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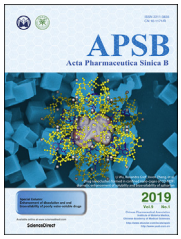

REVIEW

Nature-derived compounds modulating Wnt/β-catenin pathway: a preventive and therapeutic opportunity in neoplastic diseases

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Abstract The Wnt/β-catenin signaling is a conserved pathway that has a crucial role in embryonic and adult life. Dysregulation of the Wnt/β-catenin pathway has been associated with diseases including cancer, and components of the signaling have been proposed as innovative therapeutic targets, mainly for cancer therapy. The attention of the worldwide researchers paid to this issue is increasing, also in view of the therapeutic potential of these agents in diseases, such as Parkinson's disease (PD), for which no cure is existing today. Much evidence indicates that abnormal Wnt/β-catenin signaling is involved in tumor immunology and the targeting of Wnt/β-catenin pathway has been also proposed as an attractive strategy to potentiate cancer immunotherapy. During the last decade, several products, including naturally occurring dietary agents as well as a wide variety of products from plant sources, including curcumin, quercetin, berberin, and ginsenosides, have been identified as potent modulators of the Wnt/β-catenin signaling and have gained interest as promising candidates for the development of chemopreventive or therapeutic drugs for cancer. In this review we make an overview of the nature-derived compounds reported to have antitumor activity by modulating the Wnt/β-catenin signaling, also focusing on extraction methods, chemical features, and bio-activity assays used for the screening of these compounds.

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1. Introduction

The Wnt/β-catenin signaling cascade is an evolutionarily conserved pathway. It has a crucial role in normal embryonic development, by orchestrating a wide range of process including limb, heart, or neural development, axis specification and gastrulation[1−5]. Moreover, Wnt pathway is one of the main players in the maintenance of adult tissue homeostasis by regulating cell proliferation, migration, differentiation, survival and adhesion, as well as renewal of stem cells[6−10]. Due to its pleiotropic and essential functions in controlling a great number of process during embryonic and adult life, dysregulation of the Wnt/β-catenin signaling is associated with many types of diseases, including cancer and neurodegenerative disorders[5,11−14], fibrosis[15,16], endocrine diseases[17], and metabolic syndrome[18]. In view of this crucial role in the pathogenesis of such different kinds of diseases, in the last two decades, most of molecular components of the signaling have been proposed as innovative therapeutic targets[5,12,14,19−21]. Crucial molecules participating to the signaling seem also to possess a diagnostic/prognostic value in neoplastic diseases[22−24], and this further increases the interest of the scientific world on this pathway. Not by chance, many research groups worldwide are engaged in expanding the knowledge on this pathway and its role in the onset and progression of various diseases. Moreover, several pharmaceutical and biotech companies invested, and are currently investing, considerable funds for developing innovative drugs targeting critical steps of this signaling, or for confirming the diagnostic value of molecules participating to the Wnt/β-catenin cascade. In the last decade, a great number of Wnt pathway targeting compounds, including small molecules and biologics, have been tested as novel therapeutic agents in both preclinical and clinical studies. Most of the studies analyzed the efficacy of these compounds in anticancer therapy[14,25], since cancer has been the first disease in which a role of Wnt signaling has been demonstrated[26] and, therefore, the knowledge in this field is greater than for other pathological conditions.

Herbal preparations have been used since ancient times as the main source of therapeutic principles for world populations. In the history of medicine, there are many remarkable examples of how the discovery of natural products deeply affected advances in biology and stimulated drug discovery and therapy. Nevertheless, the interest of pharmaceutical companies toward natural compounds, as potential candidates in the drug discovery process, showed a decline during the 1990s and early 2000s, due to the advent of high-throughput screening (HTS) and combinatorial chemistry[27]. In the last years, with advances of technologies that allow to screen natural products in HTS assays, the interest in plant-derived drugs has progressively increased and a "New Golden Age" for the drug discovery of nature-derived products is emerging[28,29]. The discovery of nature-derived compounds with strong anti-cancer activity contained in many foods leads also to design chemotherapy regimen combining these compounds with conventional chemotherapeutic agents. During the last decade, several products, including naturally occurring dietary agents as well as a wide variety of products from plant sources, have been identified as potent modulators of the Wnt/β-catenin signaling and have gained interest among the researchers as promising candidates for the development of chemopreventive or therapeutic drugs for cancer[30−33].

In this review, we make an overview of the nature-derived compounds that are reported to have antitumor activity by modulating the Wnt/β-catenin signaling, also focusing on extraction methods, chemical features, and bio-activity assays used for the screening of these compounds. In addition, we briefly describe some of the preclinical studies that demonstrated, in *in vitro* and *in vivo* models of cancer, the effect of these nature-derived compounds on the signaling and some clinical trials just completed or that are ongoing, aiming to demonstrate the anti-tumor efficacy of natural agents targeting Wnt signaling components.

2. The Wnt/β-catenin signaling cascade: the current regulatory model

Wnts are secreted, cysteine-rich glycoproteins that act as ligands to promote receptor-mediated signal transduction pathways in both vertebrates and invertebrates[2,5,12,34−38]. During synthesis, Wnt proteins are modified by the attachment of an acyl group (palmitoleic acid) and this modification, brought about by the palmitoyl transferase Porcupine (membrane-bound *O*-acyltransferase family, MBOAT in Fig. 1), is crucial for Wnt secretion and for the binding to the Wnt receptor Frizzled (Fzd)[12] (Fig. 1). Wnt signals are transduced in the canonical, or β-catenin-dependent, pathway and in other two non-canonical, or β-catenin-independent, pathways (Wnt/Ca^{2+} and the planar cell polarity signaling)[35−37]. The receptor Fzd is crucial for all Wnt signaling cascades, with the N-terminal Fzd cysteine rich domain (CRD) that acts as the Wnt binding domain[35]. In addition to the Fzd, the Wnt/β-catenin pathway needs the low-density lipoprotein receptor related proteins 5 and 6 (LRP5/6) as co-receptors[39]. The formation of a Wnt−Fzd−LRP6 complex is the trigger for the Wnt/β-catenin signaling cascade.

β-Catenin was originally recognized as a cadherin-associated protein participating to the cell−cell junctions[40,41], but in the last two decades has got increasing interest as one of the most important mediators of the canonical Wnt pathway[5,6,42], mainly due to the role of this signaling in tumorigenesis. A huge number of papers on this issue are available in literature, and here we summarize the key aspects of the Wnt/β-catenin signaling, and refer to other reviews and papers for more details on the molecular mechanism[6,12,14,25,43−45]. As illustrated in Fig. 1, following the current model, in the "Wnt-OFF" state, β-catenin exists in a cadherin-bound form that regulates cell−cell junctions; the excess of β-catenin that is not segregated on the cell membrane by cadherins, is rapidly phosphorylated by glycogen synthetase kinase-3β (GSK-3β) in the adenomatous polyposis coli (APC)/axin/GSK-3β destruction complex and is then degraded by the ubiquitin−proteosome pathway (Fig. 1). Conversely, in the "Wnt ON" state, the binding of Wnt to the Fzd receptors and to the LRP5/LRP6 co-receptors inactivates the APC/axin/GSK-3β destruction complex and the results in β-catenin accumulation in the cytosol and its translocation into the nucleus. Nuclear β-catenin functions as a co-activator for TCF/LEF-mediated transcription and regulates the expression of Wnt target genes that regulates cell proliferation and survival, apoptosis, cell differentiation, cell motility and invasion, and resistance to chemotherapy. Indeed, in non-pathological conditions, β-catenin dynamically has multiple subcellular localizations, that include adherens junctions, where it participates to cell−cell contacts, the cytoplasm, where its levels are tightly controlled by the degradation at the destruction complex, and the nucleus, where it is engaged in transcriptional regulation and chromatin interactions[46]. This β-catenin dynamics is finely regulated by different factors, including growth

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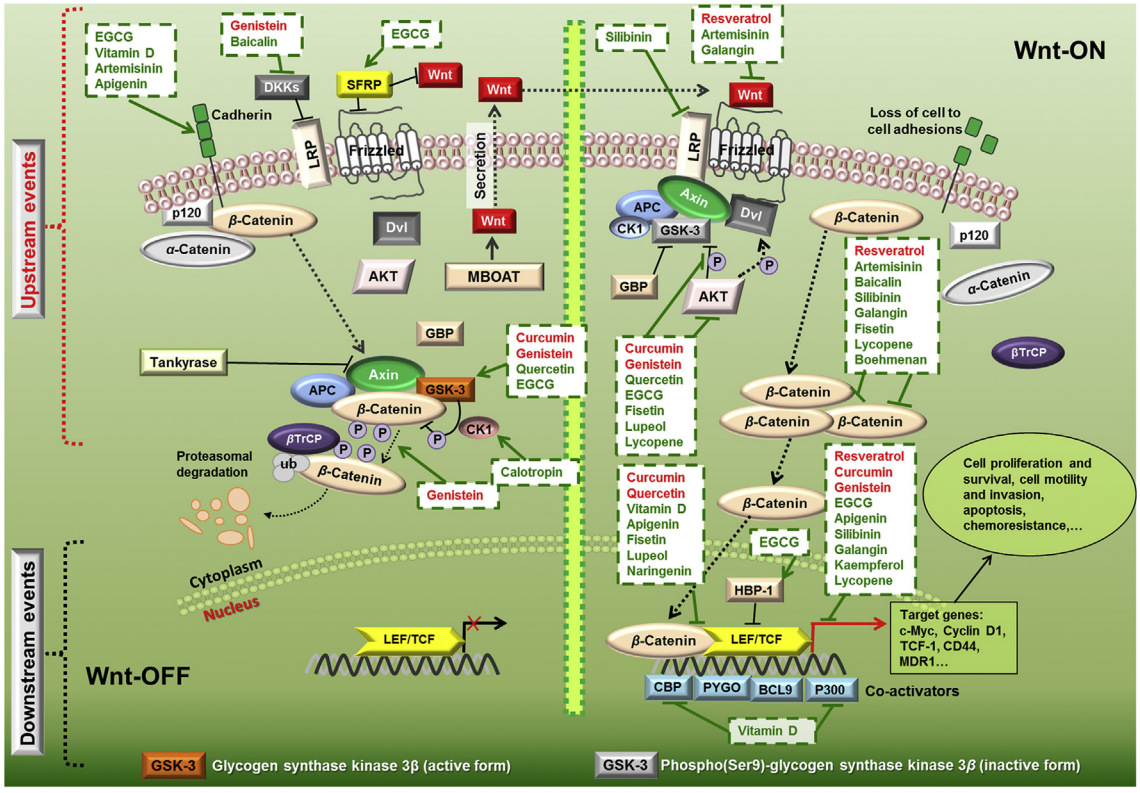

Figure 1 Schematic representation of the current regulatory model of Wnt/β-catenin signaling, and the Wnt-targeting natural compounds which are currently under investigation. AKT, serine/threonine-protein kinase; APC, adenomatous polyposis coli; BCL9, B cell lymphoma 9 co-activator; β-TrCP, E3 ubiquitin ligase; CBP, CREB-binding protein; CK, casein kinase; DKK, Dickkopf; Dvl, dishevelled; GBP, GSK3-binding protein; GSK, glycogen synthase kinase; LEF, lymphoid enhancer factor; LRP, LDL receptor-related protein; MBOAT, membrane-bound *O*-acyltransferase; P, phosphorylation; p300, E1A associated protein; PYGO, Pygopus co-activator; sFRP, secreted Frizzled-related protein; TCF, T-cell factor; ub, ubiquitination. The compounds that are currently explored in clinical studies are marked in red colour.

factors, prostaglandins, and E-cadherin levels, that play in concert to maintain a fine balancing between Wnt-OFF and Wnt-ON states. Due to the critical role of Wnt/β-catenin signaling in regulating opposite cell fates (survival *vs.* cell dead, differentiation *vs.* proliferation, etc.), the preservation of this balance in the different tissues and organs is essential, since aberrant regulation of the signaling cascade and/or the inability of the organism of restoring the right equilibrium might lead to the onset and progression of a wide range of diseases.

3. The Wnt/β-catenin pathway and cancer: therapeutic perspectives

In the two last decades, numerous studies demonstrated the key role of deregulation or constitutive activation of the Wnt/β-catenin pathway in tumor initiation, growth, metastasis and dormancy[6,12,19,21,47]. This signaling is also involved in tumor immunity and cancer stem cell maintenance in different forms of human cancer, mainly solid tumors, including colorectal, gastric, esophageal, prostate, breast, thyroid, lung, ovarian cancer, and hepatocellular carcinoma[6,12,19,21,47–50]. Therefore, many of the molecular components of the Wnt/β-catenin signaling have been proposed as innovative targets for cancer therapy[5,14,19–21,25,46,50]. Furthermore, since the Wnt/β-catenin signaling is also involved in some chronic diseases associated with an increased risk of developing cancer, such as the inflammatory bowel diseases (Chron's disease and ulcerative rectocolitis), and liver fibrosis, drug targeting this signaling has also been proposed for cancer prevention[22,51–53]. The main therapeutic approaches that are currently explored (see for review[14,25]) involve the use of drugs targeted to molecules that participate to upstream events, such as Wnt ligands, Fzd receptors, LRP5/LRP6 co-receptors, Dishevelled and others, or drugs targeting downstream events such as β-catenin/TCF or β-catenin/CBP interactions.

Several modulators of the signaling have been developed for treatment of cancer and other β-catenin-related diseases, but, up today, none of them has been yet approved or incorporated into clinical practice. The main issue for Wnt antagonists, apart the common safety aspects that could halt the development of new drugs, is the target strategies. Some drugs developed to date display a mechanism of action that involves the targeting of upstream molecules of the pathway such as tankyrase or porcupine. However, these experimental drugs failed to demonstrate a clinical efficacy in neoplastic diseases in which mutations in APC and β-catenin genes have been found, such as colorectal cancer. Even if the direct targeting of β-catenin appears the most relevant pharmacological strategy for overcoming these mutations, β-catenin seems to be an undruggable target, due the absence of ligand binding pockets usually present in enzymes and receptors[54]. These critical issues could be resolved based on the new scientific evidence about the crucial role of this pathway in the immunological response, since the Wnt/β-catenin signaling in immune cells is activated by extrinsic factors rather

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than intrinsic mutations[54]. This emerging role of the pathway is particularly noteworthy if seen in the context of the new golden age of immunotherapy in oncology[55]. The advent of immune checkpoint inhibitors (ICI) is changing the clinical paradigm and the life expectancy, with significant results for progression-free survival and overall survival for patients affected by several cancer diseases such as melanoma and non-small cell lung cancer. Despite the clinical success of these therapies, only certain types of tumors respond to ICI and others such as breast, prostate, and colon are less sensitive[54,55]. Therefore, there is a scientific and regulatory consensus that suggests the development of new research programs aiming to the optimization of the use of existing ICI in order to improve the clinical management of patients treated with these innovative medicines. Thus, the recent evidence, indicating that Wnt/β-catenin signaling plays an important role in the regulation of different tumors and their immune sensitivity, opens up new perspective in this field. Specifically, the dysregulation of this pathway seems to be deeply related to the biological function of several immune cells involved in antitumor immunity, immune evasion and exclusion mechanisms that are known to have a central role in non-responders or resistant patients treated with ICI[54]. These findings suggest that the efficacy of cancer immunotherapy by ICI treatment could be significantly improved through a combined strategy with molecules targeting Wnt/β-catenin pathway. This assumption is sustained by data from *in vitro* and *in vivo* experiments[56−60], and from clinical studies that explored the efficacy of combined treatment with Wnt/β-catenin modulators and checkpoint inhibitors have been recently started[61,62].

In this context, natural compounds could represent an alternative and important source useful for increase the availability of molecules with different pharmacological properties able to potentially overcome some critical issues that characterize synthetic and biological molecules that have been developed to date. In particular, the large number of natural compounds known for acting on the Wnt/β-catenin pathway could be precious not only for developing more efficient and less toxic preventive/therapeutic drugs but also for potentiating the efficacy of the ICI treatment, in a combined therapy. To this purpose, a phase II clinical trial (trial number: NCT03192059) is recruiting patients with advanced and/refractory cervical cancer, endometrial carcinoma or uterine sarcoma in order to study the combination of vitamin D (as co-drug) and curcumin (as supplement) with an immunomodulatory cocktail that also includes the anti PD-L1 pembrolizumab (https://clinicaltrials.gov/ct2/show/NCT03192059).

4. Natural molecules targeting Wnt/β-catenin pathway

The key role Wnt/β-catenin signaling in the mechanism of cancer development and progression has led to a significant increase of researches aimed to discover new molecule able to modulate several pharmacological target of the pathway. In this context, a large variety of natural compounds has been demonstrated to inhibit or modulate molecules involved in upstream and downstream events of the Wnt/β-catenin pathway[63]. The majority of these molecules are polyphenols, mainly flavonoids, but some terpenes and terpenoids are also included (Fig. 2). Almost all of these nature-derived compounds share the feature of having antioxidant and anti-inflammatory properties, and this could in part explain their effects on the Wnt/β-catenin signaling that is often dysregulated in condition of oxidative stress or inflammation. Conversely, up today, it has not been available to find common specificities in the chemical structures of these compounds that could be directly related to the effects on the pathway or whose direct interaction with specific targets participating to the Wnt/β-catenin signaling could be supposed. As reported in Table 1[3,25,30−33,46,63−98, 99−105], and illustrated in Fig. 1, all the mentioned compounds have multiple targets involved in both upstream and downstream events of the signaling. Indeed, all these nature-derived molecules are "modulators" of the pathway rather than specific activators or inhibitors, as reported for other synthetic Wnt-targeting drugs[14]. This means that they can act in one or in the other ways depending on the cell type and on the state of dysregulation that the signaling has in that specific disease. In other words, as modulators of the pathway, these nature-derived compounds are hypothetically able to preserve (when used in preventive strategies) or to restore (if used as therapeutics or adjuvants) the right equilibrium between Wnt ON and Wnt OFF, thus reinstating the signaling aberration that leads to the onset and progression of the diseases. Specifically, in the neoplastic disease, in which the Wnt/β-catenin pathway is generally, but not always, constitutively activated, these natural modulators act almost always as inhibitors. Conversely, in other diseases such as neurodegenerative disorders, in which the activation of the pathway has neuroprotective ability[13,14], they have beneficial effects by functioning as Wnt pathway activators[106,107]

As it concerns the use of these nature-derived compounds as a preventive and therapeutic strategy in neoplastic diseases, most of them are still in preclinical development and only three are already under clinical investigations. Therefore, in the following section we focus on the most significant natural compounds that demonstrated significant pharmacological properties in relevant model of neoplastic diseases (Table 1).

Methods used for the pharmacological screening of natural compounds described in this review include bioassay traditionally employed for the evaluation of anticancer potential of every kind of experimental drugs. Specifically, the most used screening systems are cell viability and proliferation assay, cell cycle analysis, wound healing and transwell migration assay. These methods are used to evaluate the potential cytotoxicity and anti-metastatic activity. Relating to the scope of this review, the evaluation of compounds that modulate the Wnt/β-catenin pathway was conducted through the following main techniques: Top/Fop Flash, Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blot analyses. Top flash is a cell-based luciferase assay system that involves the use of cell lines that are transfected with TCF/LEF reporter for monitoring the activity of Wnt/β-catenin signaling pathway through the employment of known inhibitor and experimental compound(s). Instead, Fop flash uses mutant TCF-binding site which does not respond to the Wnt signal[32]. These bioassays were used generally to monitor Wnt signaling pathway activity and to screen agonists or inhibitors.

4.1. *Flavonoids*

4.1.1. *Genistein*

Genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one) is the main isoflavone in soy (*Glycine max*) and was firstly extracted from *Genista tinctoria* L., a flowering plant of the family Fabaceae. It is considered a dietary phytoestrogen and the main

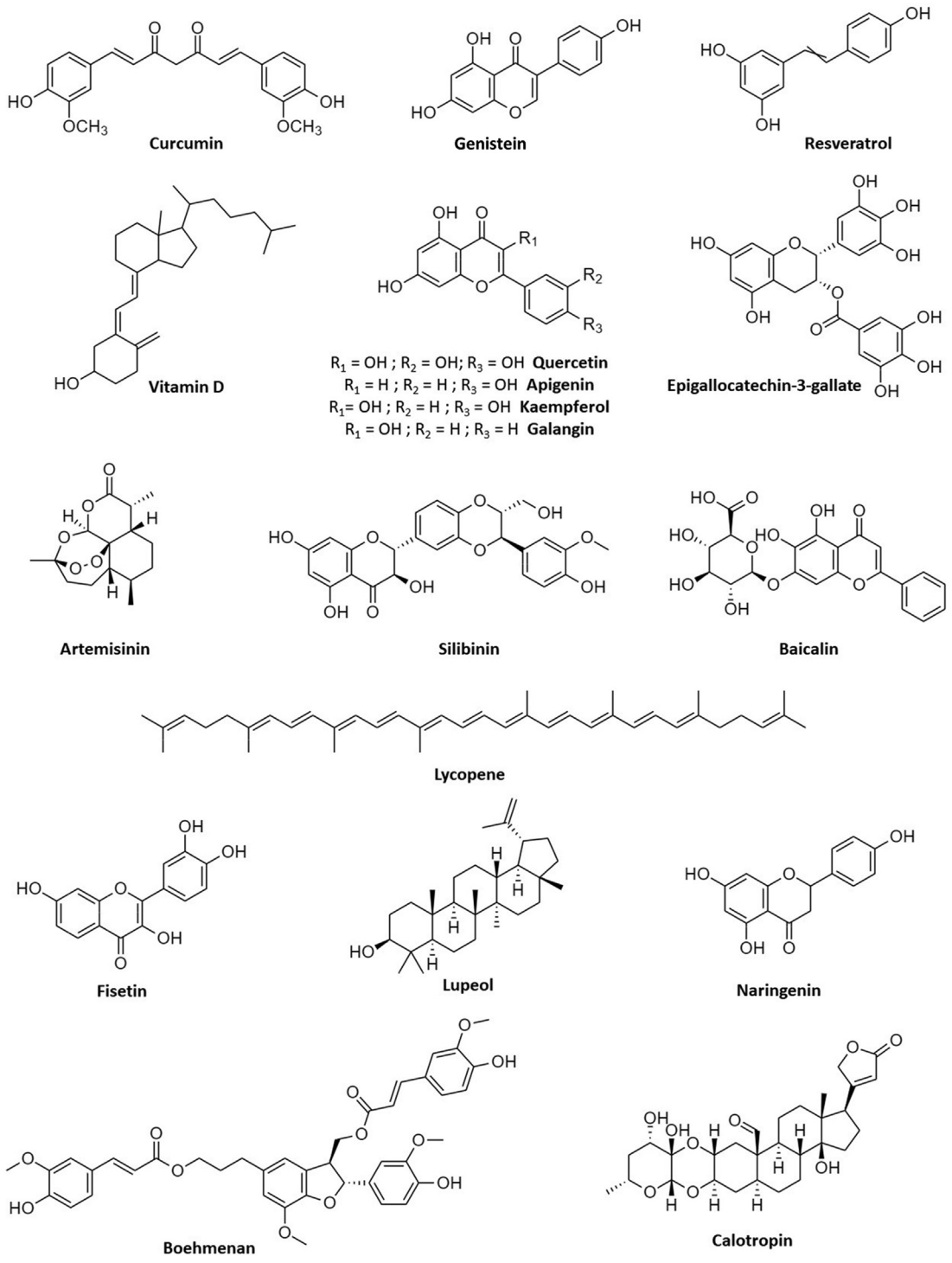

Figure 2 Molecular structures of nature-derived compounds that modulate the Wnt/β-catenin signaling.

sources of this isoflavone are soybean and soybean products[108,109]. Many different extraction techniques were reported in literature as effective for the recovery of isoflavones from natural sources and ultrasound assisted extraction and Quick Easy Cheap Effective Rugged and Safe (QuEChERS) methodology were recently reported as good strategies with high recovery yield[110]. Genistein is one of the main secondary metabolites in soy-derived food and together with other isoflavones entities and dietary phytoestrogen, it is consumed in significant quantities worldwide[111]. Moreover, genistein has different biological interesting effects, such as anti-inflammatory and pro-apoptotic effects, modulation of steroidal hormone receptors and metabolic pathways. According to literature, genistein is also able to change the expression of variety of Wnt target genes leading to the induction

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Table 1 Wnt pathway targeting natural-derived compounds that are under investigation on neoplastic diseases.

Compound and chemical class	Effect on Wnt/β-catenin pathway	Stage of development	Ref.
Curcumin Polyphenols: Curcuminoids - Diaril eptanoides	Inhibits p-GSK3β, β-catenin, E-cadherin and *N*-cadherin, cyclin D1 and the nuclear expression of dishevelled proteins. Prevents β-catenin/TCF DNA binding and transactivation.	**Phase I** in metastatic colon cancer patients in combination with 5-fluorouracil (NCT02724202) **Phase I** in advanced colorectal cancer in association with Irinotecan (NCT0185985) **Phase II** in locally advanced rectal cancer in combination with capecitabine and radiation therapy before surgery (NCT00745134) **Phase III** in locally advanced or metastatic adenocarcinoma of the colon in combination with celecoxib (NCT00295035)	46,63−65
Genistein Polyphenols: Flavonoids - Isoflavones	Inhibits of AKT phosphorylation and suppression of GSK-3β dephosphorylation. Promotes β-catenin phosphorylation Suppresses β-catenin/TCF-driven transcription. Induces the overexpression of E-cadherin, reduces the activity of WNT-1 and its targets such as c-Myc and cyclin D1. Reduces the gene expression of *Wnt-7a*. Inhibits the effect of Dkk-1. Downregulates of TCF reporter activity. Reduces the expression of Wnt5a, Sfrp1, Sfrp2 and Sfrp5.	**Phase I/II** in combination with FOLFOX or FOLFOX-Avastin for the treatment of metastatic colorectal cancer (NCT01985763)	3,31,46,63,66-71
Resveratrol Polyphenols: Stilbenoids	Decreases the expression of β-catenin, c-Myc, MMP7 and Survivin. Downregulates WNT2 and upregulate AXIN2 in Colo16 cells and Myc-tagged TCF4 protein. Upregulates of WNT1	**Phase I** in patients with suspected or documented colon cancer (NCT00256334)	30,46,63,72−74
Vitamin D Secosteroids	Decreases the binding of β-catenin to TCF. Increases E-cadherin and DKK-1	**Observational clinical study** in normal subjects and colorectal cancer patients (NCT00399607)	25,46,75,76
Quercetin Polyphenols: Flavonoids - Flavonols	Inhibits the binding between β-catenin and TCF. Inhibits GSK-3β phosphorilation. Increases the expression of E-cadherin.	**Preclinical**: *in vitro* and *in vivo*	33,46,68,77−79
EGCG (epi-gallocathechin-3-gallate) Polyphenols: Catekines	Downregulates the expression of p-GSK3β. Increases the expression of GSK3β and Reduces the level of β-catenin and its target genes. Inhibits the expression of β-catenin/TCF-4 receptor activity. Upreguates HBP1. Increases secreted frizzled-related protein expressions and E-chaderin	**Preclinical**: *in vitro* and *in vivo*	46,80−82
Artemisinin Sesquiterpenes	Decreases the expressions of Wnt5-a/b resulting in the suppression of β-catenin and in an increase of E-cadherin	**Preclinical**: *in vitro* and *in vivo*	83
Apigenin Polyphenols: Flavonoids - Flavones	Inhibits β-catenin/TCF/LEF interaction. Increases of E-cadherin and decreases nuclear β-catenin, c-Myc, and cyclin D1	**Preclinical**: *in vitro* and *in vivo*	84−88
Baicalin Polyphenols: Flavonoids	Reduces β-catenin mRNA and protein. Induces of DKK1 expression, reduces the expression of c-Myc Inhibits of microRNA-217 (negative regulator of DKK1)	**Preclinical**: *in vitro* and *in vivo*	89,90
Silibinin Polyphenols: Flavonoids	Suppresses Wnt co-receptor LRP6 expression. Reduces of β-catenin, cyclin D1 and c-Myc	**Preclinical**: *in vitro* and *in vivo*	91,92
Galangin Polyphenols: Flavonoids - Flavonols	Reduces the expression at protein and mRNA levels of Wnt3a and β-catenin. Downregulates β-catenin intracellular levels and the expression of β-catenin/TCF-dependent genes, such as cyclin D1 and c-Myc.	**Preclinical**: *in vitro* and *in vivo*	32,93,94
Fisetin Polyphenols:	Downregulates β-catenin and TCF4 and its	**Preclinical**: *in vitro* and *in vivo*	95−97

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Table 1 (*continued*)

Compound and chemical class	Effect on Wnt/β-catenin pathway	Stage of development	Ref.
Flavonoids	target genes such as cyclin D1 and MMP7. Reduces phosphorylation of GSK3-β and decreases β-catenin stabilization. Modulates the interaction between β-catenin and LEF/TCF-2 leading to the downregulation of c-Myc, Brn-2 and Mitf and its downstream targets		
Lupeol Triterpenoids	Reduces the nuclear expression of β-catenin and the formation of β-catenin/TCF4 complexes. Downregulates of the expression of GSK-3β, leading to the suppression of Akt1, PI3K, β-catenin, c-Myc and cyclin D1 mRNA expression	**Preclinical**: *in vitro*	98−100
Kaempferol Polyphenols: Flavonoids - Flavonols	Inhibits β-catenin/TCF transcriptional activity	**Preclinical**: *in vitro*	88,101
Lycopene Carotenoids	Inhibits β-catenin, c-Myc and cyclin E protein levels. Reduces activation AKT and GSK-3β phospho-inhibition. Induces a reduction in TCF/LEF reporter activity, β-catenin and cyclin D1.	**Preclinical**: *in vitro*	63,102
Naringenin Polyphenols: Flavonoids - Flavonones	Suppresses β-catenin/TCF transcriptional activity	**Preclinical**: *in vitro*	32,103
Boehmenan Polyphenols: Lignans	Decreases cytosolic and nuclear β-catenin and c-Myc	**Preclinical**: *in vitro*	104
Calotropin Carotenoids	Decreases cytosolic and nuclear β-catenin by increasing its phosphorylation mediated by CK1α	**Preclinical**: *in vitro*	105

of cell-cycle arrest, apoptosis and/or inhibition of epithelial−mesenchymal transition (EMT) and metastasis[112]. Indeed, genistein is a multitarget drug with several pharmacological activities including chemopreventive and anticancer efficacy in various type of tumors. It affects cell cycle, angiogenesis and inhibits metastasis[113]. Differently from curcumin, genistein bioavailability after oral administration does not represent a critical issue; the molecule is readily available as reported in preclinical and human trials[114].

Several *in vitro* and *in vivo* studies analyzed the effect of genistein on Wnt/β-catenin pathway and demonstrated an inhibitory effect through the modulation of different key molecules. Genistein regulates the upstream components of the β-catenin/TCF pathway by inhibiting AKT phosphorylation and suppressing GSK-3β dephosphorylation, thus facilitating the ubiquitylation and degradation of β-catenin[66]. Sarker et al.[67] showed that genistein increases the expression of GSK-3β, induces β-catenin attachment to GSK-3β and promotes β-catenin phosphorylation levels, leading to the suppression of prostate cancer growth. Other studies have also demonstrated that genistein potently suppresses β-catenin/TCF-driven transcription in AGS gastric cancer cells[33,68]. Su et al. demonstrated that genistein induces the overexpression of E-cadherin and reduces the activity of WNT-1 and its targets such as c-Myc and cyclin D1 in mouse mammary epithelial cell line HC11[115,116]. Furthermore, an *in vivo* study showed that genistein was able to inhibit Wnt pathway reducing cyclin D1 in mammary ductal epithelium of animals[31]. Genistein was also demonstrated to reduce the gene expression of Wnt-7a in endometrial adenocarcinoma cells[31,69].

Recent *in vitro* study on several renal cancer (RCC) cell lines and *ex vivo* analyses performed on tissues from 43 patients affected by clear renal cancer demonstrated that genistein induces apoptosis, inhibits proliferation and invasion through the downregulation of TCF reporter activity and miR-1260b, that has known to be overexpressed in renal cancer tissues, affecting cancer aggressiveness[70]. Its biological function was also strictly correlated with the β-catenin-dependent pathway in RCC cell lines. The study of Hirata et al.[70] demonstrated that genistein inhibited Wnt-signaling by regulating miR-1260b expression in renal cancer cells.

In another *in vivo* study on colon cancer murine model, Zhang et al.[71] demonstrated that genistein inhibited WNT target genes cyclin D1 and c-Myc and its signaling, reducing the expression of *Wnt5a*, *Sfrp1*, *Sfrp2* and *Sfrp5*. Results obtained from this study suggested that genistein possesses protective role in the development of early colon neoplasia.

As previous reported, genistein has been extensively studied as drug for cancer prevention and treatment. Over the years, 29 clinical trials have been conducted (on *ClinicalTrials.gov*), and 1 of 29 aimed to correlate the effect of genistein with the inhibition of the Wnt/β-catenin pathway (NCT01985763). This study is a phase I/II clinical trial that was conducted hypothesizing that the combination of genistein with the standard of care might reduce chemotherapy resistance and improve response rates in patients. The study was designed in order to administrate genistein with FOLFOX or FOLFOX-Avastin in 13 patients with stage IV colon or rectal neoplasms. The trial was completed in 2018 but no results have been published to date.

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Relating to the safety profile, conflicting results have been described in scientific literature. It has been reported the potential toxic effects of genistein on fertility and fetus development. Some studies demonstrated that genistein has a negative effect on ovarian differentiation, estrous cyclicity, and fertility in a rodent model. However, data from clinical trials for the determination of these potential critical issues about the use of genistein are still lacking[114].

4.1.2. Quercetin

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) is a polyphenol compound widely diffused and a common phytochemical in human diet and can be found in nuts, tea, onions, apple and in plant sources. Quercetin can also be found in dietary supplements[117]. Quercetin exhibits many beneficial activities for human health and in particular an abundant antioxidant activity due to free radicals scavenging leading to the protection of human DNA from oxidative damages[118]. Moreover, epidemiological studies highlighted that quercetin shows anti-inflammatory, antitumor, antiviral, antiallergic, and anti-edematous activities, and may provide prevention from cardiovascular pathologies[119]. Due to these multiple activities attributed to quercetin, in recent years its extraction and determination have acquired a growing interest. At date, the extraction of quercetin from natural sources can be achieved by some innovative and green techniques: molecular imprinted polymers (MIPs) were used for the recovery of this polyphenol from red wine[120] and deep eutectic solvents (DESs)-based polymeric monolithic cartridges were applied for the extraction of quercetin from *Gingko biloba*, a China native large tree[121]. Quercetin has also been detected in some traditional remedies such as in *Saururus chinesis* leaves used in Korean traditional medicine for the treatment of pain and other affections[122].

Quercetin has been considered a potential antitumor compound, based on the reported action on Wnt pathway, as described by *in vitro* and *in vivo* studies[66,123]. Specifically, *in vitro* studies demonstrated that quercetin acts by inhibiting the binding between β-catenin and TCF in SW480 colorectal cancer cell line and in HEK293 cells transfected with constitutively active mutant β-catenin gene[77,124]. The anticancer efficacy of quercetin was also confirmed in other cancer cell lines including leukemia and lymphoma cells[125], colon cancer[126], melanoma[127] and hepatocarcinoma[128].

Recent studies also suggested a promising effect of quercetin in triple negative breast cancer (TNBC) both in *in vitro* and *in vivo* models. Specifically, Srinivasan et al.[78] have shown that the treatment of MDA-MB-231 and MDA-MB-468 cells with quercetin increases the expression of E-cadherin and downregulates vimentin levels in TNBC, both markers of mesenchymal-to-epithelial transition. This effect was mediated by the modulation of β-catenin and its target genes such as cyclin D1 and c-Myc. Similar effects have been reported by Sultan et al.[79] in MDA-MB-231 and MDA-MB-157 TNBC cells and in a TNBC xenograft mouse model. The authors showed that quercetin induces apoptosis in TNBC cells by reducing the expression of different molecular markers including β-catenin. Moreover, the evaluation of anticancer activity of *in vivo* quercetin treatment in the TNBC model demonstrated the inhibition of tumor xenograft growth by 41.7% than the control group. These data support the potential use of this molecule for the treatment of TNBC[79]. Despite quercetin is being evaluated in oncologic trials as anticancer drug, none of these clinical studies evaluated its effect on the Wnt/β-catenin signaling.

4.1.3. Apigenin

Apigenin (4′,5,7-trihydroxyflavone) is a secondary metabolite from common plants, belonging to the flavone class of flavonoids. Apigenin can be found in many plants and food sources (parsley, celery, celeriac, cilantro and oregano) and even in Chinese traditional remedies (*i.e.*, *Scutellaria barbata* D. Don) and it is particularly abundant in chamomile flowers (Asteraceae)[129,130]. Throughout literature, several methods have been used for the extraction of apigenin including conventional techniques (*i.e.*, maceration and heat reflux extraction) and more advanced ones (microwave assisted extraction and ultrasound assisted extraction). Among the more innovative techniques, supercritical carbon dioxide approach gained an increasing interest due to high effectiveness and less environmental impact compared to the other methods. Most recently, a green and highly efficient technique for the recovery of apigenin from a Chinese traditional plant was developed by Yang and Mei[129] obtained by coupling ultrasound assisted extraction technology with supercritical fluid extraction. Apigenin is known as a flavonoid with many interesting beneficial biological actions including antioxidant, antimutagenic, anticarcinogenic, antiinflammatory, and antiproliferative[131]. Due to its antitumor activity and low toxicity, apigenin has been widely researched and observed to suppress different human cancer both *in vitro* and *in vivo* studies[130] and many molecular target have been described for apigenin in human cancer[131].

Apigenin was the first molecule belonging to the flavonoid class known to have an effect on Wnt pathway[33]. This natural compound possesses anticancer capacity both *in vitro* and *in vivo*, by suppressing cell migration and invasion, and inducing apoptosis and cell cycle arrest[84,132]. Its antitumor ability has been ascribed to the inhibition of β-catenin/TCF/LEF interaction, that suppresses the β-catenin nuclear entry and the expression of Wnt target genes in SW480 and HCT15 human colorectal cancer cells[85,86] and in U2OS and MG63 osteosarcoma cells[133]. *In vivo* evidence suggested that apigenin significantly reduces tumor volumes and completely inhibits lymphonodal, liver and lung metastases in a transgenic mouse model of prostatic adenocarcinoma and these effects have been related to an increase of E-cadherin and a decrease of nuclear β-catenin, c-Myc, and cyclin D1[87].

4.1.4. Baicalin

Baicalin is a flavonoid compound found in roots of different species of the genus *Scutellaria* (*S. laterifolia* and *S. galericulata*), and in particular it is the active metabolite responsible for the biological action of *Scutellaria baiacalensis* Georgi, a Chinese medicinal plant used to treat psoriasis[134]. The more common techniques applied for the extraction and isolation of baicalin are heat reflux extraction (HRE), ultrasound-assisted extraction (UAE) supercritical fluid extraction (SFE)[135]. Moreover, other more sophisticated techniques were developed for the isolation and purification of baicalin, such as deep eutectic solvents, ultrahigh pressure technology[136] and molecular imprinted polymers[137]. Baicalin exhibits an anticancer activity toward many kinds of cancers, including ovarian, prostate, breast and pancreatic cancers, esophageal squamous cell carcinoma, as well as Burkitt lymphoma[138]. The exact mechanism of action is not completely understood but it seems that it acts through multiple mechanisms involving the induction of apoptosis and the modulation of

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different pathways also including the Wnt/β-catenin signaling[89,139]. Jia et al.[89] demonstrated that the treatment with baicalin of colon cancer DLD1 and HCT-116 cells inhibits their proliferation and induces apoptosis. This effect was mediated by the induction of DKK1 expression, a relevant antagonist of Wnt signaling pathway, thus reducing the expression of β-catenin and c-Myc. The authors also demonstrated that the mechanism of action of baicalin on these cells also involves the inhibition of microRNA-217, negative regulator of DKK1. Therefore, it was shown that baicalin induces apoptosis through the miR-217/DKK1-mediated inhibition of Wnt signaling pathway[89]. Baicalin was tested also on triple negative breast cancer cell line MDA-MB-231 and mouse mammary cancer cell line 4T1[90]. The molecule did not affect cell viability but demonstrated a potential effect on migration and invasion in a dose dependently manner, and reverted epithelial-to-mesenchymal transition process by targeting β-catenin signaling. The same authors in an *in vivo* xenograft metastasis tumor model of 4T1 breast cancer cells also confirmed these results. Specifically, the compound reduced the number of liver and lung metastases through the downregulation of epithelial-to-mesenchymal transition markers and the inhibition of β-catenin in tumor tissues[90].

4.1.5. Silibinin

Silibinin is a polyphenol belonging to the flavolignan class. It is the major active compound in the extract of *Sylibum marianum* (commonly named milk thistle plant) seeds, commonly used as a medicinal plant in CTM, is recently gaining interest in western societies for the treatment of liver diseases and diabetes. In nature, Silibinin exists in a mixture of two diastereomers, silibinin A and silibinin B in a ratio of 1:1. Typically, the extraction of this polyphenol, as also described in *European Pharmacopoeia*, is achieved by two solvent steps using *n*-hexane and methanol, respectively. This procedure is achieved by means of traditional Soxhlet apparatus and requires long time of processing. In order to reduce time of extraction and to improve the recovery of silibinin, many improved methodologies have been studied, such as the pressurized liquid extraction technique developed in a recent work[140]. Silbinin has been traditionally used as nutritional supplement for hepatoprotection, but it has also demonstrated to exert anticancer activity in different *in vitro* and *in vivo* models of solid tumors such as skin, breast, lung, colon, bladder, prostate and kidney carcinomas. It has been reported that this effect are correlated with the modulation of Wnt/β-catenin pathway. Specifically, *in vitro* test showed that silibinin inhibited migration and invasiveness of PC3 prostate cancer cells through different mechanisms also including the increment of E-cadherin at cell membrane and the reduction of nuclear β-catenin[141]. Silibinin-induced suppression of cell growth correlated to the inhibition of the Wnt/β-catenin signaling was also demonstrated in the human colorectal carcinoma cell line SW480 and in the xenograft model, where the compound inhibited tumor growth by decreasing the expression of β-catenin, cyclin D1, c-Myc[142]. Another study demonstrated that silibinin acts as suppressor of the Wnt co-receptor LRP6 and that the antitumor activity is mediated by the inhibitory effect on Wnt/LRP6 signaling in in prostate and breast cancer cells[91]. The antitumor activity of silibinin was also confirmed *in vivo* in $Apc^{Min/+}$ transgenic mouse model of intestinal carcinogenesis. The natural compound prevented the polyp formation in small intestine and colon and this chemopreventive effect was mediated by the decrease of β-catenin levels and its transcriptional activity[92]. Similar results were also obtained in other *in vivo* models of colon carcinogenesis[143,144].

4.1.6. Galangin

Galangin (4*H*-1-benzopyran-4-one,3,5,7-trihydroxy-2-phenyl or 3,5,7-trihydroxyflavone) is a polyphenol compound naturally occurring in plants of the genus *Alpinia* of the Zingiberaceae family (*Alpinia officinarum*, *A. galangal*). These herbs have been widely used as traditional remedies in different regions of the world like Asian and African countries, for the treatment of many diseases. Moreover, *A. officinarum* is reported as a safe food spice for non-medical usages[145]. Extraction of bioactive compounds and in particular galangin from this genus has been purchased by the application of several techniques (*i.e.*, Soxhlet extraction, maceration, ultrasonication, and soaking) and different solvents such as petroleum ether, ethyl acetate and water[146,147]. Galangin has been suggested to have antimutagenic, antioxidant, and muscle contraction inhibitor properties[148]. In recent years, scientific studies focused on the evaluation of its beneficial effects on animal models in terms of anticancer activity[93,149] and vascular disorders treating[150]. Galangin belongs to the class of flavonol and possesses anti-oxidant, anti-inflammatory and antitumor activities as demonstrated in a variety of *in vitro* and *in vivo* models[93]. *In vitro* studies on the human oesophageal carcinoma cell lines Eca9706, TE-1, and EC109 showed that treatment with galangin resulted in cell growth inhibition, induction of apoptosis and cell cycle arrest. The molecular analysis revealed that galangin reduced the expression of *Wnt3a* and β-catenin at protein and mRNA levels. The antitumor efficacy was also confirmed in nude mice with xenograft tumors, where the treatment increased TUNEL positivity and the level of the onco-suppressor p53 and reduced the expression of Wnt3a and of the proliferation marker Ki-67[93]. Other *in vitro* studies indicated that galangin is able to suppress the proliferation of colorectal and hepatocarcinoma cell lines due to the downregulation of β-catenin intracellular levels and the transcription of β-catenin/TCF-dependent genes, such as cyclin D1 and c-Myc[94].

4.1.7. Fisetin

Fisetin (7,3′,4′-flavon-3-ol) is a bioflavonoid occurring in plants. It is found in dietary sources like strawberries, apple, persimmons and onions and in many species of plants like Eudicotylendos and Fabaceae family (genus *Acacia*), among the others[151]. As other polyphenols, fisetin shows many beneficial biological actions, for instance the ability to scavenge free radicals leading to a high antioxidant activity. Moreover, many different studies were performed to evaluate its anti-inflammatory and antiallergic activity and recently, this polyphenolic compound gained an increased interest because of its action as memory-enhancing agent in rodents[152]. Like other flavonoids, fisetin possesses antiproliferative activity against different cancers and many reviews have been published focusing on this chemopreventive potential[151,153,154]. The extraction of Fisetin from natural sources has been performed by applying acidic conditions (typically by HCl)[155] and in some cases, an ultrasonic-assisted extraction was described[155]. Scientific literature indicated that fisetin is a potent anticancer agent against different solid tumors such as lung, breast, colon and pancreatic cancer. It has been demonstrated that fisetin regulates Wnt signaling in colon cancer cell line HT-29 through the downregulation of β-catenin and TCF4 and its target genes such as cyclin D1 and matrix metalloproteinase 7[95,96]. The modulation of Wnt pathway by fisetin was also demonstrated in *in vitro* and

in vivo melanoma models[97]. Specifically, the *in vitro* study on 451Lu melanoma cells showed that fisetin induces a reduction of cell viability and G1 cell cycle arrest. This effect was correlated to the suppression of Wnt signaling and specifically to the decreased phosphorylation of GSK3-β associated with decreased β-catenin stabilization[97]. Fisetin also modulates the interaction between β-catenin and LEF/TCF-2 that resulted in the downregulation of the TCF target genes *c-Myc*, *Brn-2* and microphthalmia-associated transcription factor (*Mitf*). In the *in vivo* study, carried out on 451Lu-xenografted nude mice, fisetin induced the inhibition of tumor growth that is mediated by the inhibition of the β-catenin/Mitf signaling[97].

4.1.8. Kaempferol

Kaempferol (3,4′,5,7-tetrahydroxyflavone), is a flavonol, a kind of flavonoids, contained in many dietary and plant sources (in Angiosperm family in particular), such as grapes, tomatoes, broccoli, tea, cabbage in *Ginkgo biloba* leaves and *G. max* among the others. Typically, the extraction of this flavonoid was achieved by using solvents (mainly polar solvents like methanol, ethanol and water) and different approaches were applied (from conventional solvent extraction to ultrasound and microwave assisted extraction). One very convenient extraction recently described for kaempferol was achieved by supercritical fluid technology as reported by Ortega and co-workers in 2018[156]. Being a flavonoid, kaempferol exhibits an abundant antioxidative activity by reducing superoxide ions and lowering the formation of reactive oxygen species. Kaempferol is also a promising anticancer agent by promoting inhibition of angiogenesis and apoptosis. Kaempferol additionally exerts many others health perspectives in terms of antidiabetic, anti-inflammatory, antiaging and antiallergic activity[157]. It was demonstrated that this compound inhibits Wnt pathway in colorectal cancer cell lines SW480 and HEK293, through the suppression of the β-catenin/TCF transcriptional activity[88]. Qin et al.[101] have shown that kaempferol has antiproliferative and anti-invasive properties in retinoblastoma SO-RB50 cells. The effect was explained on the basis of the interaction with estrogen-related receptor α that induced the suppression of Wnt/β-catenin signaling[101].

4.1.9. Naringenin

Naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one) is a flavonoid belonging to the flavonone subclass. Naringenin is known as one of the most ingested flavonone by people because of its vast distribution in *Citrus* genus. Indeed, it is the most abundant flavone in grapes, tangelo, blood orange, lemons, pomelo, and tangerines, and it is found in cherries grapefruit and cocoa[158]. Naringenin possesses board biological beneficial effects for human health including antioxidant, anti-inflammatory, antidiabetic, antiproliferative and anticancer activity[159]. The extraction of naringenin has been performed by the techniques typically applied for other flavonoids and recently, a green and efficient extraction methodology based on ultrasound-assisted deep eutectic solvents extraction was developed[160]. As mentioned above, naringenin has demonstrated to have several pharmacological activities such as antioxidant, antitumor, anti-inflammatory, antiadipogenic and cardioprotective effects. These have been demonstrated in preclinical and in clinical trials. Scientific evidence on the activity that naringenin exerts on Wnt-β-catenin pathway has been also described. The natural compound demonstrated to suppress Wnt signaling in AGS human gastric cancer cells. Naringenin acts suppressing the TCF transcriptional activity in a concentration-dependent manner[103].

4.2. Other polyphenols

4.2.1. Curcumin

Curcumin [(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a diarylheptanoid (curcuminoid) extracted from the rhizome of *Curcuma longa* L., a flowering plant of the ginger family (Zingiberaceae)[161], cultivated in tropical and subtropical regions, such as Asia and Central America. *C. longa* L., also known with the common name of turmeric, is widely used in traditional medicines such as in Ayurvedica and Chinese traditional medicine (CTM). The extraction of curcuminoids, and in particular curcumin, from the rhizome of *C. longa*, firstly achieved in 1818 by Vogel and Pelletier[162], has been vastly described in literature and many different approaches were applied[163–165]. Indeed, throughout literature many conventional techniques were reported as organic solvents extraction, steam distillation, hot and cold percolation, use of hydrotrope, and alkaline solution. In addition, several advanced techniques have been studied like supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, and enzyme assisted extraction. Most recently, Patil and co-workers[166] described an advanced three-phase partitioning and batch extraction as an innovative technique suitable for the extraction of curcuminoids from natural sources. *C. longa* is utilized also in culinary uses as yellow/orange spice, therefore, curcumin can be assumed for medical purposes if utilized in traditional herbal remedies or as dietary ingredient. Curcumin expresses interesting *in vitro* and *in vivo* biological activities such as anti-inflammatory[167], antioxidant[168] and neuroprotective[169] among the others. In addition, anticancer activity of curcumin was widely investigated[64]. The biological effect is mediated by the modulation of different pathways known to have a crucial role in pathological conditions, including the Wnt/β-catenin pathway. Curcumin acts on different critical molecular components of the signaling cascade, whose overexpression is involved in cancer initiation, progression and resistance. For example, in breast cancer cells curcumin induces cell proliferation arrest and apoptosis, also inhibiting their migration and invasion of through the inhibition GSK-3β, β-catenin and its nuclear localization, E-cadherin, and cyclin D1[65,170]. Other studies demonstrated that curcumin inhibits β-catenin, p-GSK3β protein expression, cyclin D1 and c-Myc in non-small-cell lung cancer (NSCLC) and medulloblastoma cell lines and dramatically decreases proliferation and the epithelial−mesenchymal transition in SW620 human colon cancer cells, through different mechanisms including also the downregulation of the Wnt pathway[171–173]. Curcumin has also a promising antitumor activity when used to target cancer stem cells in colorectal, breast and lung cancer through a specific action on Wnt/β-catenin pathway and its target genes[174–178].

A recent *in vivo* study in a mouse model of colon cancer has demonstrated a new mechanism of regulation of Wnt/β-catenin pathway by curcumin, leading to an antitumor effect through the downregulation of β-catenin and TCF4. The authors demonstrated that the action of curcumin on the pathway was mediated by the downregulation of the microRNA (miR)-130a and miR-21. Specifically, the miR-130 was revealed as negative regulator of curcumin anticancer activity. In fact, the overexpression of miR-130a in SW480 cells abolished the curcumin-induced inhibition of cell proliferation as well as the downregulation of β-catenin, and this

suggests that curcumin regulates Wnt/β-catenin pathway by inhibiting miR-130a[177]. Another recent study conducted by Marjaneh et al.[179] explored the efficacy of a novel formulation of phytosomal curcumin in combination with 5-FU in a mouse model of colitis-associated colon cancer by evaluating the expression of cyclinD1, beclin, E-cadherin, and p-GSK3α/β. Results showed that the association strongly reduces inflammation and tumor volume and number, through modulation of the Wnt pathway and E-cadherin.

The potent antiproliferative effect observed in *in vitro* and *in vivo* model of solid tumors of lung, breast, gastrointestinal tract and central nervous system has led to explore the pharmacological potential of curcumin also in clinical trials. In the last 10 years, 33 clinical studies have been conducted in order to evaluate the antitumor potential of curcumin in cancer patients[180]. Specifically, four of them have been designed in order to use curcumin for targeting the Wnt/β-catenin pathway in CRC. They are evaluating the safety and efficacy of curcumin in association with 5-FU and with irinotecan in two phase I studies as preoperative neoadjuvant standard radiation therapy and chemotherapy in a phase II study, and in combination with celecoxib in a phase III clinical trial. These studies are in ongoing and no results have been published to date[46]. Although a potential anticancer effect has been also demonstrated in patients, further studies are warranted to validate the use of curcumin as preventive and therapeutic treatment as monotherapy or in combination with standards of care. Curcumin and its derivatives have been considered safe as shown by the clinical trials conducted until now. The main side effects reported are mild and mainly affecting the gastrointestinal system[181]. As known, the bioavailability of curcumin is a critical issue that affect antitumor efficacy in patients. New clinical development strategy aiming to define a better patient population through specific biomarkers is needed in order to optimize and potentially validate the use of curcumin as anticancer medicines.

4.2.2. *Resveratrol*

Resveratrol (3,5,4′-trihydroxy-trans-stilbene, **2**) is a stilbene contained in a wide range of food sources such as grape, blueberries and peanuts. In particular, grape peel contains high quantities of resveratrol and its glycosides[182]. Resveratrol represents one of the most interesting natural bioactive compounds because of its high beneficial potential in health applications. Indeed, several *in vitro* and *in vivo* studies have highlighted the antioxidant, anti-inflammatory, cardioprotective, neuroprotective and antitumor actions[183]. Many efforts have been faced in the extraction of resveratrol and the recovery from grape peel remain challenging because of the poor solubility of this stilbene in water and in other common solvents typically used for solvent-based extraction. Many approaches have been applied and the optimum quantitative extraction of resveratrol from grape peel (after infection with powdery mildew) has been gained by using an aqueous solution of ethanol at 60 °C[184] and other methods focused on the conversion of piceid (a resveratrol glucosyde derivative) to resveratrol to improve the extraction yield by acid hydrolysis, heat application or enzyme treatment[185−187] Recently, in order to achieve a more rapid and economic procedure, Averilla and co-workers[182], described an innovative method combining enzymes and heat to improve the extraction recovery.

As mentioned, resveratrol is a potent antioxidant and anti-inflammatory agent that has been extensively studied during the last years. It has been demonstrated that resveratrol possesses antiproliferative and antimetastatic activity through the modulation of different mechanisms[30,33,188]. In particular, it has been demonstrated that resveratrol decreases the expression of β-catenin in the nucleus of colon cancer cells and this seems to be correlated to the downregulation of molecular regulators of β-catenin localization[30,189]. Very recently, Mineda et al.[190] reported that resveratrol induces apoptosis and arrests cell proliferation in human uterine sarcoma cell line MES-SA through a dose-dependent downregualtion of β-catenin and c-Myc. Geng et al.[191] have also demonstrated that resveratrol was able to inhibits proliferation, migration and invasion of multiple myeloma cells (U266 and LP1) through a reduced expression of nuclear β-catenin, c-Myc, MMP7 and survivin. Other authors showed that resveratrol inhibits gastric cancer cell line MGC-803 through downregulation of the Wnt/β-catenin signaling pathway[192]. Specifically, the antitumor effect was mediated by the reduction of the expression of β-catenin, c-myc, and cyclin D1[192].

Cilibrasi et al.[72] demonstrated that resveratrol inhibits proliferation and motility in glioma stem cells lines isolated from patients affected by glioblastoma multiforme. The efficacy of resveratrol in these models was strongly correlated with the upregulation of WNT1 and MYC[72,193].

Resveratrol also inhibits proliferation of human osteosarcoma cells lines MG-63 and U2-OD by downregulating the expression of β-catenin[194,195]. After treatment with resveratrol, a downregulation of Wnt/β-catenin pathway target genes, such as β-catenin, c-myc, cyclin D1, MMP-2 and MMP-9 was shown, while E-cadherin level increased[195].

Resveratrol has also a promising effect on breast cancer stem-like (BSCS) cells isolated from MCF-7 and SUM159, and exhibited antitumor potential in a xenograft model of SUM159 cells in NOD SCID mice. Resveratrol inhibits the growth of tumors through different mechanisms including the reduction of the expression of β-catenin and cyclin D1 in both *in vitro* and *in vivo* models[196].

It has been also demonstrated that resveratrol improves the efficacy of temozolomide by increasing the sensitization of glioma resistant to the drug, both *in vitro* and *in vivo*. through the inhibition of Wnt2 and β-catenin expressions[197].

The antitumor effect of resveratrol has been also investigated in clinical trials. To date, 20 clinical studies were published on *ClinicalTrials.gov*. Two of 20 studies support the use of resveratrol as anticancer due to the effect of resveratrol on the Wnt/β-catenin pathway. A phase I clinical study (NCT00256334) was conducted on 11 patients with suspected or documented colon cancer. The primary outcome of the study was based on the investigation of the potential effect of resveratrol on Wnt signaling both in colon cancer and normal colonic mucosa. Results obtained from the study demonstrated that resveratrol was not able to inhibit Wnt pathway in colon cancer but showed a statistically significant inhibition of Wnt target gene, cyclinD1 and axin II, in normal colonic mucosa, suggesting a potential use of resveratrol as a preventive treatment for colon cancer[73].

As it concerns the pharmacokinetic profile, recent clinical studies reported that resveratrol is rapidly absorbed after oral administration and is well tolerated with no adverse reactions reported[198,199]. However, resveratrol has low bioavailability due to rapid and extensive metabolism in the intestine and liver but new formulations effective for improving its absorption and bioavailability have been already developed[74].

In any case, although numerous *in vitro* and *in vivo* studies and various clinical trials have been conducted up today, further clinical investigations are warranted in order to characterize the

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potential use of Resveratrol as preventive or treatment strategy in cancer.

4.2.3. Epigallocathechin-3-gallate (EGCG)

Epigallocatechin-3-gallate ([(2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromen-3-yl]3,4,5-trihydroxybenzoate, EGCG), a type of catechin, is the ester of epigallocatechin and gallic acid. EGCG is the most abundant catechin in tea (*Camellia sinensis* and *C. assanica*) and tea-based ready-to-drink beverage and can also be easily assumed with diet by the consumption of apples (apple skin), onions and hazelnuts and by the administration of dietary supplements[200]. As all the other polyphenol compounds, EGCG has a strong antioxidant activity leading to an interesting beneficial potential for human health. Indeed, due to the anti-inflammation, anti-mutagenic and anti-viral activities, EGCG has a good potential for the prevention of chronic diseases like diabetes and cancer among the others[201]. The extraction of this polyphenol was purchased by the application of always greener and more efficient techniques, such as ultrasound assisted extraction[200] and deep eutectic solvents technology[202].

EGCG is a potent antioxidant that has been extensively studied as treatment of several diseases including cancer as preventive or treatment strategies. *In vitro* and *in vivo* studies confirmed that this natural compound exerts its potential antitumoral action through different mechanism also including the modulation of the Wnt/β-catenin signaling. Studies on colorectal cancer stem cells demonstrated that EGCG suppresses the spheroid formation from the human colon cancer cells DLD-1 and SW480, by inducing apoptosis and inhibition of proliferation[80]. Specifically, EGCG downregulated the expression of p-GSK3β, increased the expression of GSK3β and reduced the level of β-catenin and its target genes[80]. The pharmacological activity of EGCG on cancer stem cells (CSCs) was also confirmed on lung cancer A549 and H1299 cells. EGCG reduced lung CSCs activity by inhibiting tumorsphere formation, decreasing lung CSCs markers, suppressing proliferation and inducing apoptosis. These effects were mediated by the downregulation of crucial molecules of the Wnt/β-catenin signaling including p-GSK3β[203]. The EGCG antitumor activity mediated by an effect on the Wnt/β-catenin pathway was also confirmed in other cell lines such as gastric and colon cancer[204.205]. Other studies showed that EGCG modulates the pathway by decreasing β-catenin levels and inhibiting the β-catenin/TCF-4 reporter activity[81], and/or by inducing HBP-1, one of the specific transcriptional repressor of the signaling[206].

The anticancer potential of EGCG was also confirmed by *in vivo* experiments. The combined administration of EGCG with fish oil significantly reduced the number of tumors through the modulation of β-catenin in $Apc^{Min/+}$ transgenic mouse model of intestinal carcinogenesis[207]. Similar results were obtained in a mouse model of liver carcinogenesis where EGCG was used in combination with theaflavin[82]. Specifically, in this mouse model, the EGCG/theaflavin association was able to reduce the expression of β-catenin and its phosphorylated form, increase secreted frizzled-related protein expressions and E-chaderin, and decrease the transcription of Wnt target genes such as cyclinD1 and cMyc[82].

4.2.4. Boehmenan

Boehmenan is a lignan from the Chinese medicinal plant *Clematis armandii*, named "Chuan-Mu-Tong" in *Chinese Pharmacopoeia* used against inflammatory conditions[208]. Lignans are fiber-associated polyphenols ubiquitous in human diet derived by phenylalanine found in plants and many common foods such as grains, nuts, seeds, legumes, vegetables, tea, coffee or wine[209,210]. Lignans are associated with many beneficial biological effects in mammals including antioxidant and antitumor actions. Indeed, numerous studies have shown that boehmenan possesses potent cytotoxic effects against many cancer cell lines[208,211]. In particular, boehmenan is able to reduce viability of colon cancer cells RKO, SW480, HCT116 through the decrease of cytosolic and nuclear β-catenin and c-Myc[104].

4.3. Terpenes and terpenoids

4.3.1. Artemisinin

Artemisin (1*R*,4*S*,5*R*,8*S*,9*R*,12*S*,13*R*)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.04,13.08,13] hexadecan-10-one) is an active sesquiterpene present in the extract of *Artemisia annua* (commonly called Qinghao), a medicinal plant included in the CTM for the treatment of fever and febrile illness[212]. Artemisinin is widely known as an antimalaric agent and was firstly extracted using a reproducible procedure (a low-temperature procedure, followed by a separation of acid portion from neutral one) by Youyou Tu and collaborators in 1971[213]. Due to its typical and unique peroxide-containing lactone structure and to the new biological properties ascribed, including antitumor and antiinfective activities, artemisinin has recently received increasing attention[214]. But the extraction of artemisinin is actually very challenging, because of the low concentration contained in the plant sources and, to fulfill the world demand of these valuable compounds, no single method alone is reliable. Therefore, many efforts have been applied in order to improve the production rate of artemisinin by using both conventional and advanced approaches[215].

Even if artemisinin is used over the years as anti-malarial treatment, in the recent years there is an increasing interest on the anticancer properties of this drug and its semi-synthetic derivatives artesunate and dihydroartemisinin. *In vitro* and *in vivo* studies revealed that these three drugs possess antitumor and antimetastatic activities even when used at very low concentrations[216–220]. The exact mechanism that supports the use of artemisinin as neoplastic agent in not completely understood. However, scientific literature hypothesizes different possible mechanisms including the modulation of Wnt/β-catenin pathway. A recent study conducted by Tong et al.[83], which evaluated the effects of artemisinin and its semi-synthetic derivatives in two different lung cancer cell lines, A549 (carcinoma *in situ*) and H1299 (adenocarcinoma from metastasis nodule) and in the A540 xenograft mice model, showed that these three drugs were able to inhibit cell proliferation, also significantly suppressing cell migration and invasion, and consistently reduce tumor volume and weight *in vivo*. The authors provided evidence that these compounds act by inhibiting the Wnt/β-catenin signaling.

4.3.2. Lupeol

Lupeol is a natural bioactive pentacyclic lupine-like triterpenodis[221]. It is found in several natural sources such as medicinal plants (*Senegalia visco*, *Abronia villosa*, *Gossampinus malabarica*, *Strobilanthes callosa*, *Ficus cordata*, *Albizia adianthifolia*, *Mimosa invisa*, *Klainedoxa gabonensis* and *Turraeanthus africanus* among the others) and dietary vegetables and fruits (mango pulp and peels)[222,223]. The extraction of lupeol has been performed by different methodologies, both conventional, such as maceration and Soxhlet, and more advanced techniques

(sonication, microwave and high hydrostatic pressures). By the comparison of the recovery yields, the most suitable approach seems to be sonication-based extractions[224,225]. Lupeol exhibits many pharmacological activities such as antioxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic and anti-mutagenic effects and exerts beneficial effects on different diseases, leading to the conclusion that this bioactive secondary metabolite is a multi-target agent with a promising pharmacological potential[221]. In particular, lupeol has selective antitumor potential on various human cancer cells. The anticancer activity of lupeol involves different mechanisms also including the suppression of Wnt/β-catenin signaling. A recent *in vitro* study conducted by Wang et al.[98] explored the effect of lupeol on two colorectal cancer cell lines: SW480 and HCT116. In these cells, lupeol was able to inhibit proliferation and migration and to promote apoptosis, and these effects were correlated with the down-regulation of transcriptional activity and protein expression mediated by the Wnt/β-catenin signaling. Specifically, lupeol decreased expression of β-catenin and TCF4 protein and reduced mRNA and protein expression of the downstream targets c-Myc and cyclin D1. The authors suggested that it is possible that potential mechanism of action could be correlated with the reduction of nuclear β-catenin expression and β-catenin/TCF4 interaction, with subsequent suppression of the signaling[98]. Zhang et al.[99] demonstrated that lupeol induced apoptosis in the hepatocellular carcinoma HCCLM3 cells in a time- and dose-dependent manner through different mechanisms including the decrease of GSK-3β phosphorylation in Ser9, with a concomitant suppression of Akt1, PI3K, β-catenin, c-Myc and cyclin D1 genes transcription[99]. That the anticancer effect of lupeol is mediated by the targeting of the Wnt/β-catenin signaling was also demonstrated in melanoma, in colorectal cancer and in prostate cancer cell lines[100,226,227].

4.3.3. *Lycopene*

Lycopene is a frequently occurring natural compound of the carotenoids class. It is an highly unsaturated hydrocarbon (11 conjugated and 2 unconjugated double bonds) found in abundant quantity in red fruits and vegetables, including tomato, watermelon, pink grapefruit, apricots, pink guava, and papaya[228], and it is responsible for the red color of these foods or plants. Lycopene exhibits important biological actions and its ability to suppress oxidation, inflammation, angiogenesis and cell migration and proliferation has been evaluated on different cell types[229]. Due to these activities, this compound was vastly studied for the treatment of different pathologies such as in the adjuvant therapy of diabetic neuropathy[230] and in the prevention of age-related macular degeneration and proliferative vitreoretinopathy[231]. Lycopene was extracted firstly in 1910 and it was usually purchased by using organic solvents. At date many extraction techniques have been developed in order to improve the reaction rate[232,233]. Indeed, Briones-Labarca and co-workers[234] have recently achieved an optimization of extraction yield of lycopene from tomato pulp by high hydrostatic pressure extraction (HHPE) technology.

Lycopene is a potent antioxidant and has antitumor activity in different type of solid cancers due to the modulation of Wnt/β-catenin signaling and others pathways. These functions are associated with an antiproliferative and pro-apoptotic effect in breast, colon, and prostate cancer cells[235]. *In vitro* studies on prostate cancer-derived stromal cells lycopene reduced Wnt/β-catenin signaling by affecting β-catenin nuclear localization. In normal prostate stromal cells treated with IGF-I, lycopene reduced the activation of AKT and the GSK3β phospho-inhibition[102]. The inhibition of Wnt/β-catenin signaling by lycopene was also confirmed in human breast cancer cell lines MCF-7 and MDA-MB-231. In these cellular models, the treatment with lycopene induced a reduction in TCF/LEF reporter activity, β-catenin and cyclin D1[236].

4.3.4. *Calotropin*

Calotropin is a toxic cardenolide, a steroid with a 5-membered lactone ring found in many plant sources such as Asclepiadoideae family and *Calotropis* genus (*C. procera* and *C. giagantea*). Calotropin, together with other cardenolides, were studied and extracted from the leaves and latex of *C. procera* by Seiber and colleagues[237] using, as extractive solvent, ethanol 95%. As a cardenolide, calotropin is considered a cardioactive compound and possesses a specific inotropic, chronotropic, and dromotropic effects that are found in natural sources[238]. Moreover, calotropin exhibits significant cytotoxicity against several cancer cells, indeed, it inhibits the proliferation of colorectal cancer cells both *in vitro* and *in vivo* tests[239]. Calotropin seems to have a potent Wnt signaling inhibitory activity. It was demonstrated in several colon cancer cell lines that this natural compound caused a substantial decrease of β-catenin in the cytosol and nucleus through the increase of its CK1-mediated phosphorylation and subsequent degradation[105].

4.4. *Secosteroids*

4.4.1. *Vitamin D*

Vitamin D is a lipophilic fat soluble vitamin produced in human body from sterols by the action of UV light on the skin, but it can be also obtained from dietary sources of both vegetal (in form of vitamin D_2 or ergocalciferol) and animal origin (in form of vitamin D_3 or cholecalciferol) and the primary sources include fatty fishes and fortified foods (milk)[240,241]. The two principal techniques for the extraction of vitamin D are liquid−liquid extraction and solid-phase extraction. On the other hand, other methods have been developed by combining different extractive procedures and adding derivatization steps in order to increase the recovery rate[242]. Vitamin D is related to many biological pathways and it is involved in bone health by promoting calcium absorption, regulation of gene expression, immunity, cardiovascular health and antioxidant regulation.

1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the active metabolite of Vitamin D_3, possesses antitumor activity as demonstrated by both *in vitro* and *in vivo* studies, as well as a protective ability in different neoplastic diseases, mainly colorectal cancer. It mediates the biological action mainly *via* the binding to the vitamin D receptor (VDR) The crosstalk between the VDR and Wnt/β-catenin signaling is known and mediates, at least in part and in certain models, the antitumor potential of 1α,25-dihydroxyvitamin D_3[75]. The use of 1,25$(OH)_2D_3$ as anticancer medicine is however hampered by its hypercalcemic effects at therapeutic doses, but several analogues that retain the antitumor actions without hypercalcemic effects are in developing[75].

Several *in vitro* studies demonstrated that 1,25$(OH)_2D_3$ inhibits the Wnt/β-catenin pathway in human colon cancer cells by three main mechanisms. First, it induces the interaction between VDR and β-catenin thus decreasing the binding of β-catenin to TCF. Second, it increases E-cadherin expression, leading the relocation of β-catenin from nucleus to plasma membrane[75,76]. Thirdly, 1,25$(OH)_2D_3$ augments the expression of Dickkopf (DKK)-1, a Wnt signaling inhibitor[243]. These molecular events lead to the

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inhibition of several β-catenin/TCF target genes such as *c-MYC*, *TCF1*, *LEF1*, *AXIN2*, *PPARγ*, and *CD44* in human colon cancer cells[76,244,245]. The antitumor efficacy of $1,25(OH)_2D_3$ in colon cancer, both *in vitro* and *in vivo*, was confirmed by others studies[246−251], and similar results were also obtained in *in vitro* and *in vivo* models of breast cancer[252,253] and in an *ex vivo* model of human uterine leiomyomas[254].

The promising results obtained in the preclinical experimentation have led to the evaluation of the potential anticancer activity of vitamin D3 also in clinical trials. However, scientific evidence obtained to date are not yet completely persuasive[75]. A large number of studies in cancer patients are ongoing in different type of cancers, as recoverable in *ClinicalTrials.gov*, but there is only one clinical trial that specifically evaluated the effect of vitamin D, alone or in combination with calcium, on the Wnt/β-catenin signaling in cancer patients. It was a randomized double blind chemoprevention trial that assessed these effects in rectal mucosa biopsies from 104 participants at baseline and one-year after (Table 1). The results obtained from this trial showed that vitamin D, alone or in association with calcium, could modify APC, β-catenin, and E-cadherin expression in patients at risk for colorectal neoplasms, supporting the use of vitamin D as potential chemopreventive treatment in colorectal cancers[255].

The safety profile of vitamin D and its synthetic derivatives is well known, and hypercalcemia is the main adverse event recognized[256]. Despite the numerous clinical trials completed and ongoing, further large and well-designed clinical studies are needed in order to evaluate the potential use of vitamin D as anticancer medicines.

5. Conclusive remarks and future perspective

As widely discussed in this review, due to the crucial role recognized to the Wnt/β-catenin pathway in cancer initiation and progression, the possibility of targeting this signaling cascade is a great opportunity to develop more effective and anticancer drugs. However, some worries are licit when we consider the risks of targeting a pathway critical in tissue homeostasis and stem cell maintenance[25]. In general, the use of drugs inhibiting signal transduction pathways crucial for embryonic development, such as the Wnt/β-catenin pathway, could be a double-edged sword, since they can act like "molecular embodiments of Dr. Jekyll and Mr. Hyde"[257]. Under this point of view, the use of nature-derived molecules that are able to modulate the pathway might limit this risk. As modulators, they could successfully revert aberrant Wnt signaling in pathological situations without interfering with the critical role of this pathway in tissue homeostasis and repair.

The discovery of new promising drugs, specifically targeting upstream and downstream events of the signaling, more and more seems to be an attractive preventive and therapeutic strategy not only for cancer but also for many other types of diseases for whose dysregulation of the Wnt/β-catenin pathway an association has been demonstrated. In particular, dysregulation of this pathway has been recently proposed as a novel pathomechanism leading to neurodegenerative disorders including Parkinson's disease (PD), Alzheimer's disease (AD) and others[13,14,258−262], and targeting the Wnt/β-catenin signaling has been suggested as new therapeutic opportunities for these brain diseases for which no cure is currently available[14,263−265]. Differently from the Wnt-targeting anticancer therapies, the knowledge about the role of Wnt/β-catenin signaling in neurodegenerative diseases is still in its infancy. There are not clinical evidences available, but only data concerning some preclinical therapeutic approaches, performed on cellular and animal models[14,258,264,265]. Among the bioactive modulators of the Wnt/β-catenin pathway that are under preclinical investigation as possible therapeutics for neurodegenerative diseases, there are many natural-derived compounds that seem to promote neuronal differentiation and for which a neuroprotective ability has been demonstrated. These include curcumin[266−268], resveratrol[107], ginsegoids[269,270], salidroside[271], and others[272,273]. Until today the therapeutic potential of natural-derived compounds against neurodegenerative disorders has been hampered by their poor bioavailability and consequent scarce delivery to the brain, but innovative delivery systems that could enhance their neuroavailability and therefore their neuroprotective activity are in developing[274], and this will reinforce their possible application as preventive and therapeutic strategy against these kind of diseases.

During the last decade, several natural compounds have been identified as modulators of the Wnt/β-catenin signaling, and most of the study aimed to demonstrate the efficacy of these modulators as tumor preventive and/or therapeutic drugs. The recently discovered involvement of this pathway in the onset of other diseases, and in particular in neurodegenerative disorders, for which a disease-modifying therapy does not exist yet, has increased the attractiveness of these natural compounds, and is stimulating for enhancing their possible use as dietary supplements or drugs in preventive and/or therapeutic new strategies against such different kind of diseases.

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Author contributions

G. Brusotti, Enrica Calleri and Annalucia Serafino conceived and designed the manuscript. G. Sferrazza, M. Corti, and Annalucia Serafino co-worked to the writing of the manuscript and figures. P. Pierimarchi, C. Temporini critically reviewed the manuscript.

Conflict of interests

The authors have no conflicts of interest to declare.

References

1. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 2004;**20**:781−810.
2. Croce JC, McClay DR. Evolution of the Wnt pathways. *Methods Mol Biol* 2008;**469**:3−18.
3. Van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. *Development* 2009;**136**:3205−14.
4. Inestrosa NC, Arenas E. Emerging roles of Wnts in the adult nervous system. *Nat Rev Neurosci* 2010;**11**:77−86.
5. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. Wnt and beta-catenin signalling: diseases and therapies. *Nat Rev Genet* 2004;**5**:691−701.
6. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. *Cell* 2012;**149**:1192−205.
7. Verkaar F, Cadigan KM, van Amerongen R. Celebrating 30 years of Wnt signaling. *Sci Signal* 2012;**5**:mr2.

8. Pinto D, Clevers H. Wnt control of stem cells and differentiation in the intestinal epithelium. *Exp Cell Res* 2005;**306**:357−63.
9. Nemeth MJ, Mak KK, Yang Y, Bodine DM. Beta-catenin expression in the bone marrow microenvironment is required for long-term maintenance of primitive hematopoietic cells. *Stem Cells* 2009;**27**: 1109−19.
10. Malhotra S, Kincade PW. Wnt-related molecules and signaling pathway equilibrium in hematopoiesis. *Cell Stem Cell* 2009;**4**:27−36.
11. Libro R, Bramanti P, Mazzon E. The role of the Wnt canonical signaling in neurodegenerative diseases. *Life Sci* 2016;**158**:78−88.
12. Nusse R, Clevers H. Wnt/beta-catenin signaling, disease, and emerging therapeutic modalities. *Cell* 2017;**169**:985−99.
13. Berwick DC, Harvey K. The importance of Wnt signalling for neurodegeneration in Parkinson's disease. *Biochem Soc Trans* 2012;**40**: 1123−8.
14. Serafino A, Sferrazza G, Colini Baldeschi A, Nicotera G, Andreola F, Pittaluga E, et al. Developing drugs that target the Wnt pathway: recent approaches in cancer and neurodegenerative diseases. *Expert Opin Drug Discov* 2017;**12**:169−86.
15. Tao H, Yang JJ, Shi KH, Li J. Wnt signaling pathway in cardiac fibrosis: new insights and directions. *Metabolism* 2016;**65**:30−40.
16. Chilosi M, Poletti V, Zamo A, Lestani M, Montagna L, Piccoli P, et al. Aberrant Wnt/beta-catenin pathway activation in idiopathic pulmonary fibrosis. *Am J Pathol* 2003;**162**:1495−502.
17. Schinner S, Willenberg HS, Schott M, Scherbaum WA. Pathophysiological aspects of Wnt-signaling in endocrine disease. *Eur J Endocrinol* 2009;**160**:731−7.
18. Schinner S. Wnt-signalling and the metabolic syndrome. *Horm Metab Res* 2009;**41**:159−63.
19. Anastas JN, Moon RT. Wnt signalling pathways as therapeutic targets in cancer. *Nat Rev Cancer* 2013;**13**:11−26.
20. Luu HH, Zhang R, Haydon RC, Rayburn E, Kang Q, Si W, et al. Wnt/beta-catenin signaling pathway as a novel cancer drug target. *Curr Cancer Drug Targets* 2004;**4**:653−71.
21. Huang P, Yan R, Zhang X, Wang L, Ke X, Qu Y. Activating Wnt/beta-catenin signaling pathway for disease therapy: challenges and opportunities. *Pharmacol Ther* 2019;**196**:79−90.
22. Serafino A, Moroni N, Zonfrillo M, Andreola F, Mercuri L, Nicotera G, et al. Wnt-pathway components as predictive markers useful for diagnosis, prevention and therapy in inflammatory bowel disease and sporadic colorectal cancer. *Oncotarget* 2014;**5**:978−92.
23. Morikawa T, Kuchiba A, Yamauchi M, Meyerhardt JA, Shima K, Nosho K, et al. Association of CTNNB1 (beta-catenin) alterations, body mass index, and physical activity with survival in patients with colorectal cancer. *J Am Med Assoc* 2011;**305**:1685−94.
24. Morikawa T, Kuchiba A, Lochhead P, Nishihara R, Yamauchi M, Imamura Y, et al. Prospective analysis of body mass index, physical activity, and colorectal cancer risk associated with beta-catenin (CTNNB1) status. *Cancer Res* 2013;**73**:1600−10.
25. Kahn M. Can we safely target the wnt pathway?. *Nat Rev Drug Discov* 2014;**13**:513−32.
26. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 1982;**31**:99−109.
27. Amirkia V, Heinrich M. Natural products and drug discovery: a survey of stakeholders in industry and academia. *Front Pharmacol* 2015;**6**:237.
28. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov* 2015;**14**:111−29.
29. Shen B. A new golden age of natural products drug discovery. *Cell* 2015;**163**:1297−300.
30. Tarapore RS, Siddiqui IA, Mukhtar H. Modulation of Wnt/beta-catenin signaling pathway by bioactive food components. *Carcinogenesis* 2012;**33**:483−91.
31. Sarkar FH, Li Y, Wang Z, Kong D. The role of nutraceuticals in the regulation of Wnt and hedgehog signaling in cancer. *Cancer Metastasis Rev* 2010;**29**:383−94.
32. Fuentes RG, Arai MA, Ishibashi M. Natural compounds with Wnt signal modulating activity. *Nat Prod Rep* 2015;**32**:1622−8.
33. Farahmand L, Darvishi B, Majidzadeh AK, Madjid Ansari A. Naturally occurring compounds acting as potent anti-metastatic agents and their suppressing effects on Hedgehog and Wnt/beta-catenin signalling pathways. *Cell Prolif* 2017;**50**:1−12.
34. Niehrs C. The complex world of Wnt receptor signalling. *Nat Rev Mol Cell Biol* 2012;**13**:767−79.
35. Angers S, Moon RT. Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol* 2009;**10**:468−77.
36. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009;**17**:9−26.
37. Willert K, Nusse R. Wnt proteins. *Cold Spring Harb Perspect Biol* 2012;**4**. a007864.
38. Gomez-Orte E, Saenz-Narciso B, Moreno S, Cabello J. Multiple functions of the noncanonical Wnt pathway. *Trends Genet* 2013;**29**: 545−53.
39. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. *Development* 2004;**131**:1663−77.
40. Ozawa M, Baribault H, Kemler R. The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J* 1989;**8**: 1711−7.
41. Vestweber D, Kemler R. Some structural and functional aspects of the cell adhesion molecule uvomorulin. *Cell Differ* 1984;**15**:269−73.
42. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 2004;**303**:1483−7.
43. Fukumoto S, Hsieh CM, Maemura K, Layne MD, Yet SF, Lee KH, et al. Akt participation in the Wnt signaling pathway through Dishevelled. *J Biol Chem* 2001;**276**:17479−83.
44. Serafino A, Moroni N, Psaila R, Zonfrillo M, Andreola F, Wannenes F, et al. Anti-proliferative effect of atrial natriuretic peptide on colorectal cancer cells: evidence for an Akt-mediated cross-talk between NHE-1 activity and wnt/beta-catenin signaling. *Biochim Biophys Acta* 2012;**1822**:1004−18.
45. Tamai K, Zeng X, Liu C, Zhang X, Harada Y, Chang Z, et al. A mechanism for Wnt coreceptor activation. *Mol Cell* 2004;**13**: 149−56.
46. Cheng X, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/beta-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother* 2019;**110**:473−81.
47. Najdi R, Holcombe RF, Waterman ML. Wnt signaling and colon carcinogenesis: beyond APC. *J Carcinog* 2011;**10**:5.
48. Fodde R, Brabletz T. Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol* 2007;**19**:150−8.
49. Monga SP. Beta-Catenin signaling and roles in liver homeostasis, injury, and tumorigenesis. *Gastroenterology* 2015;**148**:1294−310.
50. Shang S, Hua F, Hu ZW. The regulation of beta-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget* 2017;**8**: 33972−89.
51. Wang JN, Li L, Li LY, Yan Q, Li J, Xu T. Emerging role and therapeutic implication of Wnt signaling pathways in liver fibrosis. *Gene* 2018;**674**:57−69.
52. Nishikawa K, Osawa Y, Kimura K. Wnt/beta-catenin signaling as a potential target for the treatment of liver cirrhosis using antifibrotic drugs. *Int J Mol Sci* 2018;**19**.
53. Moparthi L, Koch S. Wnt signaling in intestinal inflammation. *Differentiation* 2019;**108**:24−32.
54. Wang B, Tian T, Kalland KH, Ke X, Qu Y. Targeting Wnt/beta-catenin signaling for cancer immunotherapy. *Trends Pharmacol Sci* 2018;**39**:648−58.
55. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 2017;**168**: 707−23.
56. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* 2015; **523**:231−5.

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57. Matsuda A, Ishiguro K, Yan IK, Patel T. Extracellular vesicle-based therapeutic targeting of beta-catenin to modulate anticancer immune responses in hepatocellular cancer. *Hepatol Commun* 2019;**3**:525−41.
58. Osawa Y, Kojika E, Nishikawa K, Kimura M, Osakaya S, Miyauchi H, et al. Programmed cell death ligand 1 (PD-L1) blockade attenuates metastatic colon cancer growth in cAMP-response element-binding protein (CREB)-binding protein (CBP)/beta-catenin inhibitor-treated livers. *Oncotarget* 2019;**10**:3013−26.
59. Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. Wnt/beta-catenin pathway activation correlates with immune exclusion across human cancers. *Clin Cancer Res* 2019;**25**:3074−83.
60. Ganesh S, Shui X, Craig KP, Park J, Wang W, Brown BD, et al. RNAi-mediated beta-catenin inhibition promotes T cell infiltration and antitumor activity in combination with immune checkpoint blockade. *Mol Ther* 2018;**26**:2567−79.
61. Galluzzi L, Spranger S, Fuchs E, Lopez-Soto A. Wnt signaling in cancer immunosurveillance. *Trends Cell Biol* 2019;**29**:44−65.
62. Hermel DJ, Sigal D. The emerging role of checkpoint inhibition in microsatellite stable colorectal cancer. *J Personalized Med* 2019;**9**:1−13.
63. Leow PC, Ong ZY, Ee P-LR. Natural compounds as antagonists of canonical Wnt/β-catenin signaling. *Curr Chem Biol* 2010;**4**:49−63.
64. Willenbacher E, Khan SZ, Mujica SCA, Trapani D, Hussain S, Wolf D, et al. Curcumin: new insights into an ancient ingredient against cancer. *Int J Mol Sci* 2019;**20**:1−13.
65. Prasad CP, Rath G, Mathur S, Bhatnagar D, Ralhan R. Potent growth suppressive activity of curcumin in human breast cancer cells: modulation of Wnt/beta-catenin signaling. *Chem Biol Interact* 2009;**181**:263−71.
66. Amado NG, Predes D, Moreno MM, Carvalho IO, Mendes FA, Abreu JG. Flavonoids and Wnt/beta-catenin signaling: potential role in colorectal cancer therapies. *Int J Mol Sci* 2014;**15**:12094−106.
67. Li Y, Wang Z, Kong D, Li R, Sarkar SH, Sarkar FH. Regulation of Akt/FOXO3a/GSK-3beta/AR signaling network by isoflavone in prostate cancer cells. *J Biol Chem* 2008;**283**:27707−16.
68. Park CH, Hahm ER, Lee JH, Jung KC, Yang CH. Inhibition of beta-catenin-mediated transactivation by flavanone in AGS gastric cancer cells. *Biochem Biophys Res Commun* 2005;**331**:1222−8.
69. Wagner J, Lehmann L. Estrogens modulate the gene expression of Wnt-7a in cultured endometrial adenocarcinoma cells. *Mol Nutr Food Res* 2006;**50**:368−72.
70. Hirata H, Ueno K, Nakajima K, Tabatabai ZL, Hinoda Y, Ishii N, et al. Genistein downregulates onco-miR-1260b and inhibits wnt-signalling in renal cancer cells. *Br J Canc* 2013;**108**:2070−8.
71. Zhang Y, Li Q, Zhou D, Chen H. Genistein, a soya isoflavone, prevents azoxymethane-induced up-regulation of Wnt/beta-catenin signalling and reduces colon pre-neoplasia in rats. *Br J Nutr* 2013;**109**:33−42.
72. Cilibrasi C, Riva G, Romano G, Cadamuro M, Bazzoni R, Butta V, et al. Resveratrol impairs glioma stem cells proliferation and motility by modulating the wnt signaling pathway. *PLoS One* 2017;**12**.e0169854.
73. Nguyen AV, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C, et al. Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. *Cancer Manag Res* 2009;**1**:25−37.
74. Howells LM, Berry DP, Elliott PJ, Jacobson EW, Hoffmann E, Hegarty B, et al. Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases-safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev Res* 2011;**4**:1419−25.
75. Larriba MJ, Gonzalez-Sancho JM, Barbachano A, Niell N, Ferrer-Mayorga G, Munoz A. Vitamin D is a multilevel repressor of Wnt/β-catenin signaling in cancer cells. *Cancers* 2013;**5**:1242−60.
76. Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, et al. Vitamin D_3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol* 2001;**154**:369−87.
77. Pahlke G, Ngiewih Y, Kern M, Jakobs S, Marko D, Eisenbrand G. Impact of quercetin and EGCG on key elements of the Wnt pathway in human colon carcinoma cells. *J Agric Food Chem* 2006;**54**:7075−82.
78. Srinivasan A, Thangavel C, Liu Y, Shoyele S, Den RB, Selvakumar P, et al. Quercetin regulates beta-catenin signaling and reduces the migration of triple negative breast cancer. *Mol Carcinog* 2016;**55**:743−56.
79. Sultan AS, Khalil MIM, Sami BM, Alkhuriji AF, Sadek O. Quercetin induces apoptosis in triple-negative breast cancer cells *via* inhibiting fatty acid synthase and beta-catenin. *Int J Clin Exp Pathol* 2017;**10**:156−72.
80. Chen Y, Wang XQ, Zhang Q, Zhu JY, Li Y, Xie CF, et al. (−)-Epigallocatechin-3-gallate inhibits colorectal cancer stem cells by suppressing Wnt/beta-catenin pathway. *Nutrients* 2017;**9**:1−11.
81. Dashwood WM, Orner GA, Dashwood RH. Inhibition of beta-catenin/Tcf activity by white tea, green tea, and epigallocatechin-3-gallate (EGCG): minor contribution of H_2O_2 at physiologically relevant EGCG concentrations. *Biochem Biophys Res Commun* 2002;**296**:584−8.
82. Sur S, Pal D, Mandal S, Roy A, Panda CK. Tea polyphenols epigallocatechin gallate and theaflavin restrict mouse liver carcinogenesis through modulation of self-renewal Wnt and hedgehog pathways. *J Nutr Biochem* 2016;**27**:32−42.
83. Tong Y, Liu Y, Zheng H, Zheng L, Liu W, Wu J, et al. Artemisinin and its derivatives can significantly inhibit lung tumorigenesis and tumor metastasis through Wnt/beta-catenin signaling. *Oncotarget* 2016;**7**:31413−28.
84. Ozbey U, Attar R, Romero MA, Alhewairini SS, Afshar B, Sabitaliyevich UY, et al. Apigenin as an effective anticancer natural product: spotlight on TRAIL, Wnt/beta-catenin, JAK-STAT pathways, and microRNAs. *J Cell Biochem* 2018;**120**:1060−7.
85. Xu M, Wang SS, Song Y, Yao JH, Huang K, Zhu XJ. Apigenin suppresses colorectal cancer cell proliferation, migration and invasion *via* inhibition of the Wnt/beta-catenin signaling pathway. *Oncology Letters* 2016;**11**:3075−80.
86. Lin CM, Chen HH, Lin CA, Wu HC, Sheu JJC, Chen HJ. Apigenin-induced lysosomal degradation of beta-catenin in Wnt/beta catenin signaling. *Sci Rep* 2017;**7**:1−12.
87. Shukla S, MacLennan GT, Flask CA, Fu P, Mishra A, Resnick MI, et al. Blockade of beta-catenin signaling by plant flavonoid apigenin suppresses prostate carcinogenesis in TRAMP mice. *Cancer Res* 2007;**67**:6925−35.
88. Park S, Choi J. Inhibition of beta-catenin/Tcf signaling by flavonoids. *J Cell Biochem* 2010;**110**:1376−85.
89. Jia Y, Chen L, Guo S, Li Y. Baicalin induced colon cancer cells apoptosis through miR-217/DKK1-mediated inhibition of Wnt signaling pathway. *Mol Biol Rep* 2019;**46**:1693−700.
90. Zhou T, Zhang A, Kuang G, Gong X, Jiang R, Lin D, et al. Baicalin inhibits the metastasis of highly aggressive breast cancer cells by reversing epithelial-to-mesenchymal transition by targeting beta-catenin signaling. *Oncol Rep* 2017;**38**:3599−607.
91. Lu W, Lin C, King TD, Chen H, Reynolds RC, Li Y. Silibinin inhibits Wnt/beta-catenin signaling by suppressing Wnt co-receptor LRP6 expression in human prostate and breast cancer cells. *Cell Signal* 2012;**24**:2291−6.
92. Rajamanickam S, Velmurugan B, Kaur M, Singh RP, Agarwal R. Chemoprevention of intestinal tumorigenesis in $APC^{min/+}$ mice by silibinin. *Cancer Res* 2010;**70**:2368−78.
93. Ren K, Zhang W, Wu G, Ren J, Lu H, Li Z, et al. Synergistic anticancer effects of galangin and berberine through apoptosis induction and proliferation inhibition in oesophageal carcinoma cells. *Biomed Pharmacother* 2016;**84**:1748−59.
94. Gwak J, Oh J, Cho M, Bae SK, Song IS, Liu KH, et al. Galangin suppresses the proliferation of beta-catenin response transcription-positive cancer cells by promoting adenomatous polyposis

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coli/axin/glycogen synthase kinase-3beta-independent beta-catenin degradation. *Mol Pharmacol* 2011;**79**:1014−22.
95. Suh Y, Afaq F, Johnson JJ, Mukhtar H. A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. *Carcinogenesis* 2009;**30**:300−7.
96. Gajos-Michniewicz A, Czyz M. Modulation of Wnt/beta-catenin pathway in melanoma by biologically active components derived from plants. *Fitoterapia* 2016;**109**:283−92.
97. Syed DN, Afaq F, Maddodi N, Johnson JJ, Sarfaraz S, Ahmad A, et al. Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/beta-catenin signaling and decreased Mitf levels. *J Investig Dermatol* 2011;**131**:1291−9.
98. Wang Y, Hong D, Qian Y, Tu X, Wang K, Yang X, et al. Lupeol inhibits growth and migration in two human colorectal cancer cell lines by suppression of Wnt-beta-catenin pathway. *OncoTargets Ther* 2018;**11**:7987−99.
99. Zhang L, Tu Y, He W, Peng Y, Qiu Z. A novel mechanism of hepatocellular carcinoma cell apoptosis induced by lupeol *via* brain-derived neurotrophic factor inhibition and glycogen synthase kinase 3 beta reactivation. *Eur J Pharmacol* 2015;**762**:55−62.
100. Saleem M, Murtaza I, Tarapore RS, Suh Y, Adhami VM, Johnson JJ, et al. Lupeol inhibits proliferation of human prostate cancer cells by targeting beta-catenin signaling. *Carcinogenesis* 2009;**30**:808−17.
101. Qin B, Liu JW, Liu SW, Li BJ, Ren J. Kaempferol targets estrogen-related receptor alpha and inhibits cell proliferation and invasion in retinoblastoma *via* Wnt/beta-catenin signaling pathway. *Int J Clin Exp Med* 2016;**9**:21415−23.
102. Wertz K. Lycopene effects contributing to prostate health. *Nutr Cancer* 2009;**61**:775−83.
103. Lee JH, Park CH, Jung KC, Rhee HS, Yang CH. Negative regulation of beta-catenin/Tcf signaling by naringenin in AGS gastric cancer cell. *Biochem Biophys Res Commun* 2005;**335**:771−6.
104. Shono T, Ishikawa N, Toume K, Arai MA, Ahmed F, Sadhu SK, et al. Boehmenan, a lignan from *Hibiscus ficulneus*, showed Wnt signal inhibitory activity. *Bioorg Med Chem Lett* 2015;**25**:2735−8.
105. Park HY, Toume K, Arai MA, Sadhu SK, Ahmed F, Ishibashi M. Calotropin: a cardenolide from calotropis gigantea that inhibits wnt signaling by increasing casein kinase 1alpha in colon cancer cells. *Chembiochem* 2014;**15**:872−8.
106. Zhang L, Yang X, Yang S, Zhang J. The Wnt/beta-catenin signaling pathway in the adult neurogenesis. *Eur J Neurosci* 2011;**33**:1−8.
107. Palomera-Avalos V, Grinan-Ferre C, Puigoriol-Illamola D, Camins A, Sanfeliu C, Canudas AM, et al. Resveratrol protects SAMP8 brain under metabolic stress: focus on mitochondrial function and wnt pathway. *Mol Neurobiol* 2017;**54**:1661−76.
108. Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. *Molecules* 2019;**24**. E1076.
109. Kim YS, Choi KC, Hwang KA. Genistein suppressed epithelial−mesenchymal transition and migration efficacies of BG-1 ovarian cancer cells activated by estrogenic chemicals *via* estrogen receptor pathway and downregulation of TGF-β signaling pathway. *Phytomedicine* 2015;**22**:993−9.
110. Benedetti B, Di Carro M, Magi E. Phytoestrogens in soy-based meat substitutes: comparison of different extraction methods for the subsequent analysis by liquid chromatography-tandem mass spectrometry. *J Mass Spectrom* 2018;**53**:862−70.
111. Hall JM, Powell HA, Rajic L, Korach KS. The role of dietary phytoestrogens and the nuclear receptor PPARgamma in adipogenesis: an *in vitro* study. *Environ Health Perspect* 2019;**127**:37007.
112. Tafrihi M, Nakhaei Sistani R. E-Cadherin/beta-catenin complex: a target for anticancer and antimetastasis plants/plant-derived compounds. *Nutr Cancer* 2017;**69**:702−22.
113. Chae HS, Xu R, Won JY, Chin YW, Yim H. Molecular targets of genistein and its related flavonoids to exert anticancer effects. *Int J Mol Sci* 2019;**20**:1−18.
114. Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, et al. Genistein and cancer: current status, challenges, and future directions. *Adv Nutr* 2015;**6**:408−19.
115. Su Y, Simmen RC. Soy isoflavone genistein upregulates epithelial adhesion molecule E-cadherin expression and attenuates beta-catenin signaling in mammary epithelial cells. *Carcinogenesis* 2009;**30**:331−9.
116. Su Y, Simmen FA, Xiao R, Simmen RC. Expression profiling of rat mammary epithelial cells reveals candidate signaling pathways in dietary protection from mammary tumors. *Physiol Genom* 2007;**30**:8−16.
117. Shafabakhsh R, Asemi Z. Quercetin: a natural compound for ovarian cancer treatment. *J Ovarian Res* 2019;**12**:55.
118. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol* 2008;**585**:325−37.
119. Khani R, Sheykhi R, Bagherzade G. An environmentally friendly method based on micro-cloud point extraction for determination of trace amount of quercetin in food and fruit juice samples. *Food Chem* 2019;**293**:220−5.
120. Zengin A, Badak MU, Aktas N. Selective separation and determination of quercetin from red wine by molecularly imprinted nanoparticles coupled with HPLC and ultraviolet detection. *J Sep Sci* 2018;**41**:3459−66.
121. Wang X, Li G, Ho Row K. Extraction and determination of quercetin from *Ginkgo biloba* by DESs-based polymer monolithic cartridge. *J Chromatogr Sci* 2017;**55**:866−71.
122. Nho JH, Lee HJ, Jung HK, Jang JH, Lee KH, Kim AH, et al. Effect of saururus chinensis leaves extract on type II collagen-induced arthritis mouse model. *BMC Complement Altern Med* 2019;**19**:2.
123. Temraz S, Mukherji D, Shamseddine A. Potential targets for colorectal cancer prevention. *Int J Mol Sci* 2013;**14**:17279−303.
124. Park CH, Chang JY, Hahm ER, Park S, Kim HK, Yang CH. Quercetin, a potent inhibitor against beta-catenin/Tcf signaling in SW480 colon cancer cells. *Biochem Biophys Res Commun* 2005;**328**:227−34.
125. Kawahara T, Kawaguchi-Ihara N, Okuhashi Y, Itoh M, Nara N, Tohda S. Cyclopamine and quercetin suppress the growth of leukemia and lymphoma cells. *Anticancer Res* 2009;**29**:4629−32.
126. Shan BE, Wang MX, Li RQ. Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/beta-catenin signaling pathway. *Cancer Invest* 2009;**27**:604−12.
127. Srivastava NS, Srivastava RAK. Curcumin and quercetin synergistically inhibit cancer cell proliferation in multiple cancer cells and modulate Wnt/beta-catenin signaling and apoptotic pathways in A375 cells. *Phytomedicine* 2019;**52**:117−28.
128. Chen Z, Huang C, Ma T, Jiang L, Tang L, Shi T, et al. Reversal effect of quercetin on multidrug resistance *via* FZD7/beta-catenin pathway in hepatocellular carcinoma cells. *Phytomedicine* 2018;**43**:37−45.
129. Yang YC, Wei MC. Development and characterization of a green procedure for apigenin extraction from *Scutellaria barbata* D. Don. *Food Chem* 2018;**252**:381−9.
130. Watson RR, Preedy VR, Zibadi S. *Polyphenols: prevention and treatment of human disease*. Boston: Academic press; 2018.
131. Shields M. Chapter 14—chemotherapeutics. In: Badal S, Delgoda R, editors. *Pharmacognosy*. Boston: Academic Press; 2017. p. 295−313.
132. Yan X, Qi M, Li P, Zhan Y, Shao H. Apigenin in cancer therapy: anticancer effects and mechanisms of action. *Cell Biosci* 2017;**7**:50.
133. Liu X, Li L, Lv L, Chen D, Shen L, Xie Z. Apigenin inhibits the proliferation and invasion of osteosarcoma cells by suppressing the Wnt/beta-catenin signaling pathway. *Oncol Rep* 2015;**34**:1035−41.
134. Bonesi M, Loizzo MR, Menichini F, Tundis R. *Flavonoids in treating psoriasis. Immunity and inflammation in health and disease*. Amsterdam: Elsevier; 2018. p. 281−94.

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135. Moore OA, Gao Y, Chen AY, Brittain R, Chen YC. The extraction, anticancer effect, bioavailability, and nanotechnology of baicalin. *J Nutr Med Diet Care* 2016;**2**:1−12.
136. Wang H, Ma X, Cheng Q, Wang L, Zhang L. Deep eutectic solvent-based ultrahigh pressure extraction of baicalin from *Scutellaria baicalensis* Georgi. *Molecules* 2018;**23**:1−12.
137. Liu X, Zhang W, Chen Z. Preparation of a novel molecularly imprinted polymer for the highly selective extraction of baicalin. *J Sep Sci* 2015;**38**:4233−9.
138. Li-Weber M. New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents wogonin, baicalein and baicalin. *Cancer Treat Rev* 2009;**35**:57−68.
139. Dou J, Wang Z, Ma L, Peng B, Mao K, Li C, et al. Baicalein and baicalin inhibit colon cancer using two distinct fashions of apoptosis and senescence. *Oncotarget* 2018;**9**:20089−102.
140. Wianowska D, Wisniewski M. Simplified procedure of silymarin extraction from *Silybum marianum* L. Gaertner. *J Chromatogr Sci* 2015;**53**:366−72.
141. Deep G, Gangar SC, Agarwal C, Agarwal R. Role of E-cadherin in antimigratory and antiinvasive efficacy of silibinin in prostate cancer cells. *Cancer Prev Res* 2011;**4**:1222−32.
142. Kaur M, Velmurugan B, Tyagi A, Agarwal C, Singh RP, Agarwal R. Silibinin suppresses growth of human colorectal carcinoma SW480 cells in culture and xenograft through down-regulation of beta-catenin-dependent signaling. *Neoplasia* 2010;**12**:415−24.
143. Sangeetha N, Aranganathan S, Panneerselvam J, Shanthi P, Rama G, Nalini N. Oral supplementation of silibinin prevents colon carcinogenesis in a long term preclinical model. *Eur J Pharmacol* 2010;**643**:93−100.
144. Sangeetha N, Viswanathan P, Balasubramanian T, Nalini N. Colon cancer chemopreventive efficacy of silibinin through perturbation of xenobiotic metabolizing enzymes in experimental rats. *Eur J Pharmacol* 2012;**674**:430−8.
145. Abubakar IB, Malami I, Yahaya Y, Sule SM. A review on the ethnomedicinal uses, phytochemistry and pharmacology of *Alpinia officinarum* Hance. *J Ethnopharmacol* 2018;**224**:45−62.
146. Zeng QH, Lu CL, Zhang XW, Jiang JG. Isolation and identification of ingredients inducing cancer cell death from the seeds of *Alpinia galanga*, a Chinese spice. *Food Funct* 2015;**6**:431−43.
147. Basri AM, Taha H, Ahmad N. A review on the pharmacological activities and phytochemicals of *Alpinia officinarum* (Galangal) extracts derived from bioassay-guided fractionation and isolation. *Pharmacogn Rev* 2017;**11**:43−56.
148. Bacanlı M, Başaran AA, Başaran N. Chapter 34—galangin as a plant phenolic and usage in health and disease. In: Watson RR, Preedy VR, Zibadi S, editors. *Polyphenols: prevention and treatment of human disease*. 2nd ed. Boston: Academic Press; 2018. p. 433−8.
149. Chien ST, Shi MD, Lee YC, Te CC, Shih YW. Galangin, a novel dietary flavonoid, attenuates metastatic feature *via* PKC/ERK signaling pathway in TPA-treated liver cancer HepG2 cells. *Cancer Cell Int* 2015;**15**:15.
150. Lee JJ, Lee JH, Yim NH, Han JH, Ma JY. Application of galangin, an active component of *Alpinia officinarum* Hance (Zingiberaceae), for use in drug-eluting stents. *Sci Rep* 2017;**7**:8207.
151. Kashyap D, Garg VK, Tuli HS, Yerer MB, Sak K, Sharma AK, et al. Fisetin and quercetin: promising flavonoids with chemopreventive potential. *Biomolecules* 2019;**9**:1−22.
152. Horwitz RJ. Chapter 30—the allergic patient. In: Rakel D, editor. *Integrative medicine*. 4th ed. Amsterdam: Elsevier; 2018. 300−309.e2.
153. Kashyap D, Sharma A, Sak K, Tuli HS, Buttar HS, Bishayee A. Fisetin: a bioactive phytochemical with potential for cancer prevention and pharmacotherapy. *Life Sci* 2018;**194**:75−87.
154. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. *Antioxidants Redox Signal* 2013;**19**:151−62.
155. Valianou L, Karapanagiotis I, Chryssoulakis Y. Comparison of extraction methods for the analysis of natural dyes in historical textiles by high-performance liquid chromatography. *Anal Bioanal Chem* 2009;**395**:2175−89.
156. Cid-Ortega S, Monroy-Rivera JA. Extraction of Kaempferol and its glycosides using supercritical fluids from plant sources: a review. *Food Technol Biotechnol* 2018;**56**:480−93.
157. Imran M, Rauf A, Shah ZA, Saeed F, Imran A, Arshad MU, et al. Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: a comprehensive review. *Phytother Res* 2019;**33**:263−75.
158. Ramos-Tovar E, Muriel P. Chapter 9—phytotherapy for the liver. In: Watson RR, Preedy VR, editors. *Dietary interventions in liver disease*. Boston: Academic Press; 2019. p. 101−21.
159. Salehi B, Fokou PVT, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, et al. The therapeutic potential of naringenin: a review of clinical trials. *Pharmaceuticals* 2019;**12**:11.
160. Meng Z, Zhao J, Duan H, Guan Y, Zhao L. Green and efficient extraction of four bioactive flavonoids from Pollen Typhae by ultrasound-assisted deep eutectic solvents extraction. *J Pharm Biomed Anal* 2018;**161**:246−53.
161. Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C, Ramirez-Tortosa MC. Curcumin and health. *Molecules* 2016;**21**:1−22.
162. Vogel HA, Pelletier J. Curcumin—biological and medicinal properties. *J Pharma* 1818;**2**:50.
163. Dandekar DV, Gaikar VG. Microwave assisted extraction of curcuminoids from *Curcuma longa*. *Separ Sci Technol* 2002;**37**:2669−90.
164. Kwon HL, Chung MS. Pilot-scale subcritical solvent extraction of curcuminoids from *Curcuma long* L. *Food Chem* 2015;**185**:58−64.
165. Kimthet C, Wahyudiono, Kanda H, Goto M. Extraction of curcumin from *Curcuma longa* L. using ultrasound assisted supercritical carbon dioxide. In: *AIP conference proceedings*; 2017. Available from: https://doi.org/10.1063/1.4982318.
166. Patil SS, Bhasarkar S, Rathod VK. Extraction of curcuminoids from *Curcuma longa*: comparative study between batch extraction and novel three phase partitioning. *Prep Biochem Biotechnol* 2019;**49**:407−18.
167. Kahkhaie KR, Mirhosseini A, Aliabadi A, Mohammadi A, Mosavi MJ, Haftcheshmeh SM, et al. Curcumin: a modulator of inflammatory signaling pathways in the immune system. *Inflammopharmacology* 2019:885−900.
168. Abrahams S, Haylett WL, Johnson G, Carr JA, Bardien S. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: a review. *Neuroscience* 2019;**406**:1−21.
169. Ferreira N, Saraiva MJ, Almeida MR. Uncovering the neuroprotective mechanisms of curcumin on transthyretin amyloidosis. *Int J Mol Sci* 2019;**20**:1−13.
170. Gallardo M, Calaf GM. Curcumin inhibits invasive capabilities through epithelial mesenchymal transition in breast cancer cell lines. *Int J Oncol* 2016;**49**:1019−27.
171. Zhang Z, Chen H, Xu C, Song L, Huang L, Lai Y, et al. Curcumin inhibits tumor epithelialmesenchymal transition by downregulating the Wnt signaling pathway and upregulating NKD2 expression in colon cancer cells. *Oncol Rep* 2016;**35**:2615−23.
172. Wang JY, Wang X, Wang XJ, Zheng BZ, Wang Y, Wang X, et al. Curcumin inhibits the growth *via* Wnt/beta-catenin pathway in non-small-cell lung cancer cells. *Eur Rev Med Pharmacol Sci* 2018;**22**:7492−9.
173. He M, Li Y, Zhang L, Li L, Shen Y, Lin L, et al. Curcumin suppresses cell proliferation through inhibition of the Wnt/beta-catenin signaling pathway in medulloblastoma. *Oncol Rep* 2014;**32**:173−80.
174. Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, et al. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. *Breast Canc Res Treat* 2010;**122**:777−85.
175. Li X, Wang X, Xie C, Zhu J, Meng Y, Chen Y, et al. Sonic hedgehog and Wnt/beta-catenin pathways mediate curcumin inhibition of breast cancer stem cells. *Anti Cancer Drugs* 2018;**29**:208−15.

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176. Ramasamy TS, Ayob AZ, Myint HH, Thiagarajah S, Amini F. Targeting colorectal cancer stem cells using curcumin and curcumin analogues: insights into the mechanism of the therapeutic efficacy. *Cancer Cell Int* 2015;**15**:96.
177. Dou H, Shen R, Tao J, Huang L, Shi H, Chen H, et al. Curcumin suppresses the colon cancer proliferation by inhibiting Wnt/beta-catenin pathways *via* miR-130a. *Front Pharmacol* 2017;**8**:877.
178. Zhu JY, Yang X, Chen Y, Jiang Y, Wang SJ, Li Y, et al. Curcumin suppresses lung cancer stem cells *via* inhibiting Wnt/beta-catenin and sonic hedgehog pathways. *Phytother Res* 2017;**31**:680−8.
179. Marjaneh RM, Rahmani F, Hassanian SM, Rezaei N, Hashemzehi M, Bahrami A, et al. Phytosomal curcumin inhibits tumor growth in colitis-associated colorectal cancer. *J Cell Physiol* 2018;**233**:6785−98.
180. Doello K, Ortiz R, Alvarez PJ, Melguizo C, Cabeza L, Prados J. Latest *in vitro* and *in vivo* assay, clinical trials and patents in cancer treatment using curcumin: a literature review. *Nutr Cancer* 2018;**70**:569−78.
181. Hewlings SJ, Kalman DS. Curcumin: a review of its' effects on human health. *Foods* 2017;**6**:1−11.
182. Averilla JN, Oh J, Wu Z, Liu KH, Jang CH, Kim HJ, et al. Improved extraction of resveratrol and antioxidants from grape peel using heat and enzymatic treatments. *J Sci Food Agric* 2019;**99**:4043−53.
183. Chimento A, De Amicis F, Sirianni R, Sinicropi MS, Puoci F, Casaburi I, et al. Progress to improve oral bioavailability and beneficial effects of resveratrol. *Int J Mol Sci* 2019;**20**:1−27.
184. Romero-Perez AI, Lamuela-Raventos RM, Andres-Lacueva C, de La Torre-Boronat MC. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J Agric Food Chem* 2001;**49**:210−5.
185. Vastano BC, Chen Y, Zhu N, Ho CT, Zhou Z, Rosen RT. Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. *J Agric Food Chem* 2000;**48**:253−6.
186. Parshikov IA, Netrusov AI, Sutherland JB. Microbial transformation of antimalarial terpenoids. *Biotechnol Adv* 2012;**30**:1516−23.
187. Nicotra S, Cramarossa MR, Mucci A, Pagnoni UM, Riva S, Forti L. Biotransformation of resveratrol: synthesis of trans-dehydrodimers catalyzed by laccases from *Myceliophtora thermophyla* and from *Trametes pubescens*. *Tetrahedron* 2004;**60**:595−600.
188. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat Rev Drug Discov* 2006;**5**:493−506.
189. Hope C, Planutis K, Planutiene M, Moyer MP, Johal KS, Woo J, et al. Low concentrations of resveratrol inhibit wnt signal throughput in colon-derived cells: implications for colon cancer prevention. *Mol Nutr Food Res* 2008;**52 Suppl 1**:S52−61.
190. Mineda A, Nishimura M, Kagawa T, Takiguchi E, Kawakita T, Abe A, et al. Resveratrol suppresses proliferation and induces apoptosis of uterine sarcoma cells by inhibiting the Wnt signaling pathway. *Exp Ther Med* 2019;**17**:2242−6.
191. Geng W, Guo X, Zhang L, Ma Y, Wang L, Liu Z, et al. Resveratrol inhibits proliferation, migration and invasion of multiple myeloma cells *via* NEAT1-mediated Wnt/beta-catenin signaling pathway. *Biomed Pharmacother* 2018;**107**:484−94.
192. Dai H, Deng HB, Wang YH, Guo JJ. Resveratrol inhibits the growth of gastric cancer *via* the Wnt/beta-catenin pathway. *Oncol Lett* 2018;**16**:1579−83.
193. Farooqi AA, Khalid S, Ahmad A. Regulation of cell signaling pathways and miRNAs by resveratrol in different cancers. *Int J Mol Sci* 2018;**19**:1−14.
194. Zou Y, Yang J, Jiang D. Resveratrol inhibits canonical Wnt signaling in human MG-63 osteosarcoma cells. *Mol Med Rep* 2015;**12**:7221−6.
195. Xie D, Zheng GZ, Xie P, Zhang QH, Lin FX, Chang B, et al. Antitumor activity of resveratrol against human osteosarcoma cells: a key role of Cx43 and Wnt/beta-catenin signaling pathway. *Oncotarget* 2017;**8**:111419−32.
196. Fu Y, Chang H, Peng X, Bai Q, Yi L, Zhou Y, et al. Resveratrol inhibits breast cancer stem-like cells and induces autophagy *via* suppressing Wnt/beta-catenin signaling pathway. *PLoS One* 2014;**9**.e102535.
197. Yang HC, Wang JY, Bu XY, Yang B, Wang BQ, Hu S, et al. Resveratrol restores sensitivity of glioma cells to temozolamide through inhibiting the activation of Wnt signaling pathway. *J Cell Physiol* 2019;**234**:6783−800.
198. Sergides C, Chirila M, Silvestro L, Pitta D, Pittas A. Bioavailability and safety study of resveratrol 500 mg tablets in healthy male and female volunteers. *Exp Ther Med* 2016;**11**:164−70.
199. Brown VA, Patel KR, Viskaduraki M, Crowell JA, Perloff M, Booth TD, et al. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res* 2010;**70**:9003−11.
200. Menezes Maciel Bindes M, Hespanhol Miranda Reis M, Luiz Cardoso V, Boffito DC. Ultrasound-assisted extraction of bioactive compounds from green tea leaves and clarification with natural coagulants (chitosan and *Moringa oleifera* seeds). *Ultrason Sonochem* 2019;**51**:111−9.
201. Sanlier N, Gokcen BB, Altuğ M. Tea consumption and disease correlations. *Trends Food Sci Technol* 2018;**78**:95−106.
202. Cao Q, Li J, Xia Y, Li W, Luo S, Ma C, et al. Green extraction of six phenolic compounds from Rattan (*Calamoideae faberii*) with deep eutectic solvent by homogenate-assisted vacuum-cavitation method. *Molecules* 2018;**24**:1−15.
203. Zhu J, Jiang Y, Yang X, Wang S, Xie C, Li X, et al. Wnt/beta-catenin pathway mediates (−)-epigallocatechin-3-gallate (EGCG) inhibition of lung cancer stem cells. *Biochem Biophys Res Commun* 2017;**482**:15−21.
204. Yang C, Du W, Yang D. Inhibition of green tea polyphenol EGCG((−)-epigallocatechin-3-gallate) on the proliferation of gastric cancer cells by suppressing canonical Wnt/beta-catenin signalling pathway. *Int J Food Sci Nutr* 2016;**67**:818−27.
205. Oh S, Gwak J, Park S, Yang CS. Green tea polyphenol EGCG suppresses Wnt/beta-catenin signaling by promoting GSK-3beta- and PP2A-independent beta-catenin phosphorylation/degradation. *Biofactors* 2014;**40**:586−95.
206. Kim J, Zhang X, Rieger-Christ KM, Summerhayes IC, Wazer DE, Paulson KE, et al. Suppression of Wnt signaling by the green tea compound (−)-epigallocatechin 3-gallate (EGCG) in invasive breast cancer cells. Requirement of the transcriptional repressor HBP1. *J Biol Chem* 2006;**281**:10865−75.
207. Bose M, Hao X, Ju J, Husain A, Park S, Lambert JD, et al. Inhibition of tumorigenesis in $Apc^{Min/+}$ mice by a combination of (−)-epigallocatechin-3-gallate and fish oil. *J Agric Food Chem* 2007;**55**:7695−700.
208. Pan LL, Wang XL, Luo XL, Liu SY, Xu P, Hu JF, et al. Boehmenan, a lignan from the Chinese medicinal plant *Clematis armandii*, inhibits A431 cell growth *via* blocking p70S6/S6 kinase pathway. *Integr Cancer Ther* 2017;**16**:351−9.
209. Yoder SC, Lancaster SM, Hullar MAJ, Lampe JW. Chapter 7—gut microbial metabolism of plant lignans: influence on human health. In: Tuohy K, Del Rio D, editors. *Diet-microbe interactions in the gut*. San Diego: Academic Press; 2015. p. 103−17.
210. Pathak S, Kesavan P, Banerjee A, Banerjee A, Celep GS, Bissi L, et al. Chapter 25—metabolism of dietary polyphenols by human gut microbiota and their health benefits. In: Watson RR, Preedy VR, Zibadi S, editors. *Polyphenols: mechanisms of action in human health and disease*. 2nd ed. Boston: Academic Press; 2018. p. 347−59.
211. Pan LL, Wang XL, Zhang QY, Luo XL, Xu P, Liu SY, et al. Boehmenan, a lignan from the Chinese medicinal plant *Clematis armandii*, induces apoptosis in lung cancer cells through modulation of EGF-dependent pathways. *Phytomedicine* 2016;**23**:468−76.
212. McCarthy JS, Price RN. 40—antimalarial drugs. In: Bennett JE, Dolin R, Blaser MJ, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 8th ed. Philadelphia: Content Repository Only; 2015. 495−509.e5.

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213. Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nat Med* 2011;**17**:1217−20.
214. Liu X, Cao J, Huang G, Zhao Q, Shen J. Biological activities of artemisinin derivatives beyond malaria. *Curr Top Med Chem* 2019;**19**:205−22.
215. Pandey N, Pandey-Rai S. Updates on artemisinin: an insight to mode of actions and strategies for enhanced global production. *Protoplasma* 2016;**253**:15−30.
216. Chen T, Li M, Zhang R, Wang H. Dihydroartemisinin induces apoptosis and sensitizes human ovarian cancer cells to carboplatin therapy. *J Cell Mol Med* 2009;**13**:1358−70.
217. Wang SJ, Gao Y, Chen H, Kong R, Jiang HC, Pan SH, et al. Dihydroartemisinin inactivates NF-kappaB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both *in vitro* and *in vivo*. *Cancer Lett* 2010;**293**:99−108.
218. Gomes MF, Faiz MA, Gyapong JO, Warsame M, Agbenyega T, Babiker A, et al. Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. *Lancet* 2009;**373**:557−66.
219. Rasheed SA, Efferth T, Asangani IA, Allgayer H. First evidence that the antimalarial drug artesunate inhibits invasion and *in vivo* metastasis in lung cancer by targeting essential extracellular proteases. *Int J Cancer* 2010;**127**:1475−85.
220. Odaka Y, Xu BS, Luo Y, Shen T, Shang CW, Wu Y, et al. Dihydroartemisinin inhibits the mammalian target of rapamycin-mediated signaling pathways in tumor cells. *Carcinogenesis* 2014;**35**:192−200.
221. Tsai FS, Lin LW, Wu CR. Lupeol and its role in chronic diseases. *Adv Exp Med Biol* 2016;**929**:145−75.
222. Mbaveng AT, Hamm R, Kuete V. 19—harmful and protective effects of terpenoids from African medicinal plants. In: Kuete V, editor. *Toxicological survey of African medicinal plants*. Amsterdam: Elsevier; 2014. p. 557−76.
223. Parsaeimehr A, Martinez-Chapa SO, Parra-Saldívar R. Chapter 13—medicinal plants *versus* skin disorders: a survey from ancient to modern herbalism. In: Kon K, Rai M, editors. *The microbiology of skin, soft tissue, bone and joint infections*. Boston: Academic Press; 2017. p. 205−21.
224. Ruiz-Montanez G, Ragazzo-Sanchez JA, Calderon-Santoyo M, Velazquez-de la Cruz G, de Leon JA, Navarro-Ocana A. Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.). *Food Chem* 2014;**159**:267−72.
225. Ramos-Hernandez JA, Calderon-Santoyo M, Navarro-Ocana A, Barros-Castillo JC, Ragazzo-Sanchez JA. Use of emerging technologies in the extraction of lupeol, alpha-amyrin and beta-amyrin from sea grape (*Coccoloba uvifera* L.). *J Food Sci Technol* 2018;**55**:2377−83.
226. Tarapore RS, Siddiqui IA, Saleem M, Adhami VM, Spiegelman VS, Mukhtar H. Specific targeting of Wnt/beta-catenin signaling in human melanoma cells by a dietary triterpene lupeol. *Carcinogenesis* 2010;**31**:1844−53.
227. Tarapore RS, Siddiqui IA, Adhami VM, Spiegelman VS, Mukhtar H. The dietary terpene lupeol targets colorectal cancer cells with constitutively active Wnt/beta-catenin signaling. *Mol Nutr Food Res* 2013;**57**:1950−8.
228. Ono M, Takeshima M, Nakano S. *Mechanism of the anticancer effect of lycopene (tetraterpenoids). The Enzymes*. Amsterdam: Elsevier; 2015. p. 139−66.
229. Başaran N, Bacanlı M, Başaran AA. *Lycopenes as antioxidants in gastrointestinal diseases. Gastrointestinal Tissue*. Amsterdam: Elsevier; 2017. p. 355−62.
230. Chan CH, Yusoff R, Ngoh GC, Kung FW. Microwave-assisted extractions of active ingredients from plants. *J Chromatogr A* 2011;**1218**:6213−25.
231. Chan CM, Hung CF. *Lycopene and retinal pigment epithelial cells: molecular aspects. Handbook of nutrition, diet and the eye*. Amsterdam: Elsevier; 2014. p. 587−98.
232. Naviglio D, Pizzolongo F, Ferrara L, Aragon A, Santini A. Extraction of pure lycopene from industrial tomato by-products in water using a new high-pressure process. *J Sci Food Agric* 2008;**88**:2414−20.
233. de Andrade Lima M, Kestekoglou I, Charalampopoulos D, Chatzifragkou A. Supercritical fluid extraction of carotenoids from vegetable waste matrices. *Molecules* 2019;**24**:466.
234. Briones-Labarca V, Giovagnoli-Vicuna C, Canas-Sarazua R. Optimization of extraction yield, flavonoids and lycopene from tomato pulp by high hydrostatic pressure-assisted extraction. *Food Chem* 2019;**278**:751−9.
235. Park B, Lim JW, Kim H. Lycopene treatment inhibits activation of Jak1/Stat3 and Wnt/beta-catenin signaling and attenuates hyperproliferation in gastric epithelial cells. *Nutr Res* 2018;**17**:1−12.
236. Preet R, Mohapatra P, Das D, Satapathy SR, Choudhuri T, Wyatt MD, et al. Lycopene synergistically enhances quinacrine action to inhibit Wnt-TCF signaling in breast cancer cells through APC. *Carcinogenesis* 2013;**34**:277−86.
237. Alqahtani SN, Alkholy SO, Ferreira MP. Chapter 11—antidiabetic and anticancer potential of native medicinal plants from Saudi Arabia. In: Watson RR, Preedy VR, Zibadi S, editors. *Polyphenols in human health and disease*. San Diego: Academic Press; 2014. p. 119−32.
238. Senthilkumaran S, Meenakshisundaram R, Thirumalaikolundu subramanian P. Chapter 5—plant toxins and the heart. In: Ramachandran M, editor. *Heart and toxins*. Boston: Academic Press; 2015. p. 151−74.
239. Zhou L, Cai L, Guo Y, Zhang H, Wang P, Yi G, et al. Calotropin activates YAP through downregulation of LATS1 in colorectal cancer cells. *OncoTargets Ther* 2019;**12**:4047−54.
240. Combs GF, McClung JP. Chapter 7—vitamin D. In: Combs GF, McClung JP, editors. *The vitamins*. 5th ed. Boston: Academic Press; 2017. p. 161−206.
241. Engelking LR. Chapter 45—vitamin D. In: Engelking LR, editor. *Textbook of veterinary physiological chemistry*. 3rd ed. Boston: Academic Press; 2015. p. 288−93.
242. Musteata ML, Musteata FM. Overview of extraction methods for analysis of vitamin D and its metabolites in biological samples. *Bioanalysis* 2011;**3**:1987−2002.
243. Aguilera O, Pena C, Garcia JM, Larriba MJ, Ordonez-Moran P, Navarro D, et al. The Wnt antagonist *DICKKOPF-1* gene is induced by 1alpha,25-dihydroxyvitamin D_3 associated to the differentiation of human colon cancer cells. *Carcinogenesis* 2007;**28**:1877−84.
244. Larriba MJ, Valle N, Palmer HG, Ordonez-Moran P, Alvarez-Diaz S, Becker KF, et al. The inhibition of Wnt/beta-catenin signalling by 1alpha,25-dihydroxyvitamin D_3 is abrogated by snail1 in human colon cancer cells. *Endocr Relat Cancer* 2007;**14**:141−51.
245. Larriba MJ, Ordonez-Moran P, Chicote I, Martin-Fernandez G, Puig I, Munoz A, et al. Vitamin D receptor deficiency enhances Wnt/beta-catenin signaling and tumor burden in colon cancer. *PLoS One* 2011;**6**. e23524.
246. Razak S, Afsar T, Almajwal A, Alam I, Jahan S. Growth inhibition and apoptosis in colorectal cancer cells induced by vitamin D-nanoemulsion (NVD): involvement of wnt/beta-catenin and other signal transduction pathways. *Cell Biosci* 2019;**9**:15.
247. Sun H, Jiang C, Cong L, Wu N, Wang X, Hao M, et al. CYP24A1 inhibition facilitates the antiproliferative effect of $1,25(OH)_2D_3$ through downregulation of the Wnt/beta-catenin pathway and methylation-mediated regulation of CYP24A1 in colorectal cancer cells. *DNA Cell Biol* 2018;**37**:742−9.
248. Pendas-Franco N, Garcia JM, Pena C, Valle N, Palmer HG, Heinaniemi M, et al. DICKKOPF-4 is induced by TCF/beta-catenin and upregulated in human colon cancer, promotes tumour cell invasion and angiogenesis and is repressed by 1alpha,25-dihydroxyvitamin D_3. *Oncogene* 2008;**27**:4467−77.
249. Beildeck ME, Islam M, Shah S, Welsh J, Byers SW. Control of TCF-4 expression by VDR and vitamin D in the mouse mammary gland and colorectal cancer cell lines. *PLoS One* 2009;**4**. e7872.

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250. Meyer MB, Goetsch PD, Pike JW. VDR/RXR and TCF4/beta-catenin cistromes in colonic cells of colorectal tumor origin: impact on c-FOS and c-MYC gene expression. *Mol Endocrinol* 2012;**26**:37−51.
251. Refaat B, El-Shemi AG, Kensara OA, Mohamed AM, Idris S, Ahmad J, et al. Vitamin D_3 enhances the tumouricidal effects of 5-fluorouracil through multipathway mechanisms in azoxymethane rat model of colon cancer. *J Exp Clin Cancer Res* 2015;**34**:71.
252. Zheng W, Duan B, Zhang Q, Ouyang L, Peng W, Qian F, et al. Vitamin D-induced vitamin D receptor expression induces tamoxifen sensitivity in MCF-7 stem cells *via* suppression of Wnt/beta-catenin signaling. *Biosci Rep* 2018;**38**:1−10.
253. Jeong Y, Swami S, Krishnan AV, Williams JD, Martin S, Horst RL, et al. Inhibition of mouse breast tumor-initiating cells by calcitriol and dietary vitamin D. *Mol Cancer Ther* 2015;**14**:1951−61.
254. Corachan A, Ferrero H, Aguilar A, Garcia N, Monleon J, Faus A, et al. Inhibition of tumor cell proliferation in human uterine leiomyomas by vitamin D *via* Wnt/beta-catenin pathway. *Fertil Steril* 2019;**111**:397−407.
255. Liu S, Barry EL, Baron JA, Rutherford RE, Seabrook ME, Bostick RM. Effects of supplemental calcium and vitamin D on the APC/beta-catenin pathway in the normal colorectal mucosa of colorectal adenoma patients. *Mol Carcinog* 2017;**56**:412−24.
256. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. *Nutrients* 2013;**5**:3605−16.
257. Sakata T, Chen JK. Chemical 'Jekyll and Hyde's: small-molecule inhibitors of developmental signaling pathways. *Chem Soc Rev* 2011;**40**:4318−31.
258. Colini Baldeschi A, Pittaluga E, Andreola F, Rossi S, Cozzolino M, Nicotera G, et al. Atrial natriuretic peptide acts as a neuroprotective agent in *in vitro* models of Parkinson's disease *via* up-regulation of the Wnt/beta-catenin pathway. *Front Aging Neurosci* 2018;**10**:20.
259. Inestrosa NC, Montecinos-Oliva C, Fuenzalida M. Wnt signaling: role in Alzheimer disease and schizophrenia. *J Neuroimmune Pharmacol* 2012;**7**:788−807.
260. Okerlund ND, Cheyette BN. Synaptic wnt signaling-a contributor to major psychiatric disorders?. *J Neurodev Disord* 2011;**3**:162−74.
261. Marchetti B. Wnt/beta-catenin signaling pathway governs a full program for dopaminergic neuron survival, neurorescue and regeneration in the MPTP mouse model of Parkinson's disease. *Int J Mol Sci* 2018;**19**:1−28.
262. Berwick DC, Harvey K. The regulation and deregulation of Wnt signaling by *PARK* genes in health and disease. *J Mol Cell Biol* 2014;**6**:3−12.
263. L'Episcopo F, Tirolo C, Caniglia S, Testa N, Morale MC, Serapide MF, et al. Targeting Wnt signaling at the neuroimmune interface for dopaminergic neuroprotection/repair in Parkinson's disease. *J Mol Cell Biol* 2014;**6**:13−26.
264. L'Episcopo F, Serapide MF, Tirolo C, Testa N, Caniglia S, Morale MC, et al. A Wnt1 regulated frizzled-1/beta-catenin signaling pathway as a candidate regulatory circuit controlling mesencephalic dopaminergic neuron-astrocyte crosstalk: therapeutical relevance for neuron survival and neuroprotection. *Mol Neurodegener* 2011;**6**:49.
265. Wei L, Sun C, Lei M, Li G, Yi L, Luo F, et al. Activation of Wnt/beta-catenin pathway by exogenous Wnt1 protects SH-SY5Y cells against 6-hydroxydopamine toxicity. *J Mol Neurosci* 2013;**49**:105−15.
266. Reddy PH, Manczak M, Yin X, Grady MC, Mitchell A, Tonk S, et al. Protective effects of Indian spice curcumin against amyloid-beta in Alzheimer's disease. *J Alzheimer's Dis* 2018;**61**:843−66.
267. Wang YL, Ju B, Zhang YZ, Yin HL, Liu YJ, Wang SS, et al. Protective effect of curcumin against oxidative stress-induced injury in rats with Parkinson's disease through the Wnt/beta-catenin signaling Pathway. *Cell Physiol Biochem* 2017;**43**:2226−41.
268. Huang HC, Xu K, Jiang ZF. Curcumin-mediated neuroprotection against amyloid-beta-induced mitochondrial dysfunction involves the inhibition of GSK-3beta. *J Alzheimer's Dis* 2012;**32**:981−96.
269. Zhou T, Zu G, Zhang X, Wang X, Li S, Gong X, et al. Neuroprotective effects of ginsenoside Rg1 through the Wnt/beta-catenin signaling pathway in both *in vivo* and *in vitro* models of Parkinson's disease. *Neuropharmacology* 2016;**101**:480−9.
270. Li MY, Chang CT, Han YT, Liao CP, Yu JY, Wang TW. Ginkgolide B promotes neuronal differentiation through the Wnt/beta-catenin pathway in neural stem cells of the postnatal mammalian subventricular zone. *Sci Rep* 2018;**8**:14947.
271. Wu DM, Han XR, Wen X, Wang S, Fan SH, Zhuang J, et al. Salidroside protection against oxidative stress injury through the Wnt/beta-catenin signaling pathway in rats with Parkinson's disease. *Cell Physiol Biochem* 2018;**46**:1793−806.
272. Tapia-Rojas C, Schuller A, Lindsay CB, Ureta RC, Mejias-Reyes C, Hancke J, et al. Andrographolide activates the canonical Wnt signalling pathway by a mechanism that implicates the non-ATP competitive inhibition of GSK-3beta: autoregulation of GSK-3beta *in vivo*. *Biochem J* 2015;**466**:415−30.
273. Rong Y, Liu W, Zhou Z, Gong F, Bai J, Fan J, et al. Harpagide inhibits neuronal apoptosis and promotes axonal regeneration after spinal cord injury in rats by activating the Wnt/beta-catenin signaling pathway. *Brain Res Bull* 2019;**148**:91−9.
274. Bagli E, Goussia A, Moschos MM, Agnantis N, Kitsos G. Natural compounds and neuroprotection: mechanisms of action and novel delivery systems. *In Vivo* 2016;**30**:535−47.

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