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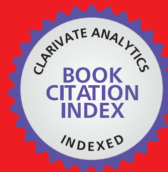

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Chapter 8

Flavonoids: Promising Natural Products for Treatment of Skin Cancer (Melanoma)

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Additional information is available at the end of the chapter

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Abstract

Melanoma, which is the most malignant skin cancer type, has got one of the fastest increasing incidence rates of all cancer types in the world. When belatedly diagnosed, melanoma is extremely invasive and metastatic. Although there are effective drugs used to treat melanoma, some cell lines have proven resistant to chemotherapy. In this context, several research groups on natural products have investigated the anticancer effect of new natural molecules in the treatment of melanoma. Flavonoids have shown to play an important role in chemoprevention and inhibition of the proliferation, migration, and invasion of melanoma cells. In this chapter, we present a systematic review performed through a literature search over a period of 20 years, using specialized databases. Analysis of all selected manuscripts demonstrated that at least 97 flavonoids have already been investigated for the treatment of melanoma using *in vitro* or *in vivo* models. Most of the bioactive flavonoids belong to the classes of flavones (38.0%), flavonols (17.5%), or isoflavonoids (17.5%). Apigenin, diosmin, fisetin, luteolin, and quercetin were considered as the most studied flavonoids for melanoma treatment. In general, flavonoids have shown to be a promising source of molecules with great potential for the treatment of melanoma.

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Keywords: cancer, melanoma treatment, herbal medicines, medicinal plants, flavonoids

1. Introduction

Natural products have contributed significantly to new drugs discovery. Historically, natural products derived from plants, microorganisms, and animals have been a promising source of medicinal preparations and molecules with therapeutic potential, for various diseases, including cancer treatment. The study of natural products also contributed to the provision of unique chemical structures, which were chemically modified, resulting in the development of new drugs [1–3].

An analysis of the new medicines approved by the US Food and Drug Administration (FDA) between 1981 and 2010 revealed that 34% of those drugs were based on small molecules from natural compounds or derivatives of natural compounds (semisynthetic products). This includes drugs such as statins, tubulin-binding anticancer, and immunosuppressant drugs. In this context, it is evident the contribution of natural products for drug discovery [3–5].

In the search for new anticancer drugs, natural products have provided many structural models with different mechanisms of action, for the treatment of melanoma regional or distant metastatic melanoma. Vinblastine from *Vinca rosea* and paclitaxel, which originates from a Chinese plant, is an example of anticancer agent obtained from natural sources. The therapy also includes drugs with different mechanisms of action, such as immunomodulatory agents, BRAF, and MEK inhibitors, and most recently, use of vaccines [6, 7]. However, even with recent advances in anticancer therapy, there is still a demand to develop new effective anticancer drugs for the melanoma treatment [8].

Despite the diversity of treatments for melanoma, the high resistance of tumor cells to conventional therapies drives the search for new anticancer agents that have less toxic effects, and greater effectiveness, incentive to develop new therapies that can be used individually or in combination with other drugs bringing therapeutic benefits for the patient. The polyphenolic compounds like flavonoids possess a large spectrum of pharmacological activity, including anticancer activity. These secondary metabolites have molecular mechanisms of action in tumor cells already understood, acting in enzymes and receptors associated and signal transduction pathways relating to cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, and metastasis [9–11].

2. Pathological aspects of skin cancer (melanoma)

2.1. Definition

Melanomas are malignant skin tumors deriving from melanocytes, the melanin-producing cells, that typically occur in the skin but may rarely occur in mucous membranes (vulva, vagina, and rectum), or uvea, the pigmented layer of the eye, lying beneath the sclera and

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cornea, and comprising the iris, choroid, and ciliary body. Melanomas account for less than 2% of skin cancers but are responsible for 80% of the mortality of patients with skin cancer [12]. They are classified in several subtypes, according to their tissue origin, tumor form, spreading and infiltrating behavior, metastatic potential, etc. These includes (a) superficial spreading melanoma, that tend to start growing outwards rather than downwards into the skin, (b) nodular melanoma, that tends to grow downwards, deeper into the skin, (c) lentigo maligna melanoma, that develops from very slow growing pigmented areas of skin called lentigo maligna or Hutchinson's melanotic freckle, (d) acral lentiginous melanoma, most commonly found on the palms of the hands and soles of the feet or around the big toenail, and (e) amelanotic melanoma, that usually have no, or very little color, occasionally are pink or red, or have light brown or gray around the edges [13].

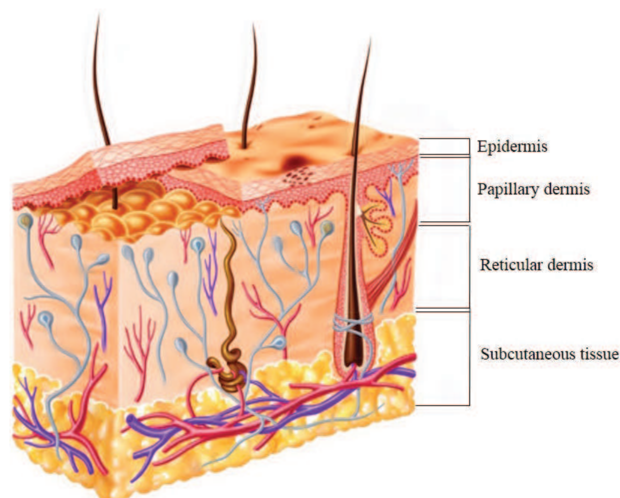
2.2. Etiology

The precise etiology of melanoma depends on several individual factors and is probably multifactorial in most cases [12]. Sun exposure (particularly UVB radiation) and genetic susceptibility (including faulty DNA repair) have been reported as major environmental and genetic factors associated with the risk of melanoma initiation and promotion [14, 15]. Precursor lesions, particularly dysplastic nevi/atypical moles, probably play a critical role in melanoma initiation [16]. Moreover, the distribution of melanoma among various work forces suggests that occupational risk factors could play an important role in the etiology of this cancer. For example, melanoma incidence is significantly higher in populations working in printing and press, petrochemical, and telecommunications industries [17].

2.3. Progression

The development of a melanoma tumor is considered a multistage process that involves various genetic and epigenetic alterations. From a histopathological point of view, the following steps can be considered: (a) common acquired nevi and dysplastic nevi, (b) radial growth phase melanoma, in which melanocytes undergo changes that enable them to survive and proliferate (c) vertical growth phase melanoma, in which tumor cells deeply invade into the dermis/hypodermis, and (d) malignant metastatic melanoma, in which the cells may eventually invade the endothelium and migrate to distant tissues [18–20]. When diagnosed in the early stages, melanoma can be easily treated by surgical excision of the primary tumor [21]. However, when the disease is at an advanced stage the treatment is very difficult because the cancer cells have a high capacity to cause metastases (including brain metastases) and acquire resistance to conventional therapy [22, 23]. The progression of cutaneous melanoma in the skin can be classified using the Clark method [18, 19] (**Figure 1**).

This classification is not very used, contrary to the TNM one, which is regularly revised by the American Joint Committee on Cancer (AJCC) [24, 25]. The TNM classification is based on the thickness of the primary tumor (T, also known as Breslow's index), presence of metastatic cells in the neighboring lymph nodes (N), and localization of metastasis in the body (M) (**Table 1**). The T criterion is subdivided into four categories from T1 to T4 discriminating melanomas from 1 –4 mm thick. Each category is subdivided into two subgroups that report the presence (a) or not (b) of ulceration of the primary tumor. The survival rate decreases with an increase in



Stages and description

I. Melanoma is found exclusively in epidermidis

II. Melanoma penetrates the upper part of the dermis (papillary dermis)

III. Melanoma penetrates to the junction of papillary dermis with the reticular dermis

IV. Melanoma penetrates the reticular dermis

V. Melanoma penetrates the subcutaneous tissue

Figure 1. Progression stages of the cutaneous melanoma, according to the Clark skin infiltration classification [18, 19].

T classification	**Tumor thickness**	**Ulceration-mitosis**
T1	≤1.0 mm	a. Without ulceration and mitosis < 1/mm^2 b. With ulceration or mitosis ≥ 1/mm^2
T2	1.01–2.0 mm	a. Without ulceration b. With ulceration
T3	2.01–4.0 mm	a. Without ulceration b. With ulceration
T4	>4.0 mm	a. Without ulceration b. With ulceration
N classification	**Metastatic lymph nodes**	**Size of metastatic lymph nodes**
N0	0	–
N1	1	a. Micrometastasis b. Macrometastasis
N2	2–3	a. Micrometastasis b. Macrometastasis c. In transit without metastatic nodule
N3	≥4	–
M classification	**Site**	**Seric LDH**
M0	0	–
M1a	Subcutaneous tissue and/or metastatic lymph nodes	Normal
M1b	Lung metastasis viscera metastasis	Normal
M1c	Distant metastasis	High

Table 1. The TNM classification of melanoma progression (adapted from Ref. [26]).

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the thickness of the tumor, which may, however, be smaller in the presence of ulceration. The classification N evaluates the number of neighboring lymph nodes containing metastatic melanoma cells. This criterion is subdivided into four categories from N0 to N3, as well as three subgroups according to the presence of small metastases detected after biopsy (a), large metastasis detected after clinical examination (b) and metastases in transit (c) (melanoma cells located between the primary tumor and the lymph node region in lymph channels). The classification M, with four categories from M0 to M1c, evaluates the localization of metastases in the organism, as well as the increase in serum lactate dehydrogenase concentration [24].

According to the TNM parameters, four melanoma progression stages can be defined (**Table 2**).

The only efficient treatment is the early surgical resection of the primary melanoma, when tumor cells have not already spread to nearby lymph nodes (stages I and II). Advanced and metastatic melanoma (stages III and IV) has very poor prognosis as most chemotherapeutical agents used to treat cancers are ineffective in killing melanoma cells, which are constitutively or adaptively resistant to proapoptotic drugs [22]. Melanoma is also resistant to radiotherapy [27, 28]. The overall positive responses to melanoma monotherapy using conventional anticancer drugs are weak and range from 4 to 26% [22]. Additionally, melanoma tumor displays pronounced neoangiogenesis [29] and a high ability to escape immune cell that explain why the 5-year survival rate for metastatic melanoma ranges from 5 to 10%, with a median survival of less than 8 months [30, 31]. Brain metastasis is present in 75% of stage IV melanoma patients, and constitutes a major cause of mortality because of the low permeability of the blood-brain barrier to chemotherapeutic drugs [30].

2.4. Epidemiology

Since 2005, World Health Organization (WHO) has identified the cutaneous melanoma as a priority public health concern as 132,000 new cases are registered every year in the world [32]. Since the early 1970s, the incidence of malignant melanoma has increased significantly, for example an average 4% every year in the United States [12, 14, 33]. The melanoma death rate in

Stage		T	N	M
I	IA	T1a	N0	M0
	IB	T1b-T2a	N0	M0
II	IIa	T2b/T3a	N0	M0
	IIb	T3b-T4a	N0	M0
	IIc	T4b	N0	M0
III	IIIa	T1 to T4b	N1a-N2a	M0
	IIIb	T1 to T4a	N1b-N2b-N2c	M0
	IIIc	T1 to T4b	N3	M0
IV	-	T1 to T4b	N1 to N3	M1

Table 2. Melanoma progression stages based on the TNM classification (adapted from Ref. [26]).

2012 was the highest in Australia and New Zealand (3.5/100,000) and Europe (2.3 per 100,000 people). In 2014, 76,100 new cases were diagnosed in the United States and 9710 patients with cutaneous melanoma died, according to the American Cancer Society. The wide disparity in melanoma incidence throughout the world depends upon the variation of early sun-exposure behaviors, recreational and vacation histories, nevus phenotypes and skin phototypes, distribution of melanoma risk genotypes, and discrepancies in epidemiological registrations between countries [34]. According to the WHO, a large number of atypical nevi (moles) are the strongest risk factor for malignant melanoma in fair-skinned populations. Malignant melanoma is more common among people with a pale complexion, blue eyes, and red or fair hair. It is over 20 times more frequent in White people compared to African-Americans and the risk increases with the age, although it also affects young adults, especially women. High, intermittent exposure to solar UV appears to be a significant risk factor for the development of malignant melanoma [14, 35], particularly for White people living in tropical regions [36, 37]. The incidence of malignant melanoma in White populations generally increases with decreasing latitude, with the highest recorded incidence occurring in Australia, where the annual rates are 10 and over 20 times the rates in Europe for women and men, respectively. Several epidemiological studies support a positive association with history of sunburn, particularly sunburn at an early age [34]. The role of cumulative sun exposure in the development of malignant melanoma is equivocal. However, malignant melanoma risk is higher in people with a history of nonmelanoma skin cancers and solar keratoses, both of which are indicators of cumulative UV exposure [38].

2.5. Immunity and immunotherapy of melanomas

Activation of genes in transformed melanocytes leads to the expression or overexpression of tumour-associated antigens. Several melanoma-associated antigens (MAA) have been identified and classified according to their tissue expression and structure [39, 40]. These include proteic antigens that can be recognized as MHC-I—peptide complexes by cytolytic T lymphocytes, membrane gangliosides, and conformational antigens inducing strong humoral responses by B-lymphocytes. MAGE-1, −2, −3 and −4 antigens are expressed by metastatic melanoma while their expression is absent in melanocytes and weak in primary melanoma tumors, indicating that the corresponding genes are activated during malignant transformation and progression [41]. Other MAA such as Melan-A/melanoma antigen recognized by T-cells (MART-1), tyrosinase, Pme117/gp100, gp75/tyrosine-related protein (TRP)-1 and AIM-2 are expressed in normally differentiated melanocytes and melanoma cells but absent in other tumor cells, suggesting the possibility to target them for a specific destruction of melanoma tumors [42, 43]. The expression of various gangliosides present in the membranes of melanocytes and melanoma cells (GM3, GD3, GM2, GD2 and *O*-acetyl GD3) is also significantly increased during malignant transformation [44]. Given that malignant melanoma is one of the most immunogenic tumor and that melanomas are highly resistant to chemotherapy and radiotherapy, immunotherapy appears as one of the most promising and relevant strategies to destroy melanoma tumors and metastatic cells.

Promising results have been reported using *ex-vivo* stimulation of tumor-infiltrating lymphocytes by cytokines and MAA, potentiation of T-cell cytotoxic activity by blocking CTL-A4

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co-inhibitory receptor (using monoclonal antibodies), CAR-T strategies, and combination of immunotherapy with chemotherapeutics (e.g., dacarbazine/CTL-A4 blockade) [22, 31, 45–49]. Interferon-α and interleukin-2 monotherapeutic treatments give an overall positive response in 13–25% patients, and constitute a first-line therapy for nonmetastatic patients. Ipilimumab, an anti-CTLA-4 monoclonal antibody, targeting a T-cell receptor decreasing T-cell activation and cytotoxicity, allows a long-term survival benefit in one-third of metastatic melanoma patients, and a complete remission in patients [50]. As a consequence, a high research effort is dedicated to the development of new antibodies activating antitumoral immunity and to the discovery of new natural drugs with cytostatic, antimetastatic, and/or antiangiogenic activity that could stimulate the immune system and be used in chemoimmunotherapy protocols to synergize with chemotherapeutic drugs and immune effectors.

In this view, only a few natural molecules have proved their efficacy to limit tumor growth and inhibit the invasiveness of highly aggressive melanoma cells in *in vitro* and *in vivo* models. The efficacy of such molecules is related to their antiangiogenic activity (e.g., resveratrol [51], curcumin [52]), to their capacity to induce melanoma cell death regardless of their apoptosis-sensitivity (e.g., narciclasine [53], carotenoids [54–59]), to their ability to target components of apoptotic pathways to overcome melanoma cells resistance to anticancer drugs (e.g., epigallocatechin gallate [60–62]), or to their strong stimulatory effect on antitumoral immunity (e.g. *Lentinula edodes* polysaccharides [63]). Considering the clinical efficacy of melanoma immunotherapy, combined to the high potential of natural compounds to limit melanoma growth and restore melanoma sensitivity to apoptosis inducers without impairing antitumoral immunity, an important research effort should be undertaken to assess the efficacy of original natural cytostatic compounds, highlight the molecular and cellular mechanisms involved in their pharmacological action, and study if these molecules favor *in vivo* melanoma rejection via their immune regulatory properties. Considering the fast growth of melanoma and failure of current treatments, the identification and clinical development of such efficient molecules will obviously have a significant impact on patient survival rate and duration.

3. Molecular and cellular pathways involved in melanoma biogenesis and progression

3.1. Implication of the MAPK pathway

The receptor tyrosine kinase MAPK pathway triggers a signaling cascade that regulates cell growth, proliferation, differentiation, and survival in response to a wide variety of extracellular stimuli including hormones, cytokines, and growth factors through the activation of tyrosine kinase receptors. As mutations of components of the MAPK pathway are associated with increased activity of ERK1/2 proteins [64], deregulation of this pathway contribute to both development and progression of melanoma. In particular, mutations in B-RAF, a member of the RAF kinase family, have been identified in up to 70% of malignant melanoma [65].

Binding of a ligand to the membrane bound tyrosine kinases receptors (RTKs) or integrins adhesion to extracellular matrix triggers the activation of the RAS GTPases which further lead

to activation/transduction of the MAPK signaling pathway. Ras GTPases are small proteins bound to the cytoplasmic membrane. The RAS gene encodes three isoforms with tissue-specific pattern: HRAS, KRAS, and NRAS [64]. Downstream targets of RAS proteins are the PI3K/Akt pathway and the serine threonine kinase RAF proteins [66, 67]. Activated B-RAF then leads to the activation of the MEK/ERKs kinases, which targets a variety of signaling pathways such as cell growth, proliferation, protein synthesis, and apoptosis.

The RAF kinase family consists of three cytoplasmic proteins (A-RAF, B-RAF, and C-RAF) which participate in the MAPK transduction pathway. Unlike, c-RAF and A-RAF, mutations in B-RAF have been identified in up to 70% of malignant melanoma [68, 65]. Most frequent activating somatic mutations in B-RAF occur at the V599E where a valine replaces a glutamic acid [68]. Identification of such activating mutations in B-RAF proteins leads to the development of new drugs, such as B-RAF inhibitors, as anticancer strategies [69].

These oncogenic B-RAF proteins are able to transform fibroblastic cell line and lead to hyperactivation of the ERK proteins [68]. Constitutive ERK leads to increased proliferation apoptosis resistance in melanoma cells [69]. Interestingly, suppression of the tumor suppressor PTEN and activating mutations in B-RAF are both necessary in melanoma development highlighting the importance of the PI3K/Akt pathway upregulation in melanoma growth and apoptosis resistance [70].

Indeed, the tumor suppressor PTEN is downregulated in melanoma and this is associated with PI3K/Akt hyperactivation [71]. Apoptosis resistance could be mediated through activation of the NF-KB pathway, target of hyperactive ERK proteins [72]. Oncogenic B-RAF also leads to inhibition of the LKB1-AMPK pathway, a central signaling pathway at a crossroad between metabolism and proliferation regulation through, in particular, inhibition of the mTOR pathway. This study highlights a new pathway in tumor growth regulation [73]. Finally, expression of MCL-1, a member of the Bcl-2 pathway, whose alternative splicing leads to proteins with either pro- and antiapoptotic activities, is increased in melanoma metastasis associated with oncogenic B-RAF [74]. Oncogenic B-RAF may also trigger the antiapoptosis pathway through inhibition of the proapoptotic Bim proteins [75].

Besides, downregulation of a downstream target of the MAPK pathway, MITF (microphthalmia-associated transcription factor), the master regulator of melanocyte development, survival, and function, is associated with poor diagnosis and melanoma progression [76]. Finally, cKIT, a tyrosine kinase receptor, might trigger proliferation signals in melanoma through activation of the MAPK pathway [77].

Others signaling pathways contribute to melanoma progression and metastasis such as the noncanonical Wnt signaling [78] and deregulation of the cyclin-dependent kinase inhibitor 2A (CDKN2A) pathway involved proliferation and apoptosis control [23].

3.2. Implication of extracellular vesicles (EV) in melanoma biogenesis and progression

Extracellular vesicles (EVs) are small vesicles released by most cell types in the extracellular environment, and as a consequence can be retrieved from various body fluids, especially plasma. EV might split into apoptotic bodies (>1 μm), microparticles (100 nm to 1 μm) released after membrane blebbing and exosomes (<100 nm), vesicles with an endosomal origin release

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after multivesicular bodies fuse with the plasma membrane [79]. EVs are biological vectors that convey lipids, different classes of proteins (cytoskeleton, adhesion, raft associated proteins, histones, chaperones [80], glycoproteins, and chemokines [81] or even morphogens such as Hedgehog proteins [82]. EVs also harbor nucleic acids [83] able to modulate the differentiation of the target cells [84]. EVs communicate with their target cells via receptor-ligand interaction, through transfer of membrane proteins [85, 86], can fuse with the plasma membrane [87], or transfer their components into target cells via phagocytosis [88] or endocytosis [89]. The ability of EVs released from antigen presenting cells to convey MHC class II proteins [90] highlights their immunomodulatory properties and their potential as therapeutic agents in anticancer strategies [91]. Moreover, as they carry tumor antigens via MCH class I proteins, EVs can initiate antitumor response *in vitro* [92] and *in vivo* [93]. Nevertheless, the composition of EVs and the message they convey depend both on the cells they originate from and the conditions triggering their release. Indeed, EVs from dendritic cells can also suppress immune response in inflammatory diseases models such as DTH (delayed-type hypersensitivity) mice [94]. This ability to attenuate immune response might be associated with the capacity of EV to induce expression of molecules able to inactivate T-cells or suppress immune response [95].

In addition to their immunomodulatory properties, the role of EVs in inflammation, angiogenesis, and proliferation has been widely demonstrated [96–98]. This suggests an implication for EVs in tumor survival and progression. In this study, exosomal markers, such as CD63, could be found also on a wide range of subpopulations of EVs, and as long as there is no determination of the cellular origin of vesicles, we chose to use the term EV to refer to both exosomes and microparticles.

3.3. Role of circulating EV in melanoma biogenesis and progression

Circulating EV can trigger inflammatory pathways in target cells [96], stimulate angiogenesis [82, 99, 100], protect against apoptosis [101], or stimulate proliferation [102].

Plasma levels of EV harboring CD63 in melanoma-engrafted SCID mice correlate to tumor size, suggesting a role of the tumor in EV secretion [103]. However, other suggests that circulating rates of EV do not differ between melanoma and healthy patients [104–106] but instead, EV protein composition might differ. In particular, plasmatic EVs from melanoma patients are enriched in platelet-derived EV involved in neovascularization (CD42a harboring EV) and antitumour immune responses (CD8 harboring EV) [105]. Furthermore, circulating endothelial and platelet derived-EV (EEV) and procoagulant EV are significantly higher in melanoma patients [107]. Such procoagulant EVs stimulate proinflammatory cytokines secretion by macrophages and drive melanoma metastasis *in vivo* [108] reinforcing the implication of EV in melanoma progression.

3.4. A role of EV in melanoma metastasis

EV release is exacerbated in human malignant [109] and murine [110] melanoma cell lines. Furthermore, in comparison with murine melanocyte cell line, metastastic melanoma cell lines secrete highly procoagulant EV harboring phosphatidylserine and enriched in tissue factor proteins suggesting that melanocyte transformation into cancer cells is associated with the secretion of such EVs [110]. Besides, Wnt5a, a noncanonical Wnt signaling ligand in involved melanoma

progression [78] induces the release of melanoma exosomes enriched in proangiogenic proteins and pro-inflammatory cytokines [111].

Proteomic analysis of human malignant melanoma cell lines A375 reveals an enrichment in proteins involved in angiogenesis and matrix remodeling such as annexin A1 and hyaluronan and proteoglycan link protein 1 (HAPLN1) [109]. Analysis of EV microRNA content reveals enrichment in miRNA involved in cell growth, proliferation, and apoptosis. Uptake of such EV promotes the invasion ability of normal melanocytes [109]. Furthermore, tumor-derived EV harbor FAS ligand involved in antitumor response through lymphocytes apoptosis [112]. Finally, human (SK-Mel28/-202/-265/-35) and mouse (B16-F10) cell line–derived exosomes are enriched in TYRP2 (tyrosinase-related protein-2), VLA-4 and Hsp90 proteins. Indeed, B16F10-derived exosomes are enriched in prooncogenic proteins such as the oncogene MET which has been described a role in cell transformation, proliferation, survival, invasion, and metastasis [113–115]. BM cell treatment with such exosomes led to an increase in tumor size compared to nontreated mice. Compared to EV derived from B16F1, a poor metastatic cell line, injection of B16F10 EV led to increased metastatic lesions and a wider tissue distribution (brain, bone) [106]. This is in agreement with previous studies suggesting that highly metastasis cells are enriched in oncogene Met72 and are more deleterious than B16F1-derived EV [116]. These data strongly suggest that EV from melanoma cells is able to suppress antitumor response and stimulate tumor progression but also their ability to trigger melanoma invasion and metastasis. However, different populations of EV have distinct procoagulant properties [117]. Thus, it is therefore necessary to identify the cell origin of EV in order to determine their role in cancer progression.

3.5. Role of microRNAs in melanoma progression

MicroRNAs are noncoding small RNAs able to bind target mRNAs, through their 3′UTRs leading to their degradation. Binding of microRNAs to their targets allows regulating a wide variety of cellular mechanisms such as proliferation, angiogenesis, inflammation, and survival.

A role for microRNAs in melanoma progression was first demonstrated through different miRNA expression signatures associated with the developmental lineage and differentiation state of solid tumors [118]. Furthermore, a microarray analysis demonstrates a specific targeting between A375 cell line and the A375 cells-derived EV of 28 miRNAs involved in cellular growth, development, and proliferation [109]. Relevance of microRNAs implication in melanoma development was illustrated by the fact that miRNAS loci are retrieved in genomic regions altered in melanoma [119]. MITF (microphthalmia-associated transcription factor) the master regulator of melanocyte development, survival, and function, which is often dysregulated in melanoma is a target of miR-137 [120] and miR-182 [121]. Finally, a number of microRNAs such as miR-214 [122] and miR-223 [123], but also miR-137, miR-182, miR-221/222, and miR-34a, have been involved in melanoma progression (for a review see [124]). In particular, miR-221 and miR-222 are involved in tumor proliferation and an increased in invasion and migration abilities through targeting of p27Kip1/CDKN1B (cyclin-dependent kinase inhibitor 1B) and the tyrosine kinase receptor c-KIT receptor [124, 125].

However, five members of the Let-7 family are downregulated in primary melanoma suggesting that these microRNAs might trigger anticancer responses. In particular, Let7b which targets cyclins exerts antitumoral responses through inhibition of cancer cycle progression [126].

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MicroRNAs can also be transported via EV and regulate the pathway in distant target cells. In particular, circulating EV from metastatic melanoma patients harbors a specific miRNA signature. Indeed, those EVs are enriched in oncogenics miRNAs mir17 and miR19a suggesting a role for miRNAs-associated EV in tumor progression and metastasis [127]. On the other hand, circulating EV in advanced melanoma patients shows a decrease in miR-125b which downregulation has been described in melanoma progression [128].

Finally, deep-RNA sequencing allows identifying an enrichment of 23 specific microRNAs in small EV including miR-199a-3p, miR-150-5p, miR-142-3p, and miR-486-5p known to be involved in melanoma progression or identified in melanoma metastasis or patient blood samples [129]. In particular, miR-214 has been associated with melanoma metastasis [122]. Interestingly, *in silico* analysis reveals that some of these miRNAs could target the BRAF pathway which is often deregulated in melanoma [129]. Metastatic cell lines secrete EV enriched in the oncogenic miR-222. Furthermore, miR-222 associated with EV can be transferred into target cells and promote tumorigenesis through activation the Akt/PI3K pathway [130].

3.6. Identification of new markers for melanoma diagnosis and prognosis

Circulating concentrations of lactate dehydrogenase [79], S100 and MIA (Melanoma Inhibitory Activity), two small proteins expressed by melanoma cells, are significantly higher in melanoma patients [131] and thus are widely used a proteins markers in order to monitor melanoma progression. LDH concentrations might be a better prognosis factor to classify advanced melanoma [132, 133].

Other circulating factors such as circulating nucleic acids or EV could be used in melanoma detection as a prognosis factor in advanced stages of diseases. Indeed, circulating EVs from stage III to stage IV are enriched TYRP2 (tyrosinase-related protein-2), a specific melanoma protein, VLA-4 (very late antigen 4) and HSP90. Furthermore, these enriched EVs correlated with poor survival prognosis [106]. These authors identified a specific exosomes protein signature that could be used as a prognosis marker in stages III and IV melanoma patients [106].

Besides, circulating EV carries melanoma markers such as S100B and MIA proteins. Concentrations of EV-S100B and EV-MIA are higher in stage IV melanoma patients and such EV was associated to poor prognosis in patients [104]. Detection of such EV could be used as an additional diagnosis and prognosis marker of melanoma patients. In contrast, these authors did not find an increase in TYRP2 containing exosomes in plasma of melanoma patients. This discrepancy could be due to difference in EV isolation, or EV concentrations/number analysis (NTA analysis vs. EV-protein concentration determination). Finally, circulating EV enriched in oncogenes miRNAs mir17 and miR19a could be used as predictive markers in melanoma patients [127].

In addition, some microRNAs detected in patient metastasis such as miR-150, miR-342-3p, miR-455-3p, miR-145, miR-155, and miR-497 could be used as a specific signature to predict postsurvival recurrence with a high expression of miR-145, miR-155 in metastatic tissue associated with longer survival [134]. Finally, identification of a specific signature of 16 differentially expressed microRNAs in patient blood samples represents a new noninvasive tool in diagnosis applications [135]. Finally, other authors suggest that microRNAs from blood patients could be used to monitor melanoma recurrence [136, 137].

4. Current melanoma treatment

The treatment options for regional or distant metastatic melanoma have expanded in recent years and are directly influenced by disease stage at diagnosis and the extent of metastases. The therapy used includes several drugs with different mechanisms of action, including chemotherapies, immunomodulatory agents, the serine/threonine protein kinase BRAF, mitogen-activated protein kinase (MEK) inhibitors, and most recently, use of vaccines [6, 7]. The primary treatment of this cancer type is surgical excision, sentinel lymph node dissection, radical lymph node dissection, and isolated limb perfusion [138, 139].

Chemotherapy may now be considered a second or third line in patients with resistance to immunotherapy and targeted therapy [140]. Tumor cells may evade the immune attack by some mechanisms, such as impaired antigen presentation, expression of factors with immunosuppressive properties, such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), interleukin-2 (IL-2), and induction of resistance to apoptosis. In addition, melanoma cells further express receptors on the cell surface which function as checkpoints to the immune system response, as the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). The ipilimumab is an anti-CTLA-4 monoclonal antibody and the nivolumab and pembrolizumab are also monoclonal antibodies directed against the PD-1 receptor, that blocking the inhibitory ligand's suppression of immune response. Thus, the main objectives of immunotherapy are to activate an immune response through the immunostimulation of IL-2, the upregulation of tumor-inhibitory T cells, and the inhibition of the immune control points [141, 142].

In addition to the immunological approach, targeted therapies have also been employed in the treatment of melanoma, such as BRAF and MEK inhibitors. The BRAF gene is responsible for encoding the B-raf protein that participates in the regulation of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway, which regulates cell proliferation, differentiation, and cell cycle progression [142]. The discovery that BRAF was mutated in about 50% of melanomas led to the development of BRAF kinase inhibitors as vemurafenib and dabrafenib. However, most patients acquire resistance mechanisms to BRAF kinase inhibition [141, 143]. In view of the development of resistance to single BRAF blockade, several combination schemes have been developed, as the combination therapy with MEK inhibitors trametinib and cobimetinib [144–146].

Despite these advances, about 80% of patients develop resistance to the current standard of treatment with the combination of a selective BRAF and MEK inhibitors, which stimulates research for new treatment alternatives. The use of triple combining therapy has also been the subject of investigations and demonstrated prolonged responses [147]. Preclinical assays performed with the triple combination of BRAF and MEK inhibitors and anti-PD-1 demonstrated high antitumor activity and phase I/II clinical studies have shown promise in BRAFV600-mutated melanoma [148]. Moreover, vaccines have also been investigated and in 2015 the Food and Drug Administration (FDA) approved the Talimogene laherparepvec (T-VEC), an oncolytic virus derived from herpes simplex type 1, which can selectively replicate within tumors and produce granulocyte macrophage colony stimulating factor (GM-CSF) which promotes increased antitumor immune response [149].

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5. Chemical and biological aspects of flavonoids

Flavonoids are phenolic constituents commonly found in a variety of fruits, vegetables, and medicinal plants. They add color, flavor, and aroma to plants, and play an important role in protection mechanisms against pathogens, ultraviolet radiation, and herbivores. Flavonoids comprise an important class of secondary metabolites, with numerous possibilities of chemical structures [150, 151].

Flavonoids have a basic phenylbenzopyrone skeleton (C_6-C_3-C_6), admitting several substitution possibilities. In accordance with the substitution pattern of A, B, and C rings of the basic structure, flavonoids can be classified as chalcones, aurones, flavones, flavonols, flavanols, flavanones, isoflavones, flavanonols, among others [10]. The most common classes of flavonoids are shown in **Figure 2**.

Flavonoids may include the polyhydroxylated or polymethoxylated form. There are more than 8000 flavonoids identified, some of the most abundant are quercetin, catechin, and kaempferol, which are often combined with glycosidic units (commonly glucose, galactose, and rhamnose) through C-C or C-O-C bonds [152, 153]. The structural diversity of flavonoids directly influences their chemical, physical and pharmacological properties.

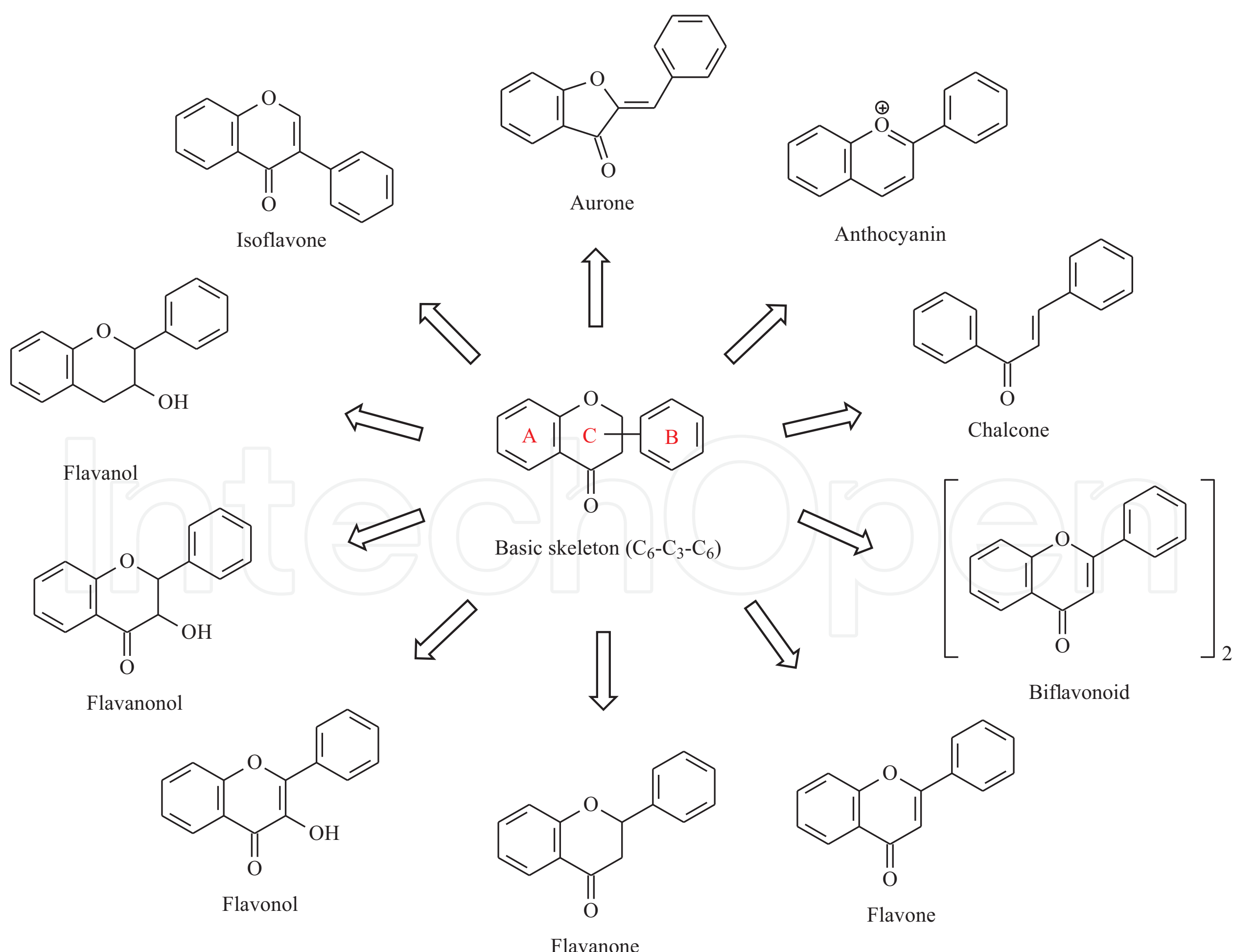

Figure 2. Basic skeleton (C_6-C_3C_6) and main classes of flavonoids. This figure was adapted from Ref. [10].

Several studies have demonstrated the therapeutic properties of flavonoids obtained from plants or through synthesis. In fact, the structural diversity of flavonoids contributes to the diverse pharmacological activities reported for these compounds. *In vitro* and *in vivo* assays have shown the antioxidant, anti-inflammatory, antinociceptive, cardioprotective, photoprotective, antidepressant, antimicrobial, and cytotoxic effects of flavonoids [154, 155].

Recently, flavonoids have been shown to be potent antitumor agents. These compounds showed promising effect against different tumor cell lines, including human melanoma cells [156, 157]. In addition, flavonoids typically exhibit low toxicity in biological systems, which make them an alternative therapy compared with traditional anticancer drugs [158–161].

6. Bioactivity of flavonoids on melanoma

In this section, the authors present a systematic review performed through a literature search over a period of 20 years (January 1996–December 2016). This literature search was performed through specialized databases (PUBMED, LILACS, SCIELO, Science Direct, and Web of Science) using different combinations of the following keywords: flavonoid, flavonoid derivative, melanoma, skin cancer, treatment, and anticancer therapy. We did not contact investigators and we did not attempt to identify unpublished data.

Manuscripts were selected based on the inclusion criteria: articles published in English, Portuguese, Spanish, or French and articles with keywords in the title, abstract, or keywords, as well as studies involving anticancer activity of natural flavonoids necessarily against melanoma in *in vitro* or *in vivo* models. Other review articles, meta-analysis, abstracts, conferences, editorial/letters, case reports, conference proceedings, or articles that did not meet the inclusion criteria were excluded from this systematic review.

For the selection of the manuscripts, two independent investigators (RGOJ and CAAF) first selected the articles according to the title, then to the abstract, and finally through an analysis of the full-text publication. A consensus between the investigators was reached as a clarification for in order to clarify all disagreements. The selected articles were manually reviewed with the purpose of identifying and excluding the works that did not fit the criteria described above.

The primary search identified 164 articles. However, among these, 39 manuscripts were indexed in two or more databases and were considered only once, resulting in 125 articles. After an initial screening of titles, abstracts, full text, and time of publication, 43 articles were selected, while the remainder did not meet the inclusion criteria (n = 82). Although many articles presented promising anticancer activity for plant extracts rich in flavonoids, we considered only articles that showed anticancer activity of the isolated flavonoids on melanoma cell lines. Investigations involving synthetic flavonoids were also excluded from this review. A flowchart illustrating the progressive study selection and numbers at each stage is shown in **Figure 3**.

Analysis of all selected manuscripts demonstrated that at least 97 flavonoids have already been investigated for the treatment of melanoma using *in vitro* or *in vivo* models. Most of the bioactive flavonoids belong to the classes of flavones (38%), flavonols (17.5%), or isoflavonoids

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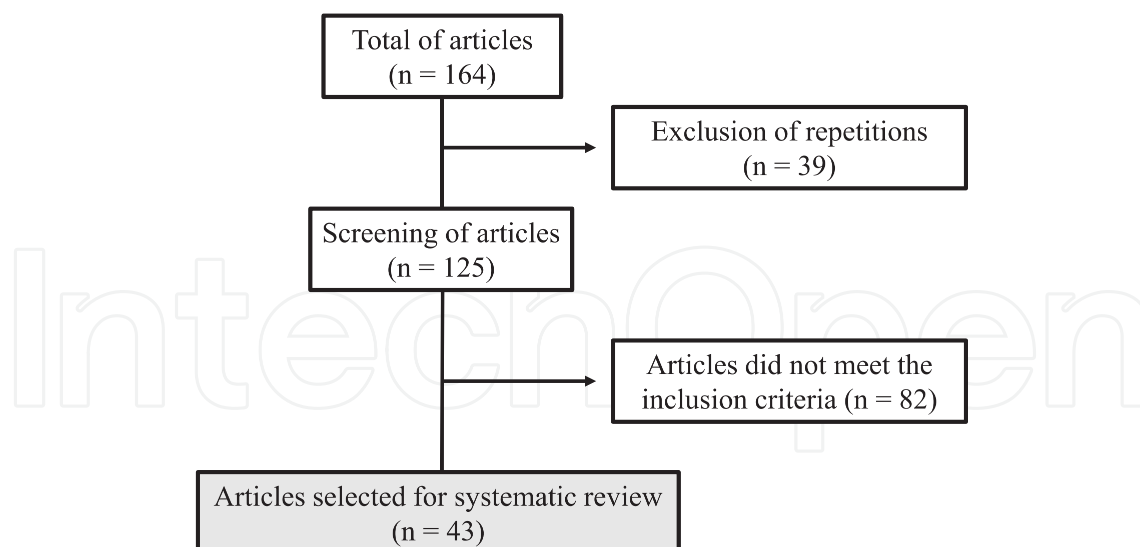

Figure 3. Flowchart of included studies for the systematic review.

(17.5%), which has aroused the interest of several research groups in natural and synthetic products in the world. All information about these flavonoids for the treatment of melanoma was reported in **Box 1**. Next, we highlight the anticancer properties of the main tested flavonoids on melanoma cells (apigenin, diosmin, fisetin, luteolin, and quercetin).

6.1. Bioactivity of apigenin on melanoma

Apigenin (4′,5,7,-trihydroxyflavone) is a nonmutagenic and low-toxicity dietary flavonoid commonly present in many fruits, vegetables, and medicinal plants. This flavone has a broad spectrum of antiproliferative activities against many types of cancer cells, including melanoma. Recent studies have demonstrated that apigenin inhibits cell growth through cell cycle arrest and apoptosis in malignant human melanoma cell lines. Hasnat et al. [169] showed that treatment with 50 μM apigenin significantly reduced viable cell percentages in A375 and A2058 human melanoma cells. Treatment with apigenin for 24 h also decreased human melanoma cell numbers in a dose-dependent manner. A similar result was observed by Spoerlein et al. [170], who evaluated the cytotoxic potential and the effect of apigenin on the cell cycle of 518A2 human melanoma cells. Apigenin also caused a dose-dependent decrease in the percentage of transwell-migrated cells, and ~90 and ~70% inhibitions of cell migration were recorded upon treatment with 20 μM of apigenin, respectively, for A2058 and A375 cells [169].

The cytotoxic effects of apigenin were related to its ability to reduce integrin protein levels and inhibit the phosphorylation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK1/2). Furthermore, apigenin treatment increased apoptotic factors such as caspase-3 and cleaved poly(ADP-ribose) polymerase in a dose-dependent manner. Cao et al. [172] have also demonstrated that apigenin suppressed STAT3 phosphorylation, decreased STAT3 nuclear localization, and inhibited STAT3 transcriptional activity. Apigenin also downregulated STAT3 target genes MMP-2, MMP-9, VEGF, and Twist1, which are involved in cell migration and invasion. In this same investigation, it was determined the *in vivo* antimetastatic effect of

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Aurones						
2,6-Dihydroxy-2-[(4-hydroxyphenyl)methyl]-3-benzofuranone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
Anthocyanins						
Cyanidin-3-*O*-β-glucopyranoside (C-3-G)	*In vitro*	M14 (HM)	5 or 10 μM	A treatment with a single dose of C-3-G decreased cell proliferation without affecting cell viability and without inducing apoptosis or necrosis. C-3-G treatment also induced increase of cAMP levels and upregulation of tyrosinase expression and activity resulting in an enhanced melanin synthesis and melanosome maturation.	Upregulation of the melanoma differentiation antigen Melan-A/MART-1 in treated cells respect to the untreated control was recorded.	[163]
Biflavonoids						
Pteridium III	*In vitro*	A375 (HM)	ND	Preliminary test determined IC_{50} equal to 106.7 μM.	NE	[164]
Chalcones						
2′,4′-Dihydroxychalcone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 44.3 μM.	NE	[162]
4,4′-Dihydroxy-2′-methoxychalcone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 56.3 μM.	NE	[162]
Isoliquiritigenin (ISL)	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 80.5 μM.	NE	[162]
	In vitro and *in vivo* (mice)	B16F0 (MM)	5–25 μg/ml	A significant concentration- and time-dependent reduction in cell proliferation was observed. The cell inhibition rate ranged from 18 to 79% and 35 to 91% after 24 and 48 h of ISL treatment (5, 10, 15, 20, and 25 μg/mL), respectively. ISL	ISL increased reactive oxygen species (ROS) formation during B16F0 cell differentiation, but no specific target was evaluated.	[165]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
				did not show significant activity in the *in vivo* model.		
Phloretin	*In vitro*	M14 (HM)	1–40 μM	Cell viability was 31.6% in M14 cells exposed to 40 μM of this compound. Phloretin induced apoptosis in a concentration dependent manner with significant effect at 20 μM after 48 h of treatment.	NE	[166]
α,2′,4,4′-tetrahydroxydihydrochalcone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} > 100 μM.	NE	[162]
Flavones						
5,3′,4′-Trihydroxy-6,7,5′-trimethoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 14 μM.	NE	[167]
5,4′-Dihydroxy-6,7,3′,5′-tetramethoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 241 μM.	NE	[167]
5,6,3′-Trihydroxy-7,4′-Dimethoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 18 μM.	NE	[167]
5,6,4′-Trihydroxy-7,3′,5′-trimethoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 39 μM.	NE	[167]
5,6,7-Trihydroxybaicalein	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 11 μM.	NE	[167]
5,6-Dihydroxy-7,3′,4′-trimethoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 29 μM.	NE	[167]
5,7-Dihydroxy-6-methoxyhispidulin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 67 μM.	NE	[167]
5,7-Dihydroxy-7-methoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 119 μM.	NE	[167]
6-Hydroxyluteolin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 13 μM.	NE	[167]
6-Methoxyflavone	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 398 μM.	NE	[167]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
6-Prenylapigenin	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 32.5 μM.	NE	[168]
Albanin	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 84.7 μM.	NE	[168]
Apigenin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 26 μM.	NE	[167]
	In vitro	A375 and A2058 (HM)	0–50 μM	Treatment with 50 μM apigenin significantly reduced viable cell percentages in both types of melanoma cells. Apigenin exhibited dose-dependent inhibition of melanoma cell migration, unlike untreated controls.	Apigenin reduced integrin protein levels and inhibited the phosphorylation of focal adhesion kinase (FAK) and extracellular signal regulated kinase (ERK1/2). Furthermore, apigenin treatment increased apoptotic factors such as caspase-3 and cleaved poly (ADP-ribose) polymerase in a dose dependent manner.	[169]
	In vitro	518A2 (HM)	ND	Preliminary test determined $IC_{50} > 50$ μM. The flavonoid also reduced cell migration.	Apigenin induced an arrest of the cell cycle of 518A2 melanoma cells at the G2/M transition and also attenuated the expression and secretion of the metastasis relevant matrix metalloproteinases MMP-2 and MMP-9.	[170]
	In vitro	MDA-MB-435 (HM)	1–50 μM	Preliminary test determined $IC_{50} > 50$ μM.	NE	[171]
	In vitro and *in vivo* (mice)	B16F10 (MM), A375 and G361 (HM)	0–40 μM (in vitro tests) and 150 mg/kg (*in vivo* tests)	Apigenin (5 and 10 μM) also dose-dependently inhibited B16F10, A375 G361 cell migration and invasion. Apigenin-treated mice had significant fewer metastatic nodules.	Apigenin suppressed STAT3 phosphorylation, decreased STAT3 nuclear localization and inhibited STAT3 transcriptional activity. Apigenin also downregulated STAT3 target genes MMP-2, MMP-9, VEGF and Twist1, which are involved in cell migration and invasion.	[172]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Artocarpin	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 10.3 μM.	NE	[168]
Baicalein	*In vitro*	B16F10 (MM)	0–200 μM	Treatment with 40 μM baicalein resulted in approximately 87% inhibition of cell growth. Baicalein also inhibited the migration and invasion of B16F10 cells.	Baicalein reduced the expression of MMPs and tightening TJ through the suppression of claudin expression, possibly in association with a suppression of the phosphoinositide 3-kinase/ Akt signaling pathway.	[173]
	In vitro	B16F10 (MM)	3.156–50 μM	Preliminary test determined IC_{50} equal to 50 μM.	NE	[174]
Brosimone I	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 10.7 μM.	NE	[168]
Chrysin	*In vitro*	518A2 (HM)	ND	Preliminary test determined IC_{50} > 50 μM. The flavonoid also reduced cell migration.	Apigenin induced an arrest of the cell cycle of 518A2 melanoma cells at the G2/M transition and also attenuated the expression and secretion of the metastasisrelevant matrix metalloproteinases MMP-2 and MMP-9.	[170]
	In vitro	B16BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 20.5 μM.	NE	[175]
	In vitro	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 51 μM.	NE	[167]
Cirsilineol	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 73 μM.	NE	[167]
Cirsiliol	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 9 μM.	NE	[167]
Cudraflavone B	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 12.5 μM.	NE	[168]
Cudraflavone C	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 9.2 μM.	NE	[168]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Desmethoxylcentaureidin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 64 μM.	NE	[167]
Diosmin	*In vivo* (mice)	B16F10 (MM)	0.2% solution (*ad libitum* as drink)	Animals treated with diosmin presented a reduction in the number of subpleural mestases in comparison to the negative control group.	NE	[176]
	In vivo (mice)	B16F10 (MM)	551 mg/kg/day Diosmin alone or combined with different doses of IFN-α	IFN-α showed a dose-dependent antiinvasive and antiproliferative activity in our study, while diosmin showed an antiinvasive activity similar to the lower dose of IFN-α used. Combination of diosmin and IFN-α have shown synergistic effect.	NE	[177]
	In vivo (mice)	B16F10 (MM)	0.2% solution (*ad libitum* as drink)	Group treated with diosmin showed the greatest reduction (52%) in the number of metastatic nodules.	NE	[178]
	In vivo (mice)	B16F10 (MM)	20 mg/day	Diosmin decreased the number of metastatic nodules (52%), implantation (79%), growth (67%) and invasion (45%) index.	NE	[179]
Eupafolin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 16 μM.	NE	[167]
Eupatilin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 58 μM.	NE	[167]
	In vitro	B16F10 (MM)	10^{-4}–10^{-8} M	Preliminary test determined IC_{50} from 33 to 85 μM.	NE	[180]
Eupatorin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 44 μM.	NE	[167]
Isolinariin A	*In vitro*	C32 (HM)	ND	Preliminary test determined IC_{50} equal to 11.76 μM.	NE	[181]

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Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Isolinariin B	*In vitro*	C32 (HM)	ND	Preliminary test determined IC_{50} equal to 21.47 μM.	NE	[181]
Jaceosidin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 27 μM.	NE	[167]
	In vitro	B16F10 (MM)	10^{-4} to 10^{-8} M	Preliminary test determined IC_{50} from 32 to 49 μM.	NE	[180]
Kuwanon C	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 14.2 μM.	NE	[168]
Linariin	*In vitro*	C32 (HM)	ND	Preliminary test determined IC_{50} equal to 12.6 μM.	NE	[181]
Luteolin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 21 μM.	NE	[167]
	In vitro	MDA-MB-435 (HM)	1–50 μM	Preliminary test determined IC_{50} equal to 30.3 μM.	NE	[171]
	In vitro	A375 (HM)	0–80 μM	Preliminary test determined IC_{50} equal to 115.1 μM. Luteolin also inhibited colony formation and induced apoptosis in a dose and time-dependent manner by disturbing cellular integrity as evident from morphological evaluation.	Accumulation of cells in G0/G1 (60.4-72.6%) phase for A375 cells after 24 h treatment indicated cell cycle arresting potential of this flavonoid, but no specific target was investigated.	[182]
	In vitro	B16F10 (MM)	3.156–50 μM	Preliminary test determined $IC_{50} > 50$ μM.	NE	[174]
	In vitro	A2058 (HM)	0–80 μg/ml	Luteolin inhibited cell proliferation (IC_{50} = 35 μg/ml) and increased apoptotic body formation. Luteolin induces apoptosis by Endoplasmic Reticulum (ER) stress via increasing Reactive Oxygen Species (ROS) levels.	Luteolin increased expression of the ER stress-related proteins; protein kinase RNA-like ER kinase, phosphor eukaryotic translation initiation factor 2α, activating transcription factor (ATF) 6, CCAAT/enhancer-binding protein-homologous protein (CHOP), and cleaved caspase 12. Furthermore, luteolin	[183]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
					increased the level of intracellular ROS, leading to ROS mediated apoptosis and ER stress.	
	In vitro and *in vivo* (mice)	B16F10	5–50 μM (in vitro tests) and 10–20 mg (*in vivo* tests)	Luteolin suppressed the hypoxia-induced changes in the cells in a dose-dependent manner. In experimental metastasis model mice, treatment with luteolin reduced metastatic colonization in the lungs by 50%.	Luteolin inhibited the hypoxia-induced epithelial-mesenchymal transition in malignant melanoma cells both *in vitro* and *in vivo* via the regulation of β3 integrin.	[184]
Morin	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 170 μM.	NE	[168]
Norartocarpin	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 7.8 μM.	NE	[168]
Pectolinarigenin	*In vitro*	B16F10 (MM)	ND	Preliminary test determined IC_{50} equal to 64 μM.	NE	[167]
Pectolinarin	*In vitro*	C32 (HM)	ND	Preliminary test determined IC_{50} equal to 7.17 μM.	NE	[181]
Tangeretin	*In vivo* (mice)	B16F10 (MM)	20 mg/day	Tangeretin decreased the number of metastatic nodules, implantation, growth and invasion index.	NE	[179]
Flavanones						
3,7-Dihydroxy-6-methoxyflavanone	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
3,7-Dihydroxyflavanone	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
7-Hydroxy-6-methoxyflavanone	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined IC_{50} equal to 6.7 μM.	NE	[162]
7-Hydroxyflavanone	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined IC_{50} equal to 99.9 μM.	NE	[162]
Alnustinol	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Artocarpanone	*In vitro*	B16 (MM)	ND	Preliminary test determined IC_{50} equal to 122.2 μM.	NE	[168]
Dihydrobaicalein	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
Dihydrooroxylin A	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined IC_{50} equal to 72.6 μM.	NE	[162]
Eriodictyol	*In vitro*	B16F10 (MM)	3.156–50 μM	Preliminary test determined $IC_{50} > 50$ μM.	NE	[174]
Garbanzol	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
Isoxanthohumol (IXN)	*In vitro*	B16 (MM) and A375 (HM)	0–100 μM	The treatment of both celllines with IXN resulted in dose-dependent decrease of cell viability (IC_{50} 21.88–24.18 μM).	PI3K/Akt and MEK-ERK signaling pathways between B16 and A375 cells were involved.	[185]
Liquiritigenin	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined IC_{50} equal to 97.7 μM.	NE	[162]
Naringenin	*In vitro*	C32 and A375 (HM)	ND	Preliminary test determined IC_{50} equal to 0.6 and 13.8 μM for C32 and A375 cells, respectively.	NE	[189]
	In vitro	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
Flavonols						
Alnusin	*In vitro*	B16-BL16 (MM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[162]
Drabanemoroside	*In vitro*	SK-MEL-2 (HM) and B16F1 (MM)	ND	Preliminary test determined IC_{50} equal to 1.9 μg/ml for SK-MEL-2. The compound was not effective against B16F1 cells ($IC_{50} > 40$ μg/ml).	NE	[189]
Fisetin	*In vitro*	A375 (HM)	20–80 μM	Preliminary test determined IC_{50} equal to 38.1 and 20.3 μM at 24 and 48 h after treatment.	Fisetin inhibited mTOR and p70S6K through direct binding while the observed inhibitory	[186]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
					effect of fisetin on AKT was mediated indirectly, through targeting interrelated pathways.	
	In vitro and *in vivo* (mice)	A375 (HM)	10 and 20 μM, in combination with sorafenib (2 and 5 μM)	Combination treatment (fisetin + sorafenib) more effectively reduced the migration and invasion of BRAF-mutated melanoma cells both in vitro and in raft cultures compared to individual agents. In addition, fisetin potentiated the antiinvasive and antimetastatic effects of sorafenib *in vivo*.	Combination treatment (fisetin + sorafenib) promoted a decrease in N cadherin, vimentin and fibronectin and an increase in E-cadherin both in vitro and in xenograft tumors. Furthermore, combination therapy effectively inhibited Snail1, Twist1, Slug and ZEB1 protein expression compared to monotherapy. The expression of MMP-2 and MMP-9 in xenograft tumors was further reduced in combination treatment compared to individual agents.	[187]
	In vitro and *in vivo* (mice)	451Lu (HM)	20–100 μM (in vitro tests) and 1 and 2 mg/kg (*in vivo* tests)	IC_{50} was estimated to be 80, 37.2, and 17.5 μM at 24, 48, and 72 hours of treatment, respectively. A smaller average tumor volume was consistently observed in mice treated with fisetin. This was more marked in animals receiving 1 mg fisetin than in animals receiving the 2 mg dose, indicating a nonlinear dose response.	Fisetin decreased cell viability with G1-phase arrest and disruption of Wnt/β-catenin signaling.	[188]
	In vitro	451Lu and A375 (HM)	20–80 μM	The efficacy of fisetin in the induction of apoptosis varied with cell type as A375 cells were more susceptible to fisetin treatment compared to 451Lu cells. Results confirm apoptosis as the primary mechanism through which fisetin inhibits melanoma cell growth.	Fisetin treatment induced endoplasmic reticulum (ER) stress in highly aggressive A375 and 451Lu human melanoma cells, as revealed by upregulation of ER stress markers including IRE1a, XBP1s, ATF4 and GRP78. Both extrinsic and intrinsic apoptosis	[189]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
					pathways are involved in fisetin cytotoxic effects.	
Galangin	*In vitro*	B16F10 (MM)	0–100 μM	Preliminary test determined IC_{50} equal to 145 μM. Galangin. Microscopical analysis revealed the reduced number of cells and morphological aberrations after a 24 h treatment. The appearance of apoptotic cells such as cell shrinking, rounding and partial detachment was evident at a galangin concentration of 100 μM.	Galangin activated apoptosis signaling cascades by cleavage of procaspase-9, procaspase-3 and PARP in B16F10 cells. Moreover, galangin significantly induced activation of phosphor-p38 MAPK in a time and dose dependent manner.	[190, 192]
	In vitro and *in vivo* (mice)	B16F10 (MM)	0–200 μM (in vitro tests) and 50 mg/kg (*in vivo* tests)	Galangin decreased the proliferation of B16F10 cells in a dose-dependent manner. The cell viabilities were 67.9% at 50 mM group, 54.5% at 100 mM group, and 48.7% at 200 mM group, respectively. A significant reduced number of migrating cells was observed when the cells were treated with galangin for 24 h. *In vivo* models showed that galangin inhibited lung metastasis of B16F10 cells.	Molecular data showed that FAK mRNA level were reduced dose-dependently. Galangin also reduced phosphorylation of FAK (Tyr397) protein and suppressed the transcription of FAK gene, indicating FAK expression is a candidate target of galangin.	[191]
	In vitro	B16 (MM)	10–250 μg/ml	Preliminary test determined IC_{50} equal to 91.65 μg/ml.	Inhibitory effect on melanin production and tyrosinase activity.	[194]
Galangin-7-methyl ether	*In vitro*	B16BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 20.8 μM.	NE	[175]
Icaritin	*In vitro*	A375S, A375R, A2058, and MEWO (all HM)	2.5–80 μM	Preliminary test determined IC_{50} equal to 2.7, 6.9, 14, and 15.6 μM in A375S, A375R, A2058, and MEWO cells, respectively, after 72h of treatment.	Icaritin suppressed p-STAT3 (tyr705) level in parallel with increases of p-STAT3 (ser727), p-ERK and p-AKT. The flavonoid significantly inhibited STAT3 nuclear translocation and reduced	[195]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
					the levels of STAT3 -targeted genes. Icaritin also inhibited IGF-1-induced STAT3 activation through downregulation of total IGF-1R level.	
Isorhamnetin	*In vitro*	SK-MEL-2 (HM) and B16F1 (MM)	ND	Preliminary test determined $IC_{50} > 40$ µg/ml for both cell lines.	NE	[189]
Isorhamnetin-3-*O*-β-D-glucoside	*In vitro*	SK-MEL-2 (HM) and B16F1 (MM)	ND	Preliminary test determined $IC_{50} > 40$ µg/ml for both cell lines.	NE	[189]
Kaempferol	*In vitro*	MDA-MB-435 (HM)	1–50 µM	Preliminary test determined IC_{50} equal to 1.5 µM.	NE	[171]
	In vitro	SK-MEL-2 (HM)	ND	Preliminary test determined IC_{50} equal to 6.9 µM.	NE	[196]
Kaempferol-3-*O*-rhamnoside	*In vitro*	SK-MEL-2 (HM)	ND	Preliminary test determined IC_{50} equal to 33.9 µM.	NE	[196]
Myricetin	*In vitro*	B16F10 (MM)	3.156–50 µM	Preliminary test determined IC_{50} equal to µM.	NE	[174]
Quercetin	*In vitro*	B16F10 (MM)	3.156–50 µM	Preliminary test determined $IC_{50} > 50$ µM.	NE	[174]
	In vitro and *in vivo* (mice)	B16F10 (MM)	25–50 µM (in vitro tests) and 7.5–15 mg/kg (*in vivo* tests), in combination with different doses of sulforaphane.	Quercetin and sulforaphane in combination inhibit the proliferation and migration of melanoma cells more effectively than either compound used alone. These compounds in combination significantly suppressed melanoma growth as compared to their individual use in a mouse model.	This combined effect was predominantly due to a decrease in MMP −9 expression in the mouse tumors.	[197]
	In vitro and	A375, A2058 (HM)	0–60 µM (in vitro tests) and 100 mg/kg (*in vivo* tests)	Exposure to quercetin resulted in inhibition of proliferation of melanoma cells, induction of cell	Quercetin inhibited the activation of STAT3 signaling by interfering with STAT3 phosphorylation, and	[198]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
	in vivo (mice)	and B16F10 (MM)		apoptosis, and suppression of migratory and invasive properties. Furthermore, quercetin suppressed A375 tumor growth and STAT3 activities in xenografted mice model, and inhibited murine B16F10 cells lung metastasis in an animal model.	reducing STAT3 nuclear localization.	
	In vitro	A375, A2058, SK-MEL-2 and MeWo (HM)	0–80 μM	Quercetin dose-dependently inhibited HGF-stimulated melanoma cell migration and invasion.	Suppression of the HGF/c-Met signaling pathway contributes to the antimetastatic action of quercetin in melanoma.	[199]
	In vitro	SK-MEL-2 (HM)	ND	Preliminary test determined IC_{50} equal to 4.7 μM.	NE	[194]
	In vitro	SK-MEL-2 (HM) and B16F1 (MM)	ND	Preliminary test determined IC_{50} equal to 21.1 and 8.2 μg/ml for SK-MEL-2 and B16F1 cell lines, respectively.	NE	[189]
Quercetin-3-*O*-rhamnoside	*In vitro*	SK-MEL-2 (HM)	ND	Preliminary test determined IC_{50} equal to 41.5 μM.	NE	[194]
Quercetin-3-*O*-β-D-glucopyranoside	*In vitro*	SK-MEL-2 (HM) and B16F1 (MM)	ND	Preliminary test determined $IC_{50} > 40$ μg/ml for both cell lines.	NE	[189]
Rhamnetin-3-*O*-rhamnoside	*In vitro*	SK-MEL-2 (HM)	ND	Preliminary test determined $IC_{50} > 100$ μM.	NE	[194]
Rhamnocitrin-3-*O*-rhamnoside	*In vitro*	SK-MEL-2 (HM)	ND	Preliminary test determined IC_{50} equal to 34.1 μM.	NE	[194]
Rutin	*In vivo* (mice)	B16F10 (MM)	20 mg/day	Rutin decreased the number of metastatic nodules, implantation, growth and invasion index.	NE	[179]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Flavanols						
Epigallocatechin	*In vitro*	CHL-1 and WM266-4 (HM)	0.1–200 μM	Preliminary test determined IC_{50} equal to 10.3 and 51.2 μM for CHL-1 and WM266-4 cells.	Expression of ER stress and apoptosis markers.	[200]
Flavanonols						
Aromadendrin	*In vitro*	M14 (HM)	1–40 μM	The compound was not able to inhibit cell proliferation at 1–40 μM concentrations.	NE	[166]
Pinobanksin	*In vitro*	B16BL6 (MM)	ND	Preliminary test determined IC_{50} > 200 μM.	NE	[175]
Pinobanksin 5-methylether	*In vitro*	B16BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 187 μM.	NE	[175]
Silymarin	*In vitro*	A375-S2 (HM)	1×10^{-5} to 1×10^{-4} M	It was assessed the effect of silymarin on anti-Fas agonistic antibody CH11 treated human malignant melanoma, A375-S2 cells. Pretreatment with silymarin significantly induced cell apoptosis in CH11-treated A375-S2 cells.	Caspase-8, −9, −3 and pan caspase inhibitors partially reversed silymarin induced apoptosis of CH11-treated cells. The expression of Fas-associated proteins with death domain (FADD), procaspase-8 and −3 was increased by silymarin pretreatment.	[201]
Isoflavonoids						
2′-Hydroxybiochanin A	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
4′-Methoxy-2′,3,7-trihydroxyisoflavanone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
7-*O*-Methylvestitol	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 24.1 μM.	NE	[162]
Biochanin A	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Calycosin	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Daidzein	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Ferreirin	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Formononetin	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Genistein	*In vitro*	518A2 (HM)	ND	Preliminary test determined IC_{50} > 50 μM. The flavonoid also reduced cell migration.	Apigenin induced an arrest of the cell cycle of 518A2 melanoma cells at the G2/M transition and also attenuated the expression and secretion of the metastasisrelevant matrix metalloproteinases MMP-2 and MMP-9.	[170]
	In vitro	M14 (HM)	12–100 μM	Genistin reduced cell proliferation in 40.9% at 100 μM dose.	Cytotoxic activity of genistin was related to its antioxidant effect, but no specific target was investigated.	[202]
Isoangustone A (IAA)	*In vitro* and *in vivo* (mice)	SK-MEL-28 and SK MEL-5 (HM)	0–20 μM (in vitro tests) and 2–10 mg/kg (*in vivo* tests)	Treatment with 20 μM of IAA inhibited the growth of SK-MEL-28 cells up to 67% as compared with untreated control cells. Moreover, in a xenograft mouse model, IAA significantly decreased tumor growth, volume, and weight of SK-MEL-28 xenografts.	IAA significantly blocked cell-cycle progression at the G1-phase and inhibited the expression of G1-phase regulatory proteins, including cyclins D1 and E in the SK-MEL-28 cell line. IAA suppressed the phosphorylation of Akt, GSK-3b, and JNK1/2. IAA also bound to phosphoinositide 3-kinase (PI3K), MKK4, and MKK7, strongly inhibiting their kinase activities in an ATP-competitive manner.	[203]
Isovestitol	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 33.6 μM.	NE	[162]
Mucronulatol	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 30.4 μM.	NE	[162]

Flavonoid	Model	Cell line	Concentration or dose	Effectiveness	Possible mechanisms of action	Reference
Pratensein	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Vestitol	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 57.4 μM.	NE	[162]
Vestitone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Violanone	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} >100 μM.	NE	[162]
Xenognosin B	*In vitro*	B16-BL6 (MM)	ND	Preliminary test determined IC_{50} equal to 34.1 μM.	NE	[162]

ND: not described; NE: not evaluated; HM: human melanoma cell line; MM: murine melanoma cell line.

Box 1. Anticancer activity of flavonoids on melanoma cell lines.

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apigenin in an experimental lung metastasis model. Apigenin-treated mice had significant fewer metastatic nodules when compared to the vehicle control group, suggesting apigenin inhibits the metastasis potential of B16F10 melanoma cells *in vivo* mouse model (**Figure 4**).

6.2. Bioactivity of diosmin on melanoma

Diosmin is a glycosylated flavonoid commonly used as an active constituent of several pharmaceutical products, mainly for cardiovascular diseases treatment. Diosmin is used in the treatment of venous insufficiency, because of its vasoprotector and venotonic properties. In addition, it acts as an antioxidant, anti-inflammatory, and antimutagenic molecule, regulating the activity of several enzymes, including cyclooxygenases and cytochrome P450 proteins [177, 179]. Interestingly, the anticancer effects of diosmin have also been studied [176, 178], suggesting that this flavonoid presents a broad spectrum of pharmacological activities.

Conesa et al. [179] performed a comparative study with three different flavonoids (tangeretin, rutin, and diosmin) using an experimental model of B16F10 melanoma cell-induced pulmonary metastasis. The greatest reduction in the number of metastatic nodules (52%) was obtained with diosmin treatment. Similarly, diosmin presented a relevant decreasing in implantation, growth, and invasion index (79.40, 67.44, and 45.23%, respectively). These results were confirmed by another study developed by Martínez et al. [178], suggesting diosmin is an effective agent against metastatic stages of melanoma.

The antimetastatic effect of diosmin has also been evaluated in combination with IFN-α [176, 177], an important cytokine that has shown the significant effect in the treatment of metastatic melanoma in high doses. In both investigations, it was verified that synergistic antiproliferative and antimetastatic effects shown by the combination of the flavonoid and the lowest dose of IFN-α, which was similar to that produced by the highest dose of the cytokine alone. These results suggest that diosmin may be used in combination with IFN-α in an attempt to reduce its therapeutic dose, thereby reducing the side effects promoted by continued cytokine use.

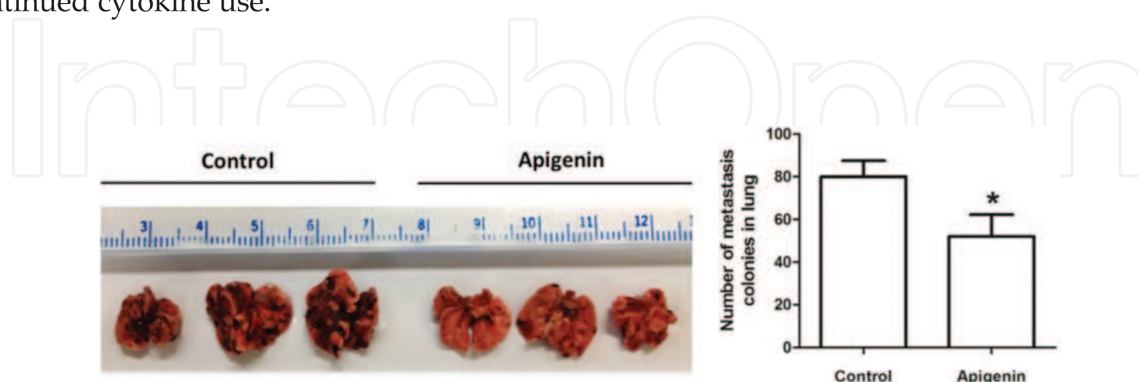

Figure 4. Apigenin inhibited murine melanoma B16F10 cell lung metastasis. B16F10 melanoma cells were injected into the tail vein of the C57BL/6 mice. These mice then received intragastric administration of vehicle or apigenin (150 mg/kg/day) for 24 consecutive days. Lung metastasis of B16F10 melanoma cells in the mouse model (upper) and the metastasis nodules number in the lungs (bottom) were shown. Data were mean ± SD, $n = 8$, $^{*}p < 0.05$. This figure was taken from Ref. [172].

6.3. Bioactivity of fisetin on melanoma

Fisetin (3,7,3′,4′-tetrahydroxyflavone) is a flavonol also found in many fruits and vegetables, such as strawberries, apples, persimmons, kiwi, onions, and cucumbers. This flavonoid has shown a relevant neuroprotective effect, aiding in memory and cognition processes, as well as reducing behavioral deficits. Recently, the effect of fisetin on anticancer therapy has also been studied [186].

Investigation conducted by Syed et al. [188] determined an IC_{50} value of 38.1 and 20.3 μM against A375 human melanoma cell line, at 24 and 48 h after treatment. In a subsequent study, Syed et al. [191] have demonstrated that fisetin induces apoptosis in melanoma cells. The efficacy of fisetin in the induction of apoptosis varied with cell type and preliminary results confirmed apoptosis as the primary mechanism through which fisetin inhibits melanoma cell growth. The possible mechanisms involved include upregulation of ER stress markers such as IRE1a, XBP1s, ATF4, and GRP78. In addition, both extrinsic and intrinsic apoptosis pathways are involved in fisetin cytotoxic effects.

The effect of fisetin was also evaluated on the growth of metastatic 451Lu human melanoma cells, which exhibit constitutive Wnt signaling in addition to harboring a mutation in the B-Raf gene. The IC_{50} value was estimated to be 17.5 μM at 72 h of treatment in the MTT assay. In an *in vivo* model, a smaller average tumor volume was consistently observed in mice treated with fisetin. This was more marked in animals receiving 1 mg fisetin than in animals receiving the 2 mg dose, indicating a nonlinear dose response. The authors attributed this effect to a decreasing of cell viability with G1-phase arrest and disruption of Wnt/β-catenin signaling mediated by fisetin [186].

A recent report evaluated the effect of fisetin in combination with sorafenib, a multi-kinase inhibitor of mutant and wild-type BRAF and CRAF kinases, on melanoma cell invasion and metastasis. In this study, fisetin potentiated the anti-invasive and antimetastatic effects of sorafenib *in vivo*, suggesting that this flavonoid can be used as an alternative agent in melanoma therapy reducing doses of anticancer drugs used for this purpose [187].

6.4. Bioactivity of luteolin on melanoma

Luteolin is a common flavone that exists in many types of plants including fruits, vegetables, and medicinal herbs. This flavonoid presents potential for cancer prevention and therapy [174]. Concerning to melanoma treatment, George et al. [182] showed that luteolin possesses relevant cytotoxicity against A375 human melanoma cell line, with an IC_{50} value of 115.1 μM in a preliminary test. Luteolin also inhibited colony formation and induced apoptosis in a dose and time-dependent manner by disturbing cellular integrity. Accumulation of cells in the G0/G1 (60.4–72.6%) phase for A375 cells after 24 h treatment indicated cell cycle arresting potential of this flavonoid, suggesting that luteolin inhibits cell proliferation and promotes cell cycle arrest and apoptosis in human melanoma cells. A similar result was demonstrated by Casagrande and Darbon [204], who highlighted the involvement of the regulation of cyclin-dependent kinases CDK2 and CDK1 in the antiproliferative effect of luteolin on OCM-1 human melanoma cells.

In a recent investigation, the inhibitory effect of luteolin on melanoma cell proliferation was related to ER stress induced. In this context, luteolin increased the expression of the ER stress-related

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proteins, such as protein kinase RNA-like ER kinase, phosphorylation eukaryotic translation initiation factor 2α, activating transcription factor (ATF) 6, CCAAT/enhancer-binding protein-homologous protein (CHOP), and cleaved caspase 12. In addition, luteolin increased the level of intracellular ROS, leading to ROS-mediated apoptosis and ER stress, suggesting that luteolin induces apoptosis by ER stress via increasing ROS levels [183].

Anticancer potential of luteolin has also evaluated *in vivo*. In experimental metastasis model, mice treatment with luteolin (10 or 20 mg/kg) reduced metastatic colonization in the lungs by 50%. This treatment increased E-cadherin expression while reduced the expression of vimentin and β3 integrin in the tumor tissues [184]. These results encourage the use of luteolin as an anticancer chemopreventive and chemotherapeutic agent.

6.5. Bioactivity of quercetin on melanoma

Quercetin is a noncarcinogenic dietary flavonoid with low toxicity, has been shown to exert antioxidant, anti-inflammatory, neuroprotective, and antimelanoma activities [193]. A preliminary study showed that quercetin presents a weak cytotoxic effect on B16F10 murine melanoma cells, with an IC_{50} value > 50 μM [174]. However, Casagrande and Darbon [204] and Kim et al. [196] showed that quercetin presents a considerable antiproliferative effect on OCM-1 and SK-MEL-2 human melanoma cells, with an IC_{50} value between 4.7 and 19 μM. In these investigations, the authors demonstrated that the presence of hydroxyl group at the 3′-position of the ring B in quercetin favors the cytotoxic effect and a G1 cell cycle arrest. The involvement of the regulation of cyclin-dependent kinases CDK2 and CDK1 may also be present in its anticancer effect.

Cao et al. [198] evaluated the involvement of STAT3 signaling in the inhibitory effects of quercetin on melanoma cell growth, migration, and invasion. Quercetin treatment promoted inhibition in proliferation of melanoma cells, induction of cell apoptosis, and suppression of migratory and invasive properties. Furthermore, mechanistic study indicated that quercetin inhibits the activation of STAT3 signaling by interfering with STAT3 phosphorylation, and reducing STAT3 nuclear localization. In an animal model, quercetin inhibited murine B16F10 cells lung metastasis, indicating that quercetin possesses antitumor potential.

7. Brief structure-activity relationship (SAR) considerations

Nagao et al. [167] evaluated the cytotoxic activity of 21 flavones and the effect of the substitution patterns on their anticancer potential, although the authors highlight that the number of compounds examined might not be sufficient to determine the structure-activity relationships. Generally, the data show that the growth inhibitory activity of one flavone against the three different tumor cell lines (including a murine melanoma cell line) is not always the same, suggesting differences in the sensitivity of tumor cells to flavones.

The influences of ring A substituents against B16F10 cells were examined. Comparing the antiproliferative activity of four 3′,4′-di-OH-flavones, the order of contribution was found to be 5-OH-6,7-di-OCH_3 > 5,6,7-tri-OH > 5,7-di-OH-6-OCH_3 > 5,7-di-OH. In contrast, in the

3′-OH-4′-OCH_3-flavones (desmethoxycentaureidin, eupatorin, and 5,6,3′-trihydroxy-7,4′-dimethoxyflavone), the order is 5,6-di-OH-7-OCH_3 > 5-OH-6,7-di-OCH_3 > 5,7-di-OH-6-OCH_3. In the 3′,4′-di-OCH_3-flavones (eupatilin and 5,6-dihydroxy-7,3′,4′-trimethoxyflavone), the order is 5,6-di-OH-7-OCH_3 > 5,7-di-OH-6-OCH_3, and in the 3′-OCH_3-4′-OH-flavones (jaceosidin and cirsilineol), it is 5,7-di-OH-6-OCH_3 > 5-OH-6,7-di-OCH_3. In addition, for ring B substituents, 3′,4′-di-OH and 3′,4′-di-OH-5′-OCH_3 showed a greater effect than the others, but the influence of 3′,5′-di-OCH_3-4′-OH appears to be not relevant [167].

Another investigation evaluated the effects of polyhydroxylated flavonoids on the growth of B16F10 melanoma cells. In general, the results suggest that the presence of a C2–C3 double bond and three adjacent hydroxyl groups in the A- or B-rings confers greater antiproliferative activity [174]. Casagrande and Darbon [204] investigated the effects of a series of flavonoids on cell proliferation and cell cycle distribution in human melanoma cells (OCM-1). Interestingly, the presence of a hydroxyl group at the 3′-position of the ring B in quercetin and luteolin was correlated to a G1 cell cycle arrest while its absence in kaempferol and apigenin was correlated to a G2 block.

The presence of isoprenoid units in the cytotoxic effect of flavonoids has also been evaluated for melanoma cells [168]. The results indicated that isoprenoid substitutions in flavonoids enhance their cytotoxic potential, and that the position of attachment and the number of isoprenoid-substituent moieties per molecule influence flavonoid cytotoxicity. This is probably related to their lipophilicity and affinity properties, which favor penetration into the cell membrane.

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