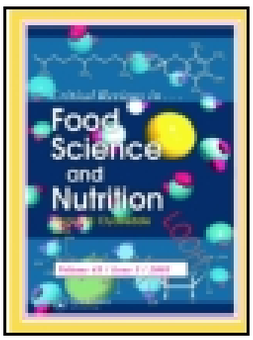

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REVIEW

Saffron anti-metastatic properties, ancient spice novel application

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ABSTRACT

Crocus sativus L. (saffron), was applied as a spice, food colorant and medicine since four millennia ago and has been used as a remedy for various maladies. In the last three decades, the anti-primary tumor properties of saffron and its main carotenoids, crocin and crocetin, have been well explored. Despite the fact that metastasis is the leading cause of death in cancer patients, the anti-metastatic potential of saffron and its carotenoids has been surveyed only this decade. This review aims to provide an unprecedented overview of the anti-metastatic effects of saffron, crocin and crocetin, and the mechanisms underlying these effects. Investigations on various cancers demonstrated the anti-migratory, anti-invasion, anti-angiogenic potentials of saffron and its carotenoids, as well as their effects suppressing cell-ECM adhesion and enhancing cell-cell attachment. Saffron and its carotenoids exert their impact through different mechanisms such as reduction of CD34 and suppression of Wnt/β-catenin, Ras/ERK, P38, DCLK1, EMT, matrix metalloproteinases and urokinases. Crocin displayed more effective anti-metastatic potency, in comparison with saffron extract and crocetin. The bioaccessibility/bioavailability, nontoxicity on normal cells, confirmed anti-tumor efficiency and the recent evidence on the anti-metastatic potential of saffron and its carotenoids, recommends them as a propitious multipotent dietary agent and herbal medicine.

KEYWORDS

saffron; crocin; crocetin; anti-invasion; anti-migration; mechanism

Introduction

Saffron, an ancient spice and food colorant, is the dried stigma of *Crocus sativus* L. and belongs to the Iridaceae family (Bolhassani, Khavari, and Bathaie 2014). Saffron comprises more than 150 components such as sugars, fats, minerals, vitamins and secondary metabolites including flavonoids, anthocyanins, terpenes and carotenoids (Winterhalter and Straubinger 2000). Among which, the carotenoids are the most essential, as they are responsible for the color and taste of the spice (Gismondi et al. 2012). Since four millennia ago saffron has been vastly prescribed as a traditional remedy for asthma, depression, inflammation, cough, colic, insomnia and cardiovascular disorder (Abdullaev and Espinosa-Aguirre 2004; Bathaie and Mousavi 2010).

Saffron extract and its main carotenoids, crocin and crocetin, possess selective toxicity against cancer cells (Milajerdi, Djafarian, and Hosseini 2016). Their nontoxicity and nonmutagenicity on normal cells (Abdullaev 2002; Alavizadeh and Hosseinzadeh 2014), as well as their bioaccessibility and bioavailability (Almodóvar et al. 2020), distinctly provides a special preference for their study as health promoting food with medicinal applications.

In the last three decades, mounting evidence has immerged supporting the anti-primary tumor properties of saffron and its two main carotenoids, crocetin and its glycosidic derivative, crocin (see Figure 1) (Hoshyar and Mollaei 2017). Numerous reviews and a plethora of *in vivo* and *in vitro* studies have described the anti-proliferative and anti-growth activity of saffron and its essential carotenoids. It has been discovered that crocin anti-cancer effects are associated with induction of apoptosis by enhancing the Bax/Bcl-2 ratio and blocking the G2/M and G1 phases of the cell cycle. Studies have elucidated that crocetin suppresses DNA synthesis and RNA polymerase II activity, which subsequently represses proliferation via impairing EGFR, phospho Cdc-2, Cdc25c, cyclin B1 and activate apoptosis by elevating the Bax/Bcl-2 ratio (Bolhassani, Khavari, and Bathaie 2014; Patel, Sarwat, and Khan 2017; Bathaie, Bolhassani, and Tamanoi 2014; Hoshyar and Mollaei 2017; Gutheil et al. 2012; Colapietro et al. 2019).

Metastasis involves a cascade of intricate processes by which the tumor cells spread from the primary neoplasm to distant organs (Hunter, Crawford, and Alsarraj 2008). The succession of events consists of the expansion of new blood vessels (angiogenesis), detachment and migration of malignant cells from the primary tumor, invasion via the basement membrane (BM) and the extra cellular matrix (ECM) encompassing the tumor, invasion of the BM adjusting the endothelium of local blood and lymphatic vessels, intravasation of the metastatic cells into the blood and/or lymphatic vessels, attachment of the mobile metastatic cells to the capillaries of the target sites, invasion of the cells through the endothelial layer and the surrounding BM

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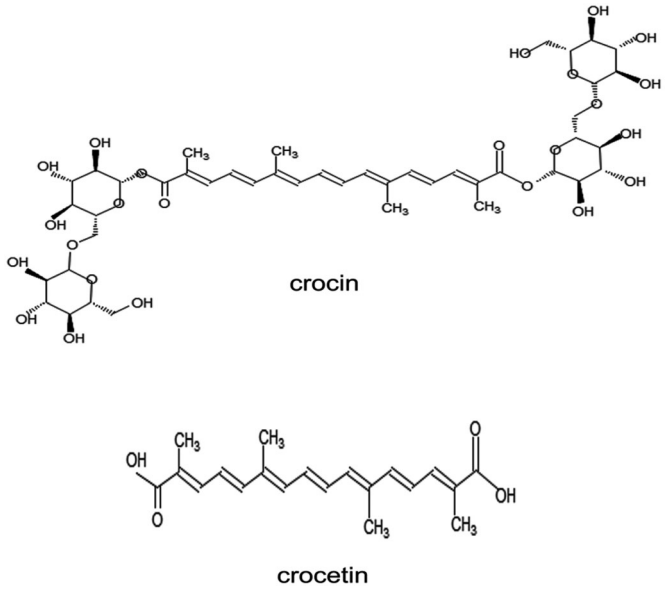

Figure 1. Chemical structure of crocin and crocetin, drawn using WinPLT.

(extravasation), and eventually the settling and developing of secondary tumors at the target organs (Guan 2015).

The majority of deaths, at least 66%, from solid tumors do not arise from the primary tumor, but are a consequence of metastasis (Dillekås, Rogers, and Straume 2019). It has been estimated that 90% of colorectal cancer and 76% of breast cancer mortality are associated with metastatic dissemination (Güllü et al. 2020; Sun et al. 2012).

Although the anti-primary tumor potency of saffron and its main carotenoids have been extensively studied and strongly confirmed, but despite the significance of metastasis, their anti-metastatic potential has been scantily explored and only recently. Therefore, this review aims to provide an unprecedented compilation of the findings on the anti-metastatic properties of saffron and its main carotenoids, and their mechanism of action.

Toxicity and safety

Emerging evidence suggests that saffron extract and its main carotenoids, crocin and crocetin, possess selective toxicity against cancer cells, with insignificant toxicity against normal cells, even in oral administration (Milajerdi, Djafarian, and Hosseini 2016). Albeit, dietary consumption toxic doses of 5 g and above, and a lethal dose of approximately 20 g has been reported (Bathaie and Mousavi 2010; Ghaffari and Roshanravan 2019), which are higher than the reported doses required for anti-metastatic efficacy. Animal experiments showed that the oral LD50 of saffron (administrated as a decoction) was the notably high dose of 20.7 g/kg (Bathaie and Mousavi 2010). Hematological and biochemical investigations showed total lack of severe toxicological indications in the liver, kidney, or bladder (Bathaie et al. 2014).

Crocin, the main carotenoid of saffron, is water soluble and perhaps this is the main cause for its safety, even at high doses (Bathaie and Mousavi 2010). It has been reported that crocin demonstrated non-mutagenicity (Bukhari, Manzoor, and Dhar 2018). Crocin at a concentration of 0.1% in the diet protected rats against hepatic damage induced by aflatoxin B1 (Alavizadeh and Hosseinzadeh 2014).

Crocetin demonstrated protective effects on hepatotoxicity and bladdertoxicity (Asai et al. 2005). Martin, Goh, and Neff (2002) suggested crocetin as an alternative for all trans retinoic acid (ATRA) to treat ATRA sensitive cancers in women of childbearing age, due to its much lower teratogenocity.

It should be mentioned that doses used for anti-metastasis studies aim to impede migration and invasion of viable cancer cells without having cytotoxic effects, therefore the doses are much less than doses used for anti-primary tumor effects, and completely fall in the safe dietary consumption range for saffron.

Bioaccessibility and bioavailability

The bioaccessibility of crocin was approximately 40% after saffron tablet digestion, and 50% from an aqueous extract of saffron under *in vitro* gastrointestinal digestion conditions (Almodóvar et al. 2020; Moratalla-López et al. 2019). In rats, crocin is stable in gastric juice for 4 h, stable enough to function there. Subsequently it is hydrolyzed to crocetin in the intestinal tract, may be through the intestinal microflora (Sheng et al. 2006). Studies on humans, rats, mice and *in vitro* digestion have demonstrated that crocin is able to reach the bloodstream, with proven functional effect, in crocetin form, with high bioavailability (Chryssanthi, Dedes, et al. 2011; Xi et al. 2007; Asai et al. 2005; Almodóvar et al. 2020).

A bioaccessibility of 66% has been reported for crocetin in *in vitro* digestion (Almodóvar et al. 2020). It has been observed that orally administered crocetin to mice is rapidly absorbed into the blood circulation and is present in plasma as an intact free form (Asai et al. 2005). Yoshino et al. (2011), showed that crocetin arrived at maximum concentrations in plasma and brain 90 minutes after the oral administration of pure crocetin to rats. The easy distribution of crocetin to human body tissues can be attributed to the weak interaction between crocetin and albumin (Umigai et al. 2011).

Anti-metastatic potential of saffron

Colorectal cancer

Recent research on colorectal cancer cells has shown that saffron crudes reduce migration of MACC1-expressing colorectal cancer cells, i.e., SW620 and SW480/MACC1 (produced by transfection of pcDNA3/MACC1), in a concentration- and MACC1-dependent manner. Whereas saffron crudes do not lead to notable alteration of migration in MACC1-nonexpressing cells SW480 and SW620/KO-MACC1 (MACC1 was knocked-out in SW620). This finding has been supported by rescue experiments, through which by eliminating saffron it is shown that these effects are reversible (Güllü et al. 2020). Metastasis-Associated Colon Cancer 1 (MACC1) is a common metastasis-promoting oncogene. Enhanced expression of MACC1 increases migration and invasiveness of cancer cells by inducing epithelial-mesenchymal transition (EMT) and increasing angiogenesis (Stein et al. 2009; Radhakrishnan

Table 1. Anti-metastatic effects of saffron against various cancers.

Type of cancers	Cell lines/animal models	Applied doses	Affected factors	Mechanism	Reference
Colorectal	SW620 SW480	1%, 2.5%, 5%, and 10% of growth medium	Migration↓	DCLK1↓⇒ MACC1↓	Güllü et al. 2020
Prostate	PC3 22rvl	0.4 mg/mL	Invasion↓ Migration↓ Cell-cell adhesion↑	MMP9↓ MMP2↓ uPA↓	Festuccia et al. 2014
	PC3 - 22rvl xenograft male mice	300 mg/kg		tPA↓ N-CAD↓ β-CAT↓ Vim↓ E-CAD↑ K18↑ ⇒EMT⊥	
Breast	MCF-7	100, 200, 400 and 800 μg/ml	Angiogenesis↓	VEGFR2↓ VEGF-A↓ MMP↓	Mousavi, Baharara, and Shahrokhabadi 2014; Mousavi and Baharara 2014; Mousavi, Baharara, and Asadi-Samani 2014

et al. 2018). Furthermore, it has been reported that MACC1 interacts with DCLK1, and that the anti-migratory potential of saffron in MACC1-expressing cells is mediated through DCLK1 down-regulation (Güllü et al. 2020). Doublecortin-like kinase 1(DCLK1) stimulates tumor initiation and metastatic spread in different tumors including breast, colorectal, and pancreas and is able to phosphorylate and activate MACC1(Gao et al. 2016; Liu et al. 2019; Ito et al. 2016).

Prostate cancer

The anti-metastatic potency of saffron extract was assessed on two aggressive prostate cell lines, PC3 and 22rv1, and xenograft mice model. The migration and invasion assays, using Transwell chamber, revealed that 0.4 mg/mL of saffron extract suppresses the mobility and invasion of PC3 and 22rvl cells. It was displayed that saffron inhibited the expression and activity of matrix metalloproteinases (MMP9, MMP2) and urokinase (tissue-derived plasminogen activator (tPA), and urokinase-type plasminogen activator (uPA)) (Festuccia et al. 2014).

The expression of MMPs has a critical role in the molecular biology of prostate carcinomas and is applied as an indicator of tumor aggressiveness (Escaff et al. 2010). Festuccia's group (2014) associated the anti-invasive property of saffron with declining MMPs expression. They found that saffron reverted epithelial-mesenchymal transition (EMT) and concluded that saffron treatment prohibited detachment of cell-cell adhesion and cell mobility, via repression of N-cadherin and β-catenin expressions (mesenchymal marker) and elevation of the expression of E-cadherin (epithelial markers). Animal experiments revealed that saffron treated PC3-bearing and 22rvl-bearing mice exhibited higher body weights, less tumor sizes and vessel counts, and delayed occurrences of tumor progression, with respect to the control group (Festuccia et al. 2014).

Breast cancer

The anti-angiogenesis response of saffron aqueous extract on the MCF7 breast cancer cell line proceeds by reduction of the expression levels of VEGFR2, VEGF-A and MMP. (Mousavi, Baharara, and Shahrokhabadi 2014; Mousavi and Baharara 2014; Mousavi, Baharara, and Asadi-Samani 2014). It should be noted that MMP is involved in regulating angiogenesis of tumors via VEGF release from ECM stores (Bergers et al. 2000). It has also been illustrated that saffron and low-frequency electromagnetic fields synergically decrease the expression of VEGFR2 and VEGF-A (Mousavi, Baharara, and Shahrokhabadi 2014; Mousavi and Baharara 2014).

In a limited clinical study Hosseini et al. (2015) evaluated the effect of saffron on response to therapy in patients suffering from liver metastasis (patients with various primary tumors such as breast, colon, ovarian and esophagus cancer). They showed that in the saffron treated group, stronger partial and complete response, and less mortality was observed compared to the placebo group.

The anti- metastatic effects of saffron is summarized in Table 1 and Figure 2.

Anti-metastatic potential of crocin

Breast cancer

Our group studied the anti-metastatic potency of crocin on cell and animal models of triple negative breast cancer (TNBC). It was found that nontoxic doses of crocin (2.5 mM and 3 mM - doses corresponding to 90% and 75% cell viability, respectively) diminished migration, invasion and cell-ECM adhesion of metastatic 4T1 cells dose dependently (Arzi, Riazi, et al. 2018). Our study on murine model of metastatic TNBC indicated that the crocin treated mice possessed more weight, higher survival rates and smaller tumors, than the control group. Histological examination detected very few metastatic colonies in the livers and lungs of the crocin treated mice (Arzi, Farahi, et al. 2018). Results of gene expression analysis showed that crocin therapy downregulated the expressions of Fzd7, MMP9, Nedd9, Vim and VEGF genes, and upregulated E-Cad in both cell and mice tissues. Therefore, it was inferred that crocin effectively inhibited metastasis via interfering with the Wnt/β-catenin pathway (Arzi, Riazi, et al. 2018; Arzi, Farahi, et al. 2018). Wnt/β-catenin signaling is immensely up-regulated and distinctly identified in metastatic TNBC and is associated with enhanced metastasis potential and poor clinical outcomes (King, Suto, and Li 2012; Dey et al. 2013).

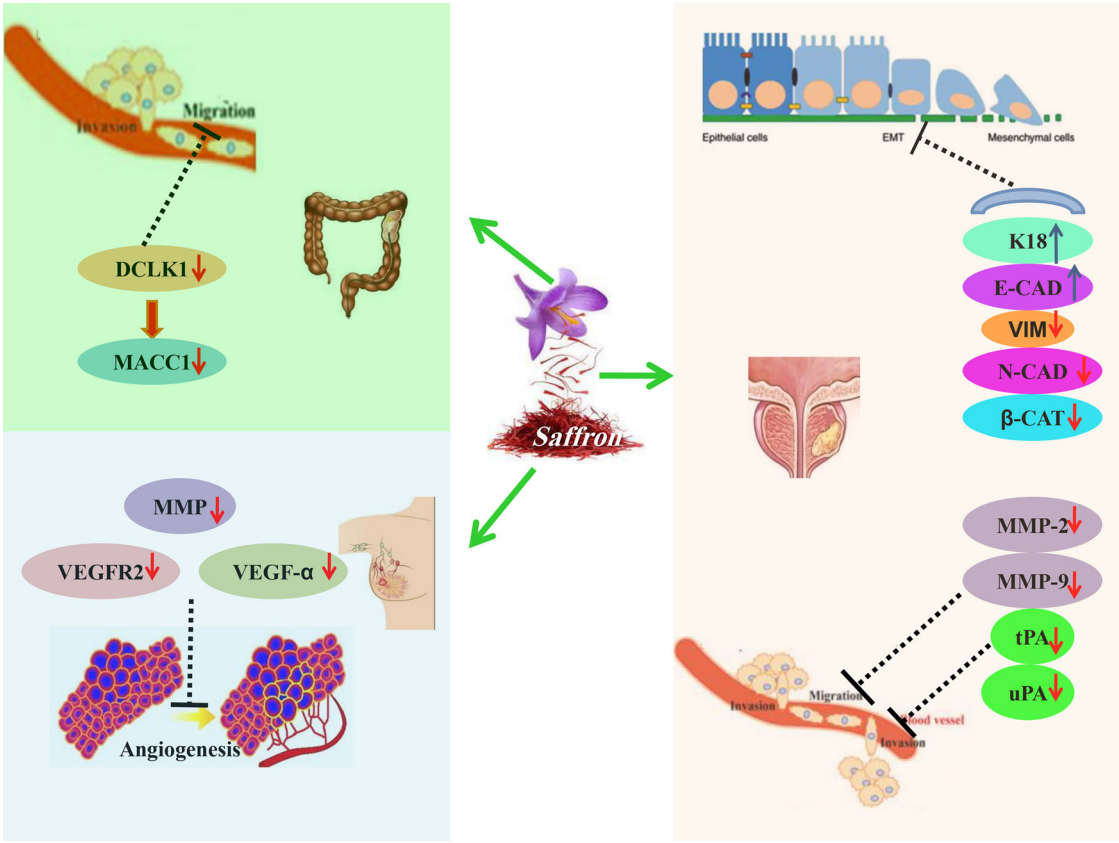

Figure 2. Schematic representation demonstrating the anti-metastatic effects of saffron against colorectal, prostate and breast cancers.

In another study on breast cancer, Chen et al. (2019) found that crocin, at a low dosage, suppressed the migration and tube formation ability of HUVEC cells. Tumor angiogenesis is critical in metastatic spread (Fox, Generali, and Harris 2007). An animal study involving the evaluation of gene expression in excised tumor tissues, induced by injecting MDA-MB-231, revealed that crocin reduced CD34 expression (Chen et al. 2019). Since $CD34^{+}$ cells are abundant in genes associated with angiogenesis (Siemerink et al. 2012), it was inferred that the anti-angiogenic potential of crocin is due to declining CD34 expression (Chen et al. 2019). High levels of CD34 expression may be a promising indicator of poor prognosis in breast cancer patients (Chen et al. 2015).

Crocin, at low concentrations, attaches to tubulin, at the same site as vinblastine, and inhibits the assembly of microtubules in breast cancer cells (MCF-7, HCC70 and HCC1806 cells) (Hire et al. 2017). The microtubule system plays a key role in metastatic spread (Fife, McCarroll, and Kavallaris 2014). Thus, it seems that crocin triggers metastasis by the same mechanism.

Gastric cancer

Crocin prevents the migration and invasion of gastric cancer cells, AGS and HGC-27. Also, crocin-treated gastric cancer cells inhibit EMT by increasing E-cadherin mRNA and protein expressions, and attenuating the expressions of Snail, and N-cadherin mRNA and protein. It was demonstrated that the expression of Krueppel-like factor 5 (KLF5) and Hypoxia-Inducible-Factor 1 A (HIF-1α) is elevated in human gastric cancer tissues - excised by surgery from gastric cancer patients, and in AGS and HGC-27 cells (compared to normal cells and tissue). Crocin was capable of reducing the elevated expressions dramatically (Zhou et al. 2019). KLF5 is a transcription factor that promotes various types of cancer, including gastric cancer. It has been positively associated with a higher cancer grade, lymph node metastasis, and poorer two-year survival rate – which proposes KLF5 as an oncogene in gastric cancer (Soon et al. 2011). HIF-1α is overexpressed in human gastric cancer, and its overexpression is correlated with the metastatic potential of gastric cancer cells (Rohwer et al. 2009). It was shown that upregulation of KLF5 reduced crocin's functions through promoting migration, invasion, and EMT, and increasing HIF-1α expression (Zhou et al. 2019). KLF5 is considered as a transactivator of HIF-1α in colon cancer cells (Lee et al. 2013). Bioinformatics analysis and dual-luciferase assays have suggested that KLF5 is a potential target of miR-320 (Zhou et al. 2019). It bears noting that miR-320 is a crucial tumor suppressor in gastric cancer. Its expression is suppressed in gastric cancer, and it inhibits the proliferation and invasion of gastric cancer (Wang et al. 2016; Zhou et al. 2017). It has been reported that crocin reduces KLF5 by increasing miR-320 expression, leading to the conclusion that crocin functions by modifying miR-320/KLF5/HIF-1α signaling (Zhou et al. 2019).

Prostate cancer

Crocin (0.4 mg/ml) impeded the migration and invasion of prostate cancer cells (PC3 and 22rvl) via reducing the

expression and activity of Matrix metalloproteinases and urokinase. Also, it was indicated that crocin induced loss of cell-cell adhesion by reverting EMT (via enhancing E-cadherin and K18 expressions and decreasing that of N-cadherin, β-catenin, and vimentin) (Festuccia et al. 2014). EMT is associated with activation of the Wnt signaling pathway (Gupta et al. 2010). Thus, it was suggested that crocin may interrupt Wnt/β-catenin. *In vivo* experiments displayed that crocin treated PC3 and 22rvl xenograft models possessed less tumor mass and vessel count, more body weight and delayed tumor progress with respect to the untreated xenograft model (Festuccia et al. 2014).

Melanoma

The survey of Bakshi et al. (2017) on melanoma bearing mice revealed that crocin therapy (2 mg/kg) improved remission parameters, as crocin treated mice had smaller tumors, higher survival times and extended delays in tumor growth and tumor silent period. In another study, Bakshi's group expanded their *in vitro* and *in vivo* investigations on melanoma metastasis. The results achieved from cellular experiments revealed that crocin (5 and 10 μg/mL) diminished cell-ECM adhesion (85.5% and 73.5%), invasion (57.2% and 29.2%), and migration (76.03% and 55.87%) of B16F-10 cells, dose dependently. Furthermore, crocin (10 μg/mL) elevated the expression of E-CAD in treated B16F-10 cells. The findings obtained from animal experiments indicated an appreciable less number of pulmonary metastatic lesions and extended survival times for crocin treated mice (250 and 500 μg/kg) in comparison with the control tumor bearing mice. No tangible variation in weights of the crocin treated mice was observed, compared to the control mice (crocin can be tolerated by mice without exhibiting toxicity). The crocin treated mice displayed activation of tissue inhibitor matrix metalloproteinase 1(TIMP1) and deactivation of MMP (2 and 9), VEGF, ERK2, and K-ras (Bakshiet al. 2018). It should be considered that VEGF, as an external stimulus, induces the expression of MMP's and can mediate migration (Wedge et al. 2002). Activation of the ERK (1/2) pathway, which functions downstream of Ras, is essential for the interaction of VEGF with its receptor (VEGFR), (Siveen and Kuttan 2011). Thus, it was concluded that crocin inhibits angiogenesis by impeding VEGF via downregulating Ras/ERK.

Crocin treatment was capable of suppressing elevated levels of TNF-α and IL-6 (Bakshi et al. 2018). TNF-α is a major cytokine mediator of cancer metastasis in mice models (Tracey et al. 1988). It was indicated that concentrations of TNF-α were remarkably higher in metastatic melanoma patients and the reduction in the number of metastasis colonies may be related to the blockage of TNF activity in melanoma cells (Ocvirk et al. 2000; Cubillos et al. 1997). Malignant melanoma cells secrete IL-6, and serum IL-6 levels are notably higher in advanced melanoma patients (Moretti et al. 2001). Moreover, crocin intensifies reduction of the levels of IL-2 and IL-10 in tumor burden mice (Bakshiet al. 2018). It has also been reported that forced expression of IL-10 in human melanoma cells restricts their tumorigenicity and metastatic capabilities (Huang, Ullrich, and Bar-Eli 1999). IL-2 has been applied as a medication for metastatic melanoma (Fewkes and Mackall 2010).

Colorectal cancer

The study of Güllü et al. (2020) on colorectal cancer cells indicated that among the three major compounds of saffron (crocetin, safranal and crocin), crocin triggered the reduction of migration of SW620, SW480/MACC1 cells (cells with high MACC1 expression). Also, it was illustrated that crocin therapy reduced DCLK1 protein levels in high MACC1-expressing cells. They concluded that the anti-migratory potential of saffron in MACC1-expressing cells is due to crocin via DCLK1down-regulation.

Crocin, in combination with 5-flurouracil (5-FU), inhibited migration and invasion in colon carcinoma cells (CT26), exhibiting improved anti-migration potency over crocin alone (Amerizadeh et al. 2018). 5-flurouracil therapy, individually or in combination with other chemotherapy cytotoxic agent, is considered as a routine standard therapy in colorectal cancer patients (D'Andre et al. 2005). Amerizadeh et al. claimed that crocin exert its anti invasion and anti migratory potential through enhancing E-cadherin expression and decreasing MMP2 and MMP9 expressions, respectively. It was reported that following crocin and combination therapy, colitis-associated colon cancer mice possessed fewer numbers of distant and middle tumors, smaller tumors and less tumor area compared to the control. Though, the combination functioned more impressively than crocin alone. Western blot findings reported that crocin inhibited PI3K expression and suppressed phosphorylation of Akt and GSK3α/β. This recent finding suggested that crocin is able to impair the Wnt/PI3K signaling pathway (Amerizadeh et al. 2018). Oncogenic PI3K/Akt signaling activates Wnt/β-catenin signaling via phosphorylation and inactivation of GSK3α/β (Vadlakonda, Pasupuleti, and Reddanna 2013).

Osteosarcoma

Crocin and the combination of crocin and cisplatin, a chemotherapeutic agent with cytotoxic effects on osteosarcoma cells, remarkably diminished the invasive behavior of osteosarcoma cells (MG63 and OS732 cells) compared with the control group. In addition, crocin in combination with cisplatin exhibited a synergistically more suppressive potential over crocin alone, It was supposed that the anti-invasion potential of the combination is related to augmentation of the expression of cleaved-caspase-3 and caspase-8 (Li et al. 2013).

The anti- metastatic effects of crocetin is summarized in Table 2 and Figure 3.

Anti-metastatic potential of crocetin

Breast cancer

It has been observed that crocetin can restrain the invasion of metastatic breast cancer cells (MDA-MB-231) dose

Table 2. Anti-metastatic effects of crocin against various cancers. antiangiogenic.

Type of cancers	Cell lines/animal models	Applied doses	Affected factors	Mechanism	Reference
Breast	4T1	2.5 & 3 mM	Invasion↓ Migration↓	FZD7↓ NEDD9↓	Arzi, Riazi, et al. 2018; Arzi, Farahi, et al. 2018; Chen et al. 2019
	4T1 xenograft female mice HUVEC	200mg/kg 1 to 6 mg/mL	Cell-ECM adhesion↓ Metastatic colonies↓	VEGF-A↓ MMP9↓ VIM↓	
	MDA-MB-231 xenograft female mice	5 mg/mL	Migration↓ Tube Formation↓ ⇒Angiogenesis⊥	E-CAD↑ ⇒Wnt/β-catenin⊥ CD34↓	
Gastric	AGS HGC-27 human gastric tumor tissues	2 & 3μM	Invasion↓ Migration↓	E-CAD↑ SNAIL↓ N-CAD↓ ⇒EMT⊥ miR-320↑ ⇓ KLF5↓ ⇓ HIF-1α↓	Zhou et al. 2019
Prostate	PC3 22rvl PC3 & 22rvl xenograft male mice	0.4 mM 200 mg/kg	Invasion↓ Migration↓ Cell-cell adhesion↑	MMP9↓ MMP2↓ uPA↓ tPA↓ N-CAD↓ β-CAT↓ VIM↓ K18↑ E-CAD↑ ⇒EMT⊥	Festuccia et al. 2014
Melanoma	B16F-10 B16F-10 xenograft male mice	5 & 10 μg/mL 250 and 500 μg/kg	Migration↓ Invasion↓ Cell-ECM adhesion↓ Pulmonary metastatic colony↓	E-Cad↑ TIMP1↑ MMP 2↓ MMP 9↓ K-Ras↓⇒ ERK2↓ ⇒VEGF↓ ⇓ Ras/ERK⊥ TNF-α↓ IL-6↓ IL-2↑ IL-10↑	Bakshi et al. 2018
Colorectal	SW620 SW480 CT-26 Colitis-associated colon cancer mice	5 mM 3 mM+5-FU* 200 ppm+ 35 mg/kg of 5-FU	Migration↓ Migration↓ invasion↓	DCLK1↓⇒ MACC1↓ E-CAD↑ MMP2↓ MMP9↓ PI3K↓⇒p-AKT↓ ⇒ p- GSK3α/β↓ ⇓ Wnt/ PI3K⊥	Güllü et al. 2020; Amerzadeh et al. 2018
Osteosarcoma	MG63 OS732	2 mmol/L+ 1 μg/m Cisplatin	Invasion↓	cleaved-caspase-3↑ caspase-8↑	Li et al. 2013

*Dose not mentioned in reference Amerizadeh et al. (2018).

dependently. Based on western blot assays, crocetin (1 and 10 μM) suppresses pro-MMP2 and MMP2 expressions. Gelatin zymography assays indicate that the same doses of crocetin notably reduce the activities of pro-MMP9, active-MMP9 and active-MMP2 in MDA-MB-231 cells (Chryssanthi, Dedes, et al. 2011). MMPs are a necessary component for invasion and metastasis of cancer cells (Deryugina and Quigley 2006). It was displayed that the mRNA and protein levels of MT1-MMP (MMP14) and more remarkably MT2-MMP (MMP15) were significantly reduced following crocetin treatment. MT1-MMP and MT2-MMP levels are augmented in cancer cells with enhanced

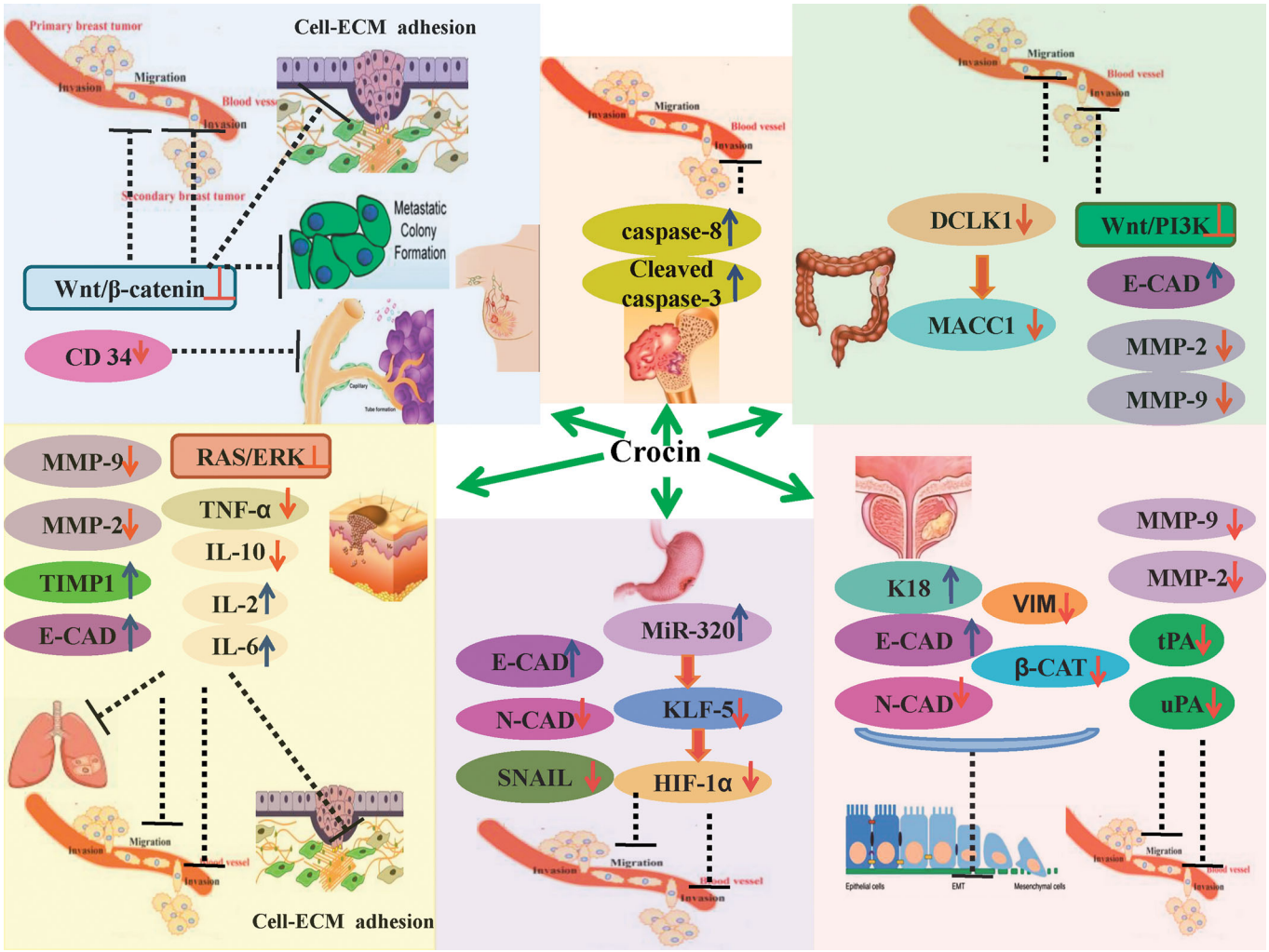

Figure 3. Schematic representation demonstrating the anti-metastatic effects of crocin against breast, gastric, prostate, colorectal cancers, and melanoma and osteosarcoma.

invasive and metastatic activities, such as MDA-MB-231 (Figueira et al. 2009; Feinberg et al. 2016). Chryssanthi's research team inferred that crocetin inhibits invasion of malignant breast cancer cells via interrupting the MMP expression and activity (Chryssanthi, Dedes, et al. 2011).

Our research on metastatic triple negative breast cancer cells (4T1) demonstrated that nontoxic doses of crocetin (0.1 and 0.14 mM respectively, corresponding to 90% and 75% cell viability) repressed migration, mobility, invasion and cell-ECM adhesion. Also, real time PCR results indicated that the expression levels of VEGF-α and Vimentin (VIM) decline following treatment with crocetin (Arzi, Riazi, et al. 2018).

Prostate cancer

It has been reported that crocetin slightly restricts migration and invasive of PC3 cells, though it functions less effectual in comparison to saffron extract and crocin. Immunohistochemical analyses showed that MMP9, MMP2, and uPA expression levels were reduced after crocetin treatment in PC3 and 22rvl tumor-bearing mice. Therefore, the moderate anti-migration and anti-invasion properties of crocetin could be associated with its effect on MMPs and uPA. It was shown that crocetin had negligible effects on the expression of EMT genes. Animal experiments revealed that in crocetin treated mice shrinkage of tumor, delay in tumor progression and reduction of vessel count was observed, even more efficiently than for saffron and crocin. Festuccia et al. (2014) declared that the anti-proliferative potential of crocetin was remarkably more evident compared to its antimetastatic effects.

Esophageal squamous carcinoma

Li et al. (2015) demonstrated that crocetin attenuated migration of esophageal squamous carcinoma cells, KYSE-150, dose dependently. Albeit, they did not mention the corresponding mechanism.

Crocetin strongly represses VEGF-induced tube formation of HUVEC cells and migration of HRMEC cells. Crocetin (3 μM) also significantly inhibits activation of P38 by inhibiting the expression of phosphorylated-p38 (Umigai et al. 2012). Activation and phosphorylation of p38 has been reported to be associated with VEGF induced migration (Rousseau et al. 1997). It has been found that levels of VE-cadherin and ZO-1 are reduced in endothelial cells, subsequent to VEGF induction and that

Table 3. Antimetastatic effects of crocetin against various cancers.

Type of cancers	Cell lines/animal models	Applied doses	Affected factors	Mechanism	Reference
Breast	MDA-MB_231	1 & 10 μM	Invasion↓	pro-MMP9↓ pro-MMP2↓ MMP9↓ MMP2↓ MT1-MMP↓	Chryssanthi, Dedes, et al. 2011; Arzi, Riazi, et al. 2018
			Invasion↓ Migration↓	MT2-MMP↓	
	4T1	0.1 & 0.14 mM	Cell-ECM adhesion↓	VIM↓ VEGF-α↓	
Prostate	PC3 22rvl	0.1 mM	Invasion↓ Migration↓	MMP9↓ MMP2↓ uPA↓	Festuccia et al. 2014
	PC3 - 22rvl xenograft male mice	100 mg/kg		tPA↓	
Esophageal squamous carcinoma	KYSE-150	100 & 200 μM/L	Migration↓	–	Li et al. 2015
–	HUVEC HRMEC	3 μM	Migration↓ Tube formation↓ ⇓ Angiogenesis⊥	p-P38↓ VE-cadherin↓	Umiga et al. 2012

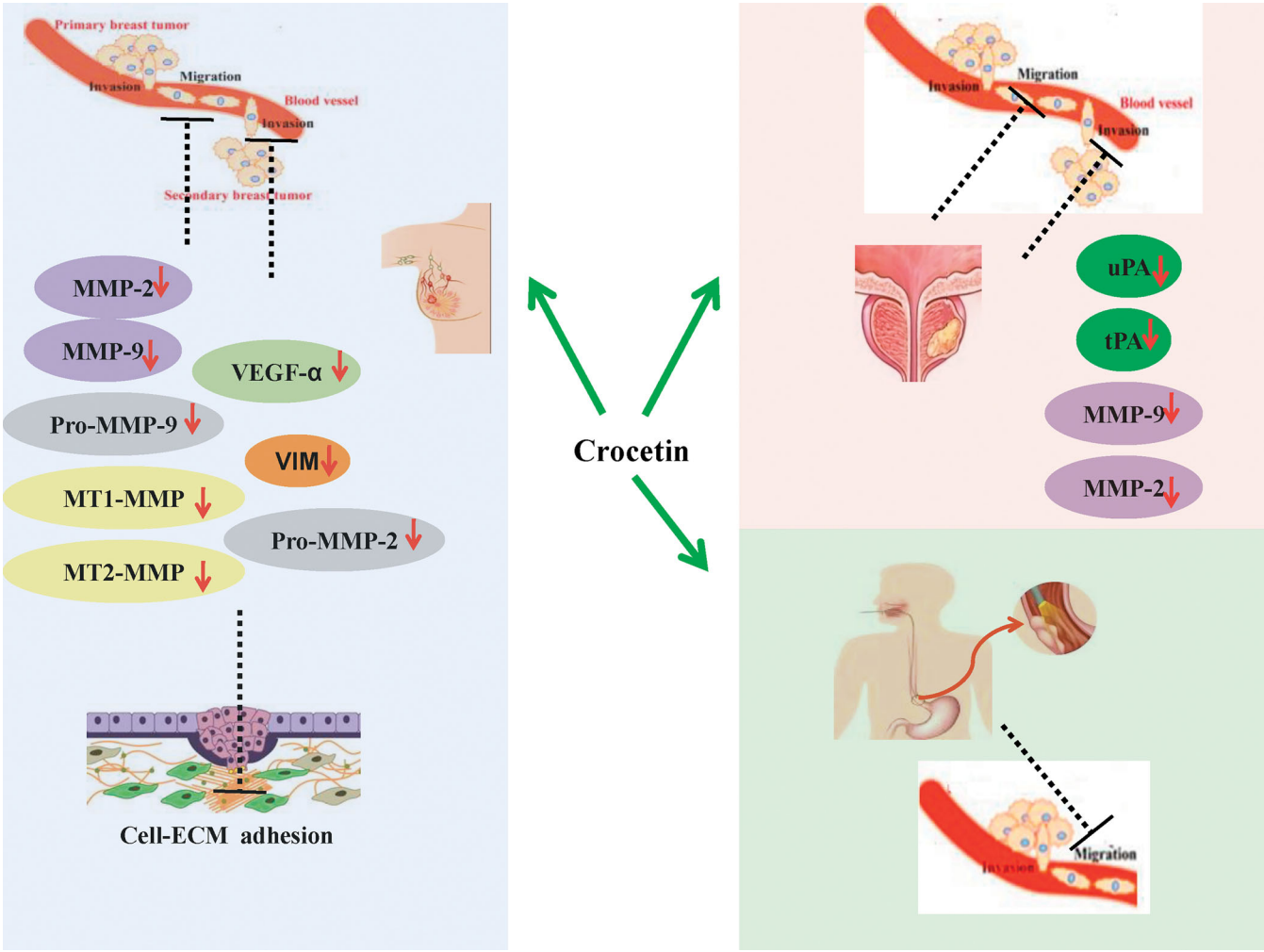

Figure 4. Schematic representation demonstrating the anti-metastatic effects of crocetin against breast, prostate cancers and esophageal squamous carcinoma.

crocetin treatment significantly prohibits the VEGF induced reduction of VE-cadherin (Umiga et al. 2012). VE-cadherin and ZO-1 are the main elements of adherens and tight junctions, and play a major role in the control of vascular permeability (Dejana, Tournier-Lasserve, and Weinstein 2009). Thus, Umigai et al. (2012) deduced that crocetin restricted VEGF-Induced angiogenesis via inhabitation of p38 phosphorylation.

The anti- metastatic effects of crocetin is summarized in Table 3 and Figure 4

Table 4. Antimetastatic effects crocin and crocetin against breast cancer.

Type of cancers	Cell lines/animal models	Applied doses	Affected factors	Mechanism	Reference
Breast	4T1	crocin 2.5 mM + crocetin 0.05 mM & crocetin 0.1 mM + crocin 2 mM	Invasion↓ Migration↓ Cell-ECM adhesion↓ Metastatic colonies↓	FZD7↓ NEDD 9↓ VEGF-A↓ MMP9↓ VIM↓ ⇒Wnt/β-catenin⊥	Arzi et al. 2020
	4T1 xenograft female mice	crocin 200 mg/kg + crocetin 5 mg/kg			

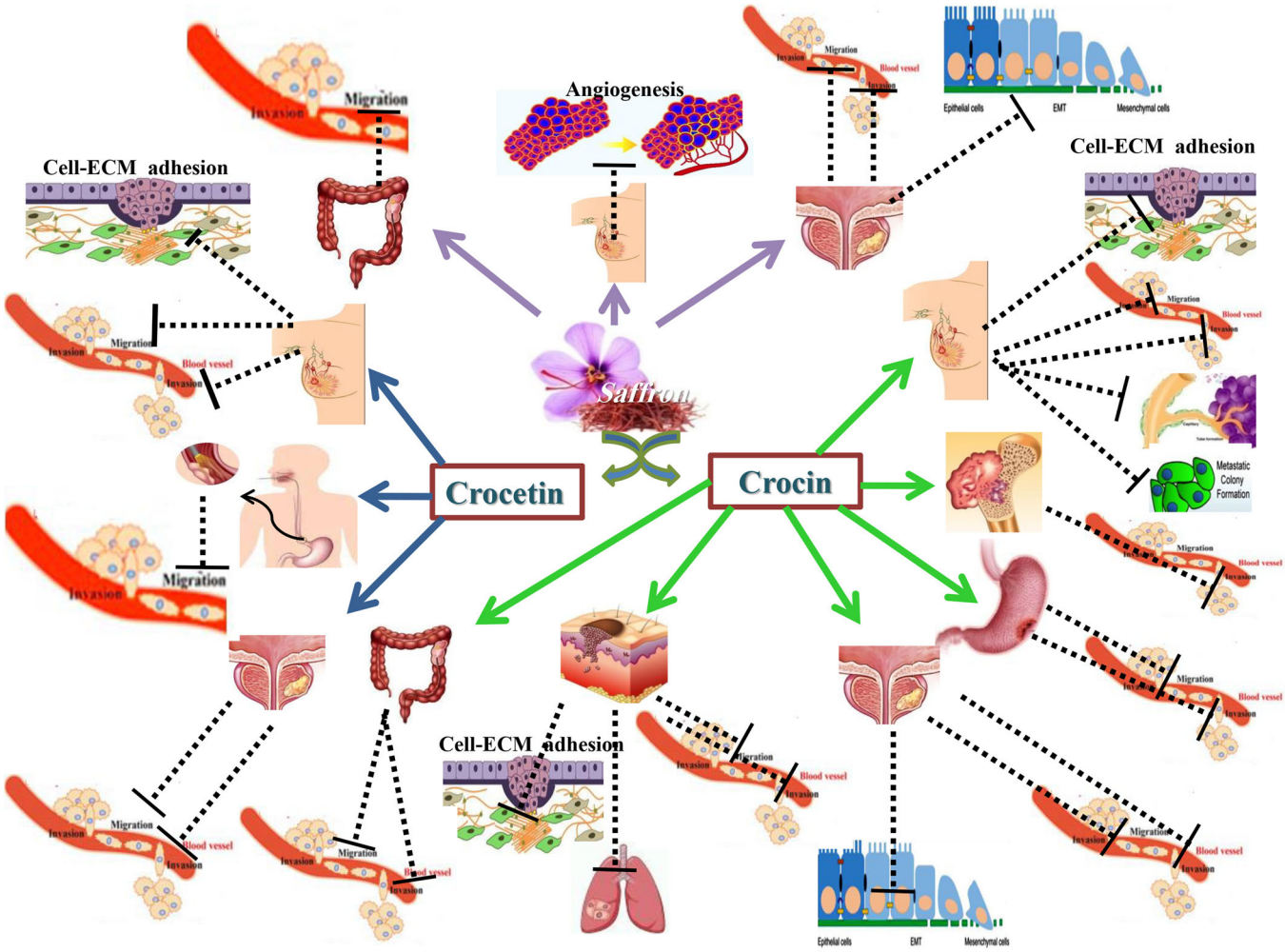

Figure 5. Schematic diagram summarizing the anti-metastatic effects of saffron, crocin and crocetin against various types of cancer.

Anti-metastatic potential of crocin and crocetin combination

Despite favorable pharmacological effects of co-administration of herbal medicines and chemotherapeutic agents, it is not without risk of adverse responses owning to pharmacodynamic and pharmacokinetic herb-chemotherapeutic interactions (Wang, Calway, and Yuan 2012). Thus, our investigation evaluated the antimetastatic effects of crocin and crocetin combination on cell and animal models of triple negative breast cancer. We prepared nontoxic combinations of crocin and crocetin according to cell viability assays. The combinations notably suppressed migration, cell mobility and invasion and cell-ECM adhesions of 4T1 cells, exhibiting a significantly stronger efficacy compared to crocin and crocetin individually.

The study on 4T1 tumor-bearing mice illustrated that the combination therapy group possessed more weight, higher survival rates and smaller tumors in comparison to the control mice. Histological analysis diagnosed significantly less metastatic colonies in the livers and lungs of the combination treated mice. Real time PCR and western blot results of cell and mice studies determined that the combinations attenuated the expression of FZD7, Nedd9, VEGF-α, Vim and MMP9. Therefore, it was concluded that the combination accomplished its anti-metastatic effects by impeding the Wnt/β-catenin pathway (Arzi et al. 2020) (See Table 4).

Conclusion

Despite extensive research on the anti-primary tumor properties of saffron and its main carotenoids, limited investigations have surveyed their antimetastatic characteristics, even though the lethal consequences of metastasis are clearly known. Thus, the present review, for the first time, accumulated and summed up evidence on the anti-metastatic potentials of saffron, crocin and crocetin. The studies on various types of cancer demonstrated the anti-migration, anti-invasion and anti-angiogenic effects of saffron, crocin and crocetin (see Figure 5). It was reported that they restricted cell-ECM attachment and elevated cell-cell adhesion. They exerted these effects via multiple mechanisms e.g., reduction of CD34 and suppression of Wnt/β-catenin, Ras/ERK, P38, DCLK1, EMT, matrix metalloproteinases and urokinase. It's worth noting that crocin illustrated remarkable anti-metastatic potency, while crocetin revealed notable anti-proliferative effects. Our investigations showed that a crocin-crocetin combination proved to have greater antimetastatic potency than crocin alone, i.e., crocetin enhances the antimetastatic properties of crocin. The anti-primary tumor and anti-metastatic potency of saffron carotenoids and their nontoxicity on normal cells and tissues has been confirmed in cell and animal model research. These data, along with adequate bioaccessibility/bioavailability, endow saffron and its carotenoids with significant medicinal value. Hence, it is recommended that they be considered as food ingredients with beneficial anti-metastatic properties and/or promising multipurpose anti cancer remedies.

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Disclosure statement

The authors have declared that there is no conflict of interest.

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