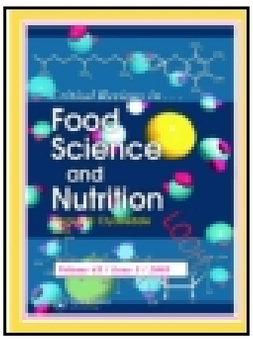

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REVIEW

In vitro evidence of the antitumor capacity of *Solanaceae* and *Cucurbitaceae* in colon cancer: A systematic review

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ABSTRACT

Colon cancer is the fourth leading cause of cancer deaths around the world. Despite advances in understanding its etiology and in diagnosis and treatment, new therapeutic strategies are still required. In this sense, the *Solanaceae* and *Cucurbitaceae* families have been widely used to treat various pathologies, including cancer, for their bioactive components. The objective of this systematic review was to analyze the antitumor activity of the bioactive components present in extracts from *Solanaceae* and *Cucurbitaceae* families using different in in vitro models of colon cancer. 241 publications have been identified (published from January 2008 to January 2020) from different electronic data base. 44 articles were included, 26 of which examined the Solanaceae family. The antitumor activity exhibited by this family was due to the withanolide-type steroid compounds they harbor. 18 articles were related to the Cucurbitaceae family. This family is characterized by their production of cucurbitacin-type triterpenoid compounds and their derivatives, which confer antitumor activity. In conclusion, the different genera belonging to both families are an important source of bioactive compounds with relevant activity against colon cancer. More experimental and in vivo studies will be required to corroborate their antitumor activity and to leverage them in future clinical practice.

KEYWORDS

Solanaceae; *Cucurbitaceae*; colon cancer; antitumor activity; bioactive compounds

Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer deaths worldwide, with an incidence of between one and two million new cases annually. In fact, its incidence increased by more than 200,000 new cases per year between 1990 and 2012, with its detection being more frequent in Western countries (Bray et al. 2018). There is evidence that the risk factors in CRC include environmental factors, age, genetic or family factors, and predisposing diseases. Among the environmental factors, diet plays an essential role and is the subject of continuous research. In fact, excessive alcohol consumption, excess weight and obesity, and certain types of foods such as processed meats have been linked to this pathology. Similarly, several studies have shown the protective properties that food, such as the foods in the Mediterranean diet, can have against CRC. The foods included in this diet (e.g., fish, olive oil, fruits, legumes, and vegetables, etc.) harbor bioactive compounds such as oleic acid, linoleic acid, tyrosol, hydroxytyrosol, catechin, quercetin, rutin, sulforaphane, and procyanidins, among others (Rotelli et al. 2015; Donovan et al. 2017).

The main treatments for CRC are surgery (if the tumor is resectable) and chemotherapy. The chemotherapies currently used are based on different cytotoxins, either as monotherapies or combination therapies (with oxaliplatin, irinotecan, 5-fluorouracil, capecitabine, TAS-102, or raltitrexed). Over the few last years, active biological drugs for advanced CRC have been developed, including the monoclonal antibodies cetuximab, panitumumab, bevacizumab, and a recombinant fusion protein (aflibercept) which has very precise indications (Loree and Kopetz 2017; Nappi et al. 2018; Bregni et al. 2020). However, the results are very limited, as clearly indicated by the average survival rates of these patients at only 15 to 20.5 months (Berrino et al. 2007). Treatments showing low tumor specificity, high toxicity in healthy tissues, and those which cause patients to develop a of multidrug resistance (MDR) phenotype, all reduce the effectiveness of the therapy. Therefore, improving patient prognosis requires the development of new strategies

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that add therapeutic activity to preventive action (Idrees and Tejani 2019; Reglero and Reglero 2019).

In this context, the activity of plant extracts or derivatives on the viability and survival of tumor cells has gained great interest in recent years (Goyal et al. 2017). Thus, more than 5,000 phytochemicals classified as phenolic compounds, carotenoids, vitamins, alkaloids, nitrogenous compounds, and organosulfur compounds have been identified in seeds, fruits, roots, tubers, and leaves (Thapliyal, Khar, and Chandra 2018). The vegetable species most widely included in diets worldwide are those belonging to the *Solanaceae* and *Cucurbitaceae* families. *Solanaceae* is one of the biggest plant families, with its genus *Solanum* being the richest in edible species (accounting for an excess of 1,500 species). Some noteworthy species of known economic and cultural relevance include potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*), eggplants (*Solanum melongena*), and peppers (*Capsicum* spp.). Since species of the genus *Solanum* contain various phytochemicals such as alkaloids, saponins, flavonoids, terpenes, lignans, sterols, phenolic compounds, and coumarins, this genus has been used since ancient times for medicinal purposes. The alkaloids present in members of this genus are of particular interest because they have extensive antimicrobial, antirheumatic, antioxidant, and anticancer biological activity (Jayakumar and Murugan 2015), in the latter case against several tumor types including breast, colon and prostate cancer (Kaunda and Zhang 2019).

The *Cucurbitaceae* family also includes numerous species used as food such as pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), and muskmelon (*Cucumis melo*) as well as other species with high levels of nutrients (proteins, minerals, fiber, etc.) and bioactive compounds (Rezig et al. 2019). In addition, specific species such as *Momordica charantia* have been studied because of their high content of bioactive compounds with anti-inflammatory, antidiabetic, and anticancer properties (Bortolotti, Mercatelli, and Polito 2019). Given the importance of developing alternative therapeutic strategies for CRC, this systematic review examined studies published in the academic literature which studied functional extracts or compounds isolated from species of the *Solanaceae* or *Cucurbitaceae* families that had selective antitumor activity, and which had also specified the molecular and cellular mechanisms involved in tumor cell death.

Methods

Study eligibility

The aim of this systematic review was to evaluate the most recent and representative information on the antitumor activity of functional extracts or bioactive compounds isolated from plant species belonging to the *Solanaceae* and *Cucurbitaceae* families, specifically in colon cancer. Following the PRISMA guidelines, this systematic review has been developed (Muka et al. 2020). For this purpose, the bibliometric analysis covered a 12-year period, considering older results obsolete. In this way, more than half of the current scientific texts published on this topic were included according to the Burton–Kebler index for obsolescence based on the median age/median production (Száva-Kováts 2002).

Inclusion criteria

The research papers included were in vitro studies published from January 2008 to January 2020 in which the effects of plant extracts or isolated compounds from either the *Solanaceae* or *Cucurbitaceae* families on colon cancer cell lines were tested, including Ic50 values and/or mechanisms of antitumor activity. All the research articles had been published in peer reviewed journals and those with fully accessible texts were selected. To reduce publication bias, other studies found in the bibliographic references of the selected articles were included in the analysis, if they met the inclusion criteria.

Exclusion criteria

Studies in which isolated extracts or compounds were not tested in colon cancer cell lines or in which the bioactive compound of interest had been synthesized or purchased rather than being derived from plant matter, were excluded. In addition, studies in which the bioactive compound extraction and/or purification methodology had not been specified were also excluded.

Data sources

The present systematic review was carried out using the electronic databases: MedLars Online International Literature, through PubMed, SCOPUS, Web of Science and Cochrane Library Plus. First, the medical subject headings (MeSH) were defined using "Solanaceae", "Cucurbitaceae", and "colonic neoplasms" as descriptive terms. The final equation was ((((“colonic neoplasms”[MeSH] OR (“colonic”[Title/Abstract] AND “neoplasms”[Title/Abstract]) OR “colonic neoplasms”[Title/Abstract] OR “colorectal cancer”[Title/Abstract] OR (“colorectal”[Title/Abstract] AND “cancer”[Title/Abstract]) OR (“colon”[Title/Abstract] AND “cancer”[Title/Abstract]) OR “colon cancer”[Title/Abstract]) AND ((“Solanaceae”[MeSH] OR “Solanaceae”[Title/Abstract]) OR (“Cucurbitaceae”[MeSH] OR “Cucurbitaceae”[Title/Abstract])) AND (“2008/01/01”[PDAT]: “2020/01/30”[PDAT])). The same strategy was used for all the databases with adaptations, as appropriate. Next, the list of studies was completed by searching the bibliographies of the selected publications, implementing the inclusion and exclusion criteria in each case.

Study selection

Two of the authors (M.F. and C.M.) carried out the literature search, reviewed the study abstracts, selected the appropriate ones for further full text examination. Bibliographic and meta-analysis reviews, epidemiological studies, communications to conferences, and book chapters were excluded

Table 1. Summary of the genera and species of the *Cucurbitaceae* family with antitumor activity, as well as the parts of the plants that had been used for the extraction or isolation of the bioactive compounds.

Genus	Species	N° articles	Parts of the plant
Momordica	*M. balsamina* L.	1	Aerial parts
	M. charantia L.	3	Fruits and seeds
Hemsleya	*H. pengxianensis* W.J Chang	1	Rhizomes
Cucumis	*C. melo* L.	1	Peel and seeds
	C. sativus L.	1	Leaves and fruits
Cucurbita	*C. moshata* D.	1	Pulp and seeds
	C. pepo L.	1	Seed
Luffa	*L. echinate* Roxb.	2	Fruit
Citrullus	*C. colocynthis* L.	1	Leaves
	C. latanus (Thunb.) Matsum. & Nakai	1	Fruit pulp
Trichosanthes	*T. cucumerina* L.	1	Root and fruit
	T. kirilowii Maxim.	2	Root and fruit
Gynostemma	*G. petnaphyllum (Thunb.) Makino*	2	Aerial plant

at this point. There were no language restrictions. In the second stage of the selection process, the authors examined all the full-text articles according to the specified inclusion and exclusion criteria. Since the objective of this study was to review existing data related to in vitro studies, articles that had only included in vivo data as well as human clinical trials were manually excluded.

Data extraction

Following the study selection process, the same two authors independently reviewed and extracted data from the selected studies. According to the Cohen Kappa statistical test (Cohen 1968) which exceeded 0.8, there was good agreement between the two researchers (Wanden-Berghe and Sanz-Valero 2012). Any discrepancies were resolved in consensus between M.F. and C.M. or between two other authors (R.M. and J.M.P.), if necessary. The quality of the selected studies was determined by using a specific two-part questionnaire for in vitro model studies. The first part includes filter questions to determine whether the studies meet the premises of an in vitro study (score $>$ 6) and the second part determines the quality of the study (0–6 = low; 7–14 = good; 15–20 = excellent) including the materials and methods, results, and conclusions. The extracted data are summarized in Tables 1 and 2 and have been classified according to their corresponding family. To facilitate the understanding of the selected studies, the publication reference, plant species studied, type of extraction, isolated compounds, cell line tested, IC_{50}, cytotoxicity assay, and mechanisms of action, and objective were included.

Results

As shown in Figure 1, 281 articles were obtained through the initial systematic search in the different electronic databases; after discarding non-original articles ($n = 15$), duplicates ($n = 49$), and articles that were not related to the subject of this review ($n = 142$), 75 articles remained. These were meticulously analyzed and a further 35 articles were discarded, 24 articles because they did not meet the inclusion criteria and another 11 failed the quality test. After this comprehensive screening, 40 articles were obtained, to which another 4 articles were added after screening their respective bibliographies. Thus, a total of 44 articles were included in this systematic review. Upon examining these 44 articles it was found that over the last 12 years, more articles had focused on the anti-tumor activity of extracts or bioactive compounds from species of the *Solanaceae* family (26 articles) than of the *Cucurbitaceae* family (18 articles). In fact, in 2011 and 2014, no articles regarding the *Cucurbitaceae* family were published, and in 2019, only one was published (Figure 2).

In this systematic review, articles that had studied and reported on a total of 13 species in the *Cucurbitaceae* family and 15 species in the *Solanaceae* family, were included. A total of 8 genera in the *Cucurbitaceae* family and 6 genera in the *Solanaceae* family had been studied. Specifically, the *Solanum* and Withania genera, both belonging to the *Solanaceae* family, had reported the most antitumor activity in colon cancer, as shown in Figure 3. The plant material most often used to obtain the functional extracts was the fruit, followed by the leaves, aerial parts, and the whole plant; seeds and roots had been less studied as sources of antitumor agents against colon cancer (Figure 4). The preferred extraction solvents had been methanol and ethanol although the methodologies used were very variable (Figure 5). In addition, the mechanisms of action by which extracts and their bioactive compounds inhibit cell proliferation were also analyzed (Figure 6).

Species of the Cucurbitaceae family with antitumor activity in Colon cancer

Of the forty four articles included in this systematic review, eighteen articles studied the in vitro colon cancer antitumor capacity of extracts from different species in the *Cucurbitaceae* family. These articles had investigated 8 genera (*Momordica, Hemsleya, Cucumis, Cucurbita, Luffa, Citrullus, Trichosanthes*, and *Gynostemma),* among which *Momordica* and *Trichosanthes* were the most frequently studied (with four and three articles, respectively). A total of 13 species had been studied, of which *Momordica charantia* had been analyzed in three articles, and *Luffa echinata*, *Trichosanthes kirilowii*, and *Gymnostemma petnaphyllum* had been analyzed in two articles each. One article each had been published in reference to the species *Momordica balsamina, Hemsleya pengxianensis, Cucumis melo, Cucumis sativus, Cucurbita moshata, Cucurbita pepo, Citrullus colocynthis, Citrullus latanus*, and *Trichosanthes cucumerina*.

Most studies had isolated bioactive compounds from plant extracts (Table 1) and these had most often been tested on the HT-29 colon cancer cell line. MTT assays had been the principal method used to determine cell antiproliferative activity, and the isolated compounds or functional extracts tested had usually been dissolved in DMSO and exhibited antitumor activity against these colon cancer cells.

Table 2. Antiproliferative activity of the functional extract or isolated compounds from Cucurbitaceae family in colon cancer lines.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Cytotoxicity assay	IC_{50} Extract solution/compound	Mechanism of action
Aerial parts of *Momordica balsamina* L. (Ramalhete et al. 2018)	Methanol	Balsaminol A Balsaminol D Balsaminol F Balsaminagenin A Balsaminagenin B Balsaminosides A-C Cucurbalsaminol A Karavilagenin C Karavoates A-R	HT-29; HT-29-RBDB; HT-29RNOV Alone dissolved in DMSO SRB	Karavoate A HT-29: 7.9 μM HT-29-RBDB: 3.1 μM HT-29RNOV: 2.3 μM Karavoate C HT-29: 13.8 μM HT-29-RBDB: 7.1 μM HT-29RNOV: 4.9 μM Karavoate E HT-29: 15.4 μM HT-29-RBDB: 6.9 μM HT-29RNOV: 4 μM	The compounds show inhibitory effect against HT-29 and its resistant variants.
Seeds of Bitter gourd *Momordica charantia* L. (Dia and Krishnan 2016)	Ethanol 70%	BG-4 (peptide)	HCT-116; HT-29 BG-4 alone MTS	HCT-116: 251.3 μg/ml (24 h) 134.4 μg/mL (48 h) HT-29: 249.2 μg/ml (24 h) 217.0 μg /mL (48 h)	BG-4 induced apoptosis reducing the expression of Bcl-2 and increasing the expression of Bax. Such changes resulted in the increased expression of caspase-3 and affected the expression of cell cycle proteins p21 and CDK2.
Bitter melon (*Momordica charantia* L.) whole fruit (Kwatra et al. 2013a)	Methanol	–	HT-29 Alone and in combination with DOX (co-treatment and pretreatment) Hexosaminidase assay	Co-treatment 1 h (μM): DOX alone: 98 DOX + 50 μg/ml BME: 85 DOX + 100 μg/ml BME: 11 DOX + 150 μg/ml BME: 3 Pretreatment (24 h with BME + 4 h with DOX (μM): DOX alone: 8 DOX +25 μg/ml BME: 1.5 Pretreatment with 1uM DOX 12 h and BME: BME alone: 45 μg/ml BME + DOX: > 100 μg/ml	DOX interacted synergistically with BME. BME reduced the expression of multidrug resistance-conferring proteins P-glycoprotein, MRP-2 and BCRP. BME is a potent inhibitor of MDR function.
Bitter Melon (*Momordica charantia* L.) (Kwatra et al. 2013b)	Methanol	BMW BMSk	HT-29, SW480 DMSO Hexosaminidase assay	BMW: HT-29: 57 μg/ml SW480: 85 μg/ml BMSk: HT-29: 105 μg/ml SW480: 108 μg/ml	BME inhibited the proliferation and colony formation of colon cancer cells. BMW showed higher antitumor activity than BMSk. BME caused S and G2/M cell cycle arrest. BME did not induce apoptosis. BME induced autophagy in colon cancer cells. BME affected energy homeostasis of cancer cells acting on cellular ATP though AMPK mediated pathway. BME holds anticancer stem cell activity.
Rhizomes of *Hemsleya pengxianensis* W.J Chang var.	Ethanol 95% by reflux	Hemslepenside J-P 16,25-O-diacetyl-cucurbitane F	HT-29 Alone dissolved in DMSO MTT	Hemslepenside K: 17.71 ± 0.95 μM 16,25-O-diacetyl-cucurbitane F:	The compounds inhibited cell proliferation. The compounds

(*continued*)

Table 2. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Cytotoxicity assay	IC_{50} Extract solution/compound	Mechanism of action
jinfushanensis (Wang et al. 2018)		25-O-acetyl-23,24-dihydrocucurbitacin F		0.69 ± 0.06 μM 25-O-acetyl-23,24-dihydrocucurbitacin F: 0.37 ± 0.025 μM	16,25-O-diacetyl-cucurbitane F and 25-O-acetyl-23,24-dihydrocucurbitacin F induced F-actin aggregation, G2/M phase cell cycle arrest and cell apoptosis.
Peel and seeds of melon (*Cucumis melo L.* reticulatus group) (Rolim et al. 2018)	Water, hydro-methanolic solution (30:70 v/v) and hydro-ethanolic solution (30:70 v/v)	–	HT-29 Peel aqueous Peel Hydroethanolic Peel Hydromethanolic Seed Aqueous Seed Hydroethanolic Seed Hydromethanolic MTT	–	The hydromethanolic and ethanolic extracts showed higher inhibition than aqueous extracts.
Leaves and fruit of *Cucumis sativus* L. (Wu et al. 2019)	Ethanol 95%	CuC	HCT-116 Alone dissolved in DMSO MTT		CuC has antitumour activity. CuC induced apoptosis and cell migration inhibition via suppression of Akt phosphorylation, followed by modulation of p21/cyclin signals.
Seed of pumpkin (*Cucurbita pepo L.* ssp. pepo var. styriaca) (Medjakovic et al. 2016)	Hydroethanolic extract (60%)	Cucurbitacin	Caco-2 Alone dissolved in distilled water or DMSO MTT	–	Curcubitacin did not transactivate the androgen receptor. Cucurbitacin showed no estrogenic activity in yeast assay. Cucurbitacin showed no activity in the yeast assay with progesterone receptor. Crude pumpkin seed extract inhibited the cell growth in colon cancer.
Pumkin pulp and seeds of *Cucurbita moshata* D., variety "long of Naplkes" (Russo et al. 2017)	Supercritical C02 extraction	Carotenoids	Caco-2 CEN CyQuant assay	–	CEN delayed cell proliferation by 50% at 96 h. Increased number of autophagic vacuoles and expression of LC3-II.
Fruit of *Luffa echinata Roxb* (Shang et al. 2016)	Methanolic		SW-480 Alone MTT	LER: 70.2 μg/ml at 24 h	LER fruit extract inhibited the proliferation. Produced the release of lactate dehydrogenase in a dose dependent manner. LER induced apoptosis, DNA fragmentation, and cellular ROS accumulation, promoted the expression of caspases 3, 8, 9, Bax, Increased Bad, and p53 proteins expression, and decreased the levels of Bcl-2 and Bcl-XL.

(*continued*)

Table 2. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Cytotoxicity assay	IC_{50} Extract solution/compound	Mechanism of action
Fruits of *Luffa echinata Roxb* (Shang et al. 2012)	Methanol	–	HT-29 Alone MTT	LER extract: 80.6 μg/ml	Methanolic extract of LER exerts its antiproliferative effects by inducing apoptotic cell death, and causing G2/M arrest in HT-29 cells. LER also promotes ROS generation and MMP loss in mitochondria and regulates the Bax and Bcl-2 genes.
Leaves of *Citrullus colocynthis* (L.) (Chawech et al. 2015)	Ethyl acetate extract	Acetyl Glucocucurbitacin E Coumaroyl-acetyl Glucocucurbitacin I Cucurbitacin E Glucocucurbitacin E Cucurbitacin I Glucocucurbitacin I	HT-29; Caco-2 Dissolved in EtOH 10% MTS	Caco-2: Acetyl Glucocucurbitacin E: 24 µg/ml Coumaroyl-acetyl Glucocucurbitacin I: 15 µg/ml Glucocucurbitacin I: 34 µg/ml	The compounds presented cytotoxic activity against Caco-2 cell line
Dried fruit pulp *Citrillus latanus* (Thunb.) var. *citriode* (Abdelwahab et al. 2012)	–	CLG	HT-29 Alone MTT	CLG: 79.76 ± 2.34 μg/ml	The apoptogenic property of CLG on HT-29 cells is related to the inhibition of reactive nitrogen and oxygen species, eventually leading to caspase-3-mediated apoptosis.
Fruit from *Trichosanthes kirilowii* Maxim. (Song, Chang, and Li 2016)	Precipitation with acetone (v/v = 1:2)	Protein TKP	DLD1; HCT-116; SW620; NCM460 TKP protein dissolved in PBS MTT	DLD1 29.00 ± 1.76 µg/ml HC-T116 37.46 ± 3.26 µg/ml SW620 68.40 ± 3.89 µg/ml NCM460 272.91 ± 8.69 µg/ml	TKP protein decreased cell viability and induced cell apoptosis via the PI3K/AKT-mediated mitochondrial pathway. There was a loss of mitochondrial membrane potential, upregulation of cytochrome c and Bax, downregulation of Bcl-2, and activation of caspase-9 and 3.
Root of Trichosanthes kirilowii Maxim. (Minh et al. 2015)	methanolic extraction	Trichobenzolignan Ligballinol (-)-pinoresin Ehletianol C Luteolin 7-O-β-D-glucopyranoside Chrysoeriol-7- O-β-D-glucopyranoside 10α-cucurbita-5,24-dien-3β-ol Arvenin I	HT-29 Alone MTT	Trichobenzolignan: 16,2 µM Ligballinol: 45,5 µM (-)-pinoresin: 60,9 µM Ehletianol C: >100µM Luteolin 7-O-β-D-glucopyranoside: 16 µM Chrysoeriol-7- O-β-D-glucopyranoside: 40,7 µM 10α-cucurbita-5,24-dien-3β-ol: 4,1µM Arvenin I: 49,4 µM	The compounds showed cytotoxic activity against HT-29 cell line
Root and fruit juice of *Trichosanthes cucumerina* L. (Kongtun et al. 2009)	Root: Petroleum ether, dichloromethane and ethanol. Fruit: diethyl ether	Root: bryonolic acid (1), bryononic acid (2), and dihydrocucurbitacin B (4). Fresh fruit: Cucurbitacin B (3)	Caco-2 Root extract, bryonolic acid, spray-dried fruit juice and cucurbitacin B. Dissolved in DMSO MTT	Root extract: 135.74 ± 0.16 μg/ml Bryonolic acid: >500 μg/ml Spray-dried fruit juice: 100.74 ± 0.24 μg/ml Cucurbitacin B: 1.49 ± 0.21 μg/ml	Bryonolic acid is the main component in *T. cucumerina* root. *T. cucumerina* fruit juice contained predominantly cucurbitacin B. Cucurbitacin B inhibited colon cancer cells, much

(*continued*)

Table 2. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Cytotoxicity assay	IC_{50} Extract solution/compound	Mechanism of action
					more strongly than bryonolic acid.
Gynostemma pentaphyllum (Thunb.) Makino 5 commercial samples coded GP1, GP2, GP3, GP4 and GP5 (Xie et al. 2010)	Ethanol 75% Ethanol 100%. Acetone 50%	–	HT-29 Alone dissolved in DMSO ATP-Lite 1 step kit	–	The 50% acetone extract had the highest content of total phenolics and flavonoids. The 100% ethanol extract had the highest total saponin content and DPPH capacity. The 50% acetone extract showed the greatest HOSC value. The 100% ethanol showed a strongest inhibitory effect, but the 5 samples differ in their antiproliferative properties. The samples significantly inhibited the LPS-stimulated TNF-R1 mRNA expression.
Herb tea of *Gynostemma pentaphyllum* (Thunb.) Makino (Yang et al. 2008)	Ethanol 95%	PSGP	SW-116; HT-29 Alone MTT	–	PSGP exerted antitumor activity by enhancing the immune function of macrophages. PSGP-Induced ROS production in Macrophages.

Half maximal inhibitory concentration (IC_{50}); dimethyl sulfoxide (DMSO); sulforhodamine B (SRB); 3-(4,5-dimethytlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); Hydroxyl Radical Scavenging Capacity (HOSC); Bitter Melon Extracts (BME); peel extracts of bitter melon (BMSk); Cucurbitacin C (CuC); Nanoemulsion oil-in water with a final carotenoid concentration of 200-400 ug/mL (CEN); Luffa echinata Roxb (LER); Cucurbitacin L 2-O-β-glucoside (CLG); Polysaccharide (PSGP); resistant to daunorubicin (RDB); resistant to mitoxantrone (RNOV); BCL2 antagonist/killer 1 (BAK1); cyclin-dependent kinase 2 (CDK2); doxorubicin (DOX); multi-drug resistance (MDR); multidrug resistance gene 1 (MDR1); P-glycoprotein (P-gp); multidrug resistance protein 2 (MRP-2); breast cancer resistance protein (BCRP); AMP-activated protein kinase (AMPK); mitochondrial membrane potential (MMP); B-cell lymphoma-extra large (Bcl-xL); lipopolysaccharide (LPS); phosphatidylinositol 3-kinase pathway (PI3K/Akt); tumor necrosis factor receptor 1 (TNF-R1); 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH); polysaccharide (PSGP); reactive oxygen species (ROS).

Momordica genus

As shown in Table 2, of the four articles studying antitumor activity in colon cancer that had focused on the *Momordica* genus, one of them had analyzed the aerial parts of *M. balsamina* (Ramalhete et al. 2018) and three had studied the seeds (Dia and Krishnan 2016), fruit (Kwatra, Subramaniam, et al. 2013a, Kwatra, Venugopal, et al. 2013b), and peel (Kwatra, Venugopal, et al. 2013b). Methanol had been used as the extraction process solvent in three of these four articles, while ethanol had been used in the other one (Dia and Krishnan 2016). Nine compounds had been isolated from the aerial parts of *M. balsamina*, of which only three (Karavoate A, Karavoate C, and Karavoate E) had anti-tumor activity in the HT-29 cell line, with IC_{50} values and resistant variants between 2 µM and 16 µM (Ramalhete et al. 2018). For *M. charantia*, the seed extract had shown the least antitumor activity, followed by the peel extract. Methanolic extracts from the fruit had low IC_{50} values between 40–60 µg/ml. In addition, this extract had caused S and G2/M-phase cell cycle arrest, interacted synergistically with the chemotherapeutic agent doxorubicin and produced autophagy in colon cancer cells as the main molecular mechanisms of action.

Hemsleya genus

Only one article had analyzed this genus in the rhizome of *H. pengxianensis* as the source of antitumor agents for colon cancer (W. Wang et al. 2018) (Table 2); three bioactive compounds had been isolated using 95% ethanol as the extraction solvent. Of these, 16,25-O-diacetyl-cucurbitane F and 25-O-acetyl-23,24-dihydrocucurbitacin F had the lowest IC_{50} values, ranging from 0.3–0.7 µM, while hemslepenside K presented the highest IC_{50}, with a value of 17.71 µM. The main molecular mechanisms of antitumor action exhibited by these aforementioned compounds were F-actin aggregation, G2/M-phase cell cycle arrest, and cell apoptosis.

Cucumis *genus*. Only one article carried out extraction process of the seeds and peel of *C. melo*, in this case using water, methanol, and a methanol-water mixture as solvents (Rolim et al. 2018). An ethanolic (95%) extract was prepared from *C. sativus L*, in which the bioactive compound cucurbitacin C (CuC) had been isolated (Wu et al. 2019). CuC, as well as the above hydro-methanolic and methanolic extracts, all exhibited antitumor activity in colon cancer cells, although none specified the IC_{50}.

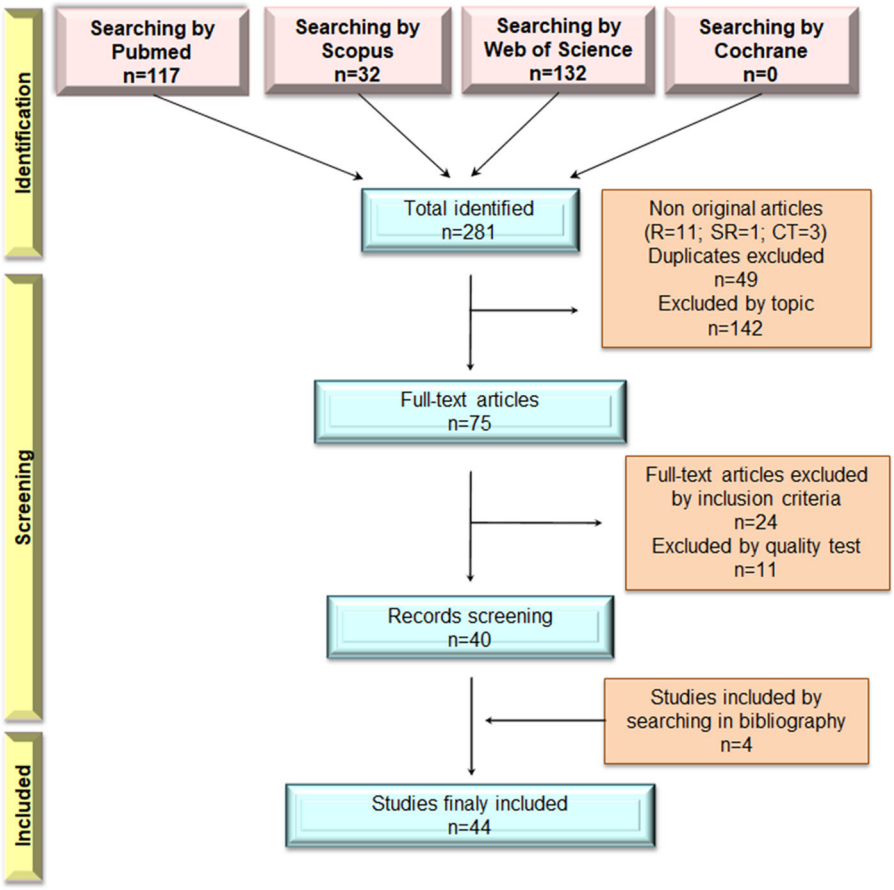

Figure 1. Flow diagram of the eligible studies included in this systematic review.

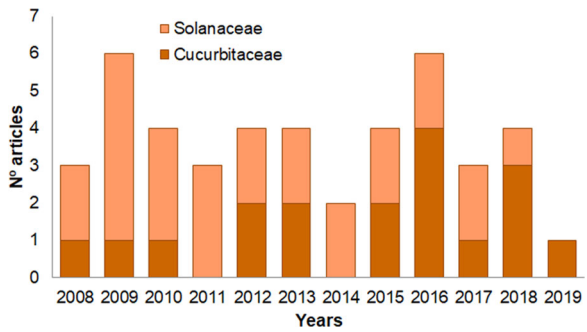

Figure 2. Representation of the number of articles published in the academic literature per year about the *Solanaceae* and *Cucurbitaceae* families.

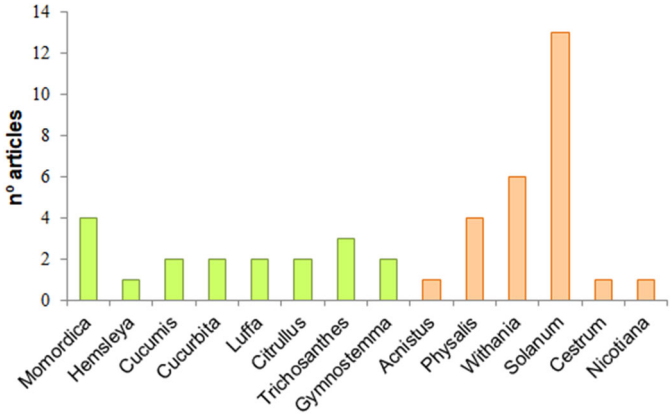

Figure 3. Representation of the number of articles from genera of the *Cucurbitaceae* family (*Momordica, Hemsleya, Cucumis, Cucurbita, Luffa, Citrullus, Trichosantes,* and *Gymnostemma*) and *Solanaceae* family (Acnistus, Physalis, Withania, Solanum, Cestrum and Nicotiana) that had reported antitumoral activity in colon cancer.

Cucurbita genus

Two articles had studied this genus, one of them had described the isolation of carotenoids from *C. pepo* seed extracts using a 60% hydroethanolic solution (Medjakovic et al. 2016), while the other had assayed extracts from *C. moshata* seeds and pulp using supercritical CO_2 extraction, finally isolating the bioactive compound cucurbitacin (Russo et al. 2017). Both reported that functional plant extracts exhibited antiproliferative activity against colon cancer cell lines, although neither specified the IC_{50} values.

Luffa *genus.* Two articles had assayed methanolic functional extracts from the fruit of *L. echinata* (L.-H. Shang et al. 2012; 2016). One of the extracts exhibited antitumor activity in HT-29 cells with an IC_{50} of 80.6 µg/ml (L.-H. Shang et al. 2012), and the other in SW480 cells with an IC_{50} value of 70.2 µg/ml after 24 hours (L.-H. Shang et al. 2016). The antiproliferative effect in HT-29 had been related to G2/M arrest, while the extract had induced apoptosis by DNA fragmentation and activation of caspase pathways in the SW480 cell line.

Citrullus *genus.* Of the two articles that had studied this genus, one had carried out a functional plant extraction

from *C. colocynthis* leaves using ethyl acetate (Chawech et al. 2015); six bioactive compounds had been isolated and dissolved in 10% ethanol to test their antiproliferative capacity. Only three (acetyl glucocucurbitacin E, coumaroyl-acetyl glucocucurbitacin I, and glucocucurbitacin I) exhibited antitumor activity against Caco-2 cells with IC_{50} values of 15–34 µg/ml. In a second study, a functional extract was obtained from *Citrullus latanus* fruit pulp to isolate a bioactive compound (cucurbitacin L 2-O-O-glucoside) with an IC_{50} value of 79.76 µg/ml which had induced apoptosis by activating caspase 3 (Abdelwahab et al. 2012).

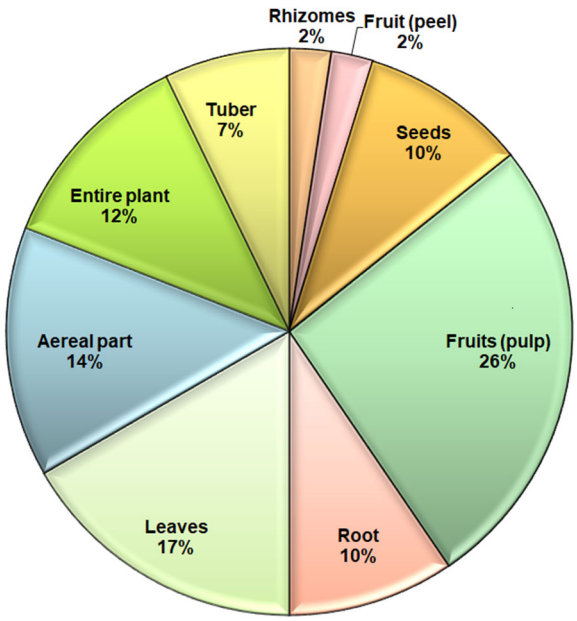

Figure 4. Representation of different parts of the plant that had been used to obtain functional extracts or in the purification of the bioactive compounds.

Trichosanthes *genus.* Of the three articles that studied this genus, two had focused on *T. kirilowii* (Song, Chang, and Li 2016; Minh et al. 2015) and one on *T. cucumerina* (Kongtun et al. 2009); all of the articles had assessed the activity of the roots and fruit. A protein extract had been prepared from the fruit, from which the TKP protein had been subsequently isolated by anion exchange and gel-exclusion chromatography (Minh et al. 2015). The bioactive compound was tested on four colon cancer cell lines (DLD1, HCT-116, SW620, and NCM460), and all of them had shown antiproliferative action, with IC_{50} values of 29–273 µg/ml. Specifically, the TKP protein caused cell death by activating caspases 9 and 3. Eight compounds had been isolated from the root of the same species using a methanolic extraction (Song, Chang, and Li 2016). All these compounds were tested on HT-29 cells, and seven had IC_{50} values of 4–61 µM, whereas ehletianol C exhibited the lowest antitumor activity with an IC_{50} exceeding 100 µM.

Three compounds (bryonolic acid, bryononic acid, and dihydrocucurbitacin B) had been isolated from the root of *T. cucumerina,* by extraction with petroleum ether, dichloromethane, and ethanol (Kongtun et al. 2009), while only one (Cucurbitacin B) had been isolated from its fruit by extracting with diethyl ether. Of all these isolated compounds, Cucurbitacin B had the lowest IC_{50} value (1.49 µg/ml), while bryonolic acid had an IC_{50} value exceeding 500 µg/ml; none of the other isolated compounds showed antiproliferative activity in Caco-2 cells. However, the root extract and spray-dried fruit juice had exhibited antiproliferative activity with IC_{50} values of 100–135 µg/ml.

Gynostemma *genus.* Of the two articles studying *G. petnaphyllum*, both had carried out functional extracts from the aerial parts of the plant (Xie et al. 2010; Yang et al. 2008) using different percentages of ethanol as a solvent (100%,

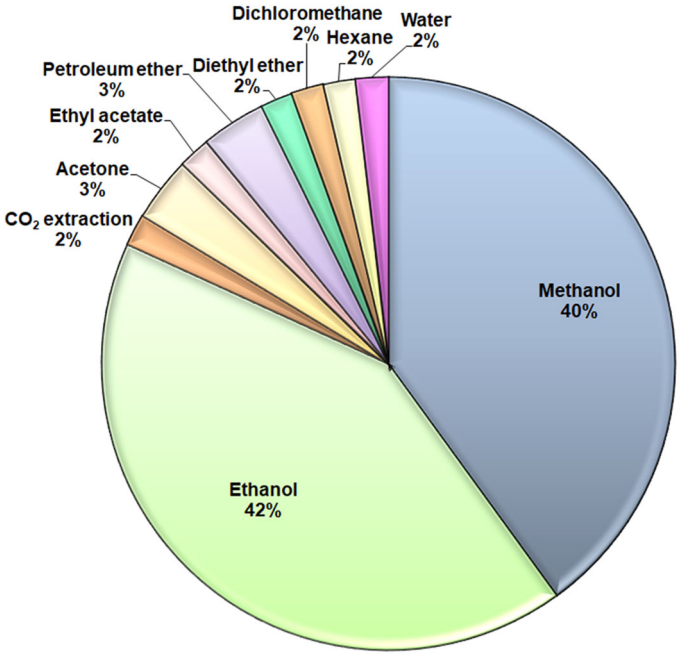

Figure 5. Representation of the main solvents that had been used in the extraction of bioactive compounds.

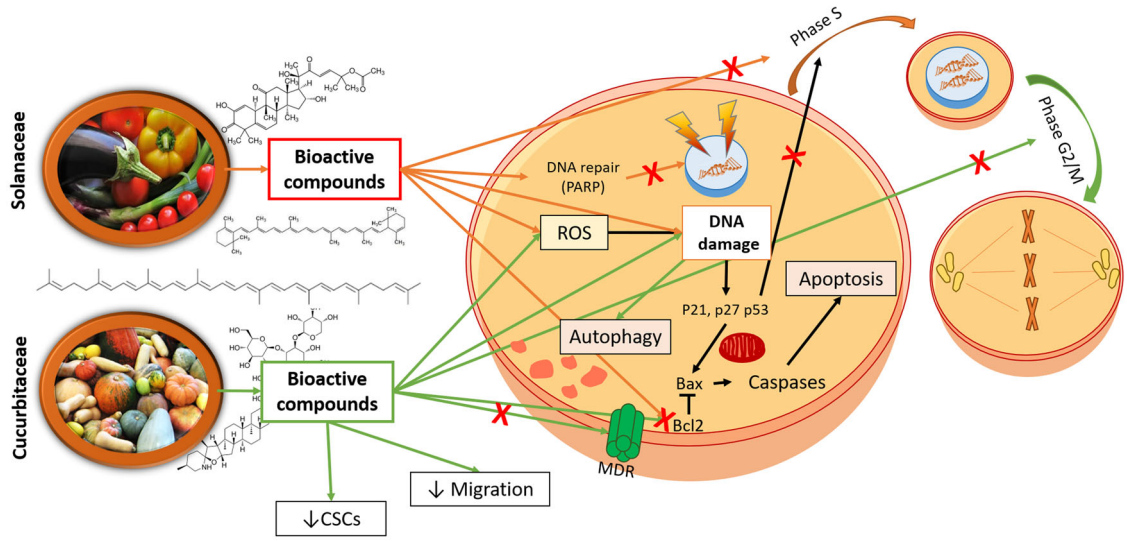

Figure 6. Schematic representation of the main mechanisms by which Solanaceae and Cucurbitaceae extracts and their bioactive compounds cause cell death in colon cancer cells. Most of the mechanisms founded are related to caspase activation, ROS modulation, autophagy or alteration of DNA repair pathway such as PARP (Poly(ADP-ribose- polymerase). In addition, some compounds, especially those from the Cucurbitaceae family, affected the CSCs (cancer stem cells) viability, migration capacity and alteration of multidrug resistance protein (MDR) efflux. In both families, there are bioactive compounds which resulted in modulation or blockage in different phases of the cell cycle.

Table 3. Summary of the genera and species of the *Solanaceae* family that exhibited antitumor activity, as well as the parts of the plants that had been used for the extraction or in the isolation of bioactive compounds.

Genus	Species	N° articles	Parts of the plant
Acnistus	*A. arborescens* (L.) Schltdl.	1	Leaves
Physalis	*P. angulate* L.	2	The entire plant
	P. peruviana L.	1	Aerial parts
	P. divericata D. Don	1	Aerial parts
Withania	*W. somnifera* (L.) Dunal	4	Root, leaves
	W. obstusifolia Täckh.	1	Leaves
	W. frutescens (L.) Pauquy	1	Leaves
Solanum	*S. lycopersicum* L.	4	Fruit
	S. lyratum Thunb.	3	The entire plant
	S. tuberosum L.	3	Tuber
	S. glabratum Dunal	1	Aerial parts
	S. xanthocarpum Schrad. & H. Wendl.	1	The entire plant
	S. verbascifolium L.	1	Leaves
Cestrum	*C. laevigatum* Schltdl.	1	Stems and roots
Nicotiana	*N. glauca* Graham	1	Leaves and stem

95%, and 75%) in addition to 50% acetone extraction. A polysaccharide with antitumor activity (by improving macrophage immune function) had been isolated using the 95% ethanol extraction solution. Of all the other plant extracts obtained with the other solvents, only those from the 100% ethanol solution had shown significant inhibitory effects, although the IC_{50} values were not provided.

Species of the Solanaceae family with antitumor activity in Colon cancer

Within the *Solanaceae* family, a total of twenty six published articles were available that described the use of extracts or isolated compounds against colon cancer cell lines; these had investigated six different genera *(Acnistus, Physalis, Withania, Solanum, Cestrum*, and *Nicotiana).* As shown in Table 3, *Solanum* was the most frequently studied genus (thirteen articles), followed by *Withania* (six articles), and *Physalis* (four articles). A total of 15 species had been investigated, of which the most frequently studied had been *W. somnifera* (four articles) and *S. lycopersicum* (four articles), followed by *S. lyratum* and *S. tuberosum* (three articles each), and *P. angulata* (two articles). Only one article had been published for each of the following: *A. arborescens*, *P. peruviana*, *P. divericata*, *W. obstusifolia*, *W. frutescens*, *S. glabratum Dunal*, *S. xanthocarpum*, *S. verbascifolium*, *C. laevigatum,* and *N. glauca* (Table 4). Most studies had tested bioactive compounds isolated from plant extracts, usually in HT-29 and HCT-116 cell lines, using anti-proliferative MTT assays. The bioactive compounds or extracts had generally been dissolved in DMSO and most tested compounds or extract solutions had exhibited in vitro antitumor activity against these colon cancer cells.

Solanum *genus.* We found thirteen articles that had tested functional extracts from six different species, obtained from different plant parts; eight of these had used ethanol as the solvent to make the extracts while methanol had been used in five others. Four articles had described extracts prepared from *S. lycopersicum* (Ramos-Bueno et al. 2017; Guil-Guerrero et al. 2011; Friedman et al. 2009; Saunders 2009) using petroleum ether or ethanol-water extraction solutions (two articles each), and one article had combined solvent extraction with in vitro digestion of the fruit (Guil-Guerrero et al. 2011). Extracts with petroleum ether showed a higher antitumoral activity against HT-29 and Caco-2 cells because these had a higher content of vitamin C, fiber, selenium, carotenoids, glycerol esters of fatty acids, flavonoids, and polyphenols (Ramos-Bueno et al. 2017). In addition, to try to identify the bioactive compound with antiproliferative activity, the glycoalkaloids dehydrotomatine and α-tomatine as well as the aglycones tomtidenol and tomatidine had been isolated from 6 green and 3 red fruits from *S. lycopersicum*. Fruits that had a higher α-tomatine content had

Table 4. Antiproliferative activity of the functional extract or isolated compounds from *Solanaceae* family in colon cancer lines.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Citotoxic assay	IC_{50} Extract solution/compound	Mechanism of action
Fruits of *Solanum lycopersicum* L. (Guil-Guerrero et al. 2011)	EtOH/water (1:1) or pure petroleum ether		HT-29; CCD18 Alone MTT	Petrolium ether extract: 62.5 μg/ml Digested tomato extract: 87 μg/ml Ethanol-water extract: not possible to determine	The petroleum ether extract and the post-digestion extract showed selective activity against HT-29 colon cancer line attributed to their content of carotenoids, flavonoids.
Solanum lycopersicum L Freezed-dried tomato pastes (Ramos-Bueno et al. 2017)	Petroleum ether Ethanol/water	Petroleum ether extracts: Glycerol FA esters (mainly derived of LA and OA). Lycopene. β-caroten. Ethanol/water extract: Carbohydrates. Fructose. Glucose.	HT-29; CDD18 Whole extract MTT and LDH	Petroleum ether extract: 150 μg/mL	The high activity against HT-29 cell viability of petroleum ether extracts was due to the presence of high amounts of vitamin C, fiber, selenium, FAs, lycopene and β-carotene.
Fruit of *Solanum lycopersicum L.* (Friedman et al. 2009)	Methanol	α-tomatine Dehydrotomatine Tomatidenol Tomatidine	HT-29 Alone dissolved in DMSO MTT	α-tomatine: 0.03 μg/ml Dehydrotomatine: 262 μg/ml Tomatidenol: 94.5 μg/ml Tomatidine: nd	From the isolated compounds, α-tomatine showed the highest anti-proliferative activity in the colon cancer line.
Fruits of *Ssolanum lycopersicum L.* (Saunders 2009)	Methanol	–	Caco-2 Alone SRB		The polyphenol extracts from all three tomato varieties were able to inhibit the growth of colon adenocarcinoma cells. Treatment with tomato extracts produced an arrest in the S phase of the cell cycle.
Whole plant of *Solanum lyratum* Thunb. (Ren et al. 2009)	Ethanol	Sesquiterpenoids: Lyratol C Lyratol D Dehydrovomifoli Blumenol A	HT-29 Alone Methylene blue dye assay	Lyratol C: 6.5 ± 1.6 μM Lyratol D: 6.4 ± 2.3 μM Dehydrovomifoliol: 5.7 ± 2.1 μM Blumenol A: 6 ± 3.1 μM	The four compounds showed significant cytotoxic activities against colon cancer cell lines.
The wole plant *Solanum lyratum* Thunb. (Yao et al. 2013)	Partioned with $CHCl_3$ and EtOAc Refluxing EtOH	Sesquiterpenoids: Compound 1 C15H24O4 Compound 2 C15H18O4 Compound 3 C15H18O3	HT-29 Alone MTT	Compound 1: 3.7 μg/ml Compound 2: 2.1 μg/ml Compound 3: 2.0 μg/ml	Compound 1, 2 and 3 (three isolated new sesquiterpenoids) exhibited significant cytotoxicity against colon cancer (HT-29 cell line).
The entire plant of *Solanum lyratum* Thunb. (Hsu et al. 2008)	Ethanol	–	Colo 205 Alone dissolved in DMSO Morphological changes	–	SLE at 300 μg/ml significantly decreased by 40% the viable colon cells. SLE induced S-phase arrest and apoptosis. SLE induced DNA fragments and apoptosis in a concentration-dependent manner. SLE induced ROS production and dreceased the $\Delta\Psi_m$. SLE increased the levels of

(*continued*)

Table 4. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Citotoxic assay	IC_{50} Extract solution/compound	Mechanism of action
					p27, p53, cyclin B1, active-caspase-3 and Bax, but decreased the levels of Cdk1, pro-caspase-9, Bcl-2, and NF-ΙB p65 and p50. SLE increased the gene expression of Cdk1, Cdc25c, Wee1 and p27 but decreased the gene expression of MDM2, Bcl-2, α-1 and topoisomerase IIα.
Whole Color-Fleshed potatos *(Solanum tuberosum L.)* (Madiwale et al. 2012)	Baked and chipped. Acidified ethanol (80%, with 0.1% v/v formic acid)	Polyphenols Anthocyanin	HCT-116; HT-29 Alone BrdU assay	–	Storage processing of potatoes did not affect the bioactivity of their bioactive compounds against colon cancer cells. Purple-fleshed potatoes can deliver health-benefiting polyphenolic compounds in levels comparable to blueberries and grapes.
Various Potatoes (Northstar, Mountain Rose, Purple Majesty, Bora Valley, *Solanum tuberosum L.*) (Wang et al. 2011)	Extraction B (80% acetone solution)		Caco-2 Alone XTT and PMS	Northstar: 144.8 ± 9.6 μg/ml Mountain Rose: 33.7 ± 13.1 μg/ml Purple Majesty: 79.6 ± 9.9 μg/ml Bora Valley: 101.3 ± 9.3 μg/ml *S. pinnatisectum*: 30.9 ± 8.8 μg/ml Chlorogenic acid: 2.8 ± 0.7 μg/ml Pelargonidin chloride: 21.6 ± 1.6 μg/ml Malvidin chloride: 46.2 ± 0.9 μg/ml	The greater anti-proliferative activity against colon cancer lines of the different potato species was directly correlated with their higher content of chlorogenic acid and polyphenols.
Colored-Flesh Potatoes (*Solanum tuberosum L.)* Four clones (Atlantic, Purple Majesty, Yukon Gold and CO97227.2P/PW) (Madiwale et al. 2011)	80% ethanol acidified with formic acid (0.1% v/v)	Polyphenols Anthocyanin	HT-29; HCT-116 Alone BrdU asssay	–	The storage period tends to decrease the content of polyphenols and anthocyanins. The different clones showed a high antioxidant and anti-proliferative activity attributed to their higher concentration of vitamins, minerals and polyphenols.
Aerial parts of Solanum glabratum Dunal var. sepicula (Abdel-Sattar, Farag, and Mahrous 2013)	Methanol	1) isorhamnetin-3-O-α-L-rhamnopyranosyl (1→6) β-D-glucopyranoside 2) 23-β-Dglucopyranosyl (23S,	HT-29 Alone dissolved in DMSO MTS	Compound 1: >32 μM Compound 2: >32 μM Compound 3: 16,7 μM Compound 4: >32 μM	Compound 3 showed the highest cytotoxic activity in HT-29 cell line

		25 R)-spirost-5-en-3, 23 diol 3-O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside 3) (25 R)-spirost-5-en-3-ol 3-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Dglucopyranosyl-(1→3)]-β-D-galactopyranoside 4) (23S, 25 R)-spirost-5-en-3, 23 diol 3-O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside			
Whole plant of *Solanum xanthocarpum Schrad. & H. Wendl.* (Bhutani et al. 2010)	Methanol	Diosgenin Diosgenone Solasodine B2-solamargine Solamargine Solasonine	HCT116 Alone SRB	–	Steroidal constituents from *S. xanthocarpum* have the capacity to induce cell death. Compounds 1 and 3 (diosgenin and solasodine) were weakly cytotoxic (70 − 80% cell viability at 50 mM),
Leaves of *Solanum verbascifolium* L. (Ohtsuki et al. 2010)	Methanol	Compound 1: New (C28H32O14) Compound 2: Isocytisoside 7-O-β-D-glucoside Compound 3: Embinoidin	DLD1/TR Alone (dissolved in DMSO) or in combination with TRAIL FMCA	–	The fractions containing compounds 1–3 were active against DLD1/TR cells at 50 mg/mL. In the DLD1/TR cells, there was no significant difference in viability between treatment with compounds alone or combined with TRAIL.
The entire plant of *Physalis angulata* L. (Chen et al. 2018)	Hexane, chloroform, ethyl acetate and methanol	Physalin F	SW480; DLD1 Alone EdU labeling Assay	–	Physalin F inhibited Wnt/β-catenin pathway.
The entire plant of Physalis angulata L. (Lee et al. 2008)	Methanol	Withangulatin A Withangulatin I	COLO 205 Alone dissolved in DMSO MTT	Withangulatin A: 16.6 ± 0.5 μM Withangulatin I: 53.6 ± 0.5 μM	Both compounds exhibited inhibitory activities against COLO 205. Withangulatin A showed stronger cytotoxic activity.
Aerial parts of *Physalis peruviana* L. (Park et al. 2016)	Methanol	4HWE Physalactone Phyperunolide E Withaperuvin C Phyperunolide F	HT-29; HCT-116; CACO-2 Alone dissolved in DMSO SRB	4-Hydroxywithanolide E: 0.1 ± 0.0 μM HT-29 0.2 ± 0.0 μM HCT-116 0.2 ± 0.1 μM CaCo-2 Physalactone: 1.3 ± 0.1 μM HT-29 1.5 ± 0.1 μM HCT-116 2.0 ± 0.4 μM Caco-2 Phyperunolide E:	4HWE showed antiproliferative effect that was accomplished via G0/G1 cell cycle arrest and induction of apoptosis. The expression of 21 genes was altered, including downregulation of PTGS2, and

(*continued*)

Table 4. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Citotoxic assay	IC_{50} Extract solution/compound	Mechanism of action
				12.7 ± 1.0 μM HT-29 16.2 ± 0.8 μM HCT-116 19.5 ± 3.9 μM Caco-2 Withaperuvin C: 2.9 ± 0.2 μM HT-29 4.1 ± 0.1 μM HCT-116 5.1 ± 1.0 μm Caco-2 Phyperunolide F: 5.8 ± 0.1 μM HT-29 7.7 ± 0.6 μM HCT-166 9.1 ± 1.2 μM Caco-2	this correlated with reduced protein levels of COX-2.
Aerial part of *Physalis divericat* D. Don (Ma et al. 2015)	Ethanol	PB	HCT-116 Alone dissolved in DMSO MTT	PB: 1.35 μM	PB induced apoptosis and the cleavage of PARP and caspase-3. PB induced autophagosome formation, and accumulation of LC3-II and p62, but decreased Beclin 1 protein level PB produced marked changes in microtubules and F-actin microfilaments. PB increased the phosphorylation of p38, ERK and JNK, and increased ROS production in the cells
Leaves of *Withania somnifera* (L.) Dunal (Mondal et al. 2012)	Methanol	WithaD	HCT-116 Alone MTT	WithaD: 0.9 μM	WithaD induced p53-dependent Bax and p53-independent Bak activation. WithaD inhibited in vitro and in vivo tumor growth in nude mice model
Root of *Withania somnifera* (L.) Dunal (Mulabagal et al. 2009)	Methanolic	Withanolide sulfoxide: Compound 1 and the dimer Compound 2	HCT-116 Alone dissolved in DMSO MTT	Compound 1: 3.59 μg/ml	Compound 1 showed 60% inhibition against COX-2 enzyme when tested at 100 μg/ml concentration. Compound 1 has potential to inhibit the proliferation of colon cancer. Compounds 1 and 2 are potential NF-κB inhibitors and antiinflammatory agents.
Leaf of *Withania somnifera* (L.) Dunal (Widodo et al. 2010)	Methanolic	i-Extract Withanone Withaferin A	HCT116 Alone WST-1	–	The compounds exhibited inhibitory effect and induced apoptosis by the accumulation of ROS at concentrations of i-Extract (6 mg/ml), Withaferin A (1 mM), and Withanone (25 mg/ml)
	Ammonia R4 and methanol	Withanolide		–	The withanolide enhanced cell death due to increased ROS

Root powder of *Withania somnifera* (L.) Dunal (Henley et al. 2017)			HT-29 Alone and in combination with cisplatin MTT		production and reduced mitochondrial function at 10 μg/mL *W. somnifera* root extract increased the effect of cisplatin through increasing ROS production and mitochondrial dysfunction.
Leaves of Withania obtusifolia Täckh. (Alali et al. 2014)	Ethanol	Obtusifonolide Sitoindoside IX 6α-chloro-5β-hydroxy withaferin A Isowithanone 2,3-dihydro-3-ethoxywithaferin A Daturataturin A Withaferin A	Sw-620 Alone dissolved in DMSO MTT	Obtusifonolide: 7.3 μM Sitoindoside IX: 5.5 μM 6α-chloro-5β-hydroxy withaferin A: 7.1 μM Isowithanone: >10 μM 2,3-dihydro-3-ethoxywithaferin A: >10 μM Daturataturin A: >10 μM Withaferin A: 0.3 μM	Withaferin A showed the highest cytotoxic action in Sw-620 cell line
Leaves of *Withania frutescens* (L.) Pauquy. (El Bouzidi et al. 2013)	Methanol	Withanolides: Compound 1 C28 H38O6 Compound 2 C28H38O7 Compound 3 C28H40O10S	HT-29 Alone MTT	CH_2Cl_2 fraction from methanol extract: 35.2 ± 4.3 μg/ml Compound 1: 13.18 ± 1.11 μM Compound 2: 25.13 ± 3.93 μM Compound 3: 1.78 ± 0.09 μM	The CH_2Cl_2 fraction was the most active against HT-29 cell line. Compounds 1, 2 and 3 showed potential anticancer activity. Compound 3 exhibited the strongest cytotoxic action against HT-29 cancer cell lines which was comparable to that of 5-Fu
Leaves of *Acnistus arborescens* (L.) Schltdl. (Cordero et al. 2009)	Ethanol 96%	Compound 1) Withanolide D Compound 2) 12β-acetoxy-4 deoxy, 5,6, deoxy-Δ5-Withanolide D	HT-29 Alone dissolved in DMSO MTT	Compound 1: 1.44 ± 0.05 μM Compound 2: No activity	Compound 1 showed cytotoxic activity against colon cancer. Compound 2 did not show cytotoxic activity even at the highest concentration evaluated 10 μM.
Stems and roots of *Cestrum laevigatum* Schltdl. (Ribeiro et al. 2016)	Hexane; Ethanol	(25R,S)-5α-spirostan-2α,3β-diol 3-O-β-Dglucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)- β-D-galactopyranoside (1/2) (25 R,S)-5α-spirostan2α,3β-diol 3-O-β-D-glucopyranosyl-(1→2)-αL-rhamnopyranosyl-(1→4)-β-D-galactopyranoside (3/4) 26-O-β-D-glucopyranosyl-(25R,S)-5α-furost-20- ene,2α,3β-diol 3-O-β-D-galactopyranoside (5/6) 26-O-βD-	HCT-116 Alone dissolved in DMSO MTT	(1/2) 7.27 μg/mL (3/4) 11.41 μg/mL (9/10) 16.50 μg/mL	The compounds showed cytotoxic activity against HCT-116 cell line

(*continued*)

Table 4. Continued.

Material (Reference)	Extraction Method	Isolated Compounds	Cell line Administration Citotoxic assay	IC_{50} Extract solution/compound	Mechanism of action
		glucopyranosyl-(25R,S)-5α-furost-20-ene,2α,3β-diol 3-O-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (7/8) (25 R,S)-5α-spirostan2α,3β-diol 3-O-β-D-galactopyranoside (9/10)			
Leaves and stem of *Nicotiana glauca* Graham (Hassan et al. 2014)	n-hexane, ethanol, methanol water	Whole Extract	HCT-116 Alone MTT	Leaves: N-hexane: 95.08 μg/ml Ethanol: 49.66 μg/ml Methanol: 19.83 μg/ml Water: 17.20 μg/ml Stem: N-hexane: 87.07 μg/ml Ethanol: 33.58 μg/ml Methanol: 43.47 μg/ml Water: 28.95 μg/ml	The extract exhibited significant antiproliferative effect against HCT-116 cell line

Half maximal inhibitory concentration (IC50); 3-(4,5-dimethytlthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; lactate dehydrogenase (LDH); fatty acids (FAs); linoleic acid (LA); oleic acid (OA); dimethyl sulfoxide (DMSO); n.d. (not defined); sulforhodamine B (SRB); chloroform ($CHCl_3$); dichloromethane (CH2Cl2); ethyl acetate (EtOAc); *Solanum lyratum* extract (SLE); reactive oxygen species (ROS); tumor protein p53 (p53); Cyclin-dependent kinase inhibitor 1B (p27); cyclin B1 (CCNB1); BCL2 antagonist/killer 1 (BAK1); B-cell lymphoma 2 (Bcl-2); cyclin-dependent kinase 1 (Cdk1); Poly(ADP-ribose) polymerase (PARP); M-phase inducer phosphatase 3 (CDC25C); mouse double minute 2 homolog (MDM2); cell Proliferation Kit II (XTT); phenazine methosulfate (PMS); 2,2-difenil-1-picrylhydrazyl (DPPH); 2, 2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS); fluorometric microculture cytotoxicity assay (FMCA); wingless and Int-1 (Wnt); 4β-hydroxywithanolide E (4HWE); prostaglandin-endoperoxide synthase 2 (PTGS2); cyclooxygenase-2 (COX-2); physalin B (PB); light chain 3-II (LC3-II); extracellular signal-regulated kinase (ERK); c-Jun N-terminal kinase (JNK); withanolide D (WithaD); nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); 5-Fluorouracil (5-FU).

exhibited a stronger antitumor effect in HT-29 cells with an IC_{50} of 0.03 µg/mL (Friedman et al. 2009).

Three articles had studied *S. lyratum* (Yao et al. 2013; Ren et al. 2009; Hsu et al. 2008) and reported the isolation of sesquiterpenoids from the whole plant using methanolic extraction. These were lyratol C, lyratol D, drovomifoliol, and blumenol A, which showed antiproliferative activity in HT-29 cells at a concentration of 6 µM (Ren et al. 2009), and solajiangxin A, B, and C, which showed cytotoxic activity in the same cell line at 1.9–3.7 µg/mL (Yao et al. 2013). Likewise, the methanolic solution of *S. lyratum* showed antitumor activity in Colo 205 cells at a concentration of 300 µg/mL by arresting the cell cycle in S-phase, increasing reactive oxygen species (ROS), and inducing apoptosis (Hsu et al. 2008).

Three articles (Madiwale et al. 2012; 2011; Q. Wang et al. 2011) had evaluated ethanolic extracts obtained from the tuber of *S. tuberosum* which had shown slight antioxidant and antiproliferative activity against HT-29 and HCT116 cells, although no IC_{50} values had been reported. Caco-2 was the only cell line in which the ethanolic extracts had exhibited antitumor activity, with an IC_{50} of 30.9 ± 8.8 µg/mL (Q. Wang et al. 2011). This activity was due to the presence of anthocyanins, total phenolics, and chlorogenic acid, and was shown to decrease over time (Madiwale et al. 2011; 2012). Three spirostan-type saponins and one flavonoid glycoside had been isolated from the active n-butanol fraction of the aerial parts of *S. glabratum Dunal var. sepicula*, but only (25 R)-spirost-5-en-3-ol3-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Dglucopyranosyl-(1→3)]-β-D-galactopyranoside had shown cytotoxic activity against HT-29 cells, with an IC_{50} of 16.7 µM (Abdel-Sattar, Farag, and Mahrous 2013).

A series of sarsasapogenin and diosgenin-derived steroidal constituents had been isolated (diosgenin, diosgenone, solasodine, B2-solamargine, solamargine, and solasonine) from the ethanolic extract from *S. xanthocarpum*. Diosgenin and solasodine were weakly cytotoxic (70–80% cell viability) against HCT-116 cells at 50 µM (Bhutani et al. 2010). Regarding *S. verbascifolium*, three flavonoid glycosides had been isolated from the methanolic extract of the leaves (7,4′-dimethyl-apigenin-6-C-β-glucopyranosyl-2″-O-α-l-arabino-pyranoside, isocytisoside 7-O-β-D-glucoside, and embinoidin), which all showed antitumoral activity in DLD1/TR cells at a concentration of 50 mg/mL (Ohtsuki et al. 2010).

Physalis *genus.* In four articles, functional extracts had been prepared from the aerial part and whole plant using methanol as the preferred solvent. Two articles had described the isolation of whitanolides—a class of steroid compounds—from the methanolic extract of the whole plant for *P. angulata*. The withanolide compound physalin F had antitumor activity against SW480 and DLD1 cells by inhibiting Wnt/β-catenin signaling by promoting YAP-mediated ubiquitination and proteasomal β-catenin degradation (Chen et al. 2018). Another two whitanolides (withangulatin I and withangulatin A) showed antiproliferative activity against the Colo-205 line with an IC_{50} of 53.6 ± 0.5 µM and IC_{50} of 16.6 ± 0.5 µM, respectively (Lee et al. 2008).

The compound 4-hydroxywithanolide E (4HWE) had been isolated by a methanolic extract of the aerial parts of *P. peruviana* and had been found to inhibit proliferation of HT-29 cells with an IC_{50} of 0.1 µM, HCT-116 cells (IC_{50} of 0.2 µM), and CaCo-2 cells (IC_{50} of 0.2 µM). The antiproliferative effect had been shown to be the result of G0/G1 arrest and the induction of apoptosis (Park et al. 2016). For *P. divericata*, physalin B had been was isolated from the ethanolic extract of the aerial part of the plants, and this had shown antiproliferative activity against HCT-116 cells with an IC_{50} of 1.35 µM by inducing apoptosis, autophagosome formation, and increased ROS production, among other mechanisms (Ma et al. 2015).

Withania *genus.* Six articles described the preparation of functional extracts from the roots and whole plant of species in the genus *Withania* using methanol as the preferred solvent. Four articles had isolated whitanolides (steroidal lactones) from *W. somnifera;* one, named Withanolide D (WithaD), had been purified from a methanolic extract of the leaves and had induced apoptosis in HCT-116 cells via a Bax/Bak-dependent pathway at concentrations of 0.5–4 µM (Mondal et al. 2012). Another two withanolide sulfoxide compounds had been isolated from a methanolic root extract, one of which had exhibited an anti-proliferative effect against HCT-116 cells at concentrations of 0.74–3.63 µg/ml. Furthermore, both of these compounds had suppressed TNF-induced NF-kb activation at 100 µM (Mulabagal et al. 2009).

Two more whitanolides (withanone and withaferin A) had been isolated from the methanolic extract of leaves, and these had induced apoptosis in HCT166 cells by increasing ROS production (Widodo et al. 2010). Finally, the methanolic root extract had also exhibited antitumor activity against HT29 cells at 10 µg/mL by increasing ROS production and had shown synergistic action with cisplatin (100 µM) using a 48 h pre-incubation step (Henley et al. 2017).

Seven withanolide-class compounds had been isolated from the ethanolic extracts of *W. obstusifolia* leaves, among which withaferin A displayed the strongest cytotoxic activity against SW-620 cells at a 0.3 µM dose (Alali et al. 2014). Finally, three whitanolides had been isolated from the methanolic extract of *W. frutescens* leaves, of which the 2,3-dihydroxywitha-ferin A-3B-O-sulfate had exhibited the greatest antitumor activity against HT-29 cells with an IC_{50} of 1.78 ± 0.09 µM (El Bouzidi et al. 2013).

Acnistus *genus.* Two whitanolides had been isolated from an ethanolic extract of leaves from *A. arborescens,* but only withanolide D had shown cytotoxic activity against the HT29 cell line, with an IC_{50} of 1.44 ± 0.05 µM (Cordero et al. 2009).

Cestrum *genus.* Four epimeric spirostanol and furostanol-type steroidal saponins had been isolated from an ethanolic extract of *C. laevigatum* roots and stems; three of them

(Table 1) showed antitumoral activity against HCT-116 cells in concentrations of 7.27–16.50 µg/mL (Ribeiro et al. 2016).

Nicotiana *genus*. Different extracts were prepared from the leaves and stem of *N. glauca* using n-hexane, ethanol, methanol, and water. All the extracts had antiproliferative activity against HCT-116 cells in concentrations of 19.83–95.08 µg/mL (Hassan et al. 2014).

Discussion

This systematic review aimed to provide a complete description of the antitumor activity against colon cancer cell lines of functional extracts or compounds isolated from plant species belonging to the *Solanaceae* or *Cucurbitaceae* family. The mechanisms of action through which these bioactive compounds or functional extracts had induced cell death were also defined, as described in the academic literature. The inverse relationship between a vegetable-rich diet and risk of CRC is widely known. Indeed, Schwingshackl et al. 2017 recently carried out a study on the effect of the Mediterranean diet (consumption of vegetables, fruit, and fiber) on the risk of mortality and cancer recurrence in 10 European countries, showing that adherence to this diet was associated with a lower risk of mortality in CCR, breast cancer, gastric cancer, and liver cancer.

After performing an exhaustive screening, data from 44 selected articles were extracted and described. Therefore, to the best of our knowledge, this is the first systematic review to collect together current knowledge of the antitumor activity of a wide variety of species in the *Solanaceae* and *Cucurbitaceae* families. These species are commonly used for nutritional purposes and are accessible to the majority of the world's population. The mechanistic emphasis of the articles finally included in this systematic review implies that in vitro studies with cell lines derived from colon cancer were preferred.

Since the extraction process is critical to the isolation of bioactive compounds of interest, the methodology used to obtain these functional extracts was particularly important. Different methods are currently available, depending on the type of plant, plant part under study, and the nature of the target compounds. Given the absence of a universal extraction methodology, the most important parameter to consider is the type of solvent used (Colvin 2018; Q.-W. Zhang, Lin, and Ye 2018). The most frequently used solvents in the studies included in this systematic review were ethanol and methanol, although with variations in the solvent ratio and the methodology used (Figure 5).

In this study, articles that had obtained functional extracts and bioactive compounds from *Solanaceae* and *Cucurbitaceae* families that exhibited in vitro antitumor activity against cell lines derived from colon cancer were included. Within the *Cucurbitaceae* family, the isolated compounds had higher antitumor activity than the functional extracts. For example, cucurbitacin C isolated from *T. cucumerina* exhibited an IC_{50} of 1.49 ± 0.21 µg/ml, while the root extract had an IC_{50} of 135.74 ± 0.16 µg/ml (Kongtun et al. 2009). Of note, some extracts had interacted synergistically with chemotherapy agents like doxorubicin (Kwatra, Venugopal, et al. 2013b), making them promising targets for future in vivo studies or clinical trials.

The most studied genus was *Momordica*. In fact, numerous studies in *M. charantia* had highlighted the presence of bioactive compounds as well as functional extracts with anti-inflammatory, antioxidant, antibacterial, antidiabetic, and antitumor activity. This antiproliferative activity had been effective for different tumor types, both in vitro and in vivo (Bortolotti, Mercatelli, and Polito 2019; Dandawate et al. 2016), via different mechanisms of action, such as increased ROS generation, cell cycle G2/M or S-phase arrest, induction of caspase or other proapoptotic gene activation, and autophagy.

Regarding the *Cucurbitaceae* family, the core bioactive compounds isolated had been triterpenoids, polysaccharides, steroids, peptides, and carotenoids; cucurbitacin and its derivatives had been among the most widely studied and had the highest reported antitumor activity levels. Different studies had shown anti-inflammatory and anticancer activity in different tumor types, both in vitro and in vivo (J. Shang et al. 2019; Kim, Park, and Kim 2014). These tetracyclic triterpenoid derivatives, of which about 40 species are known, have been administered alone or in combination in several in vitro studies and xenograft models of various cancer types such as breast (Tannin-Spitz et al. 2007; Gupta and Srivastava 2014), lung (Shukla et al. 2015), skin (Y. Zhang et al. 2011; Y.-T. Zhang et al. 2014), brain (Yin et al. 2008; Zheng et al. 2014), liver (Chan, Meng, et al. 2010), or blood cancer (Haritunians et al. 2008; Chan, Li, et al. 2010). Their mechanisms of action had included the onset of the tumor suppressor p53 pathway and inhibition of tumor progression via the Wnt/β-catenin, JAK/STAT, phospho-RB, MAPK/ERK, and PI3K-AKT pathways. They had also induced an increase in pro-apoptotic proteins, decrease in growth receptors, cell growth arrest, and apoptosis. In addition to their anti-tumor activity, other properties such as anti-inflammatory or antioxidant activity as well as effective control of cardiac, neurological, and pulmonary lesions as well as psoriasis had been reported (Alghasham 2013).

As seen in *Cucurbitaceae*, the bioactive compounds that had been isolated for the *Solanaceae* family had exhibited higher antiproliferative activity than the functional extracts had. For example, the compounds that had been isolated for *S. lyratum,* solajiangxin A, B, and C, exhibited IC_{50} values of 1.9–3.7 μg/ml, while the entire plant extract had shown an IC_{50} value of 300 μg/ml (Hsu et al. 2008). In the *Solanaceae* family, the main isolated compounds corresponded to glycoalkaloids, carotenoids, sesquiterpenoids, saponins, flavonoids, and whitanolides, with the latter ones having shown the greatest antiproliferative effect. Different studies had indicated that these compounds had shown excellent anticancer, anti-inflammatory, and neuroprotective activities and could also be combined with other anticancer compounds, thereby making them particularly interesting in the design of new cancer therapies (Hussain et al. 2018). In addition, the bioactive compounds derived from species in the *Solanaceae*

family had also exhibited interesting synergies with known antitumor drugs such as cisplatin or oxaliplatin in human pancreatic cancer, both in vitro and in vivo (Henley et al. 2017; Li et al. 2015).

Leaves and aerial parts of the different species had usually been selected to obtain the different extracts studied (Figure 4). However, numerous articles had highlighted the importance of seeds because they contain a higher content of compounds compared to roots and aerial plant parts. Recent studies (Ozuna and León-Galván 2017; Rezig et al. 2019) have pointed out the importance of pumpkin, melon, and watermelon seeds both as a source of nutrients (protein, minerals, complex carbohydrates) and of bioactive compounds with antioxidant, antifungal, and anticarcinogenic activity. Likewise, different authors have reported the isolation of seed compounds from *Solanaceae* family including sterols, phenolic compounds, terpenes, flavonoids, and benzopyrones (coumarins and its derivates), which are known for their antioxidant, antitumor, anti-inflammatory, antibacterial, anti-fungal, and antiparasitic activity (Kaunda and Zhang 2019).

Regarding in vitro experiments, of note, most of the studies had only conducted antiproliferative trials using a single colon cancer cell line. However, using several cell lines means that more information about the complexity of the polygenetic etiology of cancer and its biological mechanisms can be obtained. Furthermore, modified cell lines can be used to ascertain the mechanisms of action and resistance/sensitivity patterns of bioactive compounds in the development of more specific drugs (Bácskay et al. 2018). Similarly, functional extracts or isolated compounds should be tested on non-tumor cells to determine their antitumor selectivity. In this review, only two articles had tested bioactive compounds in a non-tumoral cell line (Ramos-Bueno et al. 2017; Guil-Guerrero et al. 2011), despite the importance of conducting experiments in these cell lines for subsequent in vivo or clinical trials. Finally, although, most articles had meticulously studied the mechanisms of action by which isolated extracts or compounds had induced cell death, further work will still be required to corroborate the antitumor activity of the compound or extract of interest (Gordon, Brown, and Reynolds 2018).

Conclusions

Despite the importance of diet in relation to colon cancer prevention, very few articles studying antitumor activity include species of the Solanaceae and Cucurbitaceae families in their analyses. Of all the species studied, it would be necessary to carry out a more in-depth study of all parts of the plant, since different bioactive compounds can be found due to their interaction with microorganisms and environments (phyllosphere, endosphere and rhizosphere), which can be used as antitumor agents. Other wild and commonly consumed spices such as eggplants (Solanum melongena) or peppers (Capsicum spp.), for which there are no conclusive studies, should also be studied. Finally, although all the studies included in this systematic review reported positive results in which the functional extracts or their isolated compounds displayed high in vitro antitumor activity for cell lines derived from colon cancer, further studies are required to delve into the specific mechanisms of action that grant them this antiproliferative activity on colon cancer-derived cell lines. These studies would facilitate their use in clinical practice as preventive agents and/or treatment of this pathology.

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