Natural Compounds in Glioblastoma Therapy

Subjects: Oncology | Neurosciences
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Glioblastoma (GBM) is a tumor of the brain or spinal cord with poor clinical prognosis. Current interventions, such as chemotherapy and surgical tumor resection, are constrained by tumor invasion and cancer drug resistance. Dietary natural substances are therefore evaluated for their potential as agents in GBM treatment. Various substances found in fruits, vegetables, and other natural products restrict tumor growth and induce GBM cell death. These preclinical effects are promising but remain constrained by natural substances' varying pharmacological properties. While many of the reviewed substances are available as over-the-counter supplements, their anti-GBM efficacy should be corroborated by clinical trials moving forward.

Keywords: glioblastoma ; brain cancer ; natural compounds ; flavonoids ; polyphenols ; carotenoids ; lignans ; coumarins ; steroids ; tannins ; terpenes ; lifestyle medicine

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Glioblastoma (GBM) is an aggressive, often fatal astrocyte-derived tumor of the central nervous system. Conventional medical and surgical interventions have greatly improved survival rates; however, tumor heterogeneity, invasiveness, and chemotherapeutic resistance continue to pose clinical challenges. As such, dietary natural substances—an integral component of the lifestyle medicine approach to chronic diseases—are examined as potential chemotherapeutic agents. These heterogenous substances exert anti-GBM effects by upregulating apoptosis and autophagy, inducing cell cycle arrest, interfering with tumor metabolism, and inhibiting proliferation, neuroinflammation, chemoresistance, angiogenesis, and metastasis. Although these beneficial effects are promising, natural substances' efficacy in GBM is constrained by their bioavailability and blood–brain barrier permeability; various chemical formulations are proposed to improve their pharmacological properties. Many of the reviewed substances are available as over-the-counter dietary supplements, underscoring their viability as lifestyle interventions. However, clinical trials remain necessary to substantiate the in vitro and in vivo properties of natural substances.

1. Glioblastoma: Occurrence, Mechanisms, Treatments, and Challenges

Glioblastoma (GBM) is a malignant tumor of the central nervous system (brain or spinal cord) that arises from astrocytes. It is the most common type of primary brain tumor, with occurrence rates of 3.19 cases per 100,000 patients in the United States, and 2.05 per 100,000 in the United Kingdom [1]. While the prognosis of GBM is often poor, two-year survival rates have improved in recent years, rising from 7% for cases diagnosed from 1993–1995 to 17% for cases diagnosed from 2005–2007 in the USA. Survival rates are also age-related: 39% of patients diagnosed between ages 20 and 44 survive, compared to only 1% of those diagnosed past age 80 [2].

While the efficacy of GBM treatment has improved, numerous challenges remain—especially concerning conventional therapeutic modalities. For instance, surgical tumor resection improves survival rates but is hindered by the extensive invasion and ill-defined tumor boundaries of GBM [3][4]. The efficacy of chemotherapeutic drugs may be reduced by the development of (multi-)drug resistance [5]. Moreover, extracranial metastasis—though rare—can greatly complicate treatment [6].

The challenges posed by GBM stem mainly from the genetic and molecular signaling pathways through which this type of tumor occurs. Genetic alterations in GBM include the amplification of the epidermal growth factor receptor (EGFR) and cyclin-dependent kinase (e.g., CDK4) genes, the deletion of the genes for cyclin-dependent kinase inhibitors (e.g., CDK2NA), and the silencing of the O-6-methylguanine-DNA methyltransferase (MGMT) gene [7]. These and other genetic changes upregulate cellular mechanisms that favor proliferation (e.g., through Akt/mTOR signaling), cell cycle progression, excessive and self-perpetuating inflammation, tumor metastasis, angiogenesis, metabolic changes (known as the Warburg effect), and chemoresistance. Simultaneously, the effectors of apoptosis and autophagy are largely downregulated or inhibited (Figure 1). As such, conventional oncologic therapies mostly aim to reverse this imbalance between growth and death by inhibiting proliferation and upregulating apoptosis.

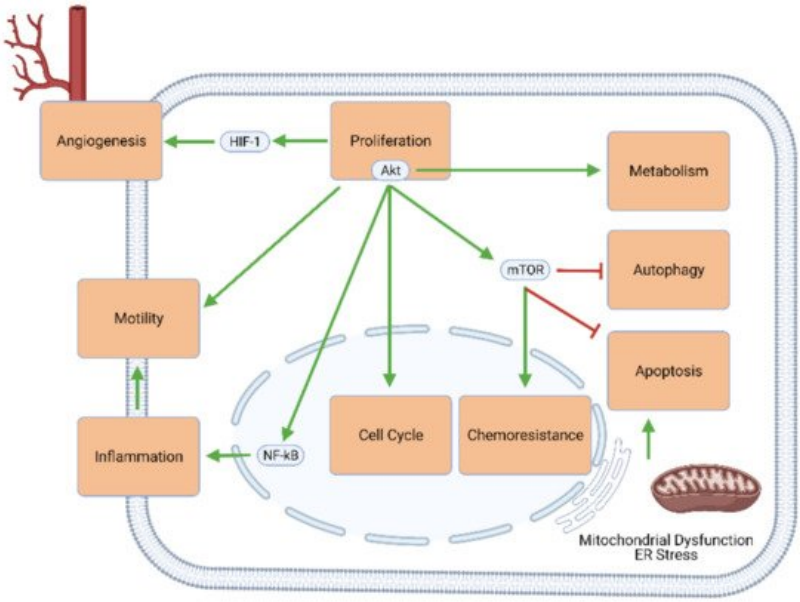

Figure 1. Intracellular signaling mechanisms involved in GBM development and progression. Elements of proliferative signaling pathways—especially Akt and mTOR—promote angiogenesis, motility and migratory potential, neuroinflammation, cell cycle progression, chemoresistance, and tumor metabolism, and concurrently inhibit GBM cell death through apoptosis and autophagy.

The molecular complexity and difficulties posed by chronic diseases such as brain cancers have encouraged some clinicians to take a holistic approach to their treatment. Lifestyle medicine focuses on lifestyle factors (e.g., diet, physical activity, and the environment) and overall health maintenance to minimize risk factors associated with chronic diseases [8]. Dietary natural substances are an essential component of lifestyle medicine and can suppress cancer or overcome challenges associated with conventional therapies. Intake of these compounds may occur through the daily diet or over-the-counter supplements. While in vitro studies are promising, they are yet to be tangibly replicated in clinical trials.

2. Natural Compounds Modulating Glioblastoma

Numerous natural substances—with established biological benefits—exert oncologic effects on GBM in vitro and/or in vivo. These include alkaloids, carboxylic acid derivatives, carotenoids, flavonoids, coumarins, curcuminoids, terpenes, lignans, natural steroids, tannins, and plant extracts (Figure 2 and Figure 3; Table 1).

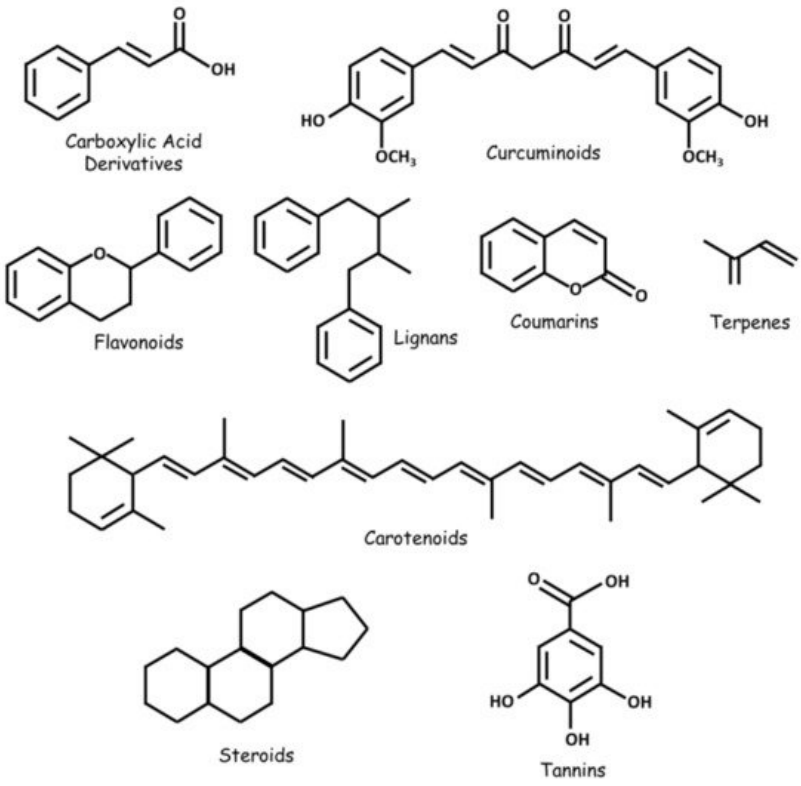

Figure 2. Some classes of natural substances with therapeutic potential in GBM.

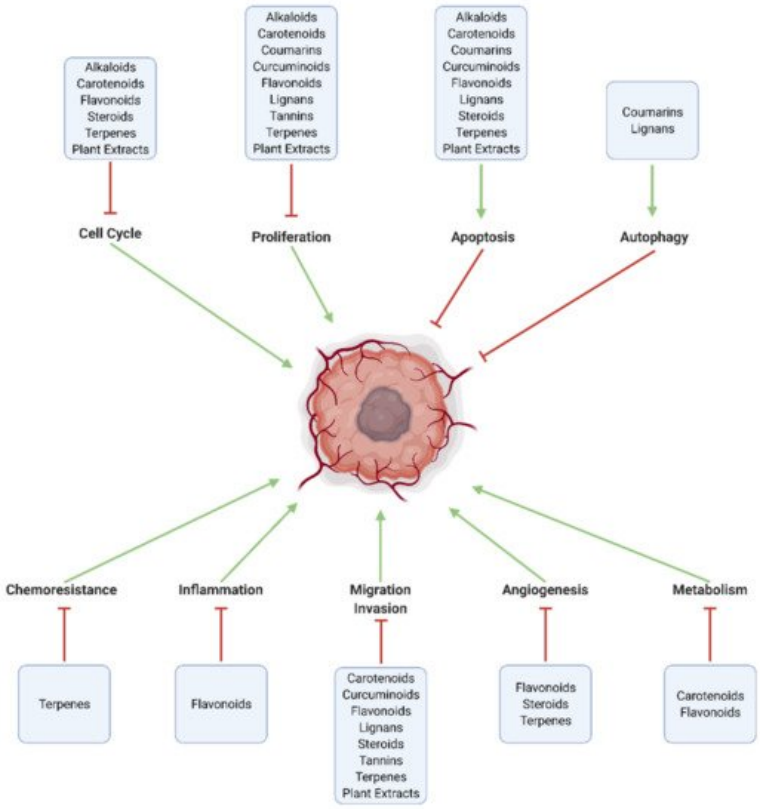

Figure 3. Major pathways modulated by natural substances in GBM. Effective chemotherapeutic substances increase cell death through apoptosis and autophagy, and inhibit intracellular mechanisms related to proliferation, cell cycle progression, tumor metabolism (Warburg effect), angiogenesis, invasion and metastasis, neuroinflammation, and chemoresistance.

Table 1. Classes and sources of natural substances with anti-GBM efficacy demonstrated in recent preclinical studies. Many of the listed compounds occur in multiple natural sources.

Substance	Class/Type	Primary Source(s)
Alkaloids		
Berberine	**Quaternary Ammonium Salt**	**Barberry (*Berberis*)**
Carboxylic Acid Derivatives		
Cinnamic Acid	**Monocarboxylic Acid**	**Cinnamon (*Cinnamomum*)**
Ferulic Acid	**Hydroxycinnamic Acid**	**Giant fennel (*Ferula communis*)**
Carotenoids		
Adonixanthin	**Carotenone**	**Derivative of astaxanthin**
Astaxanthin	**Xanthophyll**	**Chlorophyte (*Haematococcus pluvialis*)**
Crocetin	**Apocarotenoid**	**Saffron (*Crocus sativus*)**
Coumarins		
Galbanic Acid	**Sesquiterpene Coumarin**	**Celery/carrot/parsley family (*Umbelliferae*)**
Osthole	**Coumarin**	**Monnier's snowparsley (*Cnidium monnieri)***
Curcuminoids		
Curcumin	**Curcumin**	**Turmeric (*Curcuma longa*)**
Flavonoids		
Chrysin	**Dihydroxyflavone**	**Blue passion flower (*Passiflora caerulea*)**
Diosmin	**Flavone Glycoside**	**Germander (*Teucrium gnaphalodes*)**
EGCG	**Catechin**	**Green tea (*Camellia sinensis*)**

Substance	Class/Type	Primary Source(s)
Galangin	Trihydroxyflavone	Galangal (*Alpinia officinarum*)
Matteucinol	Dihydroxyflavonone	Naudin (*Miconia chamissois*)
Naringin	Flavanone Glycoside	Grapefruit (*Citrus × paradisi*)
Quercetin	Flavonol	Oak (*Quercetus*)
Resveratrol	Stilbenoid	Grape (*Vitis*)
Rutin	Flavonol Glycoside	Rue (*Ruta graveolens*)
Silymarin (Silibinin)	Flavonolignan	Milk thistle (*Silybum marianum*)
Tectorigenin	Methylated Isoflavone	Leopard lily (*Iris domestica*)
Xanthohumol	Prenylated Chalconoid	Hops (*Humulus lupulus*)
Lignans		
Arctigenin	Lignan/Polyphenol	Greater burdock (*Arctium lappa*)
Magnolol	Biphenyl	Houpu magnolia (*Magnolia officinalis*)
Steroids		
Diosgenin	Phytosteroid Sapogenin	Fenugreek (*Trigonella foenum-graecum*)
Gamabufotalin	Steroidal Lactone	Toad (*Bufo*)
N45	Steroidal Saponin	Nan chong lou (*Paris vietnamensis*)
Withaferin A	Steroidal Lactone	Ashwa-gandha (*Withania somnifera*)
Tannins		
Tannic Acid	Hydrolysable Tannin	Oak (*Quercetus*)
Terpenes		
AM01-06	Sesquiterpene Lactone	Sunflower (*Eremanthus* spp.)
Betulinic Acid	Triterpenoid	White birch (*Betula pubescens*)
Cedrol	Sesquiterpene Alcohol	Cypress (*Cupressus*); Juniper (*Juniperus*)
Coronarin D	Diterpene	White ginger lily (*Hedychium coronarium*)
Eucalyptal A	Monoterpenoid	Southern blue gum (*Eucalyptus globulus*)
Gossypol	Terpenoid Aldehyde	Cotton (*Gossypium*)
Paeoniflorin	Terpene Glycoside	Chinese peony (*Paeonia lactiflora*)
Paris saponin H	Triterpenoid Saponin	Chong Lou (*Rhizoma paridis*)
Pisosterol	Triterpene	Dead man’s foot (*Pisolithus tinctorius*)
Rupesin E	Iridoid (Monoterpenoid)	Indian valerian (*Valeriana jatamansi*)
Tubeimoside-1	Triterpenoid Saponin	Tu bei mu (*Rhizoma bolbostemmae*)
Crude/Purified Plant Extracts		
BcH, BcS	Extract-Food Supplement	Water hyssop (*Bacopa monnieri*)
CE70, CE95	Ethanol Extract	Shaggy ink cap (*Coprinus comatus*)
CP	Chloroform Partition	Johnnyberry (*Miconia chamissois*)
CW	Aqueous Extract	Shaggy ink cap (*Coprinus comatus*)
KE70, KE95	Ethanol Extract	Golden chanterelle (*Cantherellus cibarius*)
KW	Aqueous Extract	Golden chanterelle (*Cantherellus cibarius*)

Substance	Class/Type	Primary Source(s)
PE70, PE95	**Ethanol Extract**	**Puffball (*Lycoperdon perlatum*)**
PPE	**Ethanol Extract**	**Polish propolis (bee glue)**
PW	**Aqueous Extract**	**Puffball (*Lycoperdon perlatum*)**
RE70, RE95	**Ethanol Extract**	**Saffron milk cap (*Lactarius delicious*)**
RW	**Aqueous Extract**	**Saffron milk cap (*Lactarius delicious*)**
Other		
Carnosine	**Dipeptide**	**Liebig's meat extract**
CrataBL	**Protein: Lectin + Serine Protease Inhibitor**	**Beach block (*Crataeva tapia*)**
GL-PP	**Polysaccharide Peptide**	Lingzhi (*Ganoderma lucidum*)

3. Mechanistic Effects of Natural Compounds on Glioblastoma

3.1. Generalized Anti-Cancer Markers

Several generalizable effects can demonstrate the anti-GBM potential of natural compounds and highlight promising substances for further mechanistic studies (Table 2). Nearly all the substances discussed in this review decrease GBM cell viability in vitro. Cell viability assays are useful in (1) differentiating cytotoxic from biologically inert compounds and (2) identifying effective treatment concentrations to be used in further experiments. For example, decreased intracellular ATP is a marker of cell death; this effect was observed in GBM cells after treatment with curcumin, BBR, gossypol, and carnosine [9][10][11]. Several other substances, including xanthohumol and rupesin E, decreased cloning and colony formation—further indicators of cancer cell viability and malignancy—in GBM cultures.

Table 2. Generalized downstream effects of natural compounds on GBM. Many of the reviewed substances exert measurable cytotoxic effects in vitro. Moreover, several substances reduce tumor size and improve survival in-animal models of GBM.

Effect	Substance	Cell Line	Source
Increases survival	**Eucalyptal A**	**U87MG orthotopic implants, nude mice**	[12]
	Cedrol	**DBTRG-05MG subcutaneous xenografts, nude mice**	[13]
	Crocetin	**Luc-U251MG orthotopic implants, CD1 mice**	[14]
Decreases tumor area/perimeter	**Astaxanthin**	**GL261 orthotopic implants, C57BL/6J mice**	[15]
	Adonixanthin	**GL261 orthotopic implants, C57BL/6J mice**	[15]
	McC1	**U251 heterotopic xenograft, fertilized chicken eggs**	[16]
Decreases tumor volume	**Astaxanthin**	**GL261 orthotopic implants, C57BL/6J mice**	[15]
	Adonixanthin	**GL261 orthotopic implants, C57BL/6J mice**	[15]
	Naringin	**U87 subcutaneous xenograft, athymic mice**	[17]
	Xanthohumol	**U87, LN229**	[18]
	Tannic Acid	**C6 orthotopic implants, Wistar rats**	[19]
	Withaferin A	**U87 subcutaneous xenografts, nude mice**	[20]
	TBMS1	**U87 subcutaneous xenografts, NOD/SCID mice**	[21]
Decreases tumor weight	**Xanthohumol**	**U87, LN229**	[18]
	TBMS1	**U87 subcutaneous xenografts, nude mice**	[21]

Effect	Substance	Cell Line	Source
Increases cell death/dec. viability	EGCG	U251, MO59J	[22]
	Cinnamic Acid	LN-229	[23]
	Ferulic Acid	LN-229	[23]
	Astaxanthin	GL261, U251MG	[15]
	Adonixanthin	GL261, U251MG	[15]
	Cedrol	DBTRG-05MG, RG2	[13]
	AM02	U87MG, T98G	[24]
	AM04	U87MG, T98G	[24]
	AM05	U87MG, T98G	[24]
	AM06	U87MG, T98G	[24]
	Naringin	U87	[17]
	Xanthohumol	U87, T98G, LN229	[18]
	Rupesin E	GSC-3#, GSC-12#, GSC-18#	[25]
	Diosmin	U87, GBM02, GBM95	[26]
	Coronarin D	U251	[27]
	CP	GAMG, U251	[16]
	McC1	GAMG, U251	[16]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Tannic Acid	C6	[19]
	Withaferin A	U87, U251	[20]
	Betulinic Acid	U251, LN229	[28]
	TBMS1	U87, LN229	[21]
	Carnosine	U87, T98G	[11]
	CrataBL	U87	[29]
	Tectorigenin	GBM-8401, GBM-8901	[30]
	Resveratrol	U87	[31]
	Quercetin	U87	[31]
	Curcumin	U87	[32]
	Paeoniflorin	U251, T98G	[33]
	Diosgenin	C6, T98G	[34]
	CW	LN-18	[35]
	CE70	U87, LN-18	[35]
	CE95	U87, LN-18	[35]
	KW	U87, LN-18	[35]
	KE70	U87, LN-18	[35]
	KE95	U87, LN-18	[35]
	RW	U87, LN-18	[35]

Effect	Substance	Cell Line	Source
Decreases colony formation	RE70	U87, LN-18	[35]
	RE95	U87, LN-18	[35]
	PW	U87, LN-18	[35]
	PE70	U87, LN-18	[35]
	PE95	U87, LN-18	[35]
	Silymarin	U118	[36]
	BcS	U87, T98G, LN-18	[37]
	BcH	U87, T98G, LN-19	[37]
	BBR	U87	[38]
	GL-PP	U251	[39]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
	Xanthohumol	U87, T98G, LN229	[18]
	Rupesin E	GSC-3#, GSC-18#	[25]
	CP	GAMG, U251	[16]
	McC1	U251, GAMG	[16]
	Tannic Acid	C6	[19]
Decreases cloning	Arctigenin	U87MG, T98G	[41]
	AM01	U87MG, T98G	[24]
	AM02	U87MG, T98G	[24]
	AM03	U87MG, T98G	[24]
	AM04	U87MG, T98G	[24]
	AM05	U87MG, T98G	[24]
	AM06	U87MG, T98G	[24]
Decreases sphere formation	TBMS1	U87, LN229	[21]
	Gossypol	TS13-20, TS13-18	[10]
Decreases intracellular ATP	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Gossypol	Diff13-20	[10]
Upregulates p53 (mRNA)	Carnosine	U87, T98G	[11]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Upregulates p53 (protein)	BBR	U87, U251	[9]
	SLCP	U251	[9]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]

The effects of some natural substances on GBM cells in culture are replicable in vivo, underscoring their therapeutic potential. Specific terpenes, carotenoids, flavonoids, and steroids inhibit tumor growth (measured through tumor area, perimeter, weight, and volume) in murine and rat xenograft models. Interestingly, the flavonoid matteucinol also reduces the area of GBM implants in fertilized chicken eggs. These effects may improve survival rates and times in tumor-bearing animals, as is the case for eucalyptal A, cedrol, and crocetin (see Table 2).

3.2. Proliferation, Apoptosis, and Autophagy

Cell fate is regulated by a delicate balance between proliferation and death. In GBM and other tumor cells, growth factors, chemokine ligands, and other upstream signals mediate a shift towards excessive growth and proliferation (Figure 4; Table 3) [42]. Growth factors, including tumor growth factor βeta (TGF-β), insulin-like growth factor (IGF), hepatoma-derived growth factor (HDGF), and glial cell-derived neurotrophic factor (GDNF), are upregulated in GBM and contribute to downstream Ras/Raf/MEK/ERK and PI3K/Akt signaling. The upregulated chemokine (C-C motif) ligands 2 (CCL2) and 5 (CCL5) further contribute to the PI3K/Akt pathway. However, the flavonoids rutin and quercetin downregulate these proliferative signals in vitro and in vivo [43]. In the absence of natural inhibitory substances, the described growth factors and ligands bind to cell membrane receptors and activate Ras-GTP to begin the proliferative Ras/Raf/MEK/ERK pathway. In the first step, Ras-GTP activates Raf (a third degree MAPK, or MAP3K). Raf consequently activates the MAPK/ERK kinase (MEK; a second degree MAPK, or MAP2K)—an enzyme also activated by the MET proto-oncogene. MEK activates extracellular signal-regulated kinases (ERK) and their associated MAPKs in the third mechanistic step. Finally, ERK MAPKs upregulate hypoxia-inducible factor 1 αlpha (HIF-1α), whose downstream target is the epidermal growth factor receptor (EGFR). Osthole, a coumarin, may inhibit MEK activation in the second step through the downregulation of Raf [44]. TBMS1 may have a similar inhibitory function, as it downregulates MET [21]. Moreover, TBMS1, astaxanthin, and adonixanthin downregulate ERK/p-ERK to inhibit the final step of HIF-1α upregulation [15][21].

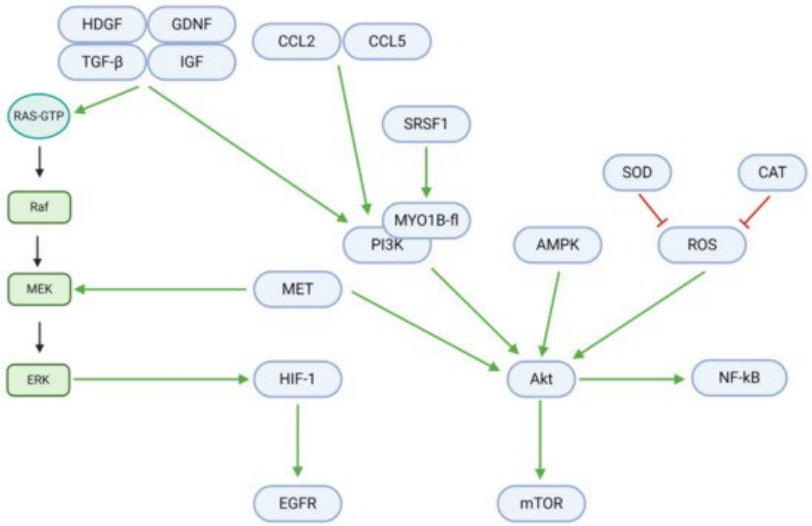

Figure 4. Intracellular mechanisms promoting proliferation in GBM. Growth factors, chemokine ligands, and other upstream signals upregulate the Ras/Raf/MEK/ERK and PI3K/Akt pathways. Downstream effectors, including HIF-1, EGFR, NF-κB, and mTOR, promote DNA synthesis, transcription, and tumor cell proliferation. Proliferative effectors notably engage in crosstalk with other signals in GBM, including those for angiogenesis (HIF-1), cell cycle progression (Akt), metabolism (Akt), motility (PI3K), apoptosis (Akt/mTOR), and autophagy (Akt/mTOR/Beclin-1).

Table 3. Natural substances decrease proliferation in GBM by downregulating upstream growth factors and chemokine ligands, components of the Ras/Raf/MEK/ERK and PI3K/Akt pathways, and downstream effectors.

Effect	Substance	Cell Line	Source
Decreases proliferation/growth	Rutin	C6	[43]
	Quercetin	C6	[43]
	Eucalyptal A	U87MG, LN229	[12]
	Rupesin E	GSC-3#, GSC-18#	[25]
	Crocetin	U87, U138, U251, U373	[14]
	Coronarin D	U251	[27]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Tannic Acid	C6	[19]
	Gossypol	Diff13-20, Diff13-18	[10]
	Betulinic Acid	U251, LN229	[28]
	CrataBL	U87	[29]
	Galbanic Acid	U87	[45]
	N45	U87	[46]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Decreases DNA synthesis	CE95	U87	[35]
	CE70	U87, LN-18	[35]
	KW	U87, LN-18	[35]
	KE95	U87, LN-18	[35]
	KE70	U87, LN-18	[35]
	PW	U87	[35]
	PE70	U87	[35]
	RW	U87, LN-18	[35]
	PPE	U87, T98G, LN-18	[37]
	BcH	U87, T98G, LN-18	[37]
Downregulates SRSF1 (mRNA)	Eucalyptal A	U87MG, LN229	[12]
Downregulates SRSF1 (protein)	Eucalyptal A	U87MG, LN229	[12]
Downregulates MYO1B-fl (protein)	Eucalyptal A	U87MG, LN229	[12]
Downregulates p-PDK1 (protein)	Eucalyptal A	U87MG, LN229	[12]
Downregulates TGF (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Downregulates TGF-β (mRNA)	Rutin	C6	[43]
	Quercetin	C6	[43]
Downregulates IGF (mRNA)	Rutin	C6, WR-U251 orthotopic implants	[43]
	Quercetin	C6, WR-U251 orthotopic implants	[43]
Downregulates CCL2 (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]

Effect	Substance	Cell Line	Source
Upregulates CCL5 (mRNA)	Rutin	C6, WR-U251 orthotopic implants	[43]
	Quercetin	C6, WR-U251 orthotopic implants	[43]
Downregulates HDGF (mRNA)	Rutin	C6, WR-U251 orthotopic implants	[43]
	Quercetin	C6, WR-U251 orthotopic implants	[43]
Downregulates GDNF (mRNA)	Rutin	C6, WR-U251 orthotopic implants	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Downregulates PI3K (protein)	SLCP	U87	[9]
	BBR	U87	[9]
	Diosgenin	C6	[34]
Downregulates (p-)PI3K (protein)	Osthole	MOGGCCM, T98	[44]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
Upregulates AMPK (protein)	Metformin	U87	[47]
Downregulates Akt (mRNA)	Arctigenin	U87MG	[41]
Downregulates Akt (protein)	Cedrol	RG2	[13]
	Metformin	U87, U251	[48]
	SLCP	U87	[9]
	BBR	U87	[9]
Downregulates p-Akt (mRNA)	Arctigenin	U87MG, T98G	[41]
Downregulates p-Akt (protein)	Eucalyptal A	U87MG, LN229	[12]
	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
	Cedrol	DBTRG-05MG, RG2	[13]
	Arctigenin	U87MG, T98G	[41]
	Xanthohumol	U87	[18]
	CP	GAMG	[16]
	McC1	GAMG, U251	[16]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Diosgenin	C6	[34]
Downregulates mTOR (protein)	Metformin	U87	[47]
	SLCP	U87	[9]
	BBR	U87, U251	[9]
Downregulates p-mTOR (mRNA)	Arctigenin	U87MG, T98G	[41]
Downregulates p-mTOR (protein)	Arctigenin	U87MG, T98G	[41]
	SLCP	U87	[9]
	BBR	U87, U251	[9]
	Diosgenin	T98G	[34]

Effect	Substance	Cell Line	Source
Downregulates Raf (protein)	Osthole	MOGGCCM, T98	[44]
Downregulates c-Myc	Eucalyptal A	U87MG, LN229	[12]
	Xanthohumol	U87, T98G, LN229	[18]
	SLCP	U87	[9]
	BBR	U87	[9]
Downregulates ROS	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
	Tannic Acid	C6	[19]
Upregulates CAT activity	Tannic Acid	C6	[19]
	BBR	U87	[38]
Upregulates SOD activity	Tannic Acid	C6	[19]
	BBR	U87	[38]
Downregulates JNK (protein)	Cedrol	DBTRG-05MG, RG2	[13]
Downregulates p-JNK (protein)	Cedrol	RG2	[13]
Downregulates p-MEK (protein)	TBMS1	U87, LN229	[21]
Downregulates p-ERK (protein)	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
	TBMS1	LN229	[21]
Downregulates p38 (protein)	Diosgenin	T98G	[34]
Upregulates p-p38 MAPK (protein)	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
Downregulates HIF-1α activity	Metformin	U251	[48]
Downregulates NF-κB	Diosgenin	C6, T98G	[34]
Downregulates MET (protein)	TBMS1	U87, LN229	[21]

In addition to the Ras-GTP pathway, proliferation is also critically induced through Akt/mTOR and NF-κB signaling. Upstream of these targets, serine/arginine-rich splicing factor 1 (SRSF1) activates myosin 1B (MYO1B), which in turn upregulates the phosphoinositide-3-kinase (PI3K). PI3K, along with MET, adenosine monophosphate-activated protein kinase (AMPK), and reactive oxygen species (ROS), upregulates Akt, a central mediator of tumor cell proliferation. This step may be hindered by TBMS1, as it downregulates MET. Superoxide dismutase (SOD) and catalase (CAT) downregulate ROS levels and could therefore also inhibit Akt activation when upregulated by tannic acid and berberine [19][38]. Finally, Akt activity can be reduced through the downregulation of PI3K. Eucalyptal A downregulates PI3K by inhibiting SRSF1 and MYO1B, while curcumin, osthole, diosgenin, and berberine downregulate PI3K directly [9][12][34][44].

Downstream, Akt upregulates the mammalian target of rapamycin (mTOR) and nuclear factor κappa of activated B cells (NF-κB), which induce proliferation. However, arctigenin, curcumin, diosgenin, and berberine downregulate (p-)mTOR, while diosgenin downregulates NF-κB [9][34][41]. Moreover, galbanic acid exerts antiproliferative, anti-metastatic, and pro-apoptotic effects via PI3K/Akt/mTOR signaling, while N45, a natural steroidal saponin, upregulates apoptosis through ROS/PI3K/Akt signaling in TMZ-resistant GBM cells [45][46].

Many natural substances' anti-cancer properties arise from the activation of cell death through apoptosis and/or autophagy. Endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and downstream caspase activity mediate the apoptotic death of GBM cells (Figure 5, Table 4). Withaferin A and EGCG upregulate activating transcription factor 4 (ATF4), an upstream effector of ER stress [20][22]. ATF4 targets ATF3, which consequently activates the C-homologous

protein (CHOP). CHOP, which is also activated by withaferin A, upregulates p21 and the apoptotic proteins Bad and Bim [20].

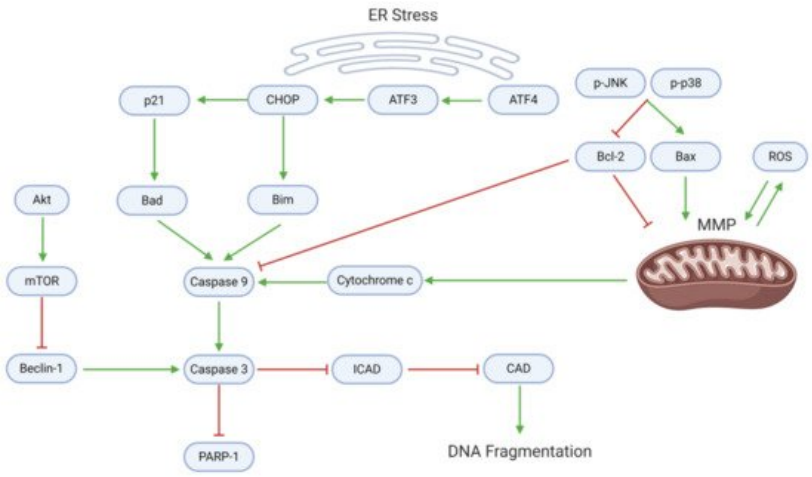

Figure 5. Proapoptotic mechanisms, which involve mitochondrial dysfunction, ER stress, and caspase activation, are suppressed in GBM. Dysregulation of mitochondrial homeostasis (often through oxidative imbalance) leads to the release of cytochrome c, a caspase activator. ER stress upregulates activating transcription factors; in turn, ATFs activate CHOP, p21, and proapoptotic proteins that enhance caspase activation. Active caspase 9 (along with Beclin-1) cleaves caspase 3, which enforces apoptosis and DNA fragmentation. In proliferating GBM cells, however, the anti-apoptotic protein Bcl-2 directly inhibits caspase 9, while mTOR inhibits Beclin-1.

Table 4. Natural substances increase apoptotic cell death in GBM by downregulating apoptotic inhibitors and upregulating active caspases, which cleave PARP-1 and induce DNA fragmentation.

Effect	Substance	Cell Line	Source
Causes apoptosis	Arctigenin	U87MG, T98G	[41]
	Osthole	MOGGCCM, T98	[44]
	Xanthohumol	U87	[18]
	Rupesin E	GSC-3#, GSC-18#	[25]
	Diosmin	GBM02, GBM95	[26]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Gossypol	TS13-20, Diff13-20	[10]
	Withaferin A	U87, U251	[20]
	Tectorigenin	GBM-8401, GBM-8901	[30]
	Diosgenin	C6, T98G	[34]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Causes DNA fragmentation	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
Upregulates (c-)caspase 9 (protein)	Cedrol	RG2	[13]
	Coronarin D	U251	[27]
	CP	GAMG	[16]
	McC1	GAMG	[16]
	Withaferin A	U87, U251	[20]
Upregulates caspase 3 (mRNA)	Pisosterol	U87, U343, AHOL1, 1231N1	[40]

Effect	Substance	Cell Line	Source
Upregulates (c-)caspase 3 (protein)	EGCG	MO59J, U251	[22]
	Cedrol	DBTRG-05MG, RG2	[13]
	Osthole	T98	[44]
	Xanthohumol	U87, T98G, LN229	[18]
	Rupesin E	GSC-3#, GSC-18#	[25]
	Crocetin	U87, U138, U251, U373	[14]
	Diosmin	GBM02, GBM95	[26]
	Coronarin D	U251	[27]
	CP	GAMG	[16]
	McC1	GAMG, U251	[16]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Withaferin A	U87, U251	[20]
	Betulinic Acid	U251, LN229	[28]
	Resveratrol	U87	[31]
	Quercetin	U87	[31]
	GL-PP	U251	[39]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Upregulates (c-)PARP (protein)	Cedrol	RG2	[13]
	Xanthohumol	U87	[18]
	Coronarin D	U251	[27]
	CP	U251	[16]
	McC1	GAMG, U251	[16]
	Gossypol	TS13-20, Diff13-20	[10]
	Withaferin A	U87, U251	[20]
Downregulates PARP-1 (protein)	Diosgenin	C6, T98G	[34]
Downregulates ICAD (protein)	Diosgenin	C6, T98G	[34]
Upregulates Bax (protein)	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
	Diosgenin	C6, T98G	[34]
Downregulates Bcl-2 (mRNA)	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Downregulates Bcl-2 (protein)	Diosgenin	C6, T98G	[34]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Upregulates Bad (protein)	Withaferin A	U87, U251	[20]
Upregulates Bim (protein)	Withaferin A	U87, U251	[20]

Effect	Substance	Cell Line	Source
Depolarizes MMP	Coronarin D	U251	[27]
	CP	U251	[16]
	McC1	U251	[16]
	Gossypol	TS13-20	[10]
	Withaferin A	U87, U251	[20]
Upregulates ROS	Coronarin D	U251	[27]
	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
Upregulates cytochrome c (protein)	SLCP	U87, U251	[9]
	BBR	U87, U251	[9]
Upregulates GRP78 (mRNA)	Withaferin A	U87, U251	[20]
Upregulates GRP78 (protein)	EGCG	MO59J	[22]
Upregulates ATF4 (mRNA)	Withaferin A	U87, U251	[20]
Upregulates ATF4 (protein)	Withaferin A	U87, U251	[20]
	EGCG	U251	[22]
Upregulates ATF6 (mRNA)	Withaferin A	U251	[20]
Upregulates XBP1 (mRNA)	Withaferin A	U87, U251	[20]
Upregulates XBP1 (protein)	Withaferin A	U87, U251	[20]
Upregulates CHOP (mRNA)	Withaferin A	U87, U251	[20]
Upregulates CHOP (protein)	Withaferin A	U87, U251	[20]
Upregulates Bax (protein)	Cedrol	DBTRG-05MG	[13]

In conjunction with ER stress, several mitochondrial mechanisms promote GBM cell apoptosis. Astaxanthin and adonixanthin upregulate (p-)p38 and associated MAPKs, which upregulate the proapoptotic Bax and downregulate the antiapoptotic Bcl-2 [15]. Alterations in the Bax:Bcl-2 ratio, mediated also by curcumin, berberine, pisosterol, and diosgenin, contribute to the depolarization of the mitochondrial membrane potential (MMP) [9][34]. Moreover, coronarin D, curcumin, and berberine upregulate intracellular ROS, further contributing to MMP depolarization [9][27][38]. MMP depolarization leads to cytochrome c release, as seen after treatment with curcumin or berberine [9].

Downstream, cytochrome c, Bad, and Bim promote the activation of caspase 9, which in turn activates caspase 3. A blockade of Akt/mTOR signaling mediated by arctigenin or osthole enhances the activity of Beclin-1, which supports caspase 3 activation [41][44]. Caspase 3 specifically blocks the inhibitor of caspase-activated DNAse (ICAD), allowing CAD to cause DNA fragmentation—an effect observed after diosgenin application [34]. Consequently, caspase 3 cleaves poly-ADP ribose polymerase 1 (PARP-1), activating apoptosis.

Autophagy is blocked in proliferating GBM cells by Akt/mTOR signaling (Figure 6, Table 5). However, arctigenin and osthole upregulate Beclin-1 mRNA and protein levels. Beclin-1 interestingly has dual roles in apoptosis and autophagy, and upregulates light chain 3B (LC3B), which promotes autophagosome formation [41][44]. Moreover, arctigenin may increase autophagy through the upregulation of phosphorylated P62 (p-P62) [41].

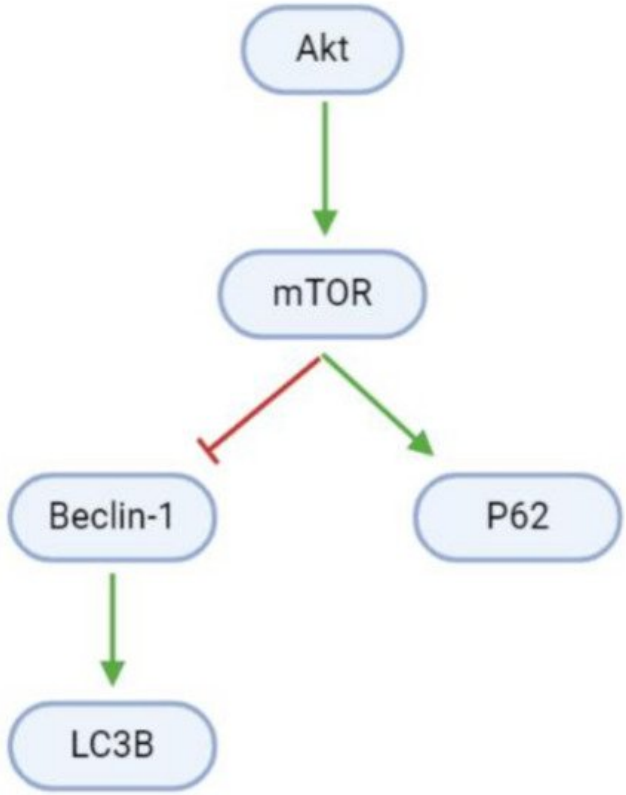

Figure 6. Pathways promoting cell death through autophagy are inhibited in GBM cells. mTOR inactivates the pro-autophagy Beclin-1 and upregulates the anti-autophagy P62.

Table 5. Arctigenin and osthole promote autophagy by upregulating Beclin-1 and LC3B-II and downregulating P62.

Effect	Substance	Cell Line	Source
Causes autophagy	**Osthole**	**MOGGCCM**	[44]
Upregulates Beclin-1 (mRNA)	**Arctigenin**	**U87MG, T98G**	[41]
Upregulates Beclin-1 (protein)	**Arctigenin**	**U87MG, T98G**	[41]
	Osthole	**MOGGCCM**	[44]
Upregulates LC3B-II (mRNA)	**Arctigenin**	**U87MG, T98G**	[41]
Upregulates LC3B-II (protein)	**Arctigenin**	**U87MG**	[41]
Downregulates P62 (mRNA)	**Arctigenin**	**U87MG, T98G**	[41]
Downregulates P62 (protein)	**Arctigenin**	**U87MG, T98G**	[41]

3.3. Cell Cycle

In addition to modulating cell proliferation and death, natural substances may also affect tumorigenesis through the induction of cell cycle arrest (Figure 7, Table 6). Uncontrolled cell cycle progression due to the Akt-mediated inhibition of cyclin-dependent kinase (CDK) inhibitors (CDKNs) causes rapid GBM cell division. However, a blockade of Akt activates (1) forkhead homeobox O (FOXO), which in turn activates the CDKN p27; and (2) the p53 tumor suppressor, which activates the CDKN p21. Elevated intracellular ROS levels mediate further upregulation of p21. ROS damages DNA, upregulating H2A family member X (H2AX) and consequently p21, as evidenced after Coronarin D, CP, and McC1 treatment [16][27].

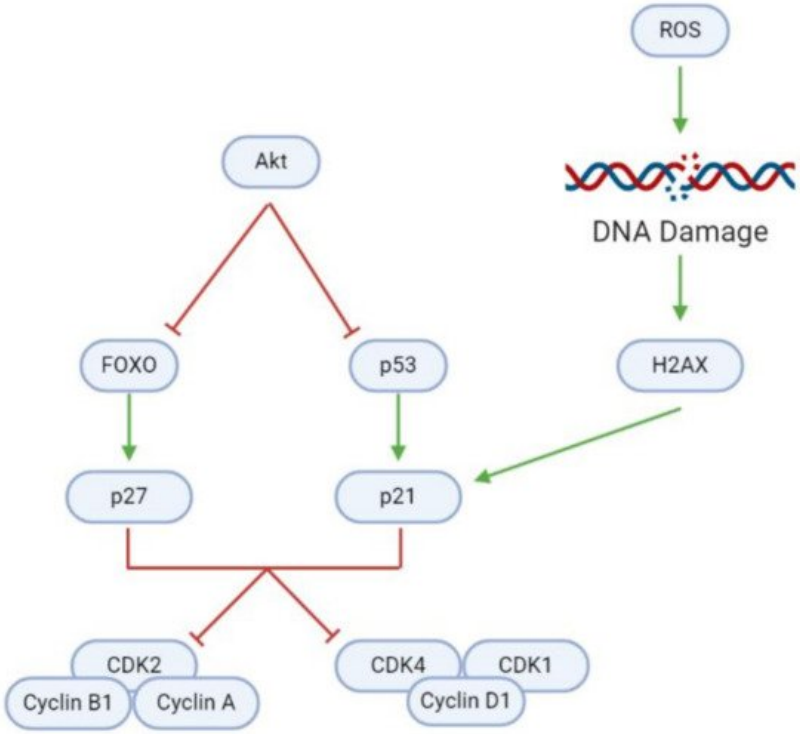

Figure 7. Inhibition of regulatory proteins allows for continuous cyclin/CDK activity and cell cycle progression in GBM cells. In healthy cells, FOXO and p53 can activate p27 and p21, respectively, and consequently induce cell cycle arrest to maintain homeostasis. DNA damage as a result of ROS accumulation is a key trigger for p21 activation. However, overactive Akt inhibits FOXO and p53, and therefore facilitates uncontrolled tumor cell growth and division.

Table 6. Natural substances induce cell cycle arrest in GBM by upregulating p53, p21, and p27, and inhibiting several cyclins and their associated CDKs.

Effect	Substance	Cell Line	Source
Causes G0/G1 phase cell cycle arrest	Cedrol	DBTRG-05MG, RG2	[13]
	Coronarin D	U251	[27]
	Tannic Acid	C6	[19]
	Tectorigenin	GBM-8401	[30]
	BBR	U87	[38]
	GL-PP	U251	[39]
Causes G2/M phase cell cycle arrest	Eucalyptal A	U87MG, LN229	[12]
	Withaferin A	U87, U251	[20]
	TBMS1	U87, LN229	[21]
	Pisosterol	U87, U343, AHOL1, 1231N1	[40]
Downregulates Cyclin D1 (protein)	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
	Cedrol	DBTRG-05MG	[13]
Downregulates CDK1 (protein)	Withaferin A	U87, U251	[20]
	TBMS1	U87	[21]
Downregulates CDK2 (protein)	Cedrol	DBTRG-05MG, RG2	[13]
Downregulates CDK4 (protein)	Tectorigenin	GBM-8401	[30]
Downregulates Cyclin A (protein)	Cedrol	DBTRG-05MG, RG2	[13]
	TBMS1	U87, LN229	[21]

Effect	Substance	Cell Line	Source
Downregulates Cyclin B1 (protein)	Cedrol	DBTRG-05MG, RG2	[13]
	TBMS1	U87, LN229	[21]
Upregulates (p-)H2AX (protein)	Coronarin D	U251	[27]
	CP	U251	[16]
	McC1	GAMG, U251	[16]
Downregulates (p-)RB (protein)	Tectorigenin	GBM-8401	[30]
Upregulates p21 (protein)	Coronarin D	U251	[27]
	Paris saponin H	U251	[49]
	Withaferin A	U87, U251	[20]
	Tectorigenin	GBM-8401	[30]
Upregulates p27 (protein)	Astaxanthin	GL261	[15]
	Paris saponin H	U251	[49]
	Adonixanthin	GL261	[15]
	AM05	T98G	[24]

As CDKNs, p21 and p27 inhibit specific cyclin-CDK complexes that are necessary for cell cycle progression. Inhibition of CDK2, Cyclin A, and Cyclin B1, as seen after treatment with cedrol or TBMS1, leads to G2/M phase cell cycle arrest [13][21]. In contrast, *Paris* saponin H upregulates p21 and p27 and downregulates Cyclin D1, eventually causing G1 phase cell cycle arrest [49]. Likewise, the inhibition of CDK1, CDK4, and Cyclin D1 by Withaferin A, TBMS1, astaxanthin, adonixanthin, and cedrol prompts G0/G1 phase arrest [13][15][20][21].

3.4. Inflammation and Immune Cell Modulation

Neuroinflammation is an essential component of GBM tumorigenesis and interacts with various pro- and anticancer mechanisms (Table 7). Bispo da Silva et al. characterized rutin and quercetin's pleiotropic effects on GBM-associated inflammation [43]. These flavonoids induce the chemotaxis and activation of microglia—resident macrophages in the nervous system—as evidenced by the immune cells' adoption of amoeboid and multipolar morphologies. Moreover, rutin and quercetin promote microglial proliferation and migration to tumor sites, where they modulate cytokine levels and thereby affect the tumor inflammatory profile. For instance, rutin and quercetin treatment upregulates interleukins 1 (IL-1), 1-βeta (IL-1β), and 18 (IL-18)—pro-inflammatory cytokines of the IL-1 family. Chemokine (C-X3-C motif) ligand 1 (CX3CL1), which promotes microglial migration, is also activated. Concurrently, interleukins 4 (IL-4), 8 (IL-8), and 10 (IL-10), which have tumorigenic properties under certain circumstances, are downregulated.

Table 7. Rutin, quercetin, and CrataBL exert pleiotropic and sometimes cell line-dependent effects on neuroinflammation.

Effect	Substance	Cell Line	Source
Activates microglia	Rutin	C6	[43]
	Quercetin	C6	[43]
Upregulates IL-1 (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Upregulates IL-1β (mRNA)	Rutin	C6	[43]
	Quercetin	C6	[43]
Downregulates IL-4 (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Upregulates IL-6 (mRNA)	Rutin	C6	[43]
	Quercetin	C6, TG1	[43]

Effect	Substance	Cell Line	Source
Downregulates IL-6 (mRNA)	Rutin	U251, TG1, WR-U251 orthotopic implants	[43]
	Quercetin	U251, WR-U251 orthotopic implants	[43]
Downregulates IL-6 (protein)	Rutin	C6	[43]
	CrataBL	U87	[29]
Downregulates IL-8 (protein)	CrataBL	U87	[29]
Downregulates IL-10 (mRNA)	Rutin	C6, U251, TG1, WR-U251 orthotopic implants	[43]
	Quercetin	C6, U251, TG1, WR-U251 orthotopic implants	[43]
Downregulates IL-10 (protein)	Rutin	C6	[43]
Upregulates IL-18 (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Upregulates TNF (mRNA)	Rutin	U251, TG1	[43]
	Quercetin	U251	[43]
Downregulates TNF (mRNA)	Rutin	U251 orthotopic implants, WR	[43]
	Quercetin	U251 orthotopic implants, WR	[43]
Upregulates TNF (protein)	Rutin	C6	[43]
Upregulates TNF-α (mRNA)	Rutin	C6	[43]
	Quercetin	C6	[43]
Upregulates CX3CL1 (mRNA)	Rutin	C6, WR-U251 orthotopic implants	[43]
	Quercetin	C6	[43]
Downregulates (p-)STAT3 (protein)	Curcumin	U87	[32]

Interestingly, the effects of natural compounds on interleukin 6 (IL-6) and tumor necrosis factor (TNF) vary between cell lines (see Table 7). Rutin and quercetin upregulate IL-6 at the mRNA level in C6 and TG1 (quercetin only) cells. However, along with CrataBL, they downregulate IL-6 at the mRNA level in U251 and TG1 (rutin only) cells and U251 xenografts in Wistar rats. They also downregulate IL-6 at the protein level in C6 and U87 cells. Similar pleiotropic effects are observed with TNF, which is upregulated at the mRNA and protein levels in U251, C6, and TG1 cells, but downregulated at the mRNA level in U251-Wistar rat xenograft models. These varying data underscore the need for further investigation into the immuno-modulatory properties of natural substances in GBM.

3.5. Migration, Invasion, and Metastasis

GBM cell migration, invasion, and metastasis are mainly mediated through the epithelial-mesenchymal transition (EMT) and modulation of the cytoskeletal actin framework (Figure 8; Table 8). To promote cell motility through actin, RhoA, a small GTPase, activates the Rho-associated protein kinase (ROCK); ROCK, in turn, activates the Lim kinase (Limk) through phosphorylation. Concurrently with RhoA/ROCK/Limk signaling, PI3K activates protein kinase A (PKA). Both Limk and PKA inhibit the activity of Cofilin (actin depolymerization factor), which ordinarily destabilizes cytoskeletal actin filaments and thereby impairs cell motility. Cofilin is active in the dephosphorylated form; as such, Limk and PKA may inhibit its activity through phosphorylation to produce p-Cofilin. Cofilin activity may be restored by paeoniflorin, which downregulates all components of the RhoA/ROCK/Limk pathway [33]. Eucalyptal A may also promote Cofilin activity, as it downregulates PKA [12]. Finally, the signal transducer and activator of transcription 3 (STAT3), a transcription factor commonly associated with inflammation, activates the actin bundling protein Fascin. Fascin acts antagonistically to Cofilin to stabilize the cytoskeleton and enhance cell motility; however, curcumin suppresses (p-)STAT3 and thereby downregulates Fascin activity [32].

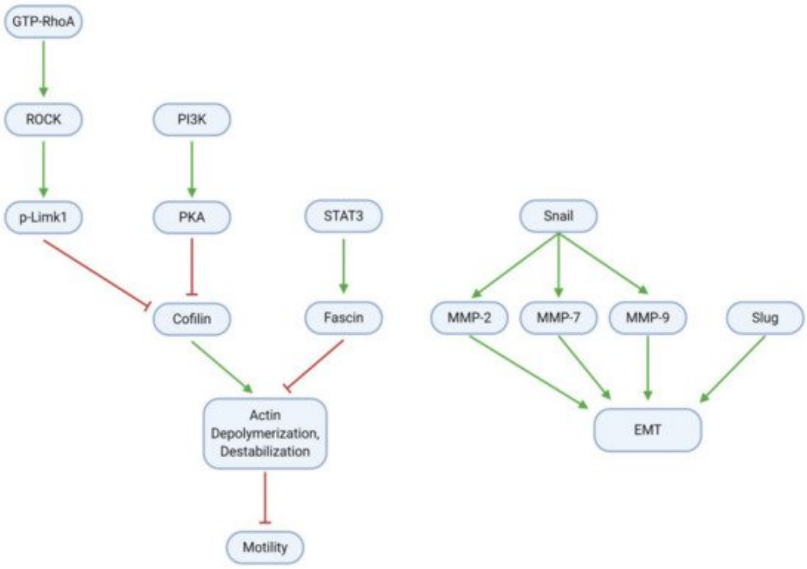

Figure 8. GBM cells gain migration and invasion abilities through EMT and modulation of the cytoskeletal actin framework. Regularization of actin filaments by STAT3/Fascin enhances cell motility; this process is reversible by Cofilin, which in tumor cells is inhibited by RhoA/ROCK/Limk and PI3K/PKA signaling. Upregulation of Snail, Slug, and MMPs further increases motility through EMT induction.

Tumor cell adhesion and motility are further influenced by EMT, a process in which tumor cells become less adhesive and more migratory, and therefore more invasive. The Snail protein is upregulated in glioblastoma and activates the matrix metalloproteinases (MMP) 2, 7, and 9, which together with Slug contribute to the EMT. Several natural compounds have anti-EMT properties in GBM. These include TBMS1 and galangin, which directly downregulate Snail (and therefore the MMPs) and Slug [21][50]. Astaxanthin, adonixanthin, and diosgenin also downregulate MMPs; however, it remains unclear whether these effects are Snail-dependent [15]. Moreover, magnolol suppresses GBM cell migration by regulating focal adhesions and N-cadherin, while gamabufotalin demonstrates antimetastatic effects by downregulating urokinase plasminogen activator (uPA) and carbonic anhydrase 9 (CA9) and upregulating tissue inhibitor of metalloproteinases 3 (TIMP-3) [51][52].

Table 8. Natural substances decrease GBM cell migration and invasion by downregulating EMT modulators (Snail, Slug, and MMPs), Cofilin inhibitors (RhoA/ROCK/Limk and PKA), and actin polymerizers (STAT3/Fascin).

Effect	Substance	Cell Line	Source
Reduces cell migration	Eucalyptal A	U87MG, LN229	[12]
	Astaxanthin	GL261, U251MG	[15]
	Adonixanthin	GL261, U251MG	[15]
	Arctigenin	U87MG, T98G	[41]
	Crocetin	U87, U251	[14]
	CP	GAMG	[16]
	McC1	U251, GAMG	[16]
	Tannic Acid	C6	[19]
	TBMS1	U87, LN229	[21]
	Curcumin	U87	[32]
	Paeoniflorin	U251, T98G	[33]
	Diosgenin	C6, T98G	[34]
	Rutin	C6	[43]
	Magnolol	LN229, U87MG	[51]
	Gamabufotalin	U87	[52]
	Quercetin	C6	[43]
Reduces cell invasion	Eucalyptal A	U87MG, LN229	[12]
	Arctigenin	U87MG, T98G	[41]
	McC1	GAMG, U251	[16]
	CrataBL	U87	[29]
	Curcumin	U87	[32]
	Paeoniflorin	U251, T98G	[33]
	Diosgenin	C6, T98G	[34]
Downregulates MMP-2 (protein)	Astaxanthin	GL261	[15]
	Adonixanthin	GL261	[15]
	Arctigenin	U87MG	[41]
	TBMS1	U87, LN229	[21]
	Diosgenin	T98G	[34]
Downregulates MMP-7 (protein)	TBMS1	U87, LN229	[21]
Downregulates MMP-9 (protein)	Arctigenin	U87MG	[41]
	Diosgenin	C6	[34]
Downregulates p-PKA 1/2/3 (prot.)	Eucalyptal A	U87MG, LN229	[12]
Downregulates p-Cofilin (protein)	Eucalyptal A	U87MG, LN229	[12]
Downregulates fibronectin (protein)	Adonixanthin	GL261	[15]
Downregulates laminin (protein)	CrataBL	U87	[29]
Downregulates Snail (protein)	TBMS1	U87, LN229	[21]
	Galangin	U87, U251	[50]
Downregulates Snail (mRNA)	Galangin	U87, U251	[50]

Effect	Substance	Cell Line	Source
Downregulates Slug (protein)	TBMS1	U87, LN229	[21]
Downregulates Fascin (protein)	Curcumin	U87	[32]
Reduces actin filament number	Paeoniflorin	T98G, U251	[33]
Downregulates GTP-RhoA (protein)	Paeoniflorin	T98G, U251	[33]
Downregulates ROCK (protein)	Paeoniflorin	T98G, U251	[33]
Downregulates (p-)Limk1 (protein)	Paeoniflorin	T98G, U251	[33]

3.6. Angiogenesis

Angiogenesis—the development of active blood vessels in and around tumor sites—is a key element of GBM progression (Table 9). Vascular endothelial growth factor (VEGF) mediates this process; it is upregulated by HIF-1 and downregulated by A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1). As such, substances such as *Paris* saponin H that inhibit HIF-1 will consequently downregulate VEGF [49]. The sesquiterpene lactone AM04 upregulates ADAMTS1 and thereby downregulates VEGF in U87 and T98G cells [24].

Table 9. Natural substances reduce angiogenesis and neovascularization primarily by downregulating VEGF.

Effect	Substance	Cell Line	Source
Decreases angiogenesis area	McC1	U251 heterotopic xenograft, fertilized chicken eggs	[16]
Decreases blood vessel junctions	McC1	U251 heterotopic xenograft, fertilized chicken eggs	[16]
Decreases tube formation	Diosgenin	C6, T98G	[34]
Upregulates ADAMTS1 (protein)	AM04	U87MG, T98G	[24]
Downregulates CD31 (mRNA)	Naringin	U87 subcutaneous xenograft, athymic mice	[17]
Downregulates CD105 (mRNA)	Naringin	U87 subcutaneous xenograft, athymic mice	[17]
Downregulates tumor hemoglobin	Naringin	U87 subcutaneous xenograft, athymic mice	[17]
Downregulates VEGF (protein)	Metformin	U251	[48]
	Paris saponin H	U251	[49]
	CrataBL	U87	[29]
	Diosgenin	C6	[34]

Reduced VEGF activity decreases tumor neovascularization; importantly, this is observable in vivo. Treatment of U87 xenografts in athymic mice with the flavonoid naringin downregulates tumor hemoglobin and the angiogenic markers cluster of differentiation 31 (CD31) and 105 (CD105) [17]. Moreover, matteucinol decreases the angiogenic area and the number of blood vessel junctions in a U251 xenograft-fertilized chicken egg model [16]. These in vivo effects demonstrate the potential applicability of specific natural substances as angiogenic modulators that inhibit GBM.

3.7. Metabolism

Cancer cells exhibit modified metabolic processes that meet the extensive energy demands of growth, proliferation, and metastasis—a phenomenon known as the Warburg effect [53]. In GBM cells, Akt promotes glucose metabolism through the upregulation of glycogen synthase kinase 3 βeta (GSK3β). GSK3β, in turn, upregulates F-box and WD-40 domain-containing protein 7 (FBW7) and c-Myc, leading to the activation of hexokinase 2 (HK2). HK2 is a major metabolic enzyme that contributes to the aerobic glycolysis observed in tumor cells by increasing glucose uptake and lactate production (Figure 9, Table 10). Xanthohumol downregulates GSK3β, and as such decreases downstream HK2 activity, glucose consumption, and lactate production [18]. In contrast, carnosine upregulates pyruvate dehydrogenase kinase 4 (PDK4), which downregulates metabolism, while crocetin downregulates fatty acid synthase (FASN), which catalyzes metabolically relevant fatty acid synthesis [11][14].

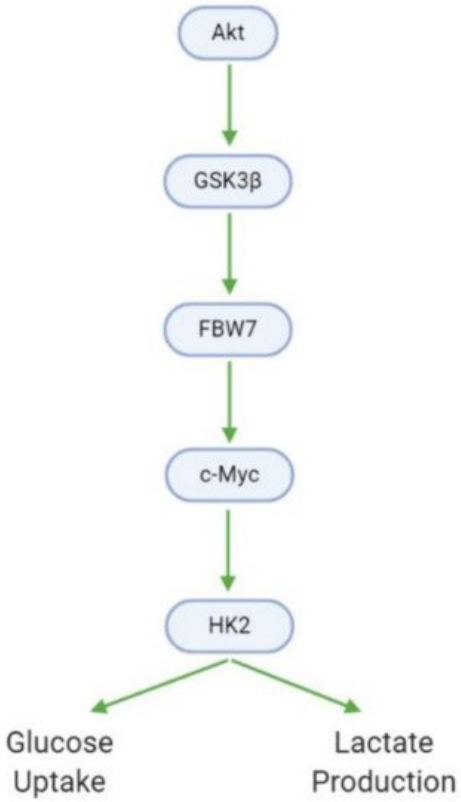

Figure 9. GBM cells utilize altered metabolic processes (Warburg effect) characterized by increased glucose uptake and lactate generation. Akt, via GSK3β, mediates the transition between the healthy and Warburg phenotypes.

Table 10. Xanthohumol, carnosine, and crocetin interfere with key enzymes in GBM cell metabolism.

Effect	Substance	Cell Line(s)	Source
Downregulates HK2 (protein)	Xanthohumol	U87, T98G, LN229	[18]
Decreases glucose consumption	Xanthohumol	U87, T98G, LN229	[18]
Decreases lactate production	Xanthohumol	U87, T98G, LN229	[18]
Downregulates (p-)GSK3β (protein)	Xanthohumol	U87	[18]
Upregulates PDK4 (mRNA)	Carnosine	U87, T98G	[11]
Downregulates FASN (protein)	Crocetin	U87, U138, U251, U373	[14]

3.8. Chemoresistance

The effects of natural substances on GBM chemoresistance remain largely uncharacterized in the recent literature. Chang et al. reported that cedrol downregulates O6-alkylguanine DNA alkyltransferase (MGMT) at the protein level in DBTRG-05MG and RG2 cells [13]. MGMT, a DNA repair protein, confers resistance to alkylating agents (e.g., temozolomide) by reversing guanine alkylation [54]. Studies from 2014 moreover indicate that pine needle extract, chrysin, and quercetin sensitize GBM cells to TMZ [55][56]. At any rate, further data are necessary to substantiate the potential of natural substances in overcoming GBM drug resistance.

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