholangiocyte (MMNK-1) (93), and breast cancer cells (MCF-7) (100,101). In *in vivo* experiments, β-sitosterol reduced tumor growth in 17 β-estradiol-treated mice (98). A nonmalignant enlargement of the prostate known as benign prostatic hyperplasia (BPH) was reduced by β-sitosterol treatment (102). β-sitosterol was also found to inhibit the proliferation and thus reduce the viability of mouse fibrosarcoma (98). β-sitosterol-3-O-glucoside from the areal part of *C. intybus* isolated by Satmbekova *et al* (26) exerted an anticancer effect against three cancer cell lines, MCF-7, HL-60 and HepG2 (91,103).

The phytosterols containing an unsaturated ring structure (Fig. 1G) are susceptible to oxidation under certain conditions. Comparison of the corresponding phytosterol and cholesterol oxidation-products (POP) in four cell lines demonstrated that phytosterol induces oxidation-independent apoptosis (104), which is in contrast to a previous report by O'Callaghan *et al* (105). These authors suggested that POP induced apoptosis by high oxidative stress, glutathione reduction, mitochondrial dysregulation and elevated caspase activity (105). From Table I, we can see that phytosterols show specificity towards fatty tissue-related cancer lines such as those derived from breast, cholangiocyte, and cervix cancer.

Delphinidin. All four delphinidins (Fig. 1E) found in *C. intybus* are di-glucoside isomers, and none has antitumor properties. However, mono-glucoside substitutes of delphinidins are cytotoxic (88). Delphinidin-3-glucoside was found to be chemopreventive against breast epithelial cells (MCF10A) (90). Derivatives of *C. intybus* derived delphinidins can serve as potent chemoprotective agents.

Anthocyanins. The aglycons are the richest anthocyanins (Fig. 1F) found in food. Cyanidin and delphinidin were found to inhibit the growth of human tumor cells *in vitro* in small quantities (84). *C. intybus* fresh leaf extract [MeOH-HCOOH (99:1, v/v)] contains malvidin-3-O-glucoside and pelargonidin-3-O-monoglucuronide (5). Malvidin-3-O-glucoside was demonstrated to have antitumor activity against MCF-7 (106) and A431 (84) cell lines. Pelargonidin-3-O-monoglucuronide increased the concentration of IL-6 and monocytes *in vitro*, affecting tumor prognosis of THP-1 cells (human monocytic leukemia) (107). Its glucoside derivative, pelargonidin-3-O-glucoside, acts as an anti-inflammatory agent (108).

Seong *et al* (109) demonstrated that delphinidin treatment induced hypoacetylation of histone acetyltransferase (HAT) and inhibited p65 acetylation in a human rheumatoid fibroblast-like synoviocyte cell line (MH7A) (Fig. 4). TNF-α stimulation increases NF-κB expression, and thus promotes the

functions of NF-κB target genes (109). Delphinidin also inhibited the release of pro-inflammatory cytokines IL-6 and TNF-α in lipopolysaccharide-treated Jurkat T lymphocytes (109). The chemotherapeutic property of delphinidin-3-glucoside against MCF10A cells is related to downregulation of non-coding RNA (lncRNA) and HOX transcript antisense RNA (HOTAIR) expression (90). Pelargonidin-3-O-monoglucuronide treatment was found to increase the IL-6 and monocytes concentration (107). Bioactivity of these compounds is related to the presence of hydroxyl group in position 3 of the C ring and 3, 4, 5 in the B ring (Fig. 1E and F) which corresponds to the report by Wang and Stoner (110), showing that methylation on these positions decreases the activity.

4-Hydroxy-3-methoxyphenyl. 4-O-Feruloylquinic acid is one of the two 4-hydroxy-3-methoxyphenyl groups (Fig. 1J) containing phytochemicals found in *C. intybus*. It was demonstrated to scavenge oxygen radical absorbance capacity (ORAC) and DPPH radical in an *in vitro* experiment (69).

Other phytochemicals. Researchers also identified several other types of phytochemicals in *C. intybus*. Artesin (also known as artemisinin, an antimalarial agent) was identified in the root extract fraction by Kisiel and Zielińska (23). Its antitumor property has been well studied (111-115). The derivatives of artesin have *in vivo* chemosensitizing effects in breast, lung, pancreas, and glioma cancer cells (112). Artesin and its derivatives activated by heme [Fe (II)] showed selective inhibition of colon (HCT116, SE480), leukemia (HL-60), breast (MCF-7), melanoma (KM, MJT3), lung (NSCLC), pancreas (PANC-1, MIAPaCa), and glioma (U87MG, A172) cancer cell lines (113-115). In primary cancer culture, cell lines and xenograft models, artesin inhibited tumor proliferation, metastasis, and angiogenesis (114). Several antitumor mechanisms of artesin have been proposed, including apoptosis, cell cycle arrest at G0/G1, and oxidative stress (111). Rapidly proliferating cancer cells express more transferrin receptors on their cell surface, leading to higher iron uptake. Artesin/artemisinin is the only phytochemical found in *C. intybus* with a peroxide bridge (Fig. 1H). When artesin binds to Fe (II), the endoperoxide bridge (Fig. 1H) is disrupted, resulting in the production of toxic C-4 and seco-C-4 free radicals that destroy tumor cells (Fig. 2) (36).

3-O-p-Coumaroyl quinic acid was isolated by Nørbaek *et al* (52) from an extract of *C. intybus* flower. 3-O-p-Coumaroyl quinic acid was demonstrated to show antiproliferative activity against PC-3 and undifferentiated non-cancerous 3T3L1 fibroblast cells (116). Usnic acid purified from the areal part extracts (26) was reported to exhibit chemoprotective effects against the wild-type p53 MCF-7 cell line along with a breast cancer cell line with non-functional p53 (MDA-MB-231), lung cancer cell line (H1299) (117) and prostate cancer cell line (LNCaP) (118). Mechanistically, (+)-usnic acid treatment dose-dependently decreases β-catenin-mediated transfection grade T-cell factor reporter plasmid activity and KAI1 COOH-terminal interacting tetraspanin-mediated AP-1 activity. In addition, (+)-usnic acid decreases the mRNA levels of CD44 (a cell-surface glycoprotein), cyclin D1 (a mitotic regulatory protein) and c-myc (a transcription factor). These are the downstream target genes of both β-catenin/LEF and c-jun/AP-1. Furthermore, (+)-usnic acid treatment was found to decrease the functionality of Rac1 and RhoA. Interestingly, cotreatment of (+)-usnic acid and cetuximab showed higher inhibition of cell proliferation then the single cetuximab treatment. These results indicate the potential antitumor activity and metastasis inhibitory quality of (+)-usnic acid and suggest (+)-usnic acid can be used for anticancer therapy with distinct mechanisms of action (119).

C. intybus root is a major source of inulin, a heterogeneous collection of fructose polymers primarily used as a prebiotic (120). The chemoprotective property of inulin has been confirmed in colon cancer colonic preneoplastic aberrant crypt foci inhibition (121). Its antitumor property was also demonstrated on transplantable liver tumor cells (TLT) and mouse mammary carcinoma cells (EMT6) (121-123). Low fermentation of inulin indicates its possibility to reach the distal part of the intestine (124). Inulin extracted from *Cichorium endivia* L. (a related species) was found to reduce the occurrence of intestinal tumors in an APC^{MIN} mouse model (121).

4. Molecular mechanisms

SARs. Most of the phytochemicals identified in *C. intybus* appear to show potent activity as inhibitors in specific tumor cell lines; only a few phytochemicals work on all cell lines. A screening study of anti-proliferative activity revealed the specificity of purified *C. intybus* phytochemicals against different cancerous cell lines. Kinjo *et al* (46) and other researchers showed that groups of phytochemicals had specificity towards specific cancer cell lines (listed in Table II). In general, polyacetylenes are potent antiproliferative agents against MK-1 cells, and compounds with 3,4-dihydroxyphenethyl against B16F10 cells, and some 6-methoxyflavone derivatives and 8-hydroxy furanocoumarins against HeLa cells are potent anti-proliferative agents (46). This indicates that the structural features of these phytochemicals directly interact with the cytochemistry of specific cancerous cells. The biological activities of the guaianolides, 6-hydroxyflavone, and anthocyanin depend broadly on the following: i) reactivity of alkylation center; ii) lipophilicity of the side chain; iii) electronic features and molecular genomics (36).

Similarly, every phytochemical has its own conserved structural features responsible for bioactivity. The antitumor property of flavonoids is partly due to their ability to counteract fatty acid synthase (FASN). Cancerous cells have an elevated metabolic rate compared with healthy cells, which enables cancer cells to grow and proliferate at a faster rate (18). Rapid cell division often leaves trails of both biochemically and structurally irregular cells. FASN is overexpressed in cancerous tissues of the breast, prostate, and colon (18), and is also related to angiogenesis and metastasis (125); therefore, FASN is recognized as an important antitumor target.

Previous studies have reported that phytochemicals of *C. intybus* exert antitumor effects by affecting many critical overactive signaling pathways in cancerous cells, such as NF-κB, p53-associated cell cycle, and CYP-mediated inactivation. The various effects of these phytochemicals confer the advantages to effectively target more than one cell type, which is often encountered during metastasis.

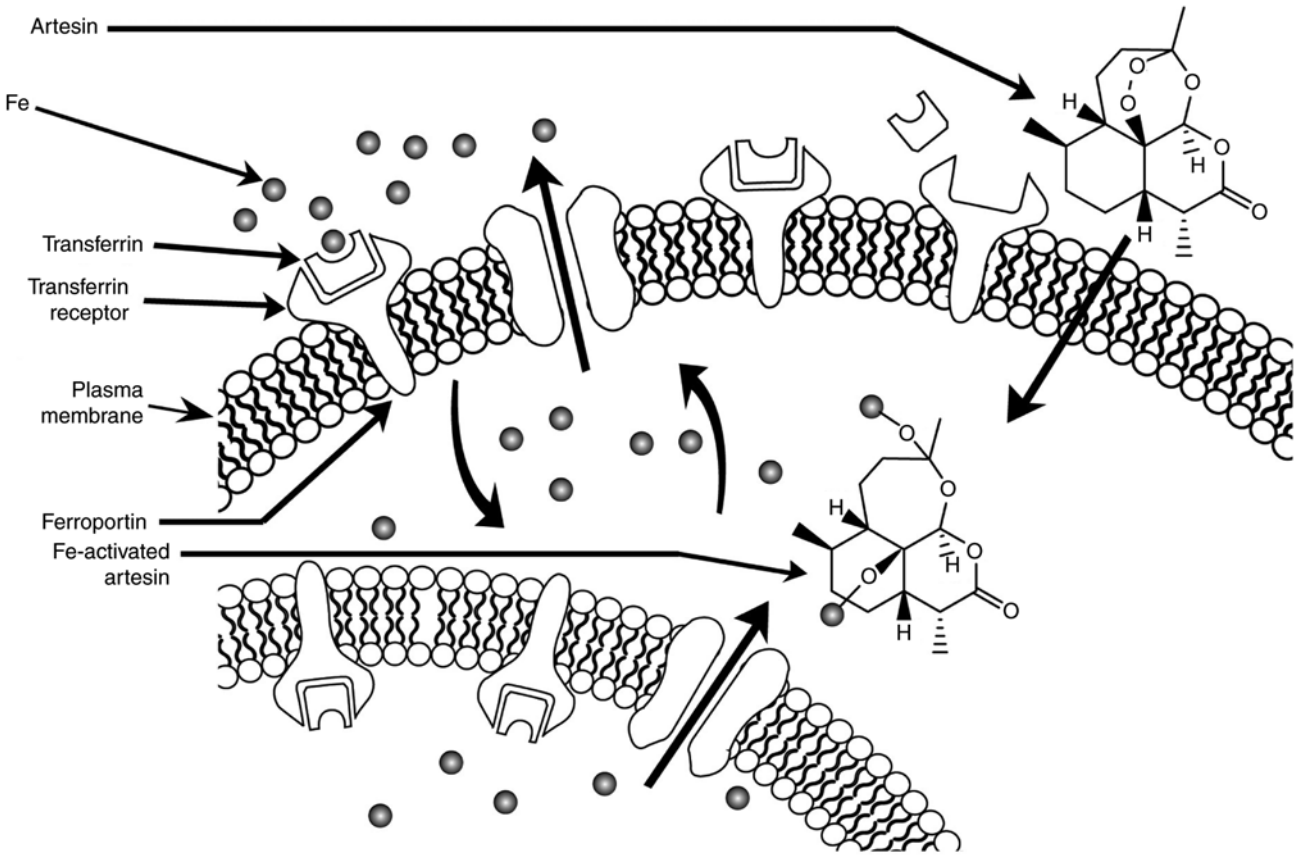


Figure 2. Iron-dependent free radical generation by artesin. Breaking of the endoperoxide bridge of artesin and binding of cytoplasmic Fe (II) to its C-4 and seco-C-4. This creates free radical-activated toxic artesin that destroys tumor cells.

Inhibition of NF-κB. The nuclear factor-κB (NF-κB) transcription factor plays a critical role in cell development, growth, and survival as well as various biological processes, including immune response and inflammation. Numerous inflammatory stimuli such as growth factors and infectious microbes lead to NF-κB activation (126). Activated NF-κB in turn regulates the expression of genes governing cell growth, proliferation, survival, and apoptosis as well as immune responses, stress responses, embryogenesis, and development of a variety of stimuli (127). Abnormal NF-κB activation causes various autoimmune, inflammatory, and malignant disorders such as rheumatoid arthritis, atherosclerosis, inflammatory bowel diseases, multiple sclerosis, and malignant tumors. Thus, inhibition of NF-κB signaling is a key target in the treatment of tumors and inflammatory diseases (127). The mammalian NF-κB family is composed of five members that form various dimeric complexes. Among these complexes, the p50/65 heterodimer is most abundant. Overexpression of this complex in cancer cells leads to the aberrant levels of cell cycle control factors. Several phytochemicals of *C. intybus* disrupt different stages of NF-κB activation and NF-κB-DNA complex formation. Guaianolides, germacranolides, heliangolides, pseudo guaianolides, hypocretenolides, and eudesmanolides are collectively classified as sesquiterpene lactones. Sesquiterpene lactones bind to the p65 dimer in the NF-κB transcription factor to prevent NF-κB-DNA binding (Fig. 3). The three-dimensional structure created by Arg 33, Arg 35, Tyr 36, Cys 38, Glu 39 and Arg 187 is crucial for DNA binding of the p65 dimer. Rüngeler *et al* (37) proposed that lactucin creates cross linkage of Cys 38 to Cys 120 in the p65 molecule (Fig. 3) that changes the DNA binding motif structure and affects subsequent transcription, eventually leading to apoptosis. This mechanism was further demonstrated in a computer-generated model by García-Piñeres *et al* (38). The authors showed the change in native confirmation of p65 caused by a sesquiterpene lactone that led to its inability of NF-κB-DNA complex formation.

Búfalo *et al* (57) demonstrated that caffeic acid mediated cell viability independent anti-inflammatory activity and proposed that caffeic acid exhibited an inhibitory effect in lipopolysaccharide (LPS)-induced NF-κB activity. The chemoprotective effect of chlorogenic acid may be accomplished through its increase of cellular antioxidant enzymes and suppression of ROS-mediated NF-κB, activator protein 1 (AP-1), and mitogen-activated protein kinase (MAPK) activation (60). 5-Caffeoylquinic acid inactivates ribosomal protein S6 kinase (p70S6K) and protein kinase B (PKB/Akt) activity and thus affects multiple cellular processes and signal transduction pathways in cancerous cells. Another study showed that 1,3-dicaffeoylquinic acid scavenged hydroxyl radical and superoxide radicals as measured by electron spin resonance (ESR) (80). Chicoric acid was found to exert its anti-inflammatory function by halting the phosphorylation of NF-κB (70). Upon co-treatment with luteolin, chicoric acid simultaneously reduced the concentration of nitric oxide and prostaglandin E2 (PGE2) in cells and also inhibited inducible

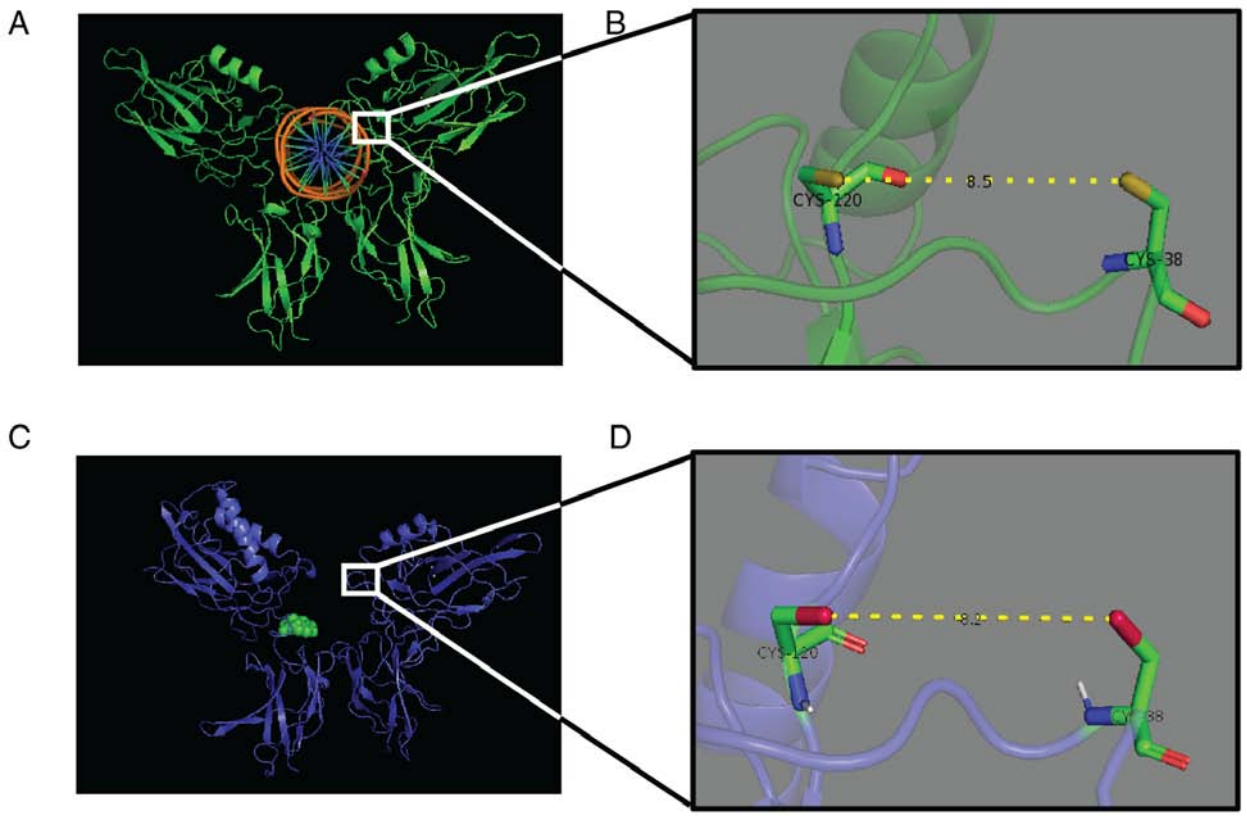


Figure 3. Mouse NF-κB p65 homodimer. Figures show (A) DNA (orange)-bound NF-κB p65 (green) homodimer (PDB ID: 1RAM), (B) distance between Cys38 and Cys120 of NF-κB p65 homodimer at the native state, (C) lactucin (green)-bound NF-κB p65 homodimer (blue), and (D) distance between Cys38 and Cys120 of NF-κB p65 homodimer at the lactucin bound state.

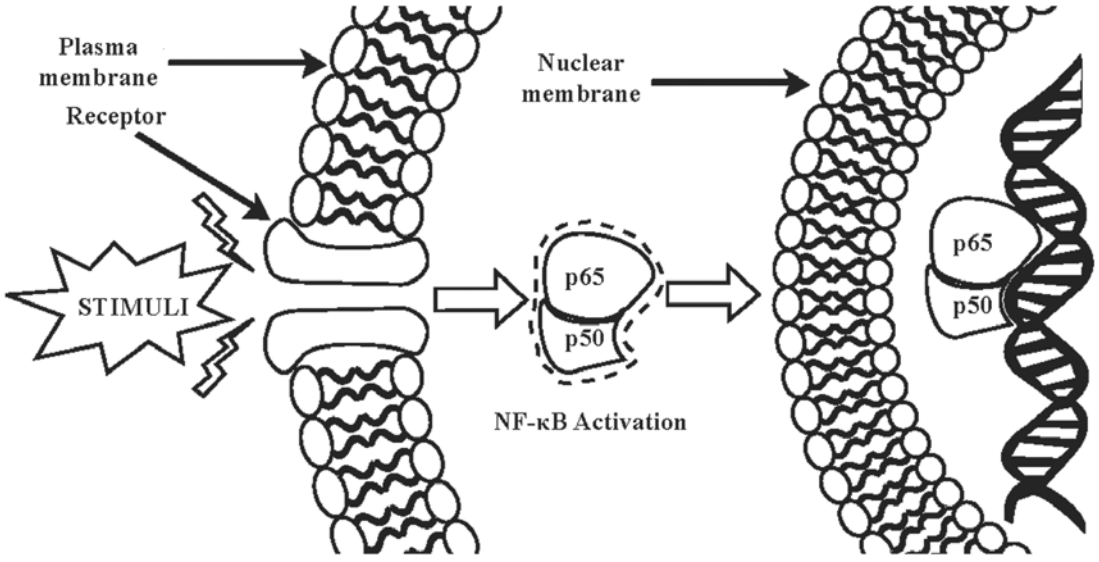


Figure 4. Activation and NF-κB-DNA binding.

nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression (70).

p53 associated cell cycle inhibition. Genomic instability is one of the fundamental cause of tumor development. p53, commonly known as TP53 or tumor protein 53, is a cell cycle regulatory protein that functions as a tumor suppressor. p53 responds to DNA damage and other types of genotoxic stress and functions to maintain genomic stability. The close involvement of p53 in maintaining genomic stability is why nearly half of human cancers lack functional p53. In the other half of cancers, the p53-independent regulatory mechanism is absent or the p53-dependent pathway gets disabled at different key points. For instance, the p53 inhibitor MDM2 is overexpressed

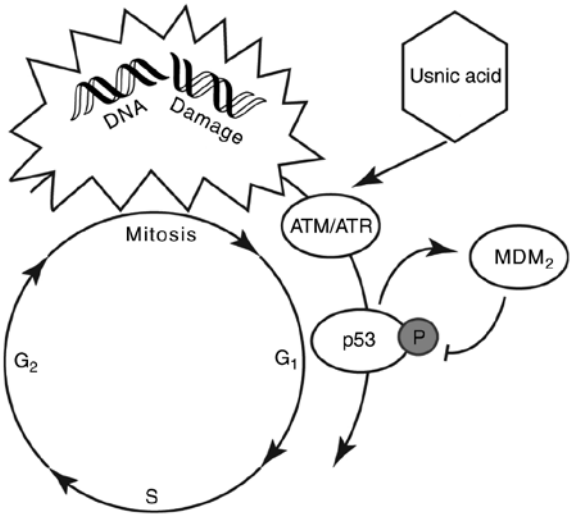


Figure 5. Cell cycle arrest at G1 phase by p53. Ataxia telangiectasia mutase (ATM)- or reductase (ATR)-mediated phosphorylation of p53, leading to G1 cell cycle arrest.

in tumors that lack p53 gene mutation. p53 is a crucial component of a complex network of signaling pathways. However, other components of this pathway can be alternately targeted for inactivation in cancer. *C. intybus* extract, in particular, usnic acid, was found to induce levels of factors such as ataxia telangiectasia mutase (ATM) or reductase (ATR) that phosphorylates p53 at the MDM2 binding site (Fig. 5) (117). However, kaempferol-7-O-glucoside arrests cell division in a p53-independent manner (87).

CYP-mediated inactivation. Cytochrome P450 enzymes (CYPs) are a superfamily of enzymes that are important for the metabolism of endobiotics and xenobiotics (128). Rodriguez-Antona and Ingelman-Sundberg (129) described the pharmacogenetics of CYPs in cancer formation and treatment. CYPs are linked to the metabolic activation of numerous pre-carcinogens and participate in the activation and inactivation of antitumor drugs (129). Hepatic CYP expression and activity can be upregulated or downregulated by bioactive phytochemicals (130). Direct foddering of dried *C. intybus* roots to pigs resulted in increased activities of CYP1A2 and CYP2A, which in turn reduced the skatole concentration in plasma and fat (128), reducing the chances for colon cancer occurrence (131). CYP1A2 is one of the class I CYPs, which are distinguished by a well-conserved sequence and lack of functional polymorph. CYP1A2 activity has interindividual (genetic) difference, and this polymorphism is triggered by external factors such as smoking (129). Although downregulated by total extract, artemisinin upregulates the mRNA expression of CYP1A2, CYP2C33, CYP2D25 and CYP3A29 in porcine hepatocyte culture (132), suggesting a shared regulatory mechanism of CYP transcription and an inverse agonist effect of *C. intybus*.

5. Toxicological studies

A toxicological study in a 28-day sub-chronic toxicity study of *C. intybus* root extract in male and female Sprague-Dawley rats revealed no adverse effects at 1,000 mg/kg/day dose (133). The *C. intybus* seed chloroform extract inhibited 50% cell growth of human colorectal adenocarcinoma cells (HCT-15) at 1,411.37 μg/ml concentration (134). The inhibition of mouse embryo-derived teratocarcinoma cells (P-19) by a methanolic extract of *C. intybus* was least bio-toxic as well as concentration and duration dependent (135). The cytotoxic activity of chlorogenic acid (CGA) at a millimolar concentration was higher in human oral squamous cell carcinoma cells (HSC-2) and a salivary gland tumor cell line (HSG) than in human gingival fibroblast cells (HGF) (136).

6. Clinical trials

The number of completed cancer or tumor-related clinical trials of *C. intybus* whole plant or crude or purified extract is inadequate. A phase 1, placebo-controlled, double-blind, dose-escalating trial was performed in patients with osteoarthritis and showed the potential of *C. intybus* root extract in the management of osteoarthritis (137). A second clinical trial reported that daily consumption of chicory coffee reduced the risk of cardiovascular disorder by lowering whole blood and plasma viscosity as well as serum MIF level but had a variable effect on platelet aggregation (138). A multi-herbal liver tonic formula called Liv-52 that contains *C. intybus* as one of the ingredients was tested in a randomized, double-blind, placebo-controlled clinical trial in cirrhotic patients. Liv-52 showed a hepatoprotective effect in cirrhotic patients due to the diuretic, anti-inflammatory, anti-oxidative, and immunomodulating properties of the component herbs (139). A spermidine-rich diet has been linked to increased survival in an animal model (140). Spermidine also reduced the overall (141) and cancer-related (142) mortality in a human clinical trial. In a difluoromethylornithine (DFMO) + sulindac colorectal adenoma prevention trial involving dietary putrescine, spermine and spermidine administration exogenous putrescine effectively increased cellular polyamine concentration and decreased the risk and reoccurrence of metachronous adenomas and advanced adenomas (143). Clinical evidence has shown that artesin derivatives (artemether and artesunate) substantially reduce tumor size, and metastasis and increase the survival of patients with laryngeal carcinoma, uveal melanomas, and pituitary macroadenomas. These derivatives are in phase I-II-III clinical trials for lupus nephritis and breast, colorectal (NCT03093129) and non-small cell lung cancer (NCT02786589) (36,111). Currently, a phase IV clinical trial in China (NCT02556814) is investigating caffeic acid combined with high-dose dexamethasone in the management of immune thrombocytopenia (ITP). Anthocyanin-rich extracts of different plant species have shown promising result in phase I clinical trials, but no convincing evidence has been shown with purified compounds (144). Several clinical trials are currently underway to investigate quercetin. One clinical trial is investigating its chemoprevention activity in squamous cell carcinoma patients (NCT03476330). Two clinical trials are examining quercetin in prostate cancer (NCT01538316), and another is studying the effect of quercetin on green tea polyphenol uptake in prostate tissue from prostate cancer patients undergoing surgery (NCT01912820). One clinical trial in Germany was previously proposed to

Table II. Chemical groups of *Cichorium intybus* L.-derived phytochemicals with cell-specific activity.

Chemical groups/structural motifs	Cell lines	Activity	(Refs.)
Polyacetylene	MK-1	Cytotoxic	(46)
3,4-Dihydroxyphenethyl	B16F10	Antiproliferative	(46)
	HSC-2, HSG	Cytotoxic	(138)
6-Methoxyflavone/6-hydroxyflavone	HeLa	Inhibitory	(46)
Delphinidin	Jurkat, MH7A	Anti-inflammatory	(109)
Phytosterol/sterol	U937	Apoptosis	(104)
	CaCo-2, HepG2	Necrosis	
Usnic acid	LNCaP	Apoptosis	(118)

study the synergic effect of dietary apigenin in combination with epigallocathechin gallate in colorectal cancer patients (NCT00609310), but this study has been suspended. A phase III clinical trial is currently investigating the effect of statins with phytosterol as a dietary intervention in breast cancer patients (NCT03971019). β-sitosterol was successfully proven to be effective in treating BPH in a phase II clinical trial (145). Inulin is used during acute radiation enteritis to prevent indigestion (146).

7. Conclusion and perspectives

C. intybus has been the subject of multiple studies examining its various bioactivities. Here we reviewed numerous reports in regards to the association of *C. intybus* whole, partial, fractionated and purified extracts, with chemotherapeutic properties. Some of the purified compounds from *C. intybus* demonstrated efficacy in *in vitro* and *in vivo* experiments as well as in clinical trials. A few of their functions are associated with the chemicals' structural features such as chemical groups and positioning. Structural activity relationship and molecular mechanisms of toxicity studies have also revealed the importance of certain chemical groups for functionality. The specificities of some phytochemicals towards some specific cell lines (Table II) also indicate structure-specific inhibition activities. Some clinical trials and cytotoxicity studies have examined the whole extract and purified compounds. However, little information is available regarding the molecular mechanism and even fewer clinical trials have investigated these properties, which is not adequate to construct a complete pathway. Further investigation of the following subject areas is crucial for optimizing the therapeutic potential of *C. intybus*. i) Identification of more phytochemicals and chemical groups or features of existing phytochemicals that interact with the key control points of tumor development. ii) From the above information, it is important to develop a complete model explaining the interaction cascade between phytochemicals and tumor cells and the associated molecular pathways for developing precision medicines. iii) It is vital to investigate the selective cytotoxicity of phytochemicals towards tumor cells and avoiding healthy cells. It is important to confirm the reproducibility of these properties in *in vitro*, *in situ*, *in vivo* and human trials.

All chemotherapeutic products currently available show various levels of indiscriminate cytotoxicity towards normal cells, hindering successful recovery. Moreover, the interpersonal and interorgan difference in metabolic profile makes generic treatment even less effective. A targeted chemotherapeutic product that does not interfere with healthy cells is thus required. Natural compounds usually target cancer cells or their metabolic pathways at the molecular level; therefore, understanding the interaction of phytochemicals with normal and cancer cells is required for designing tumor-specific personalized therapeutics. However, our review suggests that a complete molecular mechanism and clinical study information is lacking for natural bioactive compounds. In regards to the rich historical background of ethnomedicinal use and the scientific findings reported to date, *C. intybus* phytometabolites assuredly show excellent promise as a source of anticancer compounds. Future research should be focused on understanding the correlation between structure and cell specificity, phytochemical isolation and designing derivatives to formulate targeted and efficient therapeutics and prophylactics as well as establishing clinical trials to approve their mainstream use.

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Availability of data and materials

Datasets used in this review are summarized and presented with the publication as tables. Any other relevant information will be made available by the corresponding author upon reasonable request.

Authors' contributions

KMSUI and YX wrote the first draft, developed the figures and tables. YL contributed to the writing and argument

development. FW and FX jointly made critical revisions and approved the final version. All the authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

Authors disclose no potential conflicts of interests.

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