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The preventive and inhibitory effects of red raspberries on cancer

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Abstract. Red raspberries are gaining attention more and more for their nutritional and bioactive components, with potential health effects such as antitumor properties. This review aims to describe the antioxidant activities of red raspberries, emphasizing the role of anthocyanins and ellagitannins as primary contributors among red raspberry polyphenols; it also outlined the connection between red raspberries and their role in inhibiting cancer cell growth by regulating oxidative stress. Numerous studies suggest that red raspberries are able to block cancer cell progression by inhibiting proliferation, migration, and autophagy, as well as regulating the cell cycle, angiogenesis, and DNA damage repair. This review sheds light to the growing evidence supporting antioxidants as a crucial link between fruit consumption and cancer prevention.

Keywords: Red raspberry, anthocyanins, ellagitannins, oxidative stress, cancer

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1. Introduction

Cancer is a major threat to global human health and one of the leading causes of death [1]. The uncontrolled growth, proliferation and migration of cells lead to the formation of cancer [2, 3]. At present, the main tumor treatments are radiation, chemotherapy and combined therapy [4]. However, conventional chemotherapy and radiotherapy have significant toxic and side effects on healthy tissues, which affect the therapeutic effect to some extent [5]. Therefore, it is very important to develop some alternatives, such as natural products as sources of anti-tumor drugs.

The World Health Organization recommended consuming more than 400 g/day of fruits and vegetables to reduce the risk of some cancers and improve overall health [6]. The strong link between cancer prevention and a nutritious diet is often attributed to the chemopreventive and antioxidant properties exerted by bioactive compounds found in fruits and vegetables [7–9]. Fruits, particularly berries, serve as outstanding reservoirs of natural antioxidants and constitute a vital element in a wholesome diet [10].

Red raspberry is a fruit of the *Rubus genus* native to Europe [11]. Rich in nutrients and bioactive ingredients, red raspberries have the ability to block the pathogenic processes of a variety of human diseases, such as cardiovascular disease, diabetes, obesity and Alzheimer's disease, which are closely related to antioxidant and anti-inflammatory activity [12]. Red raspberries are rich in polyphenols, which are mainly composed of anthocyanins and ellagitannins [13]. Anthocyanins are natural pigments that have been shown to reduce the risk of the vast majority of cancers. Anthocyanins have strong antioxidant capacity by scavenging free radicals, reducing DNA damage, and preventing tumorigenesis [14]. To better understand the health-promoting potential of red raspberries to regulate cancer risk and their links to oxidation, we gathered recent articles from the Web of Science through separate or cross-searches using keywords: red raspberry, polyphenols, anthocyanins, ellagitannins, oxidation, cancer.

2. Nutritional and phytochemical compounds of red raspberry

The main chemical components of red raspberry include vitamins, proteins, organic acids, sugars, and polyphenols [6, 10, 15]. Red raspberries are rich in vitamins, with a vitamin C content of 26.2 mg per 100 g raw raspberries (one of the reasons for their antioxidant capabilities), and a vitamin K content of 7.8 μg. In addition, the protein content is 1.2 g/100 g, and the total sugar content is 4.42 g, mainly glucose (dextrose) (1.86 g) and fructose (2.23 g) [16]. Regarding organic acids, a study of 14 species of wild red raspberries showed that the organic acids were dominated by citric acid, with a content of 2.95 ± 0.19 to 13.85 ± 2.38 g/100 g dry weight, followed by malic acid, with a content of 0.31 ± 0.02 to 2.37 ± 0.26 g/100 g dry weight [15].

Red raspberries have been found to possess unique polyphenolic compound characteristics, especially anthocyanins and ellagitannins [10].

Anthocyanins are water-soluble pigments that give plants, fruits and vegetables their brilliant colors. Anthocyanins accounted for 78.13% of the red pigment of red raspberries [17]. The main types of anthocyanins in red raspberries include cyanidin-3-sophoroside, cyanidin-3-(2G-glucosylrutinoside), cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside, pelargonidin-3-sophoroside and pelargonidin-3-glucoside [17–19]. The anthocyanins content in red raspberries varies with different processing methods: approximately 92.1 ± 19.7 mg/100 g in fresh red raspberries [10], 13.88 ± 1.21 to 38.43 ± 2.75 mg/100 g in frozen red raspberries, 30.96 ± 5.34 to 65.29 ± 3.37 mg/100 g in red raspberry juice concentrates, and 10.89 ± 0.94 to 27.11 ± 1.15 mg/100 g in red raspberry puree samples [20].

Ellagitannins are important polyphenols in red raspberries, classified as hydrolysable tannins. They are esters of hexahydroxy biphenyls composed of a glucose or quinic acid core [21]. Ellagitannins are found in some berries especially genus *Rubus* such as raspberry, cloudberry, blackberry, arctic bramble as well as nuts and oak-aged wines [22–24]. The major ellagitannins in red raspberries are composed of sanguiin H-6 and lambertianin

C identified by ESI-MS [25]. Sanguiin H-10, monomeric casuarictin, tetrameric lambertianin D, potentillin, dimericnobotanin A, pedunculagin and other ellagitannins are also detected in red raspberries [25, 26]. Like anthocyanins, ellagitannins content are affected by variety, season, and storage method [27, 28]. Mazur, Nes [29] studied ten varieties of red raspberries and found average sanguiin H-6 and lambertianin C levels of 38 and 55 mg/100 g, respectively. Like anthocyanins, also ellagitannin contents change with storage and with different processing methods: for example, a decrease of 14 % of lambertianin C was detected in red raspberry jam after 3 months of storage in dark at 20°C compared to fresh jam, while sanguiin H6 remained stable [30].

3. Oxidative stress in cancer

Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) and the antioxidant system [31]. ROS include hydroxyl radicals ($HO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), and non-radical molecules such as hydrogen peroxide (H_2O_2) [32]. These molecules are primarily produced from oxygen in a variety of metabolic reactions that occur primarily in the mitochondria, endoplasmic reticulum (ER), and peroxisome. Mitochondria are thought to be the main source of ROS because about 2% of the oxygen consumed in mitochondria is converted to superoxide [33], while the folding of proteins in ER to form disulfide bonds requires an oxidizing environment and produces ROS [34]; peroxisomes produce ROS through β-oxidation of fatty acids and the reaction of flavin oxidase, and reduce ROS levels through catalase-mediated H_2O_2 [34]. ROS production includes both enzymatic and non-enzymatic reactions. The enzymes involved in enzymatic reactions include xanthine oxidase, NADPH oxidase, uncoupled endothelial nitric oxide synthase (eNOS), arachidonic acid and cytochrome P450 enzymes. The production of ROS by the mitochondrial respiratory chain is a non-enzymatic process [35].

The occurrence of cancer is affected by intrinsic factors, endogenous factors and exogenous factors. At present, it is generally believed that in the early stage of cancer development, the elevation of ROS has a tumor promoting effect [36]. Generally, ROS acts as signaling molecules to activate different pathways to promote cell proliferation and growth, and to activate anti-stress responses. ROS produced within cells can be counteracted by antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), or glutathione (GSH) systems, allowing oxidative lipids, proteins, and DNA to be repaired [37, 38]. However, when ROS levels exceed a cell's antioxidant regulatory capacity, extensive damage to the cell's components occurs, increasing the chance of mutations in cancer-related genes and tumor suppressor genes [39–41].

ROS activates nuclear factor-κB (NF-κB) pathway closely associated with apoptosis (by reducing levels of anti-apoptotic proteins B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-X_L)) and tumorigenesis (by regulating genes associated with cell proliferation, genes associated with angiogenesis, cancer-related pro-inflammatory factors, and antioxidant enzymes) [42–44]. H_2O_2 activates phosphoinositide 3-kinases (PI3K) pathway by inhibiting the phosphorylation of PTEN (phosphatase and tensin homolog) by oxidizing cysteine active site [45]. The activation of PI3K is closely related to cancer, and the inhibition of PI3K pathway by various inhibitors in clinical trials has been used in a variety of cancers, such as breast, pancreatic, ovarian, lung cancer, hematological malignancies and melanoma [45–47]. Mitogen-activated protein kinases (MAPK) activation phosphorylates a variety of protein kinases that control cell proliferation, differentiation, migration, and cell death [48,49]. Activation of the MAPK pathway is closely related to ROS production, and it has been shown that direct exposure to oxidative environments and elevated ROS activate MAKP and epidermal growth factor (EGF) [50]. ROS not only regulates p53, but also the downstream signal factor of p53 [51]. Hypoxia-inducible factor 1α (HIF-1α) has been extensively studied as a transcription factor in a variety of cancers, and it is regulated by oxygen and ROS [52–54], which is reported to be closely related to gene expression involved in angiogenesis, cell migration, and glucose transport periods [55, 56]. Nuclear factor erythroid 2-related factor 2 (NRF2) is also a transcription factor involved in regulating the expression of a variety of cell protection genes, including autophagy, apoptosis, proliferation, and DNA repair [57, 58]. NRF2 directly regulates ROS detoxification by regulating GSH, and also regulates ROS homeostasis by regulating free $Fe^{(ii)}$ [59].

Table 1
Antioxidant activities of red raspberry *in vitro* and *in vivo* studies

Cell line/animal model	Dose	Direct target/ interaction protein	Reference
HSC-T6 hepatic cells	250 μg/mL of red raspberries extract	Catalase↑; NRF2↑; HO-1↑	[65]
HaCaT cells	200 μg/mL of red raspberries extract	SOD↑; NRF2↑; HO-1↑; NF-κB↓; COX-2↓; DNA damage↓	[66]
Normal human dermal fibroblasts	100 μg/mL of *Rubus idaeus L.*	MAPK↓; NF-κB↓; TGF-β↓; NRF2↓	[67]
Caco-2 cells	10 μg/mL of red raspberries extract	NRF2↑; NO↑	[68]
3T3-L1 adipocytes	20 μg/mL of red raspberries extract	SOD↑; GPx↑; IL-6↓; TNF-α↓; IL-1β↓; MCP-1↓; leptin↓	[69]
3T3-L1 adipocytes	10 and 20 μM raspberry ketone	PPARγ↓; C/EBPα↓	[70]
SH-SYSY cells	50 μM of neolignans	Cell apoptosis↑; ROS↑	[71]
Wistar rats	50, 100, 200 mg/kg/day raspberry ketone for 28 days	SOD↑; GSH↑; GPx↑, SOD↑; TNF-α↓; MDA↓	[72]
C57BL/6J mice	diet supplemented with 2%, 4%, and 8% red raspberry	GSH↑; catalase↑; GPx↑	[73]
Male Swiss albino mice	Oral administration of 100 and 200 mg/kg raspberry ketone for 14 days	NF-κB↓	[74]
Wistar rats	100 and 200 mg/kg of raspberry ketone for 28 days	PPAR-α↑	[75]
Obese diabetic (db/db) mice	diet supplemented with 5.3% freeze-dried raspberry for 8 weeks	GPx↑; IL-6↓	[76]

Acronyms: nuclear factor erythroid 2-related factor 2 (NRF2); heme oxygenase-1 (HO-1); superoxide dismutase (SOD); nuclear factor-κB (NF-κB); cyclooxygenase 2 (COX-2); mitogen-activated protein kinase (MAPK); transforming growth factor β (TGF-β); glutathione peroxidase (GPx); interleukin 6 (IL-6); interleukin 1β (IL-1β); monocyte-chemoattractant protein 1 (MCP-1); proliferator-activated receptor-γ (PPARγ); CCAAT enhancer binding protein-α (C/EBPα); glutathione (GSH); malondialdehyde (MDA).

Oxidative stress is one of the most important factors which promote the occurrence of cancer. ROS could control cell proliferation, induce DNA damage, and regulate the tumor angiogenesis through modulate multiple signal transduction pathways include NF-κB, p53, MAPKs, PI3K, HIF-1α and NRF2.

4. Antioxidant activity of red raspberry

Raspberries are known to contain the highest antioxidant levels among fruits [18]. Anthocyanins and ellagitannins are the primary contributors to the antioxidant capacity of raspberries, accounting for 25% and 52% of the total, respectively [60]. Red raspberries have demonstrated the ability to influence oxidative stress in chemical assays, *in vitro* cell experiments, and *in vivo* animal models [61–64]. Here, we will elucidate the regulatory effects of red raspberries on oxidative stress across various models.

Further *in vitro* cell experiments showed that extract of red raspberry, especially polyphenols, had protective effects on oxidative stress in cells (Table 1). Red raspberries extract, containing key components such as cyanidin, ellagic acid, pelagonidin-3-sophoroside, methylquercetin-pentose conjugate, and cyanidin-3-rutinoside, attenuated HSC-T6 hepatic cells oxidative stress induced by dimethylnitrosamine. It also alleviated oxidative damage and protein carbonylation by upregulating NRF2 and heme oxygenase-1 (HO-1) levels [65]. Wang, Cheng [66] also reported that red raspberries extract up-regulated NRF2 and HO-1 to reduce UV-induced oxidative DNA

damage in HaCaT cells. Red raspberries, mainly composed of sanguiin H-6 and lambertianin C, prevented UV-induced skin photooxidation by inhibiting MAPK and NF-κB pathways and activating transforming growth factor β (TGF-β) and NRF2 genes in normal human dermal fibroblasts [67]. Red raspberries extract has a protective effect on the oxidative stress of Caco-2 cells infected with *E. coli* by enhancing the NRF2 pathway and NO production [68].

Lipid accumulation in cells leads to oxidative stress and dysregulation of adipokines. Red raspberries extract decreased ROS production by increasing SOD and glutathione peroxidase (GPx) levels in 3T3-L1 cells [69]. Consistent with this, raspberry ketone, a natural polyphenol of red raspberries, inhibited lipid accumulation in 3T3-L1 cells by inhibiting proliferator-activated receptor-γ (PPARγ) and CCAAT enhancer binding protein-α (C/EBPα) expression [70]. Similarly, neolignans from red raspberry attenuated cell apoptosis, ROS production and mitochondrial dysfunction induced by H_2O_2 in SH-SYSY cells [71].

In vivo animal studies have further deepened our understanding of the antioxidant activity of red raspberry (Table 1). In rats with nonalcoholic fatty liver disease (NAFLD), raspberry ketone increased SOD and GSH levels in the liver, decreased tumor necrosis factor-α (TNF-α), malondialdehyde (MDA) levels, and mitigated liver steatosis and inflammation [72]. Moreover, red raspberries were found to ameliorate alcoholic liver disease by attenuating alcohol-induced oxidative stress in the liver through increased levels of antioxidants such as GSH, catalase, and GPx [73]. In a lung toxicity model induced by cyclophosphamide in male Swiss albino mice, oral administration of 100 and 200 mg/kg raspberry ketone for 14 days improved lung health by inhibiting oxidative stress and the NF-κB pathway [74]. Raspberry ketone also improved cardiotoxicity, oral administration of raspberry ketone for 28 days mitigated isoproterenol-induced oxidative stress and dyslipidemia, and increased levels of proliferator-activated receptor-α (PPAR-α) in rats [75]. In an obese diabetic (db/db) mouse model, red raspberries extract reduced blood ROS levels and increased GPx activity [76].

5. Anticarcinogenic effect of red raspberry

Red raspberries have demonstrated anticancer activity *in vitro* and *in vivo* studies. They have been shown to have multiple anticancer effects including inhibiting proliferation, inducing apoptosis, inhibiting migration and angiogenesis, inhibiting tumor cell cycle progression, and repairing DNA damage (Table 2).

Concerning tumor viability and incidence, for example, red raspberries extract showed different inhibitory effects on breast cancer cells (90% death), stomach cancer cells (10% death) and colon cancer cells (10% death) [77]. Regarding *in vivo* models, in a 30-week experiment, adding 5% red raspberry to the diet reduced the incidence of esophageal cancer induced by N-nitrosomethylbenzylamine in male F344 rats [78].

The decrease or loss of apoptosis leads to wireless proliferation and metastasis of tumor cells. Apoptosis is influenced by many cytokines and mitochondria [79]. Current studies have shown that red raspberries significantly induced tumor cell apoptosis. In this context, Seeram, Adams [80] found that red raspberries extract promoted the apoptosis of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP) tumor cell lines and increased the level of cyclooxygenase 2 (COX-2) in HT-29 cells. Red raspberries extract inhibited also liver cancer cell proliferation and promoted cell apoptosis by reducing the Bcl-2/ Bcl-2-associated x (Bax) protein ratio and reducing mitochondrial membrane potential [81]. Bcl-2 protein can inhibit the opening of mitochondrial permeability transition pore, inhibit the activation of Caspase system, and prevent cell apoptosis. Ellagitannin in red raspberry also showed the effect of promoting tumor cell apoptosis. Apoptosis of Caco-2 cells was induced by red raspberry ellagitannin preparation (40–160 μg/mL), sanguiin H-6 (26.7–256) μM and lambertianin C (18.9–378 μM) [82]. After exposing A2780 human ovarian carcinoma cells to a 24-hour treatment with 40 μM sanguiin H-6, the p38 pathway was activated, facilitating apoptosis. This suggests that sanguiin H-6 may have therapeutic potential in inducing programmed cell death in ovarian carcinoma cells [83].

Cancer cells, lacking normal growth controls, demonstrate a loss of cell cycle regulation and possess unlimited reproductive potential and self-sufficiency in growth signals [84]. The cell cycle is regulated by cyclins, cyclin-

Table 2
Anti-cancer activities of red raspberry in *in vitro* and *in vivo* studies

Cell line/animal model	Dose	Effect(s)	Direct target/ interaction protein	Reference
HepG2 and Huh7 cell lines	50 mg/mL of red raspberries extract	Proliferation↓; apoptosis↑; arrested cell cycle progression at the S phase	Cyclin E1↓; cyclin A↓. CDK2↓; cyclin B1↑; methylation status of the PTEN↓; P-AKT(Ser473)↓	[85]
AGS stomach cancer cell lines; LoVo colon cancer cell lines; MCF-7 breast cancer cell lines	11 mg/mL of Meeker red raspberries extract	Proliferation↓		[77]
A549 lung cancer cell lines		Proliferation↓; migration↓; invasion↓	Cyclin D1↓; N-cadherin↓; vimentin↓; EGFR↓; STAT3↓; E-cadherin↑	[86]
Human oral (KB, CAL-27); breast (MCF-7); colon (HT-29, HCT116); prostate (LNCaP) tumor cell lines	200 μg/mL of red raspberries extract	Apoptosis↑	COX-2 ↑ in HT-29	[80]
Human microvascular endothelial cells (HMVECs)	Red raspberry phenolic compound extract (50 μg gallic acid equivalents /mL)	Endothelium viability↓; proliferation↓; migration ↓; capillary-like structures formation↓	P-VEGFR-2↓	[89]
HepG2 cells	30 and 60 mg/mL red raspberries extract	Proliferation↓; apoptosis↑	Bcl-2/Bax protein ratio↓ Caspase-3↑	[81]
Caco-2	Red raspberry ellagitannin preparation (40–160 μg/mL); sanguiin H-6 (26.7–256) μM; lambertianin C (18.9–378 μM)	Apoptosis↑	Double-strand breaks↑	[82]
A2780 human ovarian carcinoma cells	Sanguiin H-6 (40 μM)	Proliferation↓; apoptosis↑	p38↑; MAPK↑	[83]
Diethylnitrosamine induced liver injury model in rats	3.0 g/kg body weight of red raspberries extract for 20 weeks	Proliferation↓; apoptosis↑	VEGF↓; PCNA↓	[90]
Dextran sulfate sodium induced colitis in male C57BL/6J mice	Diet supplemented with 5% red raspberries for 10 weeks	Proliferation↓	COX-2↓; PCNA↓; P-STAT3↓; p53↑; Bcl2↓, Mcl1↓, cyclin D1↓	[91]
Female CD-1 mice	Diet supplemented with 5% red raspberries for 3 weeks	DNA repair↑	8-oxo-deoxyguanosine↓	[91]
N-nitrosomethylbenzylamine induced esophageal cancer in male F344 rats	Diet supplemented with 5% red raspberries for 30 weeks	Tumor incidence↓	IL-5↓	[78]

Acronyms: epidermal growth factor receptor (EGFR); signal transducer and activator of transcription 3 (STAT3); phosphorylated signal transducer and activator of transcription 3 (P-STAT3); phosphorylated activate protein kinase B (P-AKT); cyclooxygenase 2 (COX-2); vascular endothelial growth factor (VEGF); phosphorylated vascular endothelial growth factor receptor 2 (P-VEGFR-2); antiapoptotic B cell lymphoma 2 (Bcl2); Bcl-2-associated x (Bax); mitogen-activated protein kinase (MAPK); proliferating cell nuclear antigen (PCNA); myeloid cell leukemia 1 (Mcl1); interleukin 5 (IL-5).

dependent kinases (CDKs), and transcription factors. The G1 phase, where the cell readies itself for DNA synthesis, is governed by cyclin D and CDK4/6 in early G1, transitioning to the activation of the cyclin E/CDK2 complex in late G1. The cyclin E/CDK2 complex is key in transitioning from G1 to S phase, while the cyclin A/CDK2 complex controls DNA replication and propels cell cycle progression through the S phase. Loss of the cyclin A/CDK2 complex results in S phase arrest. Furthermore, the cyclin B-associated cdc2 regulates the G2/M phases [85]. Compounds targeting these processes would be beneficial in suppressing cancer progression. Red raspberries extract inhibited the proliferation of hepatocellular carcinoma cells (HepG2 and Huh7 cells) and arrested the cell cycle in the S phase by regulating the PTEN/ activate protein kinase B (AKT) pathway. PTEN is a tumor suppressor gene involved in cancer cell proliferation, migration, cell cycle progression, and differentiation. Red raspberries down-regulated levels of cell cycle-related proteins such as cyclin E1, cyclin A, cyclin B1 and CDK2 [85]. Ren, Wang [86] also reported that red raspberries extract reduced the proliferation, migration, invasion and epithelial-mesenchymal transformation of lung cancer cells A549 by inhibiting the epidermal growth factor receptor/signal transducer and activator of transcription 3 (EGFR/STAT3) pathway and reducing the expression of cyclin D1, N-cadherin and vimentin. In animal models, a 10-week diet supplemented with 5% red raspberries reduced the expression of STAT3 signaling in mouse models of colitis induced by dextran sulfate sodium, promoted the expression of tumor suppressor p53, inhibited the expression of p53 downstream signals Bcl2, myeloid cell leukemia 1 (Mcl1), and cyclin D1, and reduced the risk of colon cancer development in colitis subjects [87].

The sustained growth and metastasis of tumor cells depend on angiogenesis, the process that leads to the formation of new vessels and provides nutrients and oxygen for tumor cells [88]. Red raspberries extract has been shown to have a significant effect on angiogenesis. Red raspberry polyphenols (50 μg gallic acid equivalents /mL) inhibited cell proliferation, migration and formation of capillary-like structures in human microvascular endothelial cells (HMVECs). It also reduced the phosphorylated expression of vascular endothelial growth factor receptor 2 (VEGFR2), a major receptor for angiogenesis, in a dose-dependent manner [89]. An *in vivo* experiment has shown that after 20 weeks of administration of 3 g/kg red raspberries extract, cell proliferation and VEGF expression were inhibited and apoptosis was induced in liver lesion induced by diethylnitrosamine in rats [90].

Furthermore, DNA oxidative damage contributes to tumor development. Studies have shown that supplementing the diet with 5% red raspberries for 3 weeks increased endogenous damage in female CD-1 mice and significantly reduced levels of 8-oxo-deoxyguanosine (a marker of DNA oxidative damage) [91].

6. Conclusions

Oxidative stress is considered a cancer-promoting factor in the early stages of the disease. Studies have shown that red raspberries, particularly their polyphenols, including anthocyanins and ellagitannins, effectively reduced oxidative stress both *in vitro* and *in vivo*. Anti-cancer studies on red raspberries have demonstrated their capacity to inhibit cancer cell proliferation and migration, induce cell autophagy, regulate the cell cycle, prevent angiogenesis, and repair DNA damage through the modulation of cytokines and pathways that are closely associated with oxidative stress, thus suggesting that the anti-cancer effect of red raspberry is closely related to its antioxidant activity. However, it is worth noting that most of the current focus on *in vitro* cell line and cancer cell inhibition. Therefore, a systematic investigation into the *in vivo* antioxidant activity and anticancer effects of red raspberries is a key focus for future research.

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Conflict of interest

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