applied sciences

MDPI

Systematic Review

Plant-Derived Bioactive Compounds for Rhabdomyosarcoma Therapy In Vitro: A Systematic Review

Cristina Mesas [1,2,†], Beatriz Segura [3,†], Gloria Perazzoli [1,3], Maria Angeles Chico [1,2], Javier Moreno [1], Kevin Doello [1,2,4], Jose Prados [1,2,3,*] and Consolación Melguizo [1,2,3]

[1] Institute of Biopathology and Regenerative Medicine (IBIMER), Biomedical Research Center (CIBM), 18100 Granada, Spain; cristinam@ugr.es (C.M.); gperazzoli@ugr.es (G.P.); mchico@ugr.es (M.A.C.); javiermperez99@correo.ugr.es (J.M.); kdoello@correo.ugr.es (K.D.); melguizo@ugr.es (C.M.)
[2] Instituto Biosanitario de Granada (ibs.GRANADA), University of Granada, 18012 Granada, Spain
[3] Department of Anatomy and Embryology, University of Granada, 18071 Granada, Spain; beasegura@correo.ugr.es
[4] Service of Medical Oncology, Hospital Virgen de las Nieves, 18014 Granada, Spain
* Correspondence: jprados@ugr.es; Tel.: +34-958243535
† These authors contributed equally to this work.

Abstract: Rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children, constitutes approximately 40% of all recorded soft tissue tumors and is associated with a poor prognosis, with survival rates of less than 20% at 3 years. The development of resistance to cytotoxic drugs is a primary contributor to therapeutic failure. Consequently, the exploration of new therapeutic strategies is of vital importance. The potential use of plant extracts and their bioactive compounds emerges as a complementary treatment for this type of cancer. This systematic review focuses on research related to plant extracts or isolated bioactive compounds exhibiting antitumor activity against RMS cells. Literature searches were conducted in PubMed, Scopus, Cochrane, and WOS. A total of 173 articles published to date were identified, although only 40 were finally included to meet the inclusion criteria. Furthermore, many of these compounds are readily available and have reduced cytotoxicity, showing an apoptosis-mediated mechanism of action to induce tumor cell death. Interestingly, their use combined with chemotherapy or loaded with nanoparticles achieves better results by reducing toxicity and/or facilitating entry into tumor cells. Future in vivo studies will be necessary to verify the utility of these natural compounds as a therapeutic tool for RMS.

Keywords: Rhabdomyosarcoma; plant extract; bioactive compounds; in vitro

Citation: Mesas, C.; Segura, B.; Perazzoli, G.; Chico, M.A.; Moreno, J.; Doello, K.; Prados, J.; Melguizo, C. Plant-Derived Bioactive Compounds for Rhabdomyosarcoma Therapy In Vitro: A Systematic Review. *Appl. Sci.* **2023**, *13*, 12964. https://doi.org/10.3390/app132312964

Academic Editors: António José Madeira Nogueira and Andrea Luísa Fernandes Afonso

Received: 29 October 2023
Revised: 29 November 2023
Accepted: 30 November 2023
Published: 4 December 2023

1. Introduction

Sarcomas are a large group of rare malignant mesenchymal tumors that originate in soft tissues, bone, or nervous tissue, with an incidence of approximately 7% in children and 1% in adults [1]. Half of pediatric patients diagnosed with soft tissue sarcoma are diagnosed with rhabdomyosarcoma (RMS) [2]. There are four histological subtypes of RMS: embryonal, alveolar, pleomorphic, and spindle cell/sclerosing, with the first two being the most common, accounting for an incidence of 60% and 30%, respectively [3]. Although it depends on factors such as the site of the primary tumor, the patient's age, or the possibility of complete resection, the treatment of RMS is based on surgery, radiotherapy, and chemotherapy [4]. Despite treatment, the survival rate for this type of tumor is low, at 63% four years after diagnosis and less than 20% three years after diagnosis if metastasis occurs [5]. Due to this, the development of new treatments is necessary to improve the survival rate of these patients.

In recent years, plant extracts prepared from the bioactive components of plants have been studied due to their anti-inflammatory, antibacterial, antioxidant, and antitumor properties [6,7]. It has been shown that these bioactive components of plant origin can exhibit

selective antitumor activity, enhancing the antitumor effect of traditional chemotherapy drugs and being used as a complementary treatment. In vitro studies of extracts obtained from the plant *Christia vespertilionis* of the *Fabaceae* family showed their antitumor effect in MCF7 breast cancer cells with a selective effect against the non-tumor line MCF 10A [8]. Extracts obtained from the root of *Taraxacum* spp. have also been shown to have strong antitumor activity in pancreatic cancer, melanoma, leukemias, colorectal cancer, and gastric tumors [9].

Numerous plant extracts also have antitumor activity against different subtypes of sarcomas. This is the case for the aqueous extracts of *Salvia clandestina* tested on the osteosarcoma MG-63 cell line, showing an enhancement of the effect of free cisplatin [10]. Most of this antitumor effect is attributed to the phytochemicals in these vegetables, highlighting polyphenols, tannins, carotenoids, terpenoids, flavonoids, glycosides, or alkaloids [11]. Another plant extract with antitumor activity in osteosarcoma is the aqueous leaf extract of the mangrove plant *Rhizophora apiculate*. In addition to presenting antitumor activity, this was improved with the loading of its bioactive compounds into silver nanoparticles [12]. The ethyl acetate extract of *Caulis Spatholobi,* rich in tannins, has shown antitumor activity against the osteosarcoma Saos-2 cell line [13]. The *Artemisia annua* extract derivatives, called dihydroarteminin, demonstrated potent antitumor activity in osteosarcoma cells with IC_{50} values between 10 and 40 μM, activating apoptotic pathways and inhibiting the formation of metastasis [14]. Focusing on rhabdomyosarcoma cell lines, few studies have been found on the use of plant extracts with antitumor effects. One of them uses two extracts obtained from *Macaranga barteri* and *Calliandra portoricensis*, observing a significant reduction in the IC_{50} value compared to control cells [15]. Another article presents the cytotoxic effect of *Rosmarinus officinalis* extract on the TE671 cell line, resulting in an IC_{50} value of 0.249 mg/mL, suggesting its potential use as an antitumor in this cell line [16].

Given the low incidence of RMS, few studies have investigated this type of tumor since it is considered a rare malignant tumor. Therefore, the aim of this systematic review is the study of all research conducted to date on plant extracts or bioactive compounds from plants that have antitumor activity against RMS.

2. Materials and Methods

The search protocol of the present systematic review was previously registered on 23 October 2023, in the OSF database (https://doi.org/10.17605/OSF.IO/EUK3V (accessed on 27 November 2023)).

2.1. Study Eligibility and Data Sources

To carry out this systematic review, a bibliographic analysis of the different scientific articles published on the topic to date was carried out by searching in four different databases: PubMed, SCOPUS, Web of Science, and Cochrane. In the PubMed database, the "MeSH" terms used in the search were "Rhabdomyosarcoma", and "Plant extract", with the formula obtained: ("rhabdomyosarcoma" [MeSH Terms] OR "rhabdomyosarcoma" [All Fields] OR "rhabdomyosarcomas" [All Fields]) AND ("plant extracts" [MeSH Terms] OR ("plant" [All Fields] AND "extracts" [All Fields]) OR "plant extracts" [All Fields] OR ("plant "[All Fields] AND "extract" [All Fields]) OR "plant extract" [All Fields]). For the other electronic databases, this formula was adopted. The PRISMA guide has been systematically followed to carry out this systematic review [17].

2.2. Inclusion Criteria

Due to the scarcity of studies on the topic, there has been no time limit on the range of publication of the articles. This systematic research has included studies in which the bioactive compound was used in RMS cells and specified the IC_{50} value or the mechanism of action. Similarly, articles that used functional extracts or bioactive compounds of plant origin loaded into nanoparticles were also included due to their scientific interest. Further-

more, studies that determine the mechanism of action of bioactive compounds of plant origin were included.

It should be noted that to reduce possible publication bias, certain studies from the bibliographic references of the selected articles were analyzed and included in the bibliographic analysis if they met the inclusion criteria.

2.3. Exclusion Criteria

Articles in which the compound was not tested on RMS cells, was purchased, or the extraction method was not explained have been excluded from this systematic research. Additionally, articles that could not be read in full and those written in a language other than English, French, and Spanish have been excluded. Non-original articles, such as meta-analyses or reviews, have also been excluded from the study.

2.4. Study Selection and Data Extraction

Authors C.Me. and G.P. were responsible for determining the inclusion and exclusion criteria that characterize this systematic review. Therefore, C.Me. and B.S. were responsible for initiating the bibliographic search in the different databases described, starting by analyzing the titles of the articles and their abstracts. Thus, using the terms employed in the bibliographic search, 173 articles were obtained. The selection process to reach the final number of articles included in the study consisted of several phases.

In the first phase, duplicate articles that appeared in more than one database were excluded. In the second phase, articles not related to the topic of the review were discarded. In the last phase, a more exhaustive reading of the articles was carried out, excluding those that did not meet the inclusion and exclusion criteria. Moreover, a quality test for in vitro studies was independently conducted by C.Me. and B.S.

This quality test consisted of two phases. In the first phase, filters were specified based on the basic characteristics that must be present in a high-quality in vitro study (score 5), and those that did not reach this score were excluded. The second phase focused on the methodology, results, and conclusions sections of the scientific articles. After their analysis, they were classified according to their score into three categories: low quality (scores 0 to 5), medium quality (scores of 6 to 15), and high quality (scores of 16 to 20).

Finally, this systematic review covers a total of 40 articles. The entire process is reflected in the flow chart shown below (Figure 1).

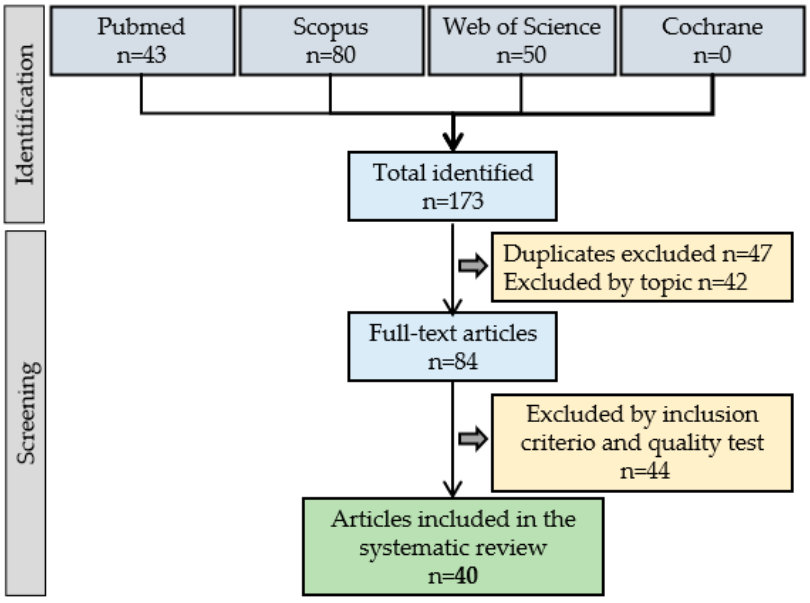

Figure 1. Schematic diagram representative of the selection process of included studies carried out for this review.

3. Results

The results have been presented by describing the mechanism of action of each plant extract or its bioactive compound. The main mechanism of action in RMS cells of all extracts or their isolated compounds was apoptosis, representing 43% of all bioactive compounds of plant origin studied in the present systematic review [18–36], followed by 19% with antioxidant activity and cytotoxicity against tumor cells [37–45]. Up to 9% of extracts or bioactive compounds induce cell death by cell cycle arrest [23,32,35,46], although some of these extracts overlap with another mechanism such as apoptosis. Minor mechanisms are the inhibition of the formation of tumor colonies, representing 3% of the total [47,48], the blockade of specific transporters important in cell viability, representing 5% of all bioactive compounds studied [36,49,50], and another 5% of them acting by inhibiting cell motility [21,23,34]. Finally, for 16% of all bioactive compounds studied in the review, the mechanism of action remains unknown [15,16,35,51–54], as no molecular studies were carried out to reveal it (Figure 2).

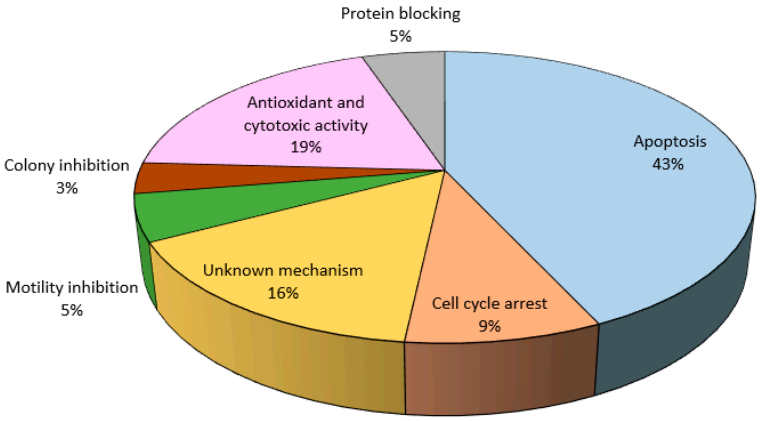

Figure 2. A graphic representation of the main mechanisms of action of plant extracts or bioactive compounds on RMS cells in vitro.

On the one hand, the most commonly used parts of the plant to obtain the bioactive component were the leaves and the whole plant, which stood out in 23% of the studies. In studies that referred to the entire plant, no reference was made to which specific parts were included. Another important group for obtaining the bioactive compound has been the aerial parts of plants. The term 'aerial parts' refers to the set of stems, leaves, flowers, fruits, or seeds. It is noteworthy that the least used plant part was the seed (Figure 3A).

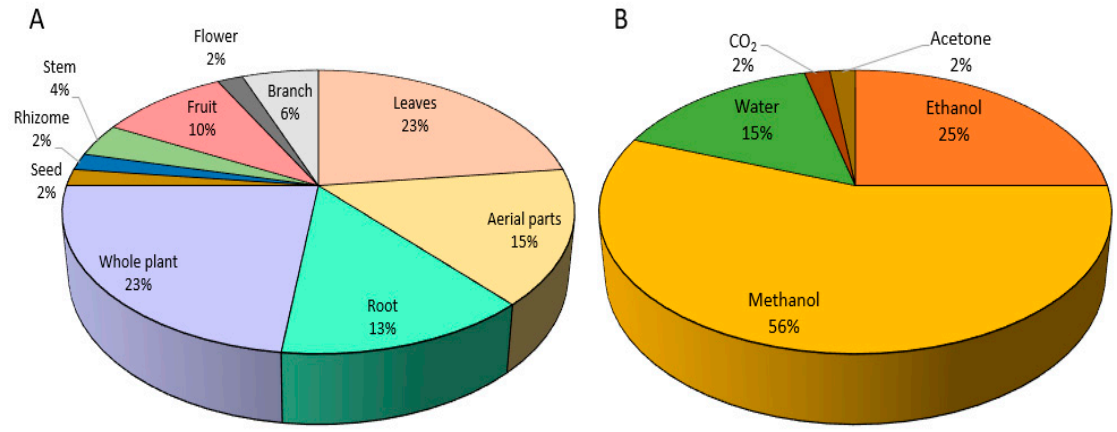

Figure 3. Graphic representation of (**A**) plant parts and (**B**) solvents that have been used to obtain the functional extracts.

On the other hand, regarding the extraction method of the plant extract or bioactive compound, the most commonly used chemical components were methanol (56%), ethanol (25%), and distilled water (15%) (Figure 3B).

Another important fact to highlight in this first part of the results regarding the relevance of the topic is reflected in the number of articles published in recent years. As previously mentioned, research on plant extracts and their possible use as a complementary treatment to the current treatment for various tumors is booming, especially for the most common cancers, such as colon cancer. Regarding RMS, a progressive increase in the number of investigations has been observed. The first article on this topic dates to 1988, and the next one is from 2003, with no articles existing in this 15 year gap. Since 2002, no publications were registered until 2012, in which we can find two articles. The highest number of publications was reached in 2020 with a total of nine articles; however, this number has decreased to six in 2022 (Figure 4).

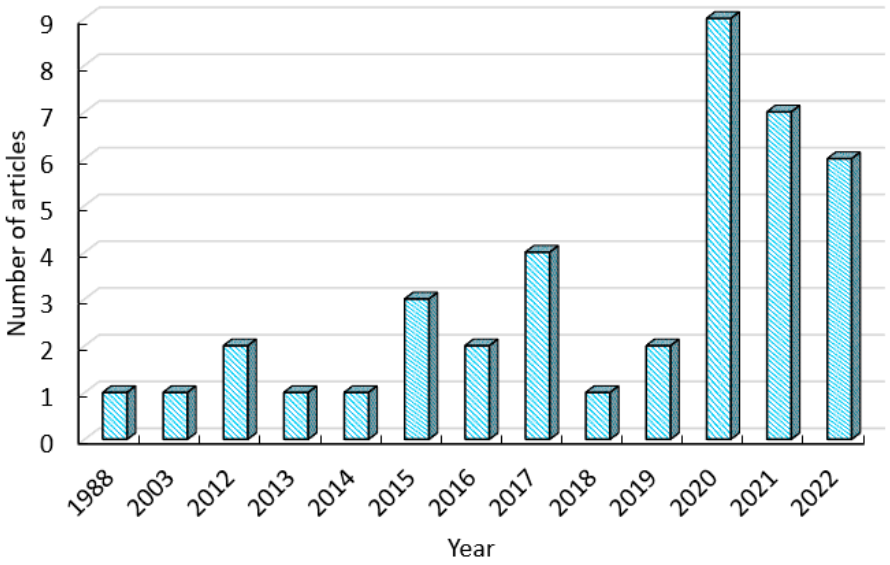

Figure 4. Graphic representation of the number of articles included in this systematic review published by year.

3.1. Plant Extracts and Isolated Bioactive Compounds That Induce Cell Death In Vitro through Apoptosis

Apoptosis is the predominant in vitro cell death mechanism in the different plant extracts and bioactive compounds analyzed in this systematic review [18–36]. Most of them induced both the intrinsic and extrinsic pathways of apoptosis, so the effect of tumor cell death was enhanced. Both pathways converge in the activation of caspases, which will carry out cell death. Apoptosis entails alterations in DNA condensation, fragmentation of the cell nucleus, or the formation of apoptotic bodies that will be degraded by phagocytic cells [55,56].

As shown in Table 1, the majority carried out this process by activating caspases 3, 8, and 9. The bioactive compounds obtained, through sonication, from the fruit of *Punica granatum* demonstrated, in a statistically significant manner, to be effective against human RMS cells versus healthy monkey cells [18]. This effectiveness was verified with the IC_{50} value, which indicates the concentration of the extract/drug necessary to inhibit 50% of the tumor population. The lowest IC_{50} value of this vegetable was obtained from its peel, being 14.8 ± 2.2 µg/mL.

Table 1. Plant extracts and isolated bioactive compounds that induce cell death in vitro by apoptosis.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Fruit of *Punica granatum* [18]	Sonication and microwaving	PL PC	RD VERO	RD: PL: 14.8 ± 2.2 µg/mL PC: 30 ± 0.9 µg/mL VERO: Both >1000 µg/mL	Inducing apoptosis by caspases 3 and 8 activation.
Leaves of *Ficus carica* [19]	Ethanol 70%	Flavonoids	RD	FC: 6.25 µg/mL FC + Dox-HCl (0.125 µM) + PDT (10 J/cm^2): 0.15 µg/mL	Changes in cell morphology suggest apoptosis. Strong synergism with chemotherapy.
Roots of *Berberis cretica* [20]	Methanol	Magnoflorin	TE671	22.83 ± 8.65 µg/mL	Inducing apoptosis by caspases 3 activation.
Roots of *Vincetoxicum arnottianum* [21]	Methanol	β- Sitosterol β Sitosterol-D-glucoside Lupeol	RH-30 HA-OH1 hMSC	RH-30: 188.75 µg/mL HA-OH1: 220.54 µg/mL hMSC: 1433.14 µg/mL	Inducing apoptosis by the PAX3-FOXO1 pathway. Proliferation reduction by PCNA protein. Motility restriction is achieved by stabilizing the cytoskeleton through S1P.
Rhizomes of *Rheum ribes* [22]	Methanol 95%	Flavonoids Alkaloids	RD	NDVE: 0.00978 mg/mL Extract: 0.3125 mg/mL	Inducing apoptosis by caspases 3 and 9 activation. Expresses the virus surface antigen on the tumor cell.
Berberis orthobotrys root [23]	Methanol		RH-30	55. 2 µg/mL	Motility inhibition occurs through stabilization of the cytoskeleton. Oncosome degradation. Cell cycle arrest in G2-apoptosis by downregulation of Bcl-2, Bax, and PCNA.
Viscum album and *Viscum TT* [24]	Ethanol	Viscotoxins Lectins Triterpenes (in *Viscum TT*).	RH-30 RMS-13		Apoptosis occurs by depolarizing the mitochondrial membrane and activating caspases.
Ripe fruits of *Fulvifomes fastuosus* [25]	Methanol	Ergone	RD CC-1 HepG-2	RD: 1.49 ± 2.74 µg/mL CC-1: 22.99 ± 2.42 µg/mL HepG-2: 68.32 ± 2.49 µg/mL	Apoptotic changes, such as fragmentation of the cell nucleus.
Curcuma amada [26]	Supercritical CO_2 extraction	(E)-Labda-8(17), 12-diene-15, 16-dial. β-myrcene. β pinene. B-caryophyllene. Ocimene.	RH-30 RD (ERMS)	CA in *RD:* 7.501 ± 0.5 µg/mL CA in SJRH30: 7.133 ± 1.2 µg/mL CA + VBL + CP in RD: 0.004 ± 0.0 µg/mL CA + VBL + CP in SJRH30: 0.045 ± 0.0 µg/mL	Apoptosis by regulating the expression of intrinsic pathway genes (Bcl-2, Bax, Bak, and p53). Inhibition of the expression of inflammation-associated genes such as COX-2 and NF-κB. Synergic effect with chemotherapy.

Table 1. *Cont.*

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Aerial parts of *Plantago lagopus* [27]	Methanol	Verbascoside Calceorioside A	HEP-2 RD MCF-7	Verbascoside in RD: 36.24 µg/mL Calceorioside A in RD: 40.28 µg/mL	Apoptotic changes in cell morphology. Antioxidant activity.
Different species of Veronica [28]	Methanol	11 types of iridoids: highlighting verminoside	HEP-2 RD L20B VERO	Verminoside in RD: 70 µM; L20B: 103 µM; HEP-2: 128 µM	Apoptotic body formation.
Chnoospora mínima [29]	Methanol 70%	Phenols Flavonoids Alkaloids	RD MCF-7 VERO	RD: 197.23 ± 68 µg/mL	Apoptosis with activation of p53 and caspases 3 and 7, morphological changes in cells, and DNA fragmentation.
Terminalia chebula *Berberis lycium* *Justicia adhatoda* *Geranium wallichianum* [30]	Distilled water	Phenols Flavonoids	RD	-	Probable apoptosis.
Leaves of *Sesamum angustifolium* and *Hibiscus articulatus* [31]	Dichloromethane, acetone, and methanol extract	-	RD	Dichloromethane extract: 106 µg/mL Methanol extract: 122 µg/mL Aqueous extract: 129 µg/mL Acetone extract: 158 µg/mL	Morphological changes in cells such as loss of cell adhesion. Apoptosis by activation of caspases 3 (increased with methanol extract) and 9 (increased with dichloromethane).
Flowers, leaves, and stem of *Agrimonia eupatoria* [32]	Distilled water Methanol	Flavonoids and tannins	RD HeLa MEF		Polyphenols induce apoptosis with cell cycle arrest at G0/G1 and antioxidant action. Flavonoids induce apoptosis with cell cycle arrest in S and inactivation of the BCL-2 gene.
Crocus sativus [33]	Methanol	Cronins DMCRT	A-172 TE-671	A-172: Crocins: 1.72 mg/mL DMCRT: 1.95 mg/mL TE-671: Crocins: 1.02 mg/mL DMCRT: 1.27 mg/mL	Apoptosis occurs through the regulation of BAX, BID, BCL-2, and MYCN.
Leaves of *Nicotiana glauca* [34]	Ethanol 96%	Palmitic acid Scopoletin	RD		Palmitic acid and scopoletin (apoptosis inducers). Reduced cell migration capacity.

Table 1. *Cont.*

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Essential oil from the aerial parts of *Cotula cinerea* [35]	Distilled water	-	RD VERO	RD: 173.05 ± 4.46 µg/mL VERO: 72.72 ± 2.18 µg/mL	Multiple mechanisms: apoptosis with activation of caspases, DNA methylation, histone acetylation, and cell cycle arrest.
Aerial parts of *Cotula cinérea* [35]	Ethanol 70% Hexane Ethyl acetate n-butanol	-	RD VERO	RD: Hexane extract: 57.21 ± 3.43 µg/mL Ethyl acetate extract: 187.52 ± 6.27 µg/mL -n-butanol extract: >500 µg/mL	Multiple mechanisms: apoptosis with activation of caspases, DNA methylation, histone acetylation, and cell cycle arrest.
Roots of *Morus alba* [36]	Methanol	SG1 SG3	T-ALL OVCAR-4 SH-EP, IMR-32 NxS2	SG1 in OVCAR-4: 34.26 µM	SG1 inhibits the XIAP protein, which leads to the initiation of apoptosis by activating caspase 9.

A-172 (Glioblastoma cells); CC-1 (Healthy rat liver cells); CA (Curcuma amada); CP (Cyclophosphamide); DMCRT (methyl ester derivative dimethyl crocetin); Dox-HCl (Doxorubicin hydrochloride); DTIC (Dacarbazine); ERMS (Embryonal rhabdomyosarcoma); FC (Ficus carica); FP (Fermented petroleum extract); FEA (Fermented ethylacetate extract); FET (Fermented ethanol extract); HeLa (Human cervical cancer cells); HEP-2 (Human epidermoid laryngeal carcinoma cells); HepG-2 (Human hepatocellular carcinoma cells); hMSC (Healthy human mesenchymal cells); L2OB (Rat fibroblasts); MCF-7 (Human breast cancer cells); MEF (Mouse fibroblasts); NDVE (Newcastle Disease Virus Extract); NFET (Non-fermented ethanol extract); NFP (Non-fermented petroleum extract); NFEA (Non-fermented ethylacetate extract); OVCAR-4 (Rhabdomyosarcoma cells); PDT (Photodynamic therapy); PL (Pomegranate peel); PC (Pericarp); PCNA (Proliferating cell nuclear antigen); RD (Embryonal rhabdomyo-sarcoma cells); RH-30 and HA-OH1 (Alveolar rhabdomyosarcoma); S1P (Transcription factor); SG1 (Sanggenon G1); SG3 (Sanggenon G3); SH-EP, IMR-32 and NxS2 (Neuroblastoma cells); T-ALL (T-cell lymphoblastic leukemia); TE671 (Rhabdomyosarcoma cells); VBL (Vinblastine); VERO (Healthy African monkey cells); Viscum TT (Viscum album + triterpenes).

The ethanolic extract of the leaves of *Ficus carica*, in combination with chemotherapy (doxorubicin hydrochloride) and photodynamic therapy, obtained an IC_{50} value of 0.15 µg/mL in RD, so the association of these three therapeutic options in vitro was better than monotherapy with the chemotherapeutic agents [19]. The same results were obtained with the ethanolic extract of rhizomes from *Rheum ribes* in combination with the Newcastle Disease Virus. The result was better than the use of the extract alone, obtaining an IC_{50} value of 0.00978 mg/mL. Cell death was produced by the activation of caspases 3 and 9 and by the expression of the virus surface antigen in the tumor cell line RD [22].

Turmeric is one of the most researched plants for its possible antitumor properties [57]. It has also been tested in RMS, both in the alveolar type (RH30 cell line) and in the embryonic type (RD cell line). Its extract was obtained using supercritical CO_2. The IC_{50} value of this extract is between 7133 and 7501 µg/mL, respectively; therefore, it was more effective in alveolar RMS. However, with the combination of Cyclophosphamide and Vinblastine, the IC_{50} value was between 0.004 and 0.045 µg/mL, making it more effective in the embryonic RMS cell line. Turmeric acts by inducing apoptosis and regulating the expression of the intrinsic pathway of genes such as Bcl-2, BAX, BAK, and p53. In addition, it has an anti-inflammatory role by inhibiting the expression of genes associated with inflammation, such as NF-κB [26].

Another extract that produces tumor cell death by regulating the intrinsic pathway of apoptosis is *Crocus sativus*, commonly called "saffron". A methanolic extraction of these compounds was tested in two cell lines: one from RMS (TE-671) and another from glioblastoma (A-172). The isolated compounds were crocins (a type of carotenoid) and a crocin ester derivative (DMCRT). The result was statistically significant for treated versus untreated cells. The IC_{50} value of both bioactive components was similar in the TE-671 cell line using crocins (1.02 mg/mL) and DMCRT (1.27 mg/mL). However, the IC_{50} of the crocin extract in the A-172 cell line was higher (1.72 mg/mL) [33].

3.2. Plant Extracts and Isolated Bioactive Compounds with Antioxidant and Cytotoxic Action

Table 2 shows several extracts and bioactive plant compounds with antioxidant and cytotoxic action against tumor cells, representing 19% of the total search [37–45].

Tradescantia pallida, commonly called "Purpurina" contains phenols, flavonoids, and anthocyanins. The extract was obtained using methanol and was studied in vitro in RDATCC and CCL-136 tumor cell lines from RMS. These phytochemicals from the plant extract led to the formation of stable silver nanoparticles. Both the extract and the nanoparticles demonstrated, in a statistically significant manner, concentration-dependent antioxidant and cytotoxic activity [37]. The TpAgNP6 nanoparticle extract showed a lower IC_{50} value than that obtained from *Tradescantia pallida* (81.5 ± 1.9 µg/mL vs. 90.59 ± 1.6 µg/mL, respectively), showing that loading them into nanoparticles increases their effectiveness. *Tecoma stans* ("thunderer") and *Narcissus tazetta* ("bunch daffodil") are two other examples of the formation of nanoparticles from plant extracts. In this case, it was observed that cell viability decreased in a statistically significant manner with the association of the extract, nanoparticles, chemotherapy, and photodynamic therapy. After this combination, the lowest IC_{50} was shown with nanoparticles obtained from *Tecoma stans* branches (2.26 ± 0.9 µg/mL) [39].

Chamomile also appears in this systematic review, obtaining its extracts through ethanolic extraction and distilled water. Apigenin is a flavonoid that was isolated from this plant. The cell lines tested were RD (RMS), Hep2c (cervical carcinoma), and L20B (rat fibroblasts). The IC_{50} range observed between the different extracts is between 9.12 and 100.92 µg/mL. The best cytotoxic activity was from the unfermented extract of chamomile flowers in the Hep2c cell line. These extracts also had antimicrobial effects, especially against *Escherichia coli* and *Candida albicans*. The antimicrobial and antioxidant activities were better with fermented extracts; however, the cytotoxic activity was better with unfermented extracts [40].

Table 2. Plant extracts and isolated bioactive compounds with antioxidant and cytotoxic actions.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Aerial parts of *Tradescantia pallida* [37]	Methanol 80%	Phenols Proanthocyanidin	RDATCC CCL-136	*T. pallida:* 90.59 ± 1.6 µg/mL TpAgNP6: 81.5 ± 1.9 µg/mL	Antioxidant action. Proliferation inhibition of malignant cells.
Roots and aerial parts of *Phyllanthus debilis* [38]	Distilled water	Phenols Proanthocyanidin	RD CC-1	AP: RD: 287.16 ± 8.39 µg/mL CC-1: 555.03 ± 4.21 µg/mL Root: RD: 216.52 ± 11.90 µg/mL CC-1: 842.01 ± 7.53 µg/mL	Antioxidant action is due to the ability of polyphenols to neutralize reactive species by donating electrons or hydrogen atoms.
Tecoma stans and *Narcissus tazetta* [39]	Methanol		RD	*N. tazetta* extract: 16.9 ± 0.6 µg/mL *N. tazetta* NPs: 4.79 ± 1.1 µg/mL *T. stans* Branch NPs: 2.26 ± 0.9 µg/mL	The methanolic extracts presented antitumor and antioxidant effects, highlighting that their loading in nanoparticles increased their effectiveness in terms of antitumor activity.
Chamaemelum nobile [40]	Ethanol Distilled water	Apigenin-7-O-β-glucoside	RD Hep2c L2OB	Non-fermented extract in RD: 12.51 ± 1.77 µg/mL Non-fermented extract in Hep2c: 9.12 ± 0.99 µg/mL Fermented extract in RD: 35.78 ± 0.32 µg/mL	Non-fermented and fermented extracts presented antitumor and antioxidant activity.
Anchusa officinalis, *Echium vulgare*, and *Echium italicum* [41]	Methanol Ethanol Chloroform Acetone	-	HEP2 RD L2OB	*RD:* *Anchusa officinalis:* 141.91 µg/mL *Echium vulgare*: 121.1 µg/mL *Echium italicum*: 129.76 µg/mL	The functional extract presented antitumor and antioxidant activity, probably due to its ability to neutralize free radicals.

Table 2. *Cont.*

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Aerial parts of *Artemisia abrotanum* [42]	Distilled water Oil extract	-	RD		Cytotoxic activity is due to oil components. Unknown mechanism of action.
Veronica peduncularis [43]	Methanol Aqueous extract		RD HEP-2	RD: 230 µg/mL HEP-2: 390 µg/mL	Cytotoxicity. Antioxidative activity, highlighting its ability to neutralize DPPH.
Onosma aucheriana aerial parts [44]	Distilled water	Flavonoids, phenols, tannins, and gallotannins.	RD Hep2c L20B	RD: 50.57 ± 0.20 µg/mL Hep2c: 40.34 ± 0.59 µg/mL L20B: 25.54 ± 0.20 µg/mL	Cytotoxic action (without specifying the exact mechanism of action). Antioxidant action, highlighting the ability of phenols to neutralize the reactive agents.
Roots of soy molasses and kudzu [45]	Methanol	Isoflavones	A-172 HOS RD	S.M extract in RD: 244.4 µg/mL KR extract in RD: 337.4 µg/mL	Antioxidative effect due to neutralization of reactive species. Antiproliferative effect.

A172 (Glioblastoma cells); AP (Aerial parts); CC-1 (Healthy rat liver cells); Hep2c (Human cervical carcinoma cells); HEP-2 (Human epidermoid laryngeal carcinoma cells); HOS (Osteosarcoma); KR (Kudzu roots); L2OB (Rat fibroblasts); NPs (Nanoparticles); RDATCC and CCL-136 (Rhabdomyosarcoma cells); RD (Embryonal rhabdomyosarcoma cells); S.M (Soy Molasses); TpAgNP6 (Extract of Tradescantia pallida load in silver NPs).

3.3. Plant Extracts and Isolated Bioactive Compounds That Induce Cell Death by Cell Cycle Arrest

Cell cycle arrest in some of its phases is another mechanism of induced death of tumor cells. In this systematic review, 9% of the isolated bioactive compounds have this mechanism of cell death [23,32,35,46] (Table 3).

Berberine and Palmatine are two alkaloids isolated from *Phellodendron amurense* using distilled water and tested in embryonic RMS cells. Although the IC_{50} value was not calculated, it was found that these compounds inhibited tumor growth depending on the dose and exposure time, reaching their greatest inhibition after 72 h of treatment. Berberine treatment reduced cell growth by cell cycle arrest in the G1 phase, decreasing the levels of the Ki67 marker, especially in the ERMS1 cell line. However, Palmatine did not induce cell cycle arrest or apoptosis, although it showed cell growth inhibition, especially in the RD cell line [46]. In this case, this cell line is a malignant strain of embryonal RMS with amplification of the MYC gene. The possible mechanism of Palmatina interaction could be tumor cell formation.

Another example of plant extracts with cell cycle-induced apoptosis was the methanolic extract of *Berberis orthonotrys* root [23]. This extract was tested in the RH-30 cell line, which belongs to the alveolar RMS. The IC_{50} value after 24 h of treatment was 55.2 µg/mL, which stopped the cell cycle in the G2 phase. Flowers, leaves, and stems of *Agrimonia eupatoria* were studied in vitro against the growth of RMS (RD cell line), cervical cancer cells (HeLa cell line), and mouse fibroblasts (MEF cell line). Both methanol and aqueous extracts showed growth inhibition in both tumor cell lines, while there was no effect in healthy cells (MEF). Although the IC_{50} value has not been studied, the antitumor effects were dose- and time-dependent. The polyphenols of both extracts induced cell cycle arrest in G0/G1, while the flavonoids produced cycle arrest in the S phase [32].

Cotula cinerea is a plant that is found mostly in north Africa. Its aerial parts were used to study its antitumor action in RD cell lines versus healthy cells (VERO). Their extracts were obtained using 70% ethanol and 30% water. The extract that showed the lowest IC_{50} value was hexane, with a value of 57.21 $\pm$ 3.43 µg/mL in RD. However, in healthy cells, the extract that showed the lowest IC_{50} value was that of essential oil (obtained with distilled water), with a value of 72.72 $\pm$ 2.18 µg/mL. The possible mechanisms of action are multiple, highlighting apoptosis through the activation of caspases or cell cycle arrest. Further studies are needed to specify the exact mechanism of action [35].

3.4. Plant Extracts and Isolated Bioactive Compounds That Inhibit the Formation of Tumor Colonies

Among all the bioactive compounds analyzed, 3% of them inhibit the formation of tumor colonies [47,48], although the most specific mechanisms on how this inhibition is carried out are not included (Table 4).

Table 3. Plant extracts and isolated bioactive compounds that induce cell death by cell cycle arrest.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Phellodendron amurense [46]	Distilled water	Berberine Palmatine	ERMS 1 KYM 1 RD	-	Berberine arrests the cell cycle in the G1 phase. Palmatine acts through a possible interaction with the formation of the extracellular matrix of the tumor cell.
Roots of *Berberis orthobotrys* [23]	Methanol	-	RH-30	55.2 µg/mL	Motility inhibition occurs through stabilization of the cytoskeleton. Oncosome degradation. Reduction of proliferation with cell cycle arrest in the G2 phase. Initiation of apoptosis through reduction of Bcl-2, Bax, and PCNA.
Flowers, leaves, and stem of *Agrimonia eupatoria* [32]	Distilled water Methanol	Polyphenols Flavonoids	RD HeLa MEF		Polyphenols induce apoptosis with cell cycle arrest at the G0/G1 phase and antioxidant action. Flavonoids induce apoptosis with cell cycle arrest in the S phase and inactivation of the BCL-2 gene.
Aerial parts of *Cotula cinerea* [35]	Ethanol 70% Ethyl acetate Hexane n-butanol	-	RD VERO	RD: HE: 57.21 ± 3.43 µg/mL EAE: 187.52 ± 6.27 µg/mL NBE: more than 500 µg/mL VERO: HE: 142.27 ± 11.33 µg/mL EAE: 212.83 ± 9.02 µg/mL NBE: 447.38 ± 6.52 µg/mL	Apoptosis involves the activation of caspases, DNA methylation, histone acetylation, and cell cycle arrest.
Essential oils from the aerial parts of *Cotula cinerea* [35]	Distilled water	-	RD VERO	RD: 173.05 ± 4.46 µg/mL VERO: 72.72 ± 2.18 µg/mL	Multiple mechanisms: apoptosis with activation of caspases, DNA methylation, histone acetylation, and cell cycle arrest.

ERMS 1 (Embryonal rhabdomyosarcoma); EAE (Ethyl acetate extract); HE (Hexane extract); HeLa (Human cervical cancer cells); KYM 1 (Rhabdo-myosarcoma cells); MEF (Mouse fibroblasts); NBE (n-butanol); RD (Embryonal rhabdomyosarcoma cells); PCNA (Proliferating cell nuclear antigen); RH-30 (Alveolar rhabdomyosarcoma cells); VERO (Healthy African monkey cells).

Table 4. Plant extracts and isolated bioactive compounds that inhibit tumor colony formation.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Leaves and stem of *Hypericum patulum* [47]	Methanol		-RD VERO HEP-2	-*H.p* leaves in RD: 117.76 ± 6.02 µg/mL -*H.p* leaves in VERO: 447.69 ± 25.19 µg/mL -*H.p* stem in RD: 0.72 ± 0.04 µg/mL -*H.p* stem in VERO: 1.82 ± 0.12 µg/mL	Inhibition of tumor colony formation.
Senecio Anonymous [48]	Ethanol 95%	12 pyrrolizidine alkaloids	A-204	Compounds 2 and 3: 120 ± 5 µg/mL	Tumor growth inhibition (not for use in vivo due to hepatotoxicity.)

A-204 (Embryonal rhabdomyosarcoma cells); HEP-2 (Human epidermoid laryngeal carcinoma cells); *H.p* (Hypericum patulum); RD (Embryonal rhabdomyosarcoma cells); VERO (Healthy African monkey cells).

The leaves and stem of *Hypericum patulum*, commonly called "St. John's Wort" were used in three cell lines: RMS (RD), healthy cells (VERO), and laryngeal squamous cell carcinoma cells (HEP-2). The extract was obtained using methanol. This extract was compared with another one from the *Hypericum* family, called *Hypericum mysorense.* The results indicated that the *Hypericum patulum* stem extract is the most potent of all the extracts tested, with an IC_{50} value in RD of 0.72 ± 0.04 µg/mL, while the least potent is that of *Hypericum patulum* leaves in VERO, with an IC_{50} value of 447.69 ± 25.19 µg/mL [47].

The *Senecio anonymous* extract was obtained using 95% ethanol and tested in vitro on the A-204 cell line (RMS). Twelve alkaloids were isolated, among which two with the lowest IC_{50} stood out with a value of 120 ± 5 µg/mL. Despite this study, none of the extracts from this plant could be used as antitumor agents due to their hepatotoxicity; therefore, in vivo studies were not carried out [48].

3.5. Plant Extracts and Isolated Bioactive Compounds That Induce Cell Death by Blocking Proteins

In total 5% of the plant extracts or bioactive compounds induce cell death by blocking proteins [36,49,50], each with a different antitumor mechanism (Table 5).

Choerospondias axillaris is a tree whose extracts were obtained using methanol. It is known to have beneficial effects at the cardiovascular level. In the research study, it was demonstrated, in different cell lines, that each of its components could inhibit proliferation and causing toxicity in malignant cells. Five compounds (Choerosponols A–E) were isolated from its fruit, with compound two (Choerosponols B) being the most effective in SJCRH30 (RMS cells) with an IC_{50} value of 0.12 ± 0.02 µM, while compound A is more effective in D283 and A-673 (RMS cell lines). Compound one (Choerosponols A) had antitumor action by inhibiting the monocarboxylate transporter type 1 (MCT1), especially in Ewing sarcoma and medulloblastoma [49].

Another extract with bioactive compounds with antitumor activity against RMS was the methanol extract from the branches and leaves of *Choerospondias axillaris*. The isolated phytochemicals were eight types of limonoids studied in RMS and hepatocarcinoma cells. The IC_{50} value was similar in both cell lines, with a result of 10.08 µg/mL in RMS. Limonoids form a gamma-lactone ring at carbons C4–C7 as an antitumor mechanism; however, more studies are needed to confirm this hypothesis [50].

Finally, in the *Morus alba* root methanol extract, a specific type of flavonoid called Sangennon G1 is highlighted among its components [36]. This flavonoid inhibits the XIAP protein, which is an apoptosis inhibitor. The inhibition of XIAP induces apoptosis in cancer cells by activating caspase 9. It is known that the extract alone had less effect than the combination with etoposide. This action was verified in vitro in RMS, T-cell lymphoblastic leukemia, and neuroblastoma cells.

3.6. Plant Extracts and Isolated Bioactive Compounds That Induce Cell Death by Restricting Motility

Only 5% of all plant extracts or bioactive compounds analyzed in this systematic review used inhibition of cell motility as a single mechanism or in combination with others [21,23,34] (Table 6).

Table 5. Plant extracts and isolated bioactive compounds induce cell death by blocking proteins.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Fruits of *Choerospondias axillaris* [49]	Methanol	Choerosponols A–E	A-673 RH30 D283 Hep293TT	RH30: Compound 2: 0.12 ± 0.02 μM Compound 1: >10 μM	Compound 1 induced inhibition of the MCT1 transporter (*SLC16A1* gene).
Branches and leaves of *Choerospondias axillaris* [50]	Methanol	Limonoids 1–8	HEP-G2 RD	HEP-G2: 8.02 μg/mL RD: 10.08 μg/mL	Limonoids induced the formation of a gamma-lactone ring at carbons C4–C7.
Root of *Morus alba* [36]	Methanol	Sanggenon G1 Sanggenon G3	T-ALL OVCAR-4 SH-EP IMR-32 NxS2	SG1 in OVCAR-4: 210 μM	SG1 inhibits the XIAP protein, leading to the initiation of apoptosis.

A-673 (Ewing sarcoma); H-EP, IMR-32, and NxS2 (Neuroblastoma cells); HepG-2 (Human hepatocellular carcinoma cells); MCT1 (monocarboxylate transporter 1); RD (Embryonal rhabdomyosarcoma cells); RH30 (Alveolar rhabdomyosarcoma cells); SG1 (Sanggenon G1); T-ALL (T-cell lymphoblastic leukemia); OVCAR-4 (Rhabdomyosarcoma cells).

Table 6. Plant extracts and isolated bioactive compounds that induce cell death by restricting motility.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds	Mechanism of Action
Roots of *Vincetoxicum arnottianum* [21]	Methanol	ß-sitosterol Lupeol	RH-30 HA-OH 1 hMSC	RH-30: 188.75 µg/mL HA-OH1: 220.54 µg/mL hMSC: 1433.14 µg/mL	Apoptosis induction by PAX3-FOXO1. Motility restriction by stabilization of the cytoskeleton through SP1.
Root of *Berberis orthobotrys* [23]	Methanol	-	RH30	55.2 µg/mL	Motility inhibition occurs through stabilization of the cytoskeleton. Oncosome degradation. Proliferation reduction with cell cycle arrest in G2. Initiation of apoptosis through reduction of Bcl-2, Bax, and PCNA.
Leaves of *Nicotiana glauca* [34]	Ethanol 96% Hexane Chloroform Ethyl acetate	Hexane: palmitic acid extract Chloroform: scopoletin extract	RD		Palmitic acid and scopoletin are inducers of apoptosis. Reduction of cell migration capacity.

hMSC (Human healthy mesenchymal cells); PAX3-FOXO1 (Fusion gene); RD (Embryonal rhabdomyosarcoma cells); RH-30 and HA-OH 1 (Alveo-lar rhabdomyosarcoma cells); SP1 (Transcription factor).

The bioactive compounds from the root of *Vincetoxicum arnottianum*, phytosterols (beta-sitosterol) and triterpenes (lupeol), were obtained using methanol. Its effect was studied in vitro in two alveolar RMS cell lines and one of the healthy human cells (RH-30, HA-OH 1, and hMSC, respectively). The combined treatment with β-sitosterol and lupeol produced a significant decrease in cell viability of 30% in RH-30 and 35% in HA-OH1. The lowest IC_{50} value was 188.75 μg/mL for the RD cell line, while in healthy cells no cytotoxicity was shown since high concentrations of the isolated compounds were needed, showing an IC_{50} greater than 1000 μg/mL. Their mechanism of action is based on apoptosis and, especially, on the restriction of motility through the stabilization of the cytoskeleton through the transcription factor SP1 [21].

Another extract previously discussed was obtained from the root of *Berberis orthobotrys*. In addition to cell cycle arrest, it inhibits cell motility by stabilizing the cytoskeleton and degrading oncosomes, which are vesicles released by tumor cells [23].

As shown in Table 3, the compounds isolated from *Nicotiana glauca* leaves using 96% ethanol were formed by palmitic acid and scopoletin. Although scopoletin showed better results in reducing cell proliferation in the RD cell line, both were inducers of apoptosis from the first three hours of treatment until the end of the trial (48 h). In addition, they reduced cell migration capacity [34].

3.7. Plant Extracts and Isolated Bioactive Compounds with Unknown Mechanism of Action

The last group is shown in Table 7, representing 16% of all bioactive compounds and plant extracts analyzed. They demonstrated the antitumor capacity, but without specifying its mechanism of action [15,16,35,51–54].

The hydroethanolic extract of the well-known "Rosemary" was capable of inhibiting cell proliferation, exerting its best activity after 72 h of treatment. Its isolated compounds were carnosic acid, carnosol, and rosmarinic acid, all phenols. It is effective in both RMS (TE671) and glioblastoma (A172) cell lines. The lowest IC_{50} value was 0.249 ± 1.09 mg/mL in TE671 after 72 h of treatment. This extract was dose- and time-dependent [16].

In addition, nanoparticles were obtained from the branches and leaves of *Datura suaveolens* and *Verbena tenuisecta*. Both plants contain flavonoids and phenols. The greatest effectiveness of the methanol extract was obtained after 48 h of in vitro treatment against RMS cells. It is known that the extract reduces cell viability; however, this effect was greater with the use of nanoparticles from *Datura suaveolens* leaves, whose IC_{50} value is 2.4 ± 0.9 μg/mL. These plant extracts also had an antibacterial effect [51].

Finally, it should be noted that the temperature at which the extracts were obtained can have a relevant role in cell viability. Extracts of *Kunzea ericoides* leaves with water in a subcritical state at 210 °C showed lower IC_{50} values than those with ethanol. The lowest IC_{50} was in L cells (healthy mouse cells) with a value of 216.8 ± 3.4 μg/mL, followed by the RD cell line, whose result was 389 ± 0.8 μg/mL. Therefore, worse results were obtained with ethanol extracts. Dose, time, and temperature are essential for the antitumor action of these extracts [54].

Table 7. Plant extracts and isolated bioactive compounds with unknown mechanism of action.

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds
Dried leaves of *Rosmarinus officinalis* [16]	Hydroethanolic solution: 70%	Carnosic acid Carnosol Rosmarinic acid	TE671 A172	TE671: 0.249 ± 1.09 mg/mL A172: 0.577 ± 0.98 mg/mL
Branches and leaves of *Datura suveolens* and *Verbena tenuisecta* [51]	Methanol Nanoparticles	Flavonoids Phenols	RD	*V. tenuisecta:* 42.5 ± 0.6 µg/mL *D. suaveolens* leaves nanoparticles: 2.4 ± 0.9 µg/mL *D. suaveolens* branches: 7.8 ± 1.1
Branches, leaves, and fruit of *Anacolosa clarkii* [52]	Methanol	*Anacolosin* A–F (1–6) *Corymbulosa:* X and Y (7–8) Two more compounds: 9–10	A-673 RH30 D283 Hep293TT	Compounds 7–10: RH30 0.3–0.8 µM Hep293TT: 0.2–0.6 µM Compounds 1–10: 0.2–4.1 µM in all cell lines
Leaves of *Macaranga barteri* [15]	Methanol	3,5-dicaffeoylquinic acid Acteoside Kaempferol Bastadin-11	RD VERO PNT2	RD: 0.22 µg/mL
Roots and leaves of *Calliandra portoricensi* [15]	Methanol	Neurolenin B Nigrosporolide Transgeranic acid	RD VERO PNT2	RD: 0.82 µg/mL
Seed of *Spondias mombin* [53]	Methanol	Phenols Flavonoids	RD	139.6 ± 0.54 µg/mL

Table 7. *Cont.*

Raw Material	Extraction Method	Isolated Compound(s)	Cell Line	IC_{50} Value of the Functional Extract or Isolated Compounds
Aerial parts of *Salvia verbenaca* [35]	Ethanol 70% Hexane Ethyl acetate n-butanol	-	RD VERO	Hexane extract in RD: 474.62 ± 1.31 µg/mL The rest of the extracts in RD: >500 µg/mL Ethyl acetate extract in VERO: 223.63 ± 1.61 µg/mL The rest of the extracts in VERO: >500 µg/mL
Leaves of *Kunzea ericoides* [54]	Distilled water Ethanol 60%	Polyphenols	RD HEP-2 L -cells	*K. ericoides* leaves extract with ethanol in RD: 3815.9 µg/mL *K. ericoides* leaves extract with water in a subcritical state at 210 °C in RD: 389 ± 0.8 µg/mL *K. ericoides* leaves extract with water in a subcritical state at 210 °C in *L cells*: 216.8 ± 3.4 µg/mL

A172 (Glioblastoma cells); A-673 (Ewing sarcoma); D283 (Medulloblastoma); HEP-2 (Human epidermoid laryngeal carcinoma cells); Hep293TT (Hepatoblastoma); L cells (Mouse cells); PNT2 (Healthy human prostate cells); RD (Rhabdomyosarcoma); SJCRH30 (Rhabdomyosarcoma cells); TE671 (Rhabdomyosarcoma cells); VERO (Healthy African monkey cells).

4. Discussion

The incidence of sarcomas is around 7% in children, and half of these patients are diagnosed with RMS [1,2]. In recent years, despite progress in the treatment of RMS, the results have not been as expected since it has metastasized in more cases. Because of this, new and more effective treatments are needed. Therapies derived from natural products are powerful current and future research tools to use in combination with classic cancer treatments to increase survival and decrease their toxicity [4,58,59].

Most of the research studies included in this systematic review are based on in vitro cell death through apoptosis. The most commonly used cell line has been RD, an embryonal RMS cell. However, they have also been used in the most malignant histological variant, that is, the alveolar, being the RH-30 cell line the most used [21,23,24,26,49]. In RH-30 and RMS-13, both alveolar RMS cell lines, it has been shown that apoptosis can occur both through the intrinsic pathway and through caspase activation [24,26].

Many of the extracts were obtained with polar solvents, for example, methanol or ethanol. In the specific case of apoptosis, it was observed that methanol is the most commonly used solvent, and its extracts have apoptotic potential against the RH-30 and RD cell lines [21,23]. On the contrary, it should be noted that, in the scientific literature on extraction methods for bioactive compounds of plant origin, ethanol is the most commonly used for the extraction of polyphenols [60]. However, for RMS cells, most of the extracts were obtained with methanol, showing high amounts of polyphenols and flavonoids.

Flavonoids and phenols are the most common bioactive compounds in plant extracts. The extraction methodology using solvents such as methanol and ethanol enhances the extraction of these bioactive compounds [61]. Although these extracts may contain other compounds, it is those of a phenolic nature that present the greatest antioxidant and antitumor bioactivity. In fact, in the case of *Phyllanthus debilis* extract [62], the antiproliferative and antioxidant activity were greater in plant roots since they contained more phenols. The IC_{50} value of the root extract in the RD cell line was 216.52 ± 11.90 µg/mL, highlighting that *Phyllanthus debilis* did not show cytotoxicity against healthy cells used as a control (CC-1).

Regarding the analysis of plants studied in this systematic review, *Choerospondias axillaris* [49,50] was the only plant species studied in two different scientific articles published in 2020. Its mechanism of action was different depending on the part of the plant used for the extract, showing better results from the extract obtained from its fruits. Therefore, it is important to know which part of the plant has been used for the extract since they may have different compounds. The genus *Veronica* appeared in two different studies, although they analyzed different species [28,43]. Some species of the genus *Berberis* were analyzed in three different articles, according to which apoptosis was the mechanism of cell death [23,24,27]. However, this does not mean that these plant extracts or isolated bioactive compounds were more relevant than the rest against RMS.

In addition to the use of the plant extract against the tumor individually, synergy studies have been carried out with the chemotherapy regimens used clinically. In seven of the articles included in this systematic review, the functional extracts were studied together with or in comparison with chemotherapy drugs. Some of them have proven that the combination of chemotherapy and plant extract achieves a greater cytotoxic effect against tumor cells and less toxicity in non-tumor cells, as shown in *Curcuma amada*, *Ficus carica*, *Tecoma stans*, and *Narcissus tazetta* [19,26,39]. In four articles, the antitumor activity of the extract was simply compared with that of the chemotherapy agent, as in the methanol extract of *Spondias mombin* seeds against the RD cell line. The IC_{50} value of the extract was 139 ± 0.54 µg/mL, while the IC_{50} value of cyclophosphamide was 0.97 ± 0.03 µg/mL. Despite having a higher IC_{50}, the extract proved, in vitro, to be 143 times less toxic than cyclophosphamide [53]. In addition, the methanol extract contains a high amount of flavonoids and phenols, which give it antioxidant properties.

In addition to extracts of plant origin, there are extracts from the Kingdom of Fungi with antitumor activity in RMS or marine species like *Anemonia sulcata* with antitumoral activity against colon cancer [63]. In, *Lenzites quercina*, whose extract was obtained from

its fruits using ethanol, presents antitumor activity against RSM in vitro. The cell lines used were RD, VERO, and HeLA (Human cervical cancer cells). The IC_{50} value of various extracts was between 0.11 µg/mL and 623 µg/mL, with the most effective being the ethyl acetate extract not fermented in HELA, although in the VERO cell line (healthy cells) high doses are needed to achieve cytotoxicity of this extract. In RD cells, the most effective IC_{50} was 0.46 µg/mL of the fermented ethanol extract, and the least effective was that of unfermented ethyl acetate (18.57 µg/mL) [64].

Another new therapeutic tool that has been included in this systematic review is the possibility of using nanoparticles against RMS. Nanoparticles are a new field of research in nanobiotechnology since they have potential applications in biomedicine. They have mainly been designed to transport drugs to specific tissues of the human body. In their use against tumor cells, they present several advantages concerning the traditional chemotherapy drugs, like greater permeability to cross-cell membranes due to their small size, biocompatibility, longer drug release time, less degradation of the drug, and toxicity of healthy cells because they specifically target malignant cells [65,66]. There are various biocompatible materials with which nanoparticles are developed, highlighting silver nanoparticles that have shown they are not toxic to human cells while they are toxic to bacteria or fungi. Therefore, they could have antimicrobial and antifungal effects [65]. In this case, nanoparticles are of interest due to their possible antitumor effect. Many studies have documented that biomolecules from plant extracts are useful for the formation of nanoparticles [66–69]. In this review, three articles were included in which the effectiveness of the extract and the nanoparticles were compared. Nanoparticles from *Tecoma stans* branches showed the best results against RD [39]. Nanoparticles are not only used against RMS but also in numerous types of tumors. This is the case of a recent article that studied silver nanoparticles from the methanol extract from the leaves and flowers of *Tecoma stans*. In this study, the antiproliferative effect was studied in vitro against colorectal cancer cells, concluding that it could be a potential antitumor tool. In addition, it presented antimicrobial and antioxidant effects. Likewise, its authors highlight the importance of carrying out in vivo studies that corroborate this [70]. Nanoparticles from the *Datura suaveolens* leaf extract [51] also demonstrated a tumor effect in vitro against RD, a cell line with a low IC_{50} value. However, there are no assays in other cell lines that can corroborate this effect. Another article that studied nanoparticles in the RD was the one that used the aerial parts of *Tradescantia pallida*. Better results were obtained with nanoparticles from the methanol extract of *Tradescantia pallida* than with the free extract [37]. However, when compared with the literature on the subject, there are no studies that analyze the antitumor effect of this plant on other cell lines. There is only one article that has analyzed the antibacterial and antioxidant effects of using the leaves of this plant to form nanoparticles [71].

Finally, another novel therapeutic option is the combination of viruses with plant extracts and chemotherapy against certain tumors. This review includes *Rheum ribes* rhizome extract, combining it with Newcastle Disease Virus [22]. The synergistic effect of both was demonstrated to trigger apoptosis in vitro in rat mammary adenocarcinoma, human RMS, and glioblastoma cells. In vivo, the same effect was demonstrated, but only in rat mammary adenocarcinoma cells. Furthermore, this combination was not toxic to healthy cells used as a control. The oncogenic power of this virus is reflected in the scientific literature of the last ten years. A recent article demonstrated the inhibition of tumor growth of glioblastoma multiforme using this virus [72] and exposed the synergistic effect of Temozolomide nanoparticles (classic chemotherapy for this tumor) and Newcastle Disease Virus inducing apoptosis, in vitro, of the cells of this brain tumor. In both articles, the mechanism of cell death is apoptosis.

Given the results obtained in this systematic review, it can be stated that functional extracts of plant origin are a source of bioactive compounds with antitumor activity for RMS, an aggressive tumor with a high childhood incidence rate. Although in recent years its study has increased, these are not enough, requiring more complete studies regarding the molecular mechanism of action. One of the limitations of the study was that of the

articles included in the systematic review, not all of them carried out experiments to understand the mechanisms of action of the functional extracts or isolated compounds. These studies are fundamental since, by knowing them, the bioactive compounds are better characterized, and synergy studies with chemotherapy or loading on nanoparticles can be carried out. Kakouri et al. (2022) studied the antiproliferative activity of dried leaves of *Rosmarinus officinalis* in RMS; however, the mechanism of action is unknown because no techniques have been used in said research to elucidate it [16]. Despite this, the isolated compounds have been studied in other types of cancer. Carnosic acid is known to induce apoptosis by activating both intrinsic and extrinsic pathways and inhibiting the Akt-mTOR signaling pathway in gastric cancer [73]. Ogbole et al. (2017) studied the roots and leaves of *Calliandra portoricensi* [15]. One of the isolated compounds, Kaempferol, has been studied in another study. Kaempferol is known to induce apoptosis in pancreatic cancer [74] and breast cancer [75]. Other research points to the induction of autophagy in non-small cell lung cancer [76]. Therefore, a more complete study of the pathways of action of extracts and bioactive compounds in RMS is necessary. In addition, there are parts of plants that are not usually studied, such as seeds, which are a source of bioactive compounds with antitumor activity for numerous types of cancer [77,78].

5. Conclusions

Throughout this systematic review, it has been proven that bioactive compounds from plant extracts or the extracts themselves are a powerful therapeutic tool against RMS, with most of them presenting lower toxicity compared to chemotherapy treatment. Although the mechanisms of cell death are varied in most of the extracts, apoptosis stands out as the most common cell death mechanism. In this systematic review, the possibility of other tools has also been studied as possible future lines of treatment for this sarcoma, such as the use of nanoparticles and viruses. This approach results in greater efficacy in co-treatment with chemotherapy. It is important to highlight that there are fewer research studies on this type of sarcoma, and most of these studies are being carried out only in vitro. Therefore, more studies are necessary to be able to transfer the information to the clinic, although the research carried out to date represents an important advance in the treatment of RMS compared to the classic approach.

Author Contributions: Conceptualization, J.P. and C.M. (Consolación Melguizo); methodology, C.M. (Cristina Mesas) and B.S.; formal analysis, C.M. (Cristina Mesas), B.S. and G.P.; investigation, C.M. (Cristina Mesas), B.S., M.A.C., J.M. and K.D.; data curation, M.A.C., J.M. and K.D.; writing—original draft preparation, C.M. (Cristina Mesas) and G.P.; writing—review and editing, J.P. and C.M. (Consolación Melguizo); visualization, J.P., C.M. (Consolación Melguizo) and G.P.; supervision, J.P.; funding acquisition, C.M. (Consolación Melguizo). All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by the Spanish Ministry of Science and Innovation (FEDER) (CPP2022-009967 and CPP2022-010017) and the Spanish Ministry of Universities and Science (RTC2019-006870-1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the main article.

Acknowledgments: M.A.C. would like to acknowledge FPU2019 grants (ref. FPU19/04967) from the Ministerio de Educación, Ciencia, Deporte, and Competitividad (Spain).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sbaraglia, M.; Bellan, E.; Dei Tos, A.P. The 2020 WHO Classification of Soft Tissue Tumours: News and Perspectives. *Pathologica* **2020**, *113*, 70–84. [CrossRef]
2. Skapek, S.X.; Ferrari, A.; Gupta, A.A.; Lupo, P.J.; Butler, E.; Shipley, J.; Barr, F.G.; Hawkins, D.S. Rhabdomyosarcoma. *Nat. Rev. Dis. Primers* **2019**, *5*, 1. [CrossRef]
3. Agaram, N.P. Evolving Classification of Rhabdomyosarcoma. *Histopathology* **2022**, *80*, 98–108. [CrossRef]
4. Dasgupta, R.; Fuchs, J.; Rodeberg, D. Rhabdomyosarcoma. *Semin. Pediatr. Surg.* **2016**, *25*, 276–283. [CrossRef]
5. Martin-Giacalone, B.A.; Weinstein, P.A.; Plon, S.E.; Lupo, P.J. Pediatric Rhabdomyosarcoma: Epidemiology and Genetic Susceptibility. *J. Clin. Med.* **2021**, *10*, 2028. [CrossRef]
6. Millán, J.; Cicero, A.F.G.; Torres, F.; Anguera, A. Effects of a Nutraceutical Combination Containing Berberine (BRB), Policosanol, and Red Yeast Rice (RYR), on Lipid Profile in Hypercholesterolemic Patients: A Meta-Analysis of Randomised Controlled Trials. *Clin. Investig. Arterioscler.* **2016**, *28*, 178–187. [CrossRef]
7. Khalid, M.; Amayreh, M.; Sanduka, S.; Salah, Z.; Al-Rimawi, F.; Al-Mazaideh, G.M.; Alanezi, A.A.; Wedian, F.; Alasmari, F.; Faris Shalayel, M.H. Assessment of Antioxidant, Antimicrobial, and Anticancer Activities of Sisymbrium Officinale Plant Extract. *Heliyon* **2022**, *8*, e10477. [CrossRef]
8. Ismail, N.Z.; Adebayo, I.A.; Mohamed, W.A.S.; Mohamad Zain, N.N.; Arsad, H. Christia Vespertilionis Extract Induced Antiproliferation and Apoptosis in Breast Cancer (MCF7) Cells. *Mol. Biol. Rep.* **2021**, *48*, 7361–7370. [CrossRef]
9. Zhu, H.; Zhao, H.; Zhang, L.; Xu, J.; Zhu, C.; Zhao, H.; Lv, G. Dandelion Root Extract Suppressed Gastric Cancer Cells Proliferation and Migration through Targeting lncRNA-CCAT1. *Biomed. Pharmacother.* **2017**, *93*, 1010–1017. [CrossRef]
10. Muscella, A.; Stefàno, E.; De Bellis, L.; Nutricati, E.; Negro, C.; Marsigliante, S. Antitumor and Antimigration Effects of *Salvia clandestina* L. Extract on Osteosarcoma Cells. *Ann. N. Y. Acad. Sci.* **2021**, *1500*, 34–47. [CrossRef]
11. Gligor, O.; Clichici, S.; Moldovan, R.; Decea, N.; Vlase, A.-M.; Fizeșan, I.; Pop, A.; Virag, P.; Filip, G.A.; Vlase, L.; et al. An In Vitro and In Vivo Assessment of Antitumor Activity of Extracts Derived from Three Well-Known Plant Species. *Plants* **2023**, *12*, 1840. [CrossRef]
12. Wen, X.; Wang, Q.; Dai, T.; Shao, J.; Wu, X.; Jiang, Z.; Jacob, J.A.; Jiang, C. Identification of Possible Reductants in the Aqueous Leaf Extract of Mangrove Plant Rhizophora Apiculata for the Fabrication and Cytotoxicity of Silver Nanoparticles against Human Osteosarcoma MG-63 Cells. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2020**, *116*, 111252. [CrossRef]
13. Liu, B.; Liu, J.; Chen, J.; Zhu, D.; Zhou, H.; Wang, X. A Study on Anticancer Activity of Caulis Spatholobi Extract on Human Osteosarcoma Saos-2 Cells. *Afr. J. Tradit. Complement. Altern. Med.* **2013**, *10*, 256–260. [CrossRef]
14. Tang, C.; Zhao, Y.; Huang, S.; Jin, Y.; Liu, J.; Luo, J.; Zheng, J.; Shi, D. Influence of Artemisia Annua Extract Derivatives on Proliferation, Apoptosis and Metastasis of Osteosarcoma Cells. *Pak. J. Pharm. Sci.* **2015**, *28*, 773–779.
15. Ogbole, O.O.; Segun, P.A.; Adeniji, A.J. In Vitro Cytotoxic Activity of Medicinal Plants from Nigeria Ethnomedicine on Rhabdomyosarcoma Cancer Cell Line and HPLC Analysis of Active Extracts. *BMC Complement. Altern. Med.* **2017**, *17*, 494. [CrossRef]
16. Kakouri, E.; Nikola, O.; Kanakis, C.; Hatziagapiou, K.; Lambrou, G.I.; Trigas, P.; Kanaka-Gantenbein, C.; Tarantilis, P.A. Cytotoxic Effect of Rosmarinus Officinalis Extract on Glioblastoma and Rhabdomyosarcoma Cell Lines. *Molecules* **2022**, *27*, 6348. [CrossRef]
17. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* **2021**, *372*, n71. [CrossRef]
18. Bandara, U.Y.; Witharana, C.; Soysa, P. The Effectiveness of the Nimali Variety of Sri Lankan Punica Granatum L. Fruit Extracts on Rhabdomyosarcoma (RD) Cells Concerning the Apoptotic Signaling Pathway. *Asian Pac. J. Cancer Prev.* **2022**, *23*, 501–510. [CrossRef]
19. Aziz, B.; Khurshid, A.; Mahmood, R.; Khan, J.A.; Javaid, S.; Alam, M.; Mujtaba Ul Hassan, S.; Ikram, M. Study of Synergistic Effects of Ficus Carica Leaves Extract Mediated Chemo-Photodynamic Therapy on Rhabdomyosarcoma Cells. *Photodiagn. Photodyn. Ther.* **2021**, *36*, 102565. [CrossRef]
20. Okon, E.; Kukula-Koch, W.; Halasa, M.; Jarzab, A.; Baran, M.; Dmoszynska-Graniczka, M.; Angelis, A.; Kalpoutzakis, E.; Guz, M.; Stepulak, A.; et al. Magnoflorine—Isolation and the Anticancer Potential against NCI-H1299 Lung, MDA-MB-468 Breast, T98G Glioma, and TE671 Rhabdomyosarcoma Cancer Cells. *Biomolecules* **2020**, *10*, 1532. [CrossRef]
21. Adamus, A.; Ali, I.; Vasileiadis, V.; Al-Hileh, L.; Lisec, J.; Frank, M.; Seitz, G.; Engel, N. Vincetoxicum Arnottianum Modulates Motility Features and Metastatic Marker Expression in Pediatric Rhabdomyosarcoma by Stabilizing the Actin Cytoskeleton. *BMC Complement. Med. Ther.* **2021**, *21*, 136. [CrossRef]
22. Al-Shammari, A.M.; Jalill, R.D.A.; Hussein, M.F. Combined Therapy of Oncolytic Newcastle Disease Virus and Rhizomes Extract of Rheum Ribes Enhances Cancer Virotherapy In Vitro and In Vivo. *Mol. Biol. Rep.* **2020**, *47*, 1691–1702. [CrossRef]
23. Adamus, A.; Peer, K.; Ali, I.; Lisec, J.; Falodun, A.; Frank, M.; Seitz, G.; Engel, N. Berberis Orthobotrys—A Promising Herbal Anti-Tumorigenic Candidate for the Treatment of Pediatric Alveolar Rhabdomyosarcoma. *J. Ethnopharmacol.* **2019**, *229*, 262–271. [CrossRef]
24. Stammer, R.M.; Kleinsimon, S.; Rolff, J.; Jäger, S.; Eggert, A.; Seifert, G.; Delebinski, C.I. Synergistic Antitumour Properties of viscumTT in Alveolar Rhabdomyosarcoma. *J. Immunol. Res.* **2017**, *2017*, 4874280. [CrossRef]
25. Fernando, D.; Adhikari, A.; Nanayakkara, C.; de Silva, E.D.; Wijesundera, R.; Soysa, P. Cytotoxic Effects of Ergone, a Compound Isolated from Fulviformes Fastuosus. *BMC Complement. Altern. Med.* **2016**, *16*, 484. [CrossRef]

26. Ramachandran, C.; Quirin, K.-W.; Escalon, E.A.; Lollett, I.V.; Melnick, S.J. Therapeutic Effect of Supercritical CO_2 Extracts of Curcuma Species with Cancer Drugs in Rhabdomyosarcoma Cell Lines. *Phytother. Res.* **2015**, *29*, 1152–1160. [CrossRef]
27. Harput, U.S.; Genc, Y.; Saracoglu, I. Cytotoxic and Antioxidative Activities of *Plantago lagopus* L. and Characterization of Its Bioactive Compounds. *Food Chem. Toxicol.* **2012**, *50*, 1554–1559. [CrossRef]
28. Saracoglu, I.; Harput, U.S. In Vitro Cytotoxic Activity and Structure Activity Relationships of Iridoid Glucosides Derived from *Veronica* Species. *Phytother. Res.* **2012**, *26*, 148–152. [CrossRef]
29. Gunathilaka, T.L.; Dilrangi, K.H.; Ranasinghe, P.; Samarakoon, K.W.; Peiris, L.D.C. Mechanistic Insight into Apoptotic Induction in Human Rhabdomyosarcoma and Breast Adenocarcinoma Cells by Chnoospora Minima: A Sri Lankan Brown Seaweed. *Pharmaceuticals* **2021**, *14*, 1154. [CrossRef]
30. Ahmad, K.S.; Rafi, M.; Kiani, B.H.; Ishtiaq, M.; Mehmood, A.; Iqbal, M.S.; Zubair, T.; Qureshi, R. Evaluation of Anticancer Activities of Selected Medicinal Plants from Ganga Choti, Lesser Himalaya, Bagh, Azad Kashmir. *Pak. J. Bot.* **2021**, *53*, 1433–1440. [CrossRef]
31. Siamayuwa, C.E.; Nyanga, L.K.; Chidewe, C. Chemopreventive Effects and Antioxidant Capacity of Combined Leaf Extracts of Sesamum Angustifolium (Oliv.) Engl. and Hibiscus Articulatus on Rhabdomyosarcoma. *Evid. Based Complement. Altern. Med.* **2020**, *2020*, 8567182. [CrossRef]
32. Ad'hiah, A.H.; Al-Bederi, O.N.H.; Al-Sammarrae, K.W. Cytotoxic Effects of *Agrimonia eupatoria* L. against Cancer Cell Lines In Vitro. *J. Assoc. Arab Univ. Basic Appl. Sci.* **2013**, *14*, 87–92. [CrossRef]
33. Hatziagapiou, K.; Nikola, O.; Marka, S.; Koniari, E.; Kakouri, E.; Zografaki, M.-E.; Mavrikou, S.S.; Kanakis, C.; Flemetakis, E.; Chrousos, G.P.; et al. An In Vitro Study of Saffron Carotenoids: The Effect of Crocin Extracts and Dimethylcrocetin on Cancer Cell Lines. *Antioxidants* **2022**, *11*, 1074. [CrossRef]
34. Musso, F.; Pronsato, L.; Milanesi, L.; Vasconsuelo, A.; Faraoni, M.B. Non-Polar Extracts of Nicotiana Glauca (Solanaceae) Induce Apoptosis in Human Rhabdomyosarcoma Cells. *Rodriguésia* **2020**, *71*, 1012019. [CrossRef]
35. Guaouguaou, F.-E.; Bebaha, M.A.A.; Taghzouti, K.; Bouyahya, A.; Bakri, Y.; Dakka, N.; Es-Safi, N.E. Cytotoxicological Investigation of the Essential Oil and the Extracts of Cotula Cinerea and Salvia Verbenaca from Morocco. *Biomed Res. Int.* **2018**, *2018*, 7163961. [CrossRef] [PubMed]
36. Seiter, M.A.; Salcher, S.; Rupp, M.; Hagenbuchner, J.; Kiechl-Kohlendorfer, U.; Mortier, J.; Wolber, G.; Rollinger, J.M.; Obexer, P.; Ausserlechner, M.J. Discovery of Sanggenon G as a Natural Cell-Permeable Small-Molecular Weight Inhibitor of X-Linked Inhibitor of Apoptosis Protein (XIAP). *FEBS Open Bio* **2014**, *4*, 659–671. [CrossRef] [PubMed]
37. Shahzadi, I.; Aziz Shah, S.M.; Shah, M.M.; Ismail, T.; Fatima, N.; Siddique, M.; Waheed, U.; Baig, A.; Ayaz, A. Antioxidant, Cytotoxic, and Antimicrobial Potential of Silver Nanoparticles Synthesized Using Tradescantia Pallida Extract. *Front. Bioeng. Biotechnol.* **2022**, *10*, 907551. [CrossRef] [PubMed]
38. Perera, D.; Soysa, P.; Wijeratne, S. Polyphenols Contribute to the Antioxidant and Antiproliferative Activity of Phyllanthus Debilis Plant In-Vitro. *BMC Complement. Altern. Med.* **2016**, *16*, 339. [CrossRef]
39. Tariq, H.; Rafi, M.; Amirzada, M.I.; Muhammad, S.A.; Yameen, M.A.; Mannan, A.; Ismail, T.; Shahzadi, I.; Murtaza, G.; Fatima, N. Photodynamic Cytotoxic and Antibacterial Evaluation of Tecoma Stans and Narcissus Tazetta Mediated Silver Nanoparticles. *Arab. J. Chem.* **2022**, *15*, 103652. [CrossRef]
40. Cvetanović, A.; Švarc-Gajić, J.; Zeković, Z.; Savić, S.; Vulić, J.; Mašković, P.; Ćetković, G. Comparative Analysis of Antioxidant, Antimicrobiological and Cytotoxic Activities of Native and Fermented Chamomile Ligulate Flower Extracts. *Planta* **2015**, *242*, 721–732. [CrossRef]
41. Boskovic, I.; Djukic, D.; Mandic, L.; Maskovic, P.; Govedarica-Lucic, A. Antioxidant and cytotoxic potential of selected plant species of the boraginaceae family. *AgricultForest* **2021**, *67*, 53–61. [CrossRef]
42. Almahdawy, S.S.; Said, A.M.; Abbas, I.S.; Dawood, A.H. The evaluation of antimicrobial and cytotoxic activity of the essential oil extracted from the aerial parts of southernwood herb (*Artemisia abrotanum* l.) that recently grown in iraq. *Asian J. Pharm. Clin. Res.* **2017**, *10*, 384–387. [CrossRef]
43. Doğan, Z.; Genç, Y.; Harput, Ü.; Karadeniz Pekgöz, A.; Saraçoğlu, İ. Chemical Profiling and Cytotoxic Activity of Aqueous Extract of Veronica Peduncularis M.Bieb.: A Chemotaxonomical Approach. *Istanb. J. Pharm.* **2021**, *51*, 372–377. [CrossRef]
44. Mašković, P.Z.; Diamanto, L.D.; Vujic, J.M.; Cvetanović, A.D.; Radojković, M.M.; Gadžurić, S.B.; Zengin, G. Onosma Aucheriana: A Source of Biologically Active Molecules for Novel Food Ingredients and Pharmaceuticals. *J. Funct. Foods* **2015**, *19*, 479–486. [CrossRef]
45. Aboushanab, S.A.; Shevyrin, V.A.; Slesarev, G.P.; Melekhin, V.V.; Shcheglova, A.V.; Makeev, O.G.; Kovaleva, E.G.; Kim, K.H. Antioxidant and Cytotoxic Activities of Kudzu Roots and Soy Molasses against Pediatric Tumors and Phytochemical Analysis of Isoflavones Using HPLC-DAD-ESI-HRMS. *Plants* **2022**, *11*, 741. [CrossRef]
46. Shinji, S.; Nakamura, S.; Nihashi, Y.; Umezawa, K.; Takaya, T. Berberine and Palmatine Inhibit the Growth of Human Rhabdomyosarcoma Cells. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 63–75. [CrossRef]
47. Vijayan, P.; Vinod Kumar, S.; Dhanaraj, S.A.; Mukherjee, P.K.; Suresh, B. In Vitro Cytotoxicity and Antitumour Properties of Hypericum Mysorense and Hypericum Patulum. *Phytother. Res.* **2003**, *17*, 952–956. [CrossRef] [PubMed]
48. Zalkow, L.H.; Asibal, C.F.; Glinski, J.A.; Bonetti, S.J.; Gelbaum, L.T.; VanDerveer, D.; Powis, G. Macrocyclic Pyrrolizidine Alkaloids from Senecio Anonymus. Separation of a Complex Alkaloid Extract Using Droplet Counter-Current Chromatography. *J. Nat. Prod.* **1988**, *51*, 690–702. [CrossRef]

49. Kil, Y.-S.; Risinger, A.L.; Petersen, C.L.; Liang, H.; Grkovic, T.; O'Keefe, B.R.; Mooberry, S.L.; Cichewicz, R.H. Using the Cancer Dependency Map to Identify the Mechanism of Action of a Cytotoxic Alkenyl Derivative from the Fruit of Choerospondias Axillaris. *J. Nat. Prod.* **2020**, *83*, 584–592. [CrossRef]
50. Phan, N.H.T.; Thuan, N.T.D.; Hien, N.T.T.; Huyen, P.V.; Hanh, T.T.H.; Vien, L.T.; Hong Quang, T.; Cuong, N.X.; Nam, N.H.; Van Kiem, P.; et al. Limonoids from Choerospondias Axillaris. *Nat. Prod. Commun.* **2020**, *15*, 1934578X20948368. [CrossRef]
51. Ashraf, A.; Fatima, N.; Shahzadi, I.; Tariq, H.; Shahzadi, A.; Yameen, M.A.; Iqbal, J.; Rafi, M. Datura Suaveolens and Verbena Tenuisecta Mediated Silver Nanoparticles, Their Photodynamic Cytotoxic and Antimicrobial Evaluation. *World J. Microbiol. Biotechnol.* **2020**, *36*, 31. [CrossRef]
52. Cai, S.; Risinger, A.L.; Petersen, C.L.; Grkovic, T.; O'Keefe, B.R.; Mooberry, S.L.; Cichewicz, R.H. Anacolosins A-F and Corymbulosins X and Y, Clerodane Diterpenes from Anacolosa Clarkii Exhibiting Cytotoxicity toward Pediatric Cancer Cell Lines. *J. Nat. Prod.* **2019**, *82*, 928–936. [CrossRef] [PubMed]
53. Abiodun, O.O.; Nnoruka, M.E.; Tijani, R.O. Phytochemical Constituents, Antioxidant Activity, and Toxicity Assessment of the Seed of *Spondias mombin* L. (Anacardiaceae). *Turk. J. Pharm. Sci.* **2020**, *17*, 343–348. [CrossRef] [PubMed]
54. Essien, S.O.; Young, B.; Baroutian, S. The Antibacterial and Antiproliferative Ability of Kānuka, Kunzea Ericoides, Leaf Extracts Obtained by Subcritical Water Extraction. *J. Chem. Technol. Biotechnol.* **2021**, *96*, 1308–1315. [CrossRef]
55. Renehan, A.G.; Booth, C.; Potten, C.S. What Is Apoptosis, and Why Is It Important? *BMJ* **2001**, *322*, 1536–1538. [CrossRef] [PubMed]
56. Jiang, M.; Qi, L.; Li, L.; Li, Y. The Caspase-3/GSDME Signal Pathway as a Switch between Apoptosis and Pyroptosis in Cancer. *Cell Death Discov.* **2020**, *6*, 112. [CrossRef] [PubMed]
57. Mesas, C.; Fuel, M.; Martínez, R.; Prados, J.; Melguizo, C.; Porres, J.M. In Vitro Evidence of the Antitumor Capacity of Solanaceae and Cucurbitaceae in Colon Cancer: A Systematic Review. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 6293–6314. [CrossRef]
58. Balach, T.; Stacy, G.S.; Haydon, R.C. The Clinical Evaluation of Soft Tissue Tumors. *Radiol. Clin. N. Am.* **2011**, *49*, 1185–1196. [CrossRef]
59. Ilaslan, H.; Schils, J.; Nageotte, W.; Lietman, S.A.; Sundaram, M. Clinical Presentation and Imaging of Bone and Soft-Tissue Sarcomas. *Cleve Clin. J. Med.* **2010**, *77*, S2-7. [CrossRef]
60. Mesas, C.; Quiñonero, F.; Doello, K.; Revueltas, J.L.; Perazzoli, G.; Cabeza, L.; Prados, J.; Melguizo, C. Active Biomolecules from Vegetable Extracts with Antitumoral Activity against Pancreas Cancer: A Systematic Review (2011–2021). *Life* **2022**, *12*, 1765. [CrossRef]
61. Salih, A.M.; Al-Qurainy, F.; Nadeem, M.; Tarroum, M.; Khan, S.; Shaikhaldein, H.O.; Al-Hashimi, A.; Alfagham, A.; Alkahtani, J. Optimization Method for Phenolic Compounds Extraction from Medicinal Plant (Juniperus Procera) and Phytochemicals Screening. *Molecules* **2021**, *26*, 7454. [CrossRef]
62. Cabeza, L.; Peña, M.; Martínez, R.; Mesas, C.; Galisteo, M.; Perazzoli, G.; Prados, J.; Porres, J.M.; Melguizo, C. Anemonia Sulcata and Its Symbiont Symbiodinium as a Source of Anti-Tumor and Anti-Oxoxidant Compounds for Colon Cancer Therapy: A Preliminary In Vitro Study. *Biology* **2021**, *10*, 134. [CrossRef]
63. Ogidi, O.C.; Oyetayo, V.O.; Akinyele, B.J.; Ogbole, O.O.; Adeniji, J.A.; Oluremi, B.B. Molecular Identity and Cytotoxicity of *Lenzites quercina* Macrofungus Extracts toward Cancer Cell Lines. *BioTechnologia* **2017**, *98*, 25–32. [CrossRef]
64. Mikhailova, E.O. Silver Nanoparticles: Mechanism of Action and Probable Bio-Application. *J. Funct. Biomater.* **2020**, *11*, 84. [CrossRef] [PubMed]
65. Altammar, K.A. A Review on Nanoparticles: Characteristics, Synthesis, Applications, and Challenges. *Front. Microbiol.* **2023**, *14*, 1155622. [CrossRef] [PubMed]
66. Hussain, I.; Singh, N.B.; Singh, A.; Singh, H.; Singh, S.C. Green Synthesis of Nanoparticles and Its Potential Application. *Biotechnol. Lett.* **2016**, *38*, 545–560. [CrossRef] [PubMed]
67. Carrillo-González, R.; Martínez-Gómez, M.A.; González-Chávez, M.D.C.A.; Mendoza Hernández, J.C. Inhibition of Microorganisms Involved in Deterioration of an Archaeological Site by Silver Nanoparticles Produced by a Green Synthesis Method. *Sci Total Environ.* **2016**, *565*, 872–881. [CrossRef]
68. Krobthong, S.; Yingchutrakul, Y.; Sittisaree, W.; Tulyananda, T.; Samutrtai, P.; Choowongkomon, K.; Lao-On, U. Evaluation of Potential Anti-Metastatic and Antioxidative Abilities of Natural Peptides Derived from *Tecoma stans* (L.) Juss. Ex Kunth in A549 Cells. *PeerJ* **2022**, *10*, e13693. [CrossRef]
69. Lavudi, K.; Harika, G.V.S.; Thirunavukarasou, A. Green Synthesis of Tecoma Stans Flower and Leaf Extracts: Characterization and Anti-Proliferative Activity in Colorectal Cancer Cell Lines. *Lett. Appl. NanoBioSci.* **2022**, *12*, 61. [CrossRef]
70. Naaz, R.; Siddiqui, V.U.; Ahmad, A.; Qadir, S.U.; Siddiqi, W.A. Study of Antibacterial and Antioxidant Activities of Silver Nanoparticles Synthesized from Tradescantia Pallida (Purpurea) Leaves Extract. *J. Dispers. Sci. Technol.* **2023**, 1–11. [CrossRef]
71. Kadhim, Z.A.; Sulaiman, G.M.; Al-Shammari, A.M.; Khan, R.A.; Al Rugaie, O.; Mohammed, H.A. Oncolytic Newcastle Disease Virus Co-Delivered with Modified PLGA Nanoparticles Encapsulating Temozolomide against Glioblastoma Cells: Developing an Effective Treatment Strategy. *Molecules* **2022**, *27*, 5757. [CrossRef] [PubMed]
72. El-Huneidi, W.; Bajbouj, K.; Muhammad, J.S.; Vinod, A.; Shafarin, J.; Khoder, G.; Saleh, M.A.; Taneera, J.; Abu-Gharbieh, E. Carnosic Acid Induces Apoptosis and Inhibits Akt/mTOR Signaling in Human Gastric Cancer Cell Lines. *Pharmaceuticals* **2021**, *14*, 230. [CrossRef] [PubMed]

73. Wang, F.; Wang, L.; Qu, C.; Chen, L.; Geng, Y.; Cheng, C.; Yu, S.; Wang, D.; Yang, L.; Meng, Z.; et al. Kaempferol Induces ROS-Dependent Apoptosis in Pancreatic Cancer Cells via TGM2-Mediated Akt/mTOR Signaling. *BMC Cancer* **2021**, *21*, 396. [CrossRef]
74. Zhu, L.; Xue, L. Kaempferol Suppresses Proliferation and Induces Cell Cycle Arrest, Apoptosis, and DNA Damage in Breast Cancer Cells. *Oncol. Res.* **2019**, *27*, 629–634. [CrossRef] [PubMed]
75. Wang, R.; Deng, Z.; Zhu, Z.; Wang, J.; Yang, X.; Xu, M.; Wang, X.; Tang, Q.; Zhou, Q.; Wan, X.; et al. Kaempferol Promotes Non-Small Cell Lung Cancer Cell Autophagy via Restricting Met Pathway. *Phytomedicine* **2023**, *121*, 155090. [CrossRef]
76. Lagariya, L.; Soni, K.; Shah, J.S. Antitumor Effects of Polyphenol-Rich Extract of Euphoria Longana Seed by Vascular Endothelial Growth Factor and Transforming Growth Factor-Beta Signaling Inhibition in Experimentally Induced Oral Cancer in Rats. *Indian J. Pharmacol.* **2022**, *54*, 329–337. [CrossRef]
77. Narayanan, N.K.; Kunimasa, K.; Yamori, Y.; Mori, M.; Mori, H.; Nakamura, K.; Miller, G.; Manne, U.; Tiwari, A.K.; Narayanan, B. Antitumor Activity of Melinjo (*Gnetum gnemon* L.) Seed Extract in Human and Murine Tumor Models in Vitro and in a Colon-26 Tumor-Bearing Mouse Model In Vivo. *Cancer Med.* **2015**, *4*, 1767–1780. [CrossRef]
78. Gupta, M.; Mazumder, U.K.; Rath, N.; Mukhopadhyay, D.K. Antitumor Activity of Methanolic Extract of *Cassia fistula* L. Seed against Ehrlich Ascites Carcinoma. *J. Ethnopharmacol.* **2000**, *72*, 151–156. [CrossRef]