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Chapter- 7

Unveiling the Potentials of *Withania somnifera* (L.) Dunal as a Precise Therapeutic Intervention Against Glioblastoma Multiforme

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Keywords: Glioblastoma Multiforme, *Withania somnifera*, phytotherapy, Withaferin A, Withanolides

Abstract:

Glioblastoma multiforme (GBM) is the most severe and fatal form of brain tumor, leading to a poor survival rate in patients and making a significant contribution to cancer-related deaths. The typical approaches to treating GBM involve surgical procedures followed by chemotherapy, targeting molecular pathways involving receptors like Epidermal Growth Factor Receptor (EGFR, EGFRvIII) and Vascular Endothelial Growth Factor Receptor (VEGFR) to modulate various cell signaling pathways. However, the effectiveness of current GBM treatments is notably constrained. *Withania somnifera* (WS) (L.) Dunal, commonly known as Ashwagandha, has a history spanning over 3,000 years in Ayurvedic and traditional medicine. This medicinal plant has diverse properties, encompassing anti-inflammatory, anticancer and antioxidant attributes. Recent advancements in the field of herbal and traditional medicines have explored its potential in managing deadly diseases like cancer. Ashwagandha or *W. somnifera*, mostly found in dry, sub-tropical regions of the world including India, is a well-known source of traditional and herbal medicines, and has many specific phytochemicals, viz. Withaferin A, Withanolide etc. This review discusses the potential of *W. somnifera*, supported by several research reports dealing with the extracts and phytochemicals from different parts of the plant, showing effectiveness against

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GBM in vitro and in vivo. Therefore, studying the effectiveness of *W. somnifera*- derived phytochemicals on specific therapeutic targets of GBM could unveil a new avenue in managing GBM. This review has exhaustively surveyed and analyzed several research reports from various literature databases on *W. somnifera*, its phytochemicals, and its leaf or root extracts as potential therapeutic interventions against GBM. It is evident that GBM management requires precise therapeutic intervention to achieve better overall survival rates in patients. *W. somnifera* holds promise in addressing the molecular targets associated with the disease, integrating precision medicine into phytotherapy. However, further research is imperative to establish these phytocompounds as nutritional supplements and to develop promising therapeutic strategies for combating GBM.

Introduction:

Glioblastoma multiforme (GBM) is considered the most aggressive and lethal type of brain tumor (Hanif et al., 2017). The reported 5-year patient survival has been consistently poor, varying to about 5% in the past three decades (Ledger et al., 1999; Youlden et al., 2012). There are 100,000 new instances of glioblastoma diagnosed annually worldwide. Despite having a low incidence rate of less than 6 per 100,000 people, glioblastoma accounts for 2.5% of all cancer-related fatalities and with the leading cause of mortality from cancer in those between the ages of 15 and 34 (Hanif et al., 2017). Managing recurrent cases of the illness has been challenging and unpredictable. Traditional chemotherapeutic agents have included drugs like temozolomide, lomustine, carboplatin, and carmustine in various dosages, with typical response rates less than 20%, 6-month progression-free survival around 15%, and overall survival typically less than 6 months, although outcomes across studies have shown variability (Wong et al., 1999; Batchelor et al., 2013).

Chemotherapeutic drugs frequently result in complications and increased malignancy with recurrent cases of GBM. Targeting these recurrent cases based on their molecular profiles has proven to be challenging, thus necessitating a personalized approach. Researchers have found that effectively targeting the tumor involves identifying the underlying cause of recurrence and detecting the signaling pathways involved. Over the years, an expanding array of phytochemicals has been employed due to their potential to target specific pathways and receptors, alongside their efficacy in penetrating the Blood-Brain Barrier (BBB). Indian traditional medicine has a notable history of employing different kinds of plants to cure a variety of diseases, and one such widely used plant in Ayurvedic studies is *Withania somnifera* (WS), commonly known as Ashwagandha or Winter Cherry (Andallu and Radhika, 2000). According to Ayurvedic medical tradition, the Rishi (sage) Punarvasu Atriya is credited with discovering the plant's therapeutic properties over 3,000 years ago (Tiwari et al., 2014). Ashwagandha possesses immunomodulatory, hemopoietic, anti-inflammatory, anticancer, anti-stress, and antioxidant potentials, also benefitting the endocrine, cardiovascular, and central nervous systems (Mishra et al., 2000). With over twenty-nine bioactive compounds found in different parts of the plant, mostly alkaloids, steroidal lactones, and saponins, preparations of *W. somnifera* have been discovered to have a therapeutic role in practically all CNS-related disorders, neurodegenerative diseases, neuropsychiatric diseases, and drug addiction (Kulkarni and Dhir, 2008). In combating prostate cancer, the

modulation of antitumor immunity with the help of Ashwagandha is promising (Dubey et al., 2021). Withaferin A (an important withanolide of WS) has been shown to trigger triple-negative breast cancer cell-specific clinically relevant anticancer effects at certain pharmacological levels (Szarc et al., 2014). WS water extracts proved to be a potential candidate in differentiation-based therapy of human neuroblastomas by simultaneously inducing downregulation of MMP 2 & 9 and apoptosis (Kataria et al., 2013). The anticancer activity of Ashwagandha and its ability to target and suppress tumors inspired researchers to find its effect on GBM.

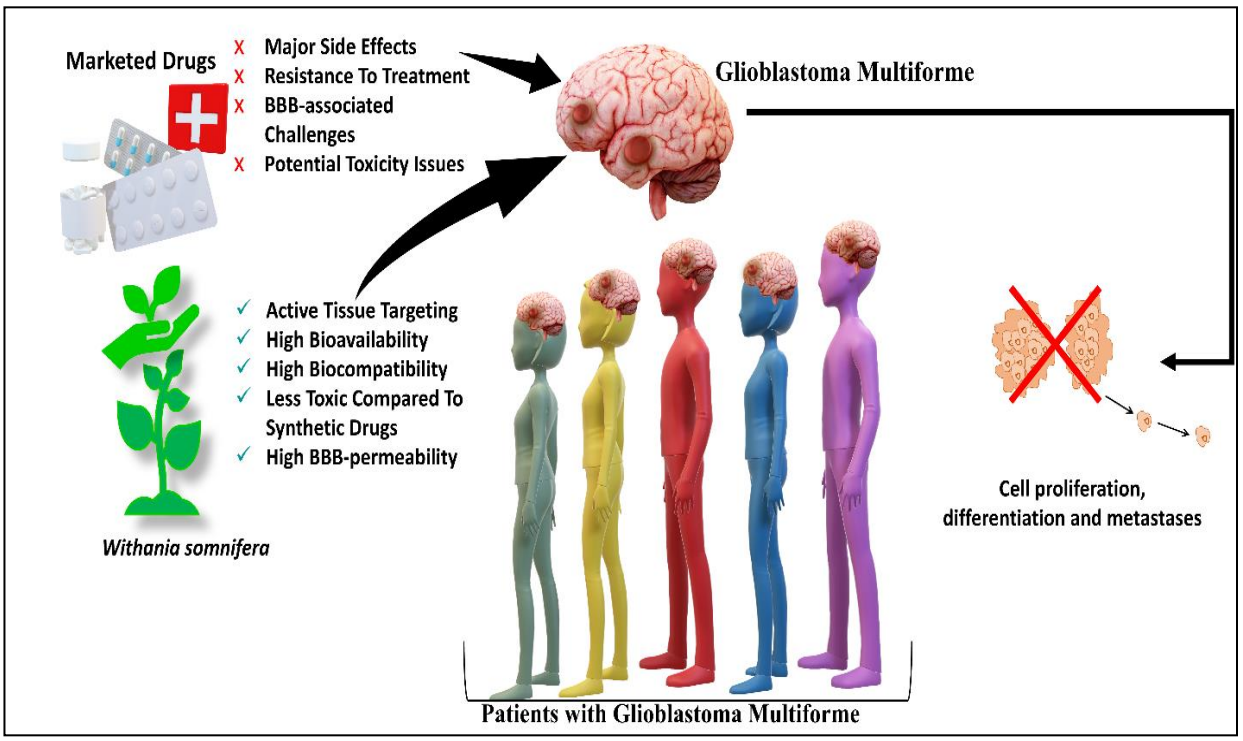

Figure 1. Graphical description of the study

Potential Targets for Glioblastoma Multiforme:

Glioblastoma Multiforme, the most malignant form of Glioma, poses a challenge in terms of complexity and management. The current treatment strategies mostly include surgical interventions followed by chemotherapy, along with the exploration of novel molecular targets such as EGFR, EGFRvIII, and VEGFR. Several potential targets for GBM are yet to be addressed. Typically, patients diagnosed with Glioblastoma undergo conventional treatment involving surgery wherever feasible, followed by field radiation combined with temozolomide chemotherapy up to six maintenance cycles (Weller et al., 2017; Stupp et al., 2005). No alternative treatment strategy has demonstrated an increase in overall survival for newly diagnosed patients, except for tumor-treating fields (Stupp et al., 2017). Although strictly limited in the newly diagnosed context, the methylation of the O6-methylguanine DNA methyltransferase (MGMT) promoter has been proven as a predictive biomarker for the benefit of treatment with temozolomide (Hegi et al., 2005; Hegi et al., 2019). The bulk of clinical trial strategies that aim at intrinsic targets of glioblastoma, address tyrosine kinase-mediated

oncogenic signaling, cell cycle regulation, and vulnerability to apoptosis induction (Fig. 2). In this context, epidermal growth factor receptor (EGFR) is amplified by 40-50%, included with mutations at EGFRvIII in 50% of these, and without amplification by 10-20% can also be seen in GBM which stimulates proliferation, invasion, and apoptosis resistance (Brennan et al., 2014; Felsberg et al., 2017). By activating phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutations or lack of tumor suppressor phosphatase and tensin homolog on chromosome ten (PTEN) function almost uniformly activates PI3K/AKT/mTOR pathway in IDH-wildtype GBM inducing metabolism, proliferation, and migration (Brennan et al., 2014). Activation of the Phosphorylated Protein Kinase B (AKT) pathway often observed in recurrent GBM occurs due to the higher levels of p-AKT phosphorylated by PDK-1 (3-phosphoinositide-dependent kinase-1). Fusion of fibroblast growth factor receptor (FGFR) with acidic coiled-coil (TACC) gene and sometimes by mutation causes proliferation of GBM (Singh et al., 2012; Di Stefano et al., 2015). Deletion or mutation in the p53 gene (35%) or neutralization of the p53 function by amplification of MDM2 or MDM4 gene (20%) has been reported in GBM (Brennan et al., 2014). In 90% of IDH-wild type GBM mutation in TERT promoter amplifies TERT transcription, which immortalizes tumor cells (Brennan et al., 2014; Killela et al., 2013; Sharma et al., 2022). Isocitrate dehydrogenase (IDH) mutations have also been observed where isoforms of IDH present in the peroxisomes and cytoplasm of glial cells exhibit increased oxidative damage and epigenetic alterations resulting in tumor growth (Horn et al., 2013). It has been revealed that altered proteasome activity may make cancer cells more vulnerable and has also been examined as a therapeutic target in recurrent GBM (Kong et al., 2019; Friday et al., 2012). Fusion in Neurotrophic tyrosine receptor kinases (NTRK) coding genes NTRK1/2/3 has been reported in 1-2% of GBM which stimulates proliferation (Ferguson et al., 2018). Up to 50% of epithelioid glioblastomas carry the uncommon BRAFV600E mutation, which induces proliferation. The proliferation-promoting MAP kinase/ERK signaling pathway is fed by a Raf family of kinase member BRAF (Korshunov et al., 2018). Overexpression of the Mesenchymal Epithelial Transition (MET) gene and infrequent amplification causes migration, invasion, and wound healing of GBM cells. It has been demonstrated that owing to homozygous CDKN2A/B deletion, RB1 gene mutations, or CDK4 or CDK6 amplification, most of the IDH-wild type glioblastomas have altered pRB cell cycle regulatory pathway (Brennan et al., 2014). Several other gene regulators were reported to be overexpressed. The Eukaryotic Initiation Factor 4A-3 (EIF4A3) is one such RNA binding protein, which is known to promote several oncogenes and their circularization, interact with miRNAs that regulate the cell cycle regulators, ultimately inducing the aggressive phenotype of GBM (Zhao et al., 2021). EIF4A3 promotes circular RNA Matrix metalloproteinase 9 (circMMP9), which in turn upregulates CDK4 and AURKA (Wang et al., 2020). EIF4A3 causes Temozolomide resistance by upregulation of NRAS (Wei et al., 2021). There are also a few potential microenvironmental targets in glioblastoma. Vascular endothelial growth factor (VEGF) forms new blood vessels where hypoxia triggers angiogenesis and becomes a potential factor for glioblastoma survival (Szabo et al., 2016; Plate et al., 1994). Cell surface molecule integrins and WNT proteins which integrate signals between cells and the

extracellular matrix, play a crucial role in cellular activities like adhesion, invasion, migration, and angiogenesis determining the fate of the cell. World Health Organization (WHO) reports showed the increased localization of WNT signaling protein β-catenin and its complex with T-cell factor (TCF4) as higher in glioma and results in increased activation of transcription factors, thus promoting resistance to chemotherapeutic treatments (Sharma et al., 2022). In glioblastoma and associated vasculature, the involvement of specific subtypes of integrins has been established (Plate et al., 1994).

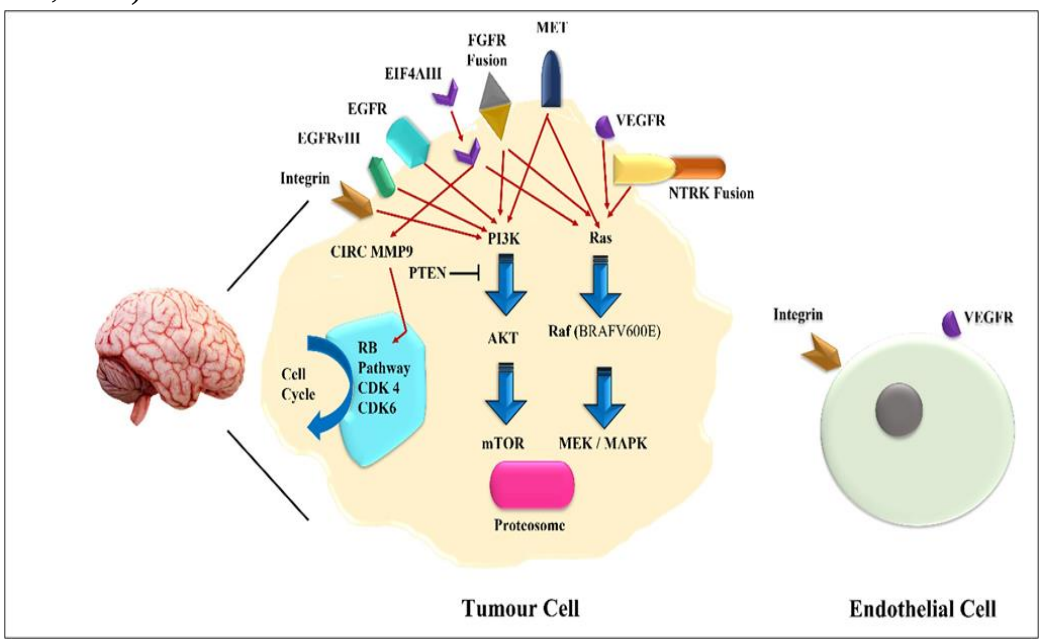

Figure 2. Several potential molecular targets in Glioblastoma Multiforme

Onco-medicinal Aspects of *Withania somnifera:*

WS is among the most prevalent Indian medicinal plants used in traditional medicine. Several bioactive compounds along with plant-specific phytochemicals, are present in various plant parts of WS, which are reported to exhibit potent activity against different types of cancers. Numerous studies have suggested that the use of WS's leaves, roots, seeds, and bark may derive benefits in cancer treatment (Kumar et al., 2017; Islam et al., 2014; Sinkar and Samarth, 2019; Li et al., 2016). Reports have confirmed the use of WS to be effective against breast cancer (Umair et al., 2019; Dar et al., 2019; Thaiparambil et al., 2011; Rah et al., 2016; Gowtham et al., 2022; Kim et al., 2015; Jawarneh and Talib, 2022; Li et al., 2015), alcoholic root extracts of WS have displayed inhibitory effects on the growth of the human breast cancer cell line (MCF7), hepatocellular carcinoma cell line (HepG2) consisting mutation in the TERT promoter, colon cancer cell line (CaCo2) with mutations in APC and β-catenin genes, and human non-small cell lung cancer cell line (A549) harboring KRAS (Kirsten rat sarcoma) mutant (Ahmed and El-Darier, 2022). Withaferin A (WA), a phytochemical present in different plant parts of WS, displays anticancer activity in human pancreatic cancer cell Panc-1 xenograft in nude mice, coupled with oxaliplatin, which causes reactive oxygen species-mediated inhibition of the PI3K/AKT pathway (Jawarneh

and Talib, 2022). In pancreatic cancer cells, heat shock protein 90 (HSP90) is targeted by WA, which stimulates antiproliferative activity (Yu et al., 2010). Withaferin A in low doses has been found to be an effective inhibitor of HSP90, which resulted in growth arrest at the G2/M phase (Wang et al., 2019). Inhibition of HSP90 by WA was also observed in A20 B-cell lymphoma allograft in Balb/c female mice (McKenna et al., 2015). In Non-Small Cell Lung Cancer, WA inhibited cancer stem cell development by impacting numerous targets of the mammalian Target of Rapamycin (mTOR)/ Signal Transducer and Activator of Transcription 3 (STAT3) pathways (Hsu et al., 2019). In the development of human prostate cancer, the alcoholic leaf extracts of WS targeted interleukin-8 (IL-8) and cyclooxygenase-2 (COX-2) in the PC-3 prostate cancer cell line. Chronic inflammation, enhanced angiogenesis, proliferation, migration, and apoptosis inhibition occur in conjunction with overexpression of IL-8 and COX-2. Moreover, the disease's transition from an androgen-dependent to an androgen-independent state is promoted by their elevated circulating levels (Setty et al., 2017). Withaferin A has been reported to induce apoptosis and inhibition of the proteasome system in nude mice PC-3 prostate cancer xenograft (Srinivasan et al., 2007; Yang et al., 2007). By inhibiting the expression of the RET (REarranged during Transfection) gene, WA is a prospective inhibitor of medullary thyroid carcinomas (MTC) cell line DRO-81-1 xenograft in nude mice (Samadi et al., 2010). On the other hand, WA induced p53-dependent apoptosis in human cervical cancer cell CaSki xenograft in nude mice by suppressing HPV oncogenes and upregulating tumor suppressor proteins (Munagala et al., 2011). Aqueous extracts of WS root are highly cytotoxic to human malignant melanoma A375 cells (Halder et al., 2015). Recent evidence suggests that WA suppresses cell growth of human myeloma cells U266B1 and IM-9 by causing intrinsic apoptosis mediated by the induction of the reactive oxygen species (ROS) pathway (Li et al., 2022). Furthermore, it is reported that Eukaryotic Translation Initiation Factor 2A (EIF2A)-dependent inhibition of translation was observed in neurogenic locus notch homolog protein 1 (NOTCH1) mutated T-cell acute lymphoblastic leukemia (T-ALL) in xenograft NRG mice (Sanchez-Martin et al., 2017). By targeting putative cancer stem cells, WA alone or in combination with cisplatin inhibited the growth and spread of ovarian cancer in A2780 xenograft in nude mice (Kakar et al., 2014). By inhibiting AKT and c-MET activation, WA also promotes apoptosis in uveal melanoma cell 92-1 xenograft in SCID mice (Samadi et al., 2021).

Among the numerous withanolides present in the plant, Withaferin A, Withanolide A and Withanone have proved evidence for affinity towards GBM and other types of tumors. These steroidal lactones have been extensively studied for their Absorption, Distribution, Metabolism, and Excretion (ADME) properties to evaluate their antagonistic activities. Structurally, Withanone is an epoxy compound with oxygen atoms at positions C6 and C7, having hydroxyl groups at C5 and C17. In contrast, Withaferin A is an epoxy compound with oxygen atoms at C5 and C6 positions, accompanied by hydroxyl groups at C4 and C27. Early studies have shown the superiority of Withaferin A in inducing apoptosis in cancer cells at concentrations <0.5µg/mL (Vaishnavi et al., 2012). Extraction of Withanolides has enabled researchers to identify pathways and targets in the management of tumor cells. With the presence of structurally different

phytochemicals in WS, it can address the tumor-inducing molecular targets in different types of cancer (Fig. 4).

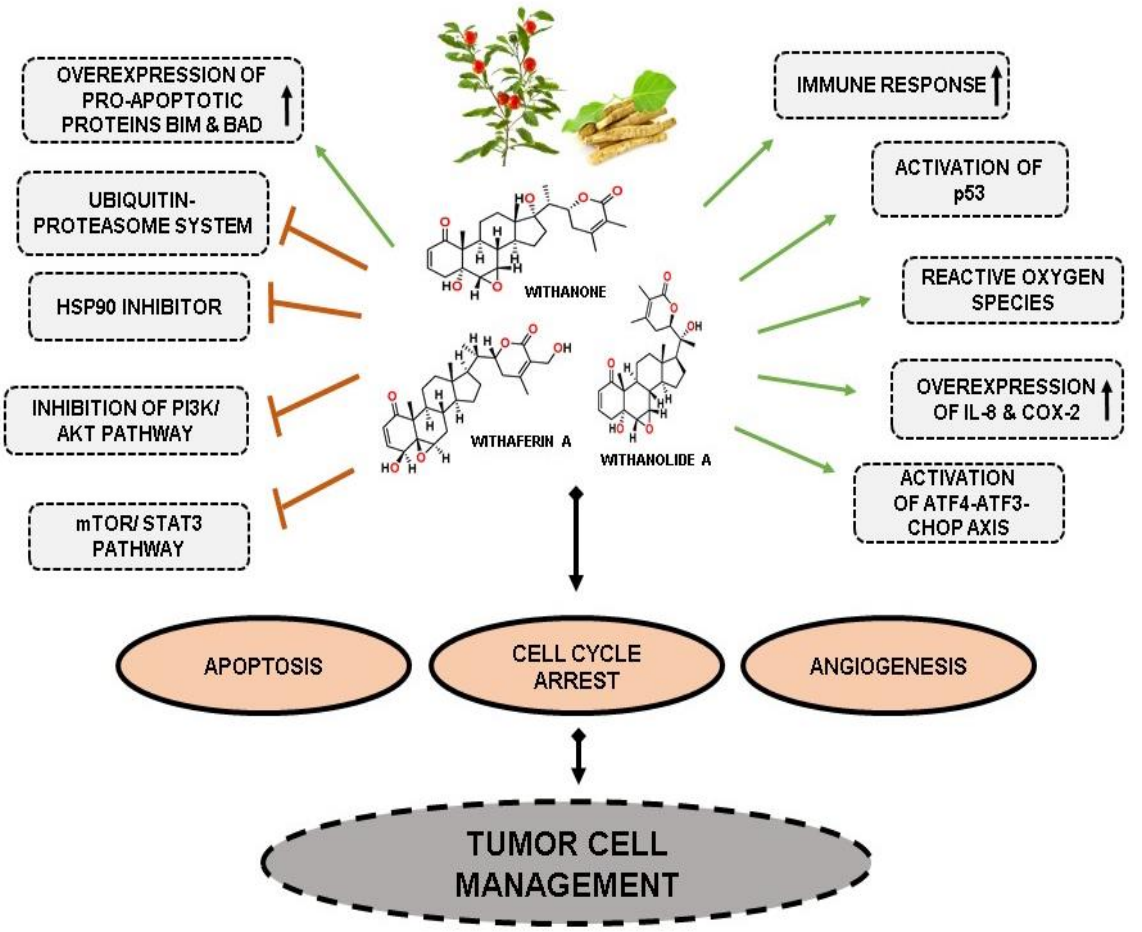

Figure 3. Several potential molecular targets of WS phytochemicals in the management of tumor cells

Phytochemical Profile of *Withania somnifera:*

Withania somnifera and its phytochemicals have been found effective in glioblastoma management (Kataria et al., 2016; Shah et al., 2009; Tang et al., 2020; Chang et al., 2016; Garg et al., 2018). An abundant number of phytochemicals have been found in the WS plant with a countable one specific to the *Withania* sp. While looking for a therapeutic cure for GBM, the effect of *Withania* sp.-specific phytochemicals existing in WS (Table 1) has been considered.

Table 1: Phytochemical profiling of different parts of WS.

Plant Part	Phytochemical Name	Reference	Plant Part	Phytochemical Name	Reference

Leaf	Withanolide Q	(Mohanraj et al., 2018)		Withaferin A	(Mohanraj et al., 2018; Khajuria et al., 2004; Mirjalili et al., 2009; Doma et al., 2012; Nakajima et al., 2011; Ali and Meitei, 2012)
	Withanolide R	(Mohanraj et al., 2018)		Withanolide E	(Mohanraj et al., 2018)
	Withanolide M	(Mohanraj et al., 2018)		Withasomnine	(Mohanraj et al., 2018)
	Withanolide D	(Mohanraj et al., 2018; Mondal et al., 2012)		Withanolide G	(Mohanraj et al., 2018)
	Withaferin A	(Mohanraj et al., 2018; Khajuria et al., 2004; Misra et al., 2005; Pramanick et al., 2008; Tong et al., 2011; Jayaprakasam et al., 2002; Mirjalili et al., 2009; Xu et al., 2011; Yoneyama et al., 2015)		Withanolide P	(Mohanraj et al., 2018)
	Withanolide E	(Mohanraj et al., 2018)		Withanone	(Mohanraj et al., 2018; Khajuria et al., 2004)
	Withasomnine	(Mohanraj et al., 2018)		Withanolide S	(Mohanraj et al., 2018)
	Withanolide G	(Mohanraj et al., 2018)		Withanolide C	(Mohanraj et al., 2018)
	Withanolide O	(Mohanraj et al., 2018)		Withanolide L	(Mohanraj et al., 2018)
	Withanolide P	(Mohanraj et al., 2018)		Withanolide J	(Mohanraj et al., 2018)
	27-Deoxywithaferin A	(Mohanraj et al., 2018)		Withanolide A	(Mohanraj et al., 2018; Khajuria et al., 2004; Soman et al., 2012; Doma et al., 2012; Zhao et al., 2002; Tohda et al., 2005; Kour et al., 2009; Nagella and Murthy, 2011; Soman et al., 2013; Devi et al., 1996)
	Withanolide N	(Mohanraj et al., 2018)		Withanoside XI	(Mohanraj et al., 2018; Zhao et al., 2002)
	Withanone	(Mohanraj et al., 2018; Khajuria et al., 2004; Misra et al., 2005;		WithanosideVIII	(Mohanraj et al., 2018; Zhao et al., 2002)

		Tong et al., 2011; Siddique et al., 2014)			
	17alpha-hydroxywithanolide D	(Mohanraj et al., 2018)		Withanoside X	(Mohanraj et al., 2018; Zhao et al., 2002)
	Withanolide S	(Mohanraj et al., 2018)		Ashwagandhanolide	(Mohanraj et al., 2018; Subbaraju et al., 2006)
	Withanolide C	(Mohanraj et al., 2018)		(-)-Anaferine	(Mohanraj et al., 2018)
	Withanolide L	(Mohanraj et al., 2018)		(2R)-2-[(1S)-1-[(8R,9S,10R,13S,14R,17S)-14,17-dihydroxy-10,13-dimethyl-1-oxo-4,7,8,9,11,12,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-1-hydroxyethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	(Mohanraj et al., 2018)
	Withanolide H	(Mohanraj et al., 2018)		Sitoindoside IX	(Garg et al., 2018)
	Withanolide I	(Mohanraj et al., 2018)		Anahygrine	(Garg et al., 2018)
	Withanolide K	(Mohanraj et al., 2018)		4-Hydroxy-1,26-dioxo-5,6:22,26-diepoxyergosta-2,24-dien-27-yl 6-o-hexadecanoylhexopyranoside	(Mohanraj et al., 2018)
	Withanolide J	(Mohanraj et al., 2018)		Coagulin Q	(Mohanraj et al., 2018; (Zhao et al., 2002)
	Withanolide A	(Mohanraj et al., 2018; Khajuria et al., 2004; Misra et al., 2005; Pramanick et al., 2008; Soman et al., 2012; Doma et al., 2012)		Withanoside IV	(Mohanraj et al., 2018; Tohda et al., 2005)
	Withanoside X	(Mohanraj et al., 2018; Tong et al., 2011)		Withanoside VI	(Mohanraj et al., 2018; Tohda et al., 2005)
	Withanolide B	(Mohanraj et al., 2018; Khajuria et al., 2004; Pramanick et al., 2008)		Withanolide B	(Mohanraj et al., 2018; Khajuria et al., 2004)
	(2R)-2-[(1S)-1-[(8R,9S,10R,13S,14R,17S)-14,17-dihydroxy-10,13-dimethyl-1-oxo-4,7,8,9,11,12,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-1-hydroxyethyl]-4,5-dimethyl-2,3-dihydropyran-6-one	(Mohanraj et al., 2018)	**Seed**	Withaferin A	(Mohanraj et al., 2018)
	Sitoindoside IX	(Mohanraj et al., 2018; Jayaprakasam et al., 2003)		Anahygrine	(Mohanraj et al., 2018)

	Physagulin-d	(Mohanraj et al., 2018; Jayaprakasam et al., 2003)	**Stem**	Withaferin A	(Mohanraj et al., 2018; Khajuria et al., 2004; Mirjalili et al., 2009)
	Viscosalactone B	(Mohanraj et al., 2018; Jayaprakasam et al., 2003)		Withanolide B	(Mohanraj et al., 2018; Khajuria et al., 2004)
	Withanoside IV	(Mohanraj et al., 2018; Jayaprakasam et al., 2003)		Withanolide A	(Mohanraj et al., 2018; Khajuria et al., 2004)
Root	Withasomidienone	(Mohanraj et al., 2018)		Withanone	(Mohanraj et al., 2018; Khajuria et al., 2004)
	Withanolide M	(Mohanraj et al., 2018)			
	Withanolide D	(Mohanraj et al., 2018; Zhao et al., 2002)			

Pharmacological Effects of *Withania somnifera* on GBM Cell Behavior:

Effect of *Withania somnifera* on Proliferation and Migration of GBM cells:

Alcoholic leaf extracts of WS, Withaferin-A, Withanone, and Withanolide-A have demonstrated the ability to inhibit the growth or proliferation of glioma cells in a dose-dependent manner established in mice and human cell lines. Under moderate dosage administration, the growths of both human and mice cells were found to be severely arrested at S and G2/M phase by all four treatments with maximum effect within 48 to 72 hours. Strong reduction in the migration of mice glioma cells and human glioma cells were found to be more sensitive than mice (Shah et al., 2009). Withaferin-A also has the property to arrest cell growth at the G2/M phase in the human GBM cell line in low doses between 12 to 48 hours (Tang et al., 2020). Withaferin-A and AshwaMAX (a root extract with 4.3% w/w Withaferin-A content) inhibited the formation of neurosphere even in patient-derived glioblastoma cell lines while being treated in a dose-dependent manner within 24 hours of treatment. They also exhibited anticancer potentials in decreasing cell viability by almost 4-folds to a fewer than 20% ± 10 viable glioblastoma cells. The same study displayed the effect of AshwaMAX on nude mice with intracranial xenografts of GBM. Three types of GBM cells were used for xenografting, viz. U87-MG, GBM2, and GBM39, wherein GBM2 and GBM39 were patient-derived cell lines. The growth rate of tumor stabilized within 25 to 28 days of implantation, and subsequently 40 mg/kg/day AshwaMAX was delivered orally on every other day. All mice with U87-MG implantations experienced huge weight loss and therefore had to be sacrificed immediately after a month of surgical implantations. However, the GBM39 xenograft mice survived a month longer. In these cases, the growth of GBM cells were observed to be inhibited to some extent, nonetheless their migration could not be halted. For GBM2 xenograft mice, the tumors began to regress rapidly within one week of initiation of treatment and almost disappeared in one month. Surprisingly, after one-month, GBM reappeared due to resistance or migration of cells and76% of the GBM2 xenograft mice survived 7 months following implantation (Chang et al., 2016).

ASH-WEX (a leaf extract containing withanolides with Withaferin A as its major constituent in distilled water) has been found to be effective against C6 rat glioma intracranial allograft in Wistar strain male albino rat. Such rats with C6 intracranial allograft were administered with ASH-WEX equivalent to 140 mg/kg/day for consecutive 21 days. Reduced activity of MMP2 and MMP9 activities were however found following ASH-WEX treatment (Kataria et al., 2016), which further indicated the inhibition of metastasis of respective cancer cells.

Effect of *Withania somnifera* on GBM Cell Differentiation:

Low dosages of alcoholic leaf extracts of WS, Withaferin-A, Withanone, and Withanolide-A led to morphological alterations in cells that ranged from polygonal structure with a small number of cytoplasmic processes to spindle form with lengthy cytoplasmic processes, resembling those generally seen in differentiated astrocytic cells (Shah et al., 2009). Aqueous leaf extracts of WS and Withanone induced differentiation in rat glioblastoma cells at low doses (Shah et al., 2015). Cucurbitacin (*Helicteres angustifolia*) - Withanone combined application at low doses caused differentiation in rat glioma cells (Garg et al., 2018) (Table 2).

Effect of *Withania somnifera* on Apoptosis of GBM Cells:

High doses of alcoholic leaf extracts of WS, WA, Withanone, and Withanolide - A have the property to induce apoptosis in both mice and human glioma cells (Shah et al., 2009). WA has been shown to display apoptotic activity on GBM cells. Following 6 hours of WA treatment, the expression of the pro-apoptotic proteins, Bim and Bad, were dramatically elevated; expression of the anti-apoptotic proteins BclxL and Bcl2 were marginally altered, along with the pro-apoptotic proteins, Bak and Bax, further indicating that the intrinsic apoptotic pathways might have initiated by the main regulators, Bim and Bad. Silencing Bim and Bad expressions alternately enhanced the viability of WA-treated GBM cell lines, thus validating the role of WA in the suppression of GBM cells (Tang et al., 2020) (Table 2).

Table 2: Cellular reactions of glioblastoma cells in response to *Withania somnifera* treatment.

Cell line	Bioactive Compound (IC50 value)	Effect	Reference
C6 (rat glioma)	Alcoholic leaf extract (low dose)	Differentiation	(Shah et al., 2009)
	Alcoholic leaf extract (5 µg/mL)	Growth inhibition	(Shah et al., 2009)
	Alcoholic leaf extract (high dose)	Apoptosis	(Shah et al., 2009)
C6 (rat glioma)	Withaferin-A (low dose)	Differentiation	(Shah et al., 2009)
	Withaferin-A (0.2 µmol/L)	Growth inhibition	(Shah et al., 2009)
	Withaferin-A (high dose)	Apoptosis	(Shah et al., 2009)
	Withanone (low dose)	Differentiation	(Shah et al., 2009)
	Withanone (40 µg/mL)	Growth inhibition	(Shah et al., 2009)
	Withanone (high dose)	Apoptosis	(Shah et al., 2009)
	Withanolide-A (low dose)	Differentiation	(Shah et al., 2009)
	Withanolide-A (35 µg/mL)	Growth inhibition	(Shah et al., 2009)
	Withanolide-A (high dose)	Apoptosis	(Shah et al., 2009)

	Cucurbitacin (Helicteres angustifolia) (5 nmol/L) Withanone (2.5 µmol/L)	Differentiation	(Tang et al., 2020)
YKG1 (human glioma)	Alcoholic leaf extract (low dose)	Differentiation	(Shah et al., 2009)
	Alcoholic leaf extract (2.5µg/mL)	Growth inhibition	(Shah et al., 2009)
	Alcoholic leaf extract (high dose)	Apoptosis	(Shah et al., 2009)
	Withaferin-A (low dose)	Differentiation	(Shah et al., 2009)
	Withaferin-A (0.1 µmol/L)	Growth inhibition	(Shah et al., 2009)
	Withaferin-A (high dose)	Apoptosis	(Shah et al., 2009)
	Withanone (low dose)	Differentiation	(Shah et al., 2009)
	Withanone (30 µg/mL)	Growth inhibition	(Shah et al., 2009)
	Withanone (high dose)	Apoptosis	(Shah et al., 2009)
	Withanolide-A (low dose)	Differentiation	(Shah et al., 2009)
	Withanolide-A (20 µg/mL)	Growth inhibition	(Shah et al., 2009)
	Withanolide-A (high dose)	Apoptosis	(Shah et al., 2009)
	Withaferin-A (0.01 µmol/L) + Withanone (5 µg)	Best Differentiation	(Shah et al., 2009)
U87	Withaferin-A (4.61 µmol/L)	Growth inhibition	(Shah et al., 2009)
	Withaferin-A (10 µmol/L)	Apoptosis	(Tang et al., 2020)
U87-MG	Withaferin-A (0.31 µmol/L)	Growth inhibition	(Garg et al., 2018)
	AshwaMAX (1.40 µmol/L)		
U251	Withaferin-A (1.37 µmol/L)	Growth inhibition	(Tang et al., 2020)
	Withaferin-A (10 µmol/L)	Apoptosis	(Tang et al., 2020)
HA1800	Withaferin-A (9.13 µmol/L)	Growth inhibition	(Tang et al., 2020)
GBM2 (Patient Derived) (Human parietal-cortical glioblastoma cells)	Withaferin-A (0.28 µmol/L)	Growth inhibition	(Chang et al., 2016)
	AshwaMAX (root extract containing 4.3% w/w Withaferin-A) (0.19 µmol/L)	Growth inhibition	(Chang et al., 2016)
GBM39 (Patient Derived) (Human parietal-cortical glioblastoma cells)	Withaferin-A (0.25 µmol/L)	Growth inhibition	(Chang et al., 2016)
	AshwaMAX (root extract containing 4.3% w/w Withaferin-A) (0.22 µmol/L)	Growth inhibition	(Chang et al., 2016)

Therefore, Ashwagandha is a plant that has been well-researched and studied against GBM. It not only provides a promising scope for generic treatment of GBM but also opens an avenue for a precise or personalized line of treatment by targeting specific proteins whose overexpression leads to GBM progression.

Effect of *Withania somnifera* on Tumor Suppression Pathways Associated with GBM:

The p53 tumor suppressor pathway is activated by the alcoholic leaf extracts of WS and Withanone (i-Factor), which results in the selective death of cancer cells (Shah et al., 2009; Widodo et al., 2007; Widodo et al., 2008). Treatment with WS greatly reduced the expression of cytokines that are pro-inflammatory such as Interleukin (IL)-1β, IL-6, chemokine IL-8, STAT-2, and Heat shock protein, HSP-70. On the other hand, reciprocal induction was observed for cyclin D and cyclin C, p38MAPK, caspase 6, and PI3K. Treatment with WS dramatically changed the JAK-STAT pathway, which controls MAP kinase signaling as well as the apoptotic process

(Shah et al., 2009; Aalinkeel et al., 2010). Furthermore, by modulating the p21-Bad pathway, Withaferin-A caused apoptosis in human GBM cell lines which is independent of p53. In addition, ATF4, XBP1, and CHOP were upregulated by WA which induced p21 expression. Therefore, WS and its phytochemicals can actually modulate several signaling pathways in order to induce apoptosis in human GBM cells as given in Figure 4 (Tang et al., 2020).

Discussion:

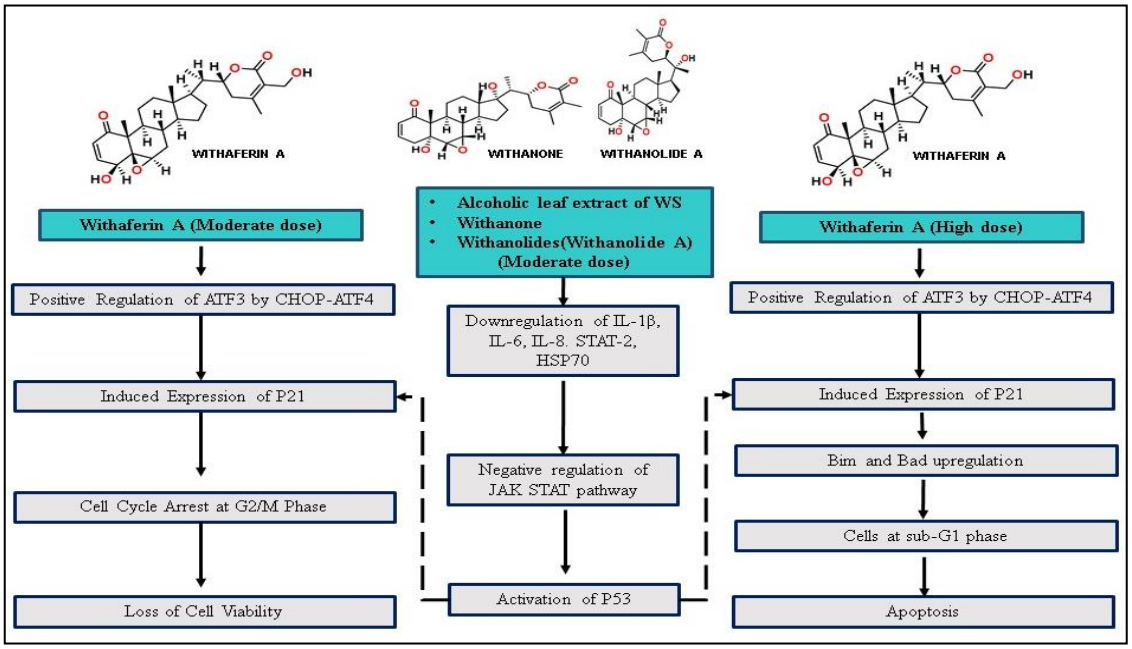

Figure 4. Mechanism of action of Withania somnifera specific phytochemicals in Glioblastoma Multiforme Management

Glioblastoma multiforme is regarded as the worst form of Glioblastoma with an exceptionally low survival rate with existing therapies indicating limited effectiveness for long-term patient survival. Due to the variety of molecular alterations in GBM cases, drug-based treatment has a significant challenge as no single therapeutic medicine can target GBM (Madhu et al., 2023). The presence of both inter-tumor and intra-tumor heterogeneity in GBM cases poses difficulties for diagnosis and therapy. Phytochemicals, being an integral part of our regular diet, hold great potential in disease management if used wisely. There have been instances of several Indian medicinal plants which have plant-specific phytochemicals, viz., Ashwagandha, cinnamon, black pepper, turmeric etc. The molecular targets that are now being aimed at in clinical trials with drugs may also be focused on by phytochemicals from WS. Furthermore, phytochemical therapy has the potential to reduce and alleviate adverse side effects of anticancer drugs. WS and its phytochemicals have been found effective against a broad range of cancers like skin carcinoma (Kumar et al., 2017; Bhattacharjee, 2020; Sinkar and Samarth, 2019; Li et al., 2016), breast cancer (Umair et al., 2019; Dar et al., 2019; Thaiparambil et al., 2011; Sarkar et al., 2022; Rah et al., 2016; Gowtham et al., 2022; Kim et al., 2014; Jawarneh et al., 2022; Li et al., 2015), hepatocellular carcinoma, colon cancer, non-small cell lung cancer (Ahmed et al., 2022; Madhu

et al., 2022; Hsu et al., 2019), pancreatic cancer (Li et al., 2015; Yu et al., 2010), lymphoma (McKenna et al., 2015), prostate cancer (Setty et al., 2017; Srinivasan et al., 2007; Sanyal et al., 2018; Yang et al., 2007), thyroid carcinoma (Samadi et al., 2010) ovarian cancer (Kakar et al., 2014), melanoma (Halder et al., 2015) myeloma (Li et al., 2022), leukemia (Sanchez-Martin et al., 2017), and glioblastoma (Kataria et al., 2016; Shah et al., 2009; Tang et al., 2020; Chang et al., 2016; Garg et al., 2018) *in-vitro* and *in-vivo*. Few of the cancer types share some common pathways i.e., the JAK-STAT signaling study in prostate cancer cells. Presently, several reports suggest that abnormal JAK/STAT signaling plays a key role in glioma genesis and resistance to treatment (Aakinkeel et al., 2010; Ou et al., 2021). Negative regulation of the JAK-STAT pathway in turn stimulates the p53 pathway. Since a major cause of cancer is inactivation of p53, a mechanism of action of WS and its phytochemicals in GBM management is highly focused on the activation of p53 and p21. Alcoholic leaf extracts of WS, Withanone, and Withanolides force negative regulation of the JAK-STAT pathway which in turn stimulates activation of p53 followed by induced p21 expression resulting in growth inhibition of GBM cells (Shah et al., 2009). Withaferin A induces expression of p21 independent of p53, by positive regulation of ATF3 by CHOP-ATF4 (Tang et al., 2020). The activation of ATF4-ATF3-CHOP axis is important for the WA based therapy involving inhibition of cell cycle and apoptosis at the G2/M level (Kumar et al., 2023). The WA in low doses cause differentiation, in moderate doses they cause growth inhibition, and in high doses, they induce cell apoptosis by upregulating pro-apoptotic proteins Bim and Bad (Fig. 4). Though the *in-vivo* model depicted an increase in survival time, it also displayed formation of resistant sublines of GBM and migration towards microenvironments where WS root extracts were not effective (Chang et al., 2016). While evaluating the capability of WS phytochemicals in targeting signaling pathways, the structural advantages of steroidal lactones have paved the way for their affinity towards the target. Molecular docking, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), and molecular dynamics studies indicate stronger affinity and inhibition of the regulating pathways in Glioblastoma stem cells (Dhami et al., 2017; Swati et al., 2023; Arsalan et al., 2023; Lee et al., 2022). The utilization of WS's phytocompounds presents an opportunity for the development of targeted medications in the fight against GBM. Recent research has also paved the way for fractionalization of many novel glycoside compounds from WS, providing experimental evidence by testing its anti-angiogenic properties against various tumors such as Hepatocellular carcinoma (Lee et al., 2022). Though there have been limited studies on the high-dosage side effects of Withanolides, few reports have suggested the possibility of liver damage in human patients occasionally with increased and regular dosages of WS extracts, with Withanone being the source of DNA damage. Although, cases showed quick recovery with drug withdrawal (Malik et al., 2013; Siddiqui et al., 2021). Persistent progress and discoveries in precision medicine, particularly in the realm of GBM treatments, it is essential to explore the WS-specific extracts domain. Extending the scope of research can enhance the prognosis and overall condition of GBM patients. The scientific community's continued investigation and the

potential breakthroughs offered by precision medicine hold great promise for revolutionizing GBM treatment (Iyer et al., 2023).

Conclusion:

This study provides a complete insight on the use of Withanolides as a potential and unexplored approach for the treatment of Glioblastoma multiforme. The study focuses on different withanolides present in WS by highlighting the preliminary results obtained from their screening against GBM and other cancer cell lines. The massive scope for several other formulations like AshwaMAX that focus merely on the WA content has shown inhibition of growth in glioblastoma cells. The effects of other WS-specific phytochemicals and their potential on current clinical target molecules of GBM are yet to be explored. The lack of pre-clinical or *in-vivo* studies on the effect of WS phytochemicals in glioblastoma multiforme management opens a scope for in-depth studies and research with WS-specific phytochemicals. With developed ample pre-clinical research data demonstrating favorable outcomes, along with therapeutic effects of WS phytochemicals on resistant GBM sublines and tumor microenvironments, there is a considerable prospect for WS to be recognized as a revolutionary advancement in glioblastoma multiforme management. Summarizing, the combinational or synergistic effect of the *Withania*-specific phytochemicals could be another alternative approach to the therapeutic use of WS in glioblastoma multiforme therapy. As the quest to find a novel and cheap therapeutic strategy against GBM persists, interventions by means of various extracts and phytochemicals from WS may ultimately emerge as a precise management system for GBM.

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Data availability statement:

The data utilized in this review article are obtained from publicly accessible sources or referenced publications. The original resources can be accessed through the provided references for further analysis or replication purposes.

Author Contributions:

All authors participated in data curation, developing the methodology and writing the original draft of the article. Sohini Kulavi, Karan Iyer, and Debajit Dhar were responsible for conceptualization, formal analysis, investigation and reviewing, and editing subsequent versions of the article. Sohini Kulavi was responsible for visualization, while Arnab Kumar Ghosh and Jaya Bandyopadhyay were solely responsible for supervision and validation of the work.

References:

Aalinkeel, R., Hu, Z., Nair, B. B., Sykes, D. E., Reynolds, J. L., & Mahajan, S. D. (2010). Genomic analysis highlights the role of the JAK-STAT signaling in the anti-proliferative effects of dietary flavonoid-'ashwagandha' in prostate cancer cells. *Evid Based Complement Alternat Med*., 7(2), 177-87.

Ahmed, H. A., & El-Darier, S. M. (2022). Phytochemistry, allelopathy and anticancer potentiality of Withania somnifera (L.) Dunal (Solanaceae). *Braz J Biol*., 84, e263815.

Ali, S. A., & Meitei, K. V. (2005). Withania somnifera root extracts induce skin darkening in wall lizard melanophores via stimulation of cholinergic receptors. *Nat Prod Res*., 26(17), 1645-8.

Andallu, B., & Radhika, B. (2000). Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root. *Indian J. Exp Biol*., *38*(6), 607-9.

Arsalan, B., Masarat, N., Nahida, T., Suhaib, A., & Mehrose, A. (2023). An updated review on phytochemistry and molecular targets of Withania somnifera (L.) Dunal (Ashwagandha). *Frontiers in Pharmacology*, *14*, 1663-9812.

Batchelor, T. T., Mulholland, P., Neyns, B., Nabors, L. B., Campone, M., & Wick, A. (2013). Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol*., 31(26), 3212-8.

Bhattacharjee, P. (2020). Effects of cold stress, alprazolam and phytomedicine in combination with stress on blood glucose and haematogical parameter of the male albino rat. *Int. J. Exp. Res. Rev.*, *22*, 37-44. https://doi.org/10.52756/ijerr.2020.v22.005

Brennan, C. W., Verhaak, R. G., McKenna, A., Campos, B., Noushmehr, H., & Salama, S. R. (2013). The somatic genomic landscape of glioblastoma. *Cell*, *155*(2), 462-77.

Chang, E., Pohling, C., Natarajan, A., Witney, T. H., Kaur, J., & Xu, L. (2016). AshwaMAX and Withaferin A inhibits gliomas in cellular and murine orthotopic models. *J Neurooncol*., *126*(2), 253-64.

Dar, P. A., Mir, S. A., Bhat, J. A., Hamid, A., Singh, L. R., & Malik, F. (2019). An anti-cancerous protein fraction from Withania somnifera induces ROS-dependent mitochondria-mediated apoptosis in human MDA-MB-231 breast cancer cells. *Int J Biol Macromol*., *135*, 77-87.

Devi, P. U., Akagi, K., Ostapenko, V., Tanaka, Y., & Sugahara, T. (1996). Withaferin A: a new radiosensitizer from the Indian medicinal plant Withania somnifera. *Int J Radiat Biol*., 69(2), 193-7.

Dhami, J., Chang, E., & Gambhir, S. S. (2017). Withaferin A and its potential role in glioblastoma (GBM). *Journal of neuro-oncology*, 131(2), 201–211.

Di Stefano, A. L., Fucci, A., Frattini, V., Labussiere, M., Mokhtari, K., & Zoppoli, P. (2015). Detection, characterization, and inhibition of FGFR-TACC fusions in IDH wild-type glioma. *Clin Cancer Res*., 21(14), 3307-17.

Doma, M., Abhayankar, G., Reddy, V. D., & Kavi Kishor, P. B. (2012). Carbohydrate and elicitor enhanced withanolide (withaferin A and withanolide A) accumulation in hairy root cultures of Withania somnifera (L.). *Indian J Exp Biol*., *50*(7), 484-90.

Dubey, S., Singh, M., Nelson, A., & Karan, D. (2021). A Perspective on *Withania somnifera* Modulating antitumor Immunity in Targeting Prostate Cancer. *J Immunol Res*., *2021*, 9483433.

Felsberg, J., Hentschel, B., Kaulich, K., Gramatzki, D., Zacher, A., & Malzkorn, B. (2017). Epidermal growth factor receptor variant III (EGFRvIII) positivity in *EGFR*-amplified glioblastomas: prognostic role and comparison between primary and recurrent tumors. *Clin Cancer Res*., *23*(22), 6846-55.

Ferguson, S. D., Zhou, S., Huse, J. T., de Groot, J. F., Xiu, J., & Subramaniam, D. S. (2018). Targetable gene fusions associate with the IDH wild-type astrocytic lineage in adult gliomas. *J Neuropathol Exp Neurol*., *77*(6), 437-42.

Friday, B. B., Anderson, S. K., Buckner, J., Yu, C., Giannini, C., & Geoffroy, F. (2012). Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. *Neuro Oncol*., *14*(2), 215-21.

Garg, S., Kaul, S. C., & Wadhwa, R. (2018). Anti-stress and glial differentiation effects of a novel combination of cucurbitacin B and withanone (CucWi-N): experimental evidence. *Ann Neurosci*., *25*(4), 201-9.

Gowtham, H. G., Murali, M., Singh, S. B., Shivamallu, C., Pradeep, S., & Shivakumar, C. S. (2022). Phytoconstituents of Withania somnifera unveiled Ashwagandhanolide as a potential drug targeting breast cancer: investigations through computational, molecular docking and conceptual DFT studies. *PLOS ONE*, *17*(10), e0275432.

Halder, B., Singh, S., & Thakur, S. S. (2015). Withania somnifera Root Extract Has Potent cytotoxic Effect against Human Malignant Melanoma Cells. *PLOS ONE*, *10*(9), e0137498.

Hanif, F., Muzaffar, K., Perveen, K., Malhi, S. M., & Simjee, S. H. U. (2017). Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pac J Cancer Prev*., *18*(1), 3-9.

Hegi, M. E., Diserens, A. C., Gorlia, T., Hamou, M. F., de Tribolet, N., & Weller. M. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*., *352*(10), 997-1003.

Hegi, M. E., Genbrugge, E., Gorlia, T., Stupp, R., Gilbert, M. R., & Chinot, O. L. (2019). *MGMT* promoter methylation cutoff with safety margin for selecting glioblastoma patients into trials omitting temozolomide: A pooled analysis of four clinical trials. *Clin Cancer Res*., *25*(6),1809-16.

Horn, S., Figl, A., Rachakonda, P. S., Fischer, C., Sucker, A., & Gast, A. (2013). Tert promoter mutations in familial and sporadic melanoma. *Science*, *339*(6122), 959-61.

Hsu, J. H. M., Chang, P. M. H., Cheng, T. S., Kuo, Y. L., Wu, A. T. H., & Tran, T. H. (2019). Identification of withaferin A as a potential candidate for anti-cancer therapy in non-small cell lung cancer. *Cancers*, *11*(7), 1003.

Islam, M. K., Saha, S., Mahmud, I., Mohamad, K., Awang, K., & Jamal Uddin, S. J. (2014). An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. *J Ethnopharmacol*., *151*(2), 921-30.

Iyer, K., Saini, S., Bhadra, S., Kulavi, S., & Bandyopadhyay, J. (2023). Precision medicine advancements in glioblastoma: A systematic review. *Biomedicine*, *13*(2), 1.

Jawarneh, S., & Talib, W. H. (2022). Combination of Ashwagandha water Extract and Intermittent Fasting as a Therapy to Overcome cisplatin Resistance in Breast Cancer: an *in vitro* and *in vivo* Study. *Front Nutr*., *9*, 863619.

Jayaprakasam, B., Zhang, Y., Seeram, N. P., & Nair, M. G. (2003). Growth inhibition of human tumor cell lines by withanolides from Withania somnifera leaves. *Life Sci*., 74(1), 125-32.

Kakar, S. S., Ratajczak, M. Z., Powell, K. S., Moghadamfalahi, M., Miller, D. M., & Batra, S. K. (2014). Withaferin a alone and in combination with cisplatin suppresses growth and metastasis of ovarian cancer by targeting putative cancer stem cells. *PLOS ONE*, *9*(9), e107596.

Kataria, H., Kumar, S., Chaudhary, H., & Kaur, G. (2016). Withania somnifera Suppresses Tumor Growth of Intracranial Allograft of glioma Cells. *Mol Neurobiol*., *53*(6), 4143-58.

Kataria, H., Wadhwa, R., Kaul, S. C., & Kaur, G. (2013). Withania somnifera water extract as a potential candidate for differentiation based therapy of human neuroblastomas. *PLOS ONE*, *8*(1), e55316.

Khajuria, R. K., Suri, K. A., Gupta, R. K., Satti, N. K, Amina, M., & Suri, O. P. (2004). Separation, identification, and quantification of selected withanolides in plant extracts of Withania somnifera by HPLC-UV(DAD)-positive ion electrospray ionisation-mass spectrometry. *J Sep Sci*., *27*(7-8), 541-6.

Killela, P. J., Reitman, Z. J., Jiao, Y., Bettegowda, C., Agrawal, N., & Diaz, L. A. Jr. (2013). TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci U S A*, *110*(15), 6021-6.

Kim, S. H., & Singh, S. V. (2014). Mammary cancer chemoprevention by withaferin AIs accompanied by in vivo suppression of self-renewal of cancer stem cells. *Cancer Prev Res (Phila)*, *7*(7), 738-47.

Kong, X. T., Nguyen, N. T., Choi, Y. J., Zhang, G., Nguyen, H. N., & Filka, E. (2018). Phase 2 study of bortezomib combined with temozolomide and regional radiation therapy for upfront treatment of patients with newly diagnosed glioblastoma multiforme: safety and efficacy assessment. *Int J Radiat Oncol Biol Phys*., *100*(5), 1195-203.

Korshunov, A., Chavez, L., Sharma, T., Ryzhova, M., Schrimpf, D., & Stichel, D. (2018). Epithelioid glioblastomas stratify into established diagnostic subsets upon integrated molecular analysis. *Brain Pathol*., *28*(5), 656-62.

Kour, K., Pandey, A., Suri, K. A., Satti, N. K., Gupta, K. K., & Bani, S. (2009). Restoration of stress-induced altered T cell function and corresponding cytokines patterns by Withanolide A. *Int Immunopharmacol*., *9*(10), 1137-44.

Kulkarni, S. K., & Dhir, A. (2008). Withania somnifera: an Indian ginseng. *Prog Neuropsychopharmacol Biol Psychiatry*, *32*(5), 1093-105.

Kumar, S., Mathew, S. O., Aharwal, R. P., Tulli, H. S., Mohan, C. D., Sethi, G., Ahn, K. S., Webber, K., Sandhu, S. S., & Bishayee, A. (2023). Withaferin A: A Pleiotropic Anticancer Agent from the Indian Medicinal Plant *Withania somnifera* (L.) Dunal. *Pharmaceuticals (Basel, Switzerland)*, *16*(2), 160.

Kumar, S., Singh, B. S., & Singh, R. B. (2017). Ethnomedicinal plants uses to cure different human diseases by rural and tribal peoples of Hathras district of Uttar Pradesh. *J Pharmacogn Phytochem.*,*6*(2), 346-8.

Lee, D., Yu, J. S., Ha, J. W., Lee, S. R., Lee, B. S., Kim, J. C., Kim, J. K., Kang, K. S., & Kim, K. H. (2022). Antitumor Potential of Withanolide Glycosides from Ashwagandha (*Withania somnifera*) on Apoptosis of Human Hepatocellular Carcinoma Cells and Tube Formation in Human Umbilical Vein Endothelial Cells. *Antioxidants (Basel, Switzerland)*, *11*(9), 1761.

Legler, J. M., Ries, L. A., Smith, M. A., Warren, J. L., Heineman, E. F., & Kaplan, R. S. (1999). Cancer surveillance series [corrected]: Brain and other central nervous system cancers: recent trends in incidence and mortality. *J Natl Cancer Inst*., *91*(16), 1382-90.

Li, L., Niu, B., Zhang, W., Hou, L., & Zheng, Y. (2022). Withaferin A inhibits cell proliferation of U266B1 and IM-9 human myeloma cells by inducing intrinsic apoptosis. *Acta Biochim Pol*., *69*(1), 197-203.

Li, W., Zhang, C., Du, H., Huang, V., Sun, B., & Harris, J. P. (2016). Withaferin A suppresses the up-regulation of acetyl-coA carboxylase 1 and skin tumor formation in a skin carcinogenesis mouse model. *Mol Carcinog*, *55*(11), 1739-46.

Li, X., Zhu, F., Jiang, J., Sun, C., Wang, X., & Shen, M. (2015). Synergistic antitumor activity of withaferin A combined with oxaliplatin triggers reactive oxygen species-mediated inactivation of the PI3K/AKT pathway in human pancreatic cancer cells. *Cancer Lett*., *357*(1), 219-30.

Madhu, N.R., Sarkar, B., Biswas, P., Roychoudhury, S., Behera, B.K., & Acharya, C.K. (2023). Therapeutic potential of melatonin in glioblastoma: Current knowledge and future prospects. Biomarkers in Cancer Detection and Monitoring of Therapeutics, Volume-2. Elsevier Inc., pp. 371-386. ISBN 978-0-323-95114-2. https://doi.org/10.1016/B978-0-323-95114-2.00002-9

Madhu, N.R., Sarkar, B., Roychoudhury, S., Behera, B.K. (2022). Melatonin Induced in Cancer as a Frame of Zebrafish Model. © Springer Nature Singapore Pte Ltd. 2022, S. Pathak et al. (eds.), Handbook of Animal Models and its Uses in Cancer Research, pp. 1-18. ISBN: 978-981-19-1282-5 https://doi.org/10.1007/978-981-19-1282-5_61-1

Malik, T., Pandey, D. K., & Dogra, N. (2013). Ameliorative potential of aqueous root extract of *Withania somnifera* against paracetamol induced liver damage in mice. *Pharmacologia*, *4*, 89–94.

McKenna, M. K., Gachuki, B. W., Alhakeem, S. S., Oben, K. N., Rangnekar, V. M., & Gupta, R. C. (2015). Anti-cancer activity of withaferin A in B-cell lymphoma. *Cancer Biol Ther*. 16(7), 1088-98.

Mirjalili, M. H., Fakhr-Tabatabaei S. M., Alizadeh, H., Ghassempour, A., & Mirzajani, F. (2009). Genetic and withaferin A analysis of Iranian natural populations of Withania somnifera and W. *coagulans* by RAPD and HPTLC. *Nat Prod Commun*., *4*(3), 337-46.

Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev*., 5(4), 334-46.

Misra, L., Lal, P., Sangwan, R. S., Sangwan, N. S., Uniyal, G. C., & Tuli, R. (2005). Unusually sulfated and oxygenated steroids from Withania somnifera. *Phytochemistry*, 66(23), 2702-7.

Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., & Mangalapandi, P. (2018). IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. *Sci Rep*., *8*(1), 4329.

Mondal, S., Bhattacharya, K., Mallick, A., Sangwan, R., & Mandal, C. (2012). Bak compensated for Bax in p53-null cells to release cytochrome c for the initiation of mitochondrial signaling during Withanolide D-induced apoptosis. *PLOS ONE, 7*(3), e34277.

Munagala, R., Kausar, H., Munjal, C., & Gupta, R. C. (2011). Withaferin A induces p53-dependent apoptosis by repression of HPV oncogenes and upregulation of tumor suppressor proteins in human cervical cancer cells. *Carcinogenesis, 32*(11), 1697-705.

Nagella, P., & Murthy, H. N. (2010). Establishment of cell suspension cultures of Withania somnifera for the production of withanolide A. *Bioresour Technol*., 101(17). 6735-9.

Nakajima, H., Wakabayashi, Y., Wakamatsu, K., & Imokawa, G. (2011). An extract of Withania somnifera attenuates endothelin-1-stimulated pigmentation in human epidermal equivalents through the interruption of PKC activity within melanocytes. *Phytother Res*., 25(9),1398-411.

Ou, A., Ott, M., Fang, D., & Heimberger, A. B. (2021). The role and therapeutic targeting of JAK/STAT signaling in glioblastoma. *Cancers (Basel),* 13(3), 437.

Plate, K. H., Breier, G., Weich, H. A., Mennel, H. D., & Risau, W. (1994). Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. *Int J Cancer*, *59*(4), 520-9.

Pramanick, S., Roy, A., Ghosh, S., Majumder, H. K., & Mukhopadhyay, S. (2008). Withanolide Z, a new chlorinated withanolide from Withania somnifera. *Planta Med*., *74*(14),1745-8.

Rah, B., Amin, H., Nayak, D., Chakraborty, S., Rawoof, A., & Mintoo, M. J. (2016). Dual modulation of Ras-Mnk and PI3K-AKT-mTOR pathways: a novel c-FLIP inhibitory mechanism of 3-AWA mediated translational attenuation through dephosphorylation of eIF4E. *Sci Rep*., 6(1), 1-16.

Roth. P., Silginer, M., Goodman, S. L., Hasenbach, K., Thies, S., & Maurer, G. (2013). Integrin control of the transforming growth factor-β pathway in glioblastoma. *Brain*., *136*(2), 564-76.

Samadi, A. K., Cohen, S. M., Mukerji, R., Chaguturu, V., Zhang, X., Timmermann, B. N., et al. (2012). Natural withanolide withaferin A induces apoptosis in uveal melanoma cells by suppression of Akt and c-MET activation. *Tumour Biol*., 33(4), 1179-89.

Samadi, A. K., Mukerji, R., Shah, A., Timmermann, B. N., & Cohen, M. S. (2010). A novel RET inhibitor with potent efficacy against medullary thyroid cancer in vivo. *Surgery*, 148(6), 1228-36.

Sanchez-Martin, M., Ambesi-Impiombato, A., Qin, Y., Herranz, D., Bansal, M., Girardi, T., et al. (2017). Synergistic antileukemic therapies in NOTCH1-induced T-ALL. *Proc Natl Acad Sci U S A*, *114*(8), 2006-11.

Sanyal, R., Mallick, S., & Mazumder, A. (2018). Indigenous Knowledge of Ethnic Community on Usage of Kripa (*Lumnitzera racemosa*) and its preliminary screening. *Int. J. Exp. Res. Rev.*, *15*, 44-50. https://doi.org/10.52756/ijerr.2018.v15.007

Sarkar, B., Bhattacharya, P., Yen Chen, C., Maity, J., & Biswas, T. (2022). A comprehensive characterization and therapeutic properties in ripened Noni fruits (*Morinda citrifolia* L.). *Int. J. Exp. Res. Rev.*, *29*, 10-32. https://doi.org/10.52756/ijerr.2022.v29.002

Setty Balakrishnan, A., Nathan, A. A., Kumar, M., Ramamoorthy, S., & Ramia Mothilal, S. K. (2017). Withania somnifera targets interleukin-8 and cyclooxygenase-2 in human prostate cancer progression. *Prostate Int*., *5*(2), 75-83.

Shah, N., Kataria, H., Kaul, S. C., Ishii, T., Kaur, G., & Wadhwa, R. (2009). Effect of the alcoholic extract of Ashwagandha leaves and its components on proliferation, migration, and differentiation of glioblastoma cells: combinational approach for enhanced differentiation. *Cancer Sci*., *100*(9),1740-7.

Shah, N., Singh, R., Sarangi, U., Saxena, N., Chaudhary, A., & Kaur, G. (2015). Combinations of Ashwagandha leaf extracts protect brain-derived cells against oxidative stress and induce differentiation. *PLOS ONE*, 10(3):e0120554.

Sharma, P., Mondal, H., Mondal, S., & Majumder, R. (2022). Recent updates on the role of phytochemicals in the treatment of glioblastoma multiforme. *Journal of Cancer Research and Therapeutics*.

Siddique, A. A., Joshi, P., Misra, L., Sangwan, N. S., & Darokar, M. P. (2014). 5,6-de-epoxy-5-en-7-one-17-hydroxy withaferin A, a new cytotoxic steroid from Withania somnifera L. Dunal leaves. *Nat Prod Res*., 28(6), 392-8.

Siddiqui, S., Ahmed, N., Goswami, M., Chakrabarty, A., & Chowdhury, G. (2021). DNA damage by Withanone as a potential cause of liver toxicity observed for herbal products of *Withania somnifera* (Ashwagandha). *Current research in toxicology*, *2*, 72–81.

Singh, D., Chan, J. M., Zoppoli, P., Niola, F., Sullivan, R., & Castano, A. (2012). Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science*, *337*(6099), 1231-5.

Sinkar, S. R., & Samarth, V. D. (2019). Medicinal plants used for the treatment of various skin disorders by a rural community in warud region of Maharashtra. *The Pharm Innov J.*, *8*(5), 791-4.

Soman, S., Anju, T. R., Jayanarayanan, S., Antony, S., & Paulose, C. S. (2013). Impaired motor learning attributed to altered AMPA receptor function in the cerebellum of rats with temporal lobe epilepsy: ameliorating effects of *Withania somnifera* and withanolide A. *Epilepsy Behav.*, *27*(3), 484-91.

Soman, S., Korah, P. K., Jayanarayanan, S., Mathew, J., & Paulose, C. S. (2012). Oxidative stress induced NMDA receptor alteration leads to spatial memory deficits in temporal lobe epilepsy: ameliorative effects of Withania somnifera and Withanolide A. *Neurochem Res.*, *37*(9), 1915-27.

Srinivasan, S., Ranga, R. S., Burikhanov, R., Han, S. S., & Chendil, D. (2007). Par-4-dependent apoptosis by the dietary compound withaferin A in prostate cancer cells. *Cancer Res.*, *67*(1), 246-53.

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., & Taphoorn, M. J. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.*, *352*(10), 987-96.

Stupp, R., Taillibert, S., Kanner, A., Read, W., Steinberg, D., Lhermitte, B., et al. (2017). Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: A randomized clinical trial. *JAMA*, *318*(23), 2306-16.

Subbaraju, G. V., Vanisree, M., Rao, C. V., Sivaramakrishna, C., Sridhar, P., & Jayaprakasam, B. (2006). Ashwagandhanolide, a bioactive dimeric thiowithanolide isolated from the roots of Withania somnifera. *J Nat Prod.*, *69*(12), 1790-2.

Swati, K., Quazi, S., Srivastava, R., Panda, S.P., Agrawal, K., Parkash, A., & Kumar, D. (2023). Exploring Silybin B as a FOXM1 Inhibitor in Glioblastoma Stem Cells: Molecular Docking, ADMET, and Molecular Dynamics Approaches.

Szabo, E., Schneider, H., Seystahl, K., Rushing, E. J., Herting, F., Weidner, K. M., et al. (2016). Autocrine VEGFR1 and VEGFR2 signaling promotes survival in human glioblastoma models in vitro and in vivo. *Neuro Oncol.*, 18(9),1242-52.

Szarc vel Szic, K., Op de Beeck, K., Ratman, D., Wouters, A., Beck, I. M., & Declerck, K. (2014). Pharmacological levels of Withaferin A (Withania somnifera) trigger clinically relevant anticancer effects specific to triple negative breast cancer cells. *PLOS ONE*, 9(2), e87850.

Tang, Q., Ren, L., Liu, J., Li, W., Zheng, X., & Wang, J. (2020). Withaferin A triggers G2/M arrest and intrinsic apoptosis in glioblastoma cells via ATF4-ATF3-CHOP axis. *Cell Prolif.*, *53*(1), e12706.

Thaiparambil, J. T., Bender, L., Ganesh, T., Kline, E., Patel, P., & Liu, Y. (2011). Withaferin A inhibits breast cancer invasion and metastasis at sub-cytotoxic doses by inducing vimentin disassembly and serine 56 phosphorylation. *Int J Cancer*, *129*(11), 2744-55.

Tiwari, R., Chakraborty, S., & Saminathan, M. (2014). Ashwagandha [Withania somnifera]: role in safeguarding health, immunomodulatory efects, combating infections and therapeutic application: a review. *J Biol Sci*., *2*, 77-94.

Tohda, C., Kuboyama, T., & Komatsu, K. (2005). Search for natural products related to regeneration of the neuronal network. *Neurosignals*,*14*(1-2), 34-45.

Tong, X., Zhang, H., & Timmermann, B. N. (2011). Chlorinated Withanolides from *Withania somnifera*. *Phytochem Lett*., *4*(4), 411-4.

Umair, M., Altaf, M., Bussmann, R. W., & Abbasi, A. M. (2019). Ethnomedicinal uses of the local flora in Chenab riverine area, Punjab Province Pakistan. *J Ethnobiol Ethnomed*., *15*(1), 7.

Vaishnavi, K., Saxena, N., Shah, N., Singh, R., Manjunath, K., Uthayakumar, M., Kanaujia, S. P., Kaul, S. C., Sekar, K., & Wadhwa, R. (2012). Differential activities of the two closely related withanolides, Withaferin A and Withanone: bioinformatics and experimental evidences. *PloS one*, *7*(9), e44419.

Wang, H. C., Hu, H. H., Chang, F. R., Tsai, J. Y., Kuo, C. Y., & Wu, Y. C. (2019). Different effects of 4β-hydroxywithanolide E and withaferin A, two withanolides from Solanaceae plants, on the Akt signaling pathway in human breast cancer cells. *Phytomedicine*, *53*,213-22.

Wang, R., Zhang, S., Chen, X., Li, N., Li, J., & Jia, R. (2018). EIF4A3-induced circular RNA MMP9 (circMMP9) acts as a sponge of miR-124 and promotes glioblastoma multiforme cell tumorigenesis. *Mol Cancer*, *17*(1), 166.

Wei, Y., Lu, C., Zhou, P., Zhao, L., Lyu, X., Yin, J., et al. (2021). EIF4A3-induced circular RNA ASAP1 promotes tumorigenesis and temozolomide resistance of glioblastoma via NRAS/MEK1/ERK1-2 signaling. *Neuro Oncol*., *23*(4), 611-24.

Weller, M., van den Bent, M., Tonn, J. C., Stupp, R., Preusser, M., Cohen-Jonathan-Moyal, E., et al. (2017). European Association for Neuro-Oncology (EANO) Task Force on Gliomas. European Association for Neuro-Oncology (EANO) guideline on the diagnosis and treatment of adult astrocytic and oligodendroglial gliomas. *Lancet Oncol*., *18*(6), e315-29.

Widodo, N., Kaur, K., Shrestha, B. G., Takagi, Y., Ishii, T., Wadhwa, R., et al. (2007). Selective killing of cancer cells by leaf extract of Ashwagandha: identification of a tumor-inhibitory factor and the first molecular insights to its effect. *Clin Cancer Res*., *13*(7), 2298-306.

Widodo, N., Takagi, Y., Shrestha, B. G., Ishii, T., Kaul, S. C., & Wadhwa, R. (2008). Selective killing of cancer cells by leaf extract of Ashwagandha: components, activity and pathway analyses. *Cancer Lett*., *262*(1), 37-47.

Wong, E. T., Hess, K. R., Gleason, M. J., Jaeckle, K. A., Kyritsis, A. P., & Prados, M. D. (1999). Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol*., *17*(8), 2572-8.

Xu, Y. M., Gao, S., Bunting, D. P., & Gunatilaka, A. A. (2011). Unusual withanolides from aeroponically grown Withania somnifera. *Phytochemistry*, *72*(6), 518-22.

Yang, H., Shi, G., & Dou, Q. P. (2007). The tumor proteasome is a primary target for the natural anticancer compound Withaferin A isolated from 'Indian winter cherry'. *Mol Pharmacol*., *71*(2), 426-37.

Yoneyama, T., Arai, M. A., Sadhu, S. K., Ahmed, F., & Ishibashi, M. (2015). Hedgehog inhibitors from Withania somnifera. *Bioorg Med Chem Lett*., *25*(17), 3541-4.

Youlden, D. R., Baade, P. D., Valery, P. C., Ward, L. J., Green, A. C., & Aitken, J. F. (2012). Childhood cancer mortality in Australia. *Cancer Epidemiol*., *36*(5), 476-80.

Yu, Y., Hamza, A., Zhang, T., Gu, M., Zou, P., & Newman, B. (2010). Withaferin A targets heat shock protein 90 in pancreatic cancer cells. *Biochem Pharmacol*., *79*(4), 542-51.

Zhao, J., Jiang, Y., Chen, L., Ma, Y., Zhang, H., & Zhou, J. (2021). The EIF4A3/CASC2/RORA feedback loop regulates the aggressive phenotype in glioblastomas. *Front Oncol*., *11*, 699933.

Zhao, J., Nakamura, N., Hattori, M., Kuboyama, T., Tohda, C., & Komatsu, K. (2002). Withanolide derivatives from the roots of Withania somnifera and their neurite outgrowth activities. *Chem Pharm Bull (Tokyo)*, *50*(6), 760-5.

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