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Review Article

Indian Ayurvedic medicine: Overview and application to brain cancer

Herbert B. Newton [a,b,*]

[a] Neuro-Oncology Center and Brain Tumor Institute, University Hospitals of Cleveland Medical Center, Seidman Cancer Center, Cleveland, Ohio, USA
[b] Molecular Oncology Program, Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

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ABSTRACT

Ayurveda is the traditional medicine system of India, and has been in practice for millennia. It is a traditional approach that uses 1000's of different plant preparations in various combinations for treatment of human ailments, including cancer. Ethnopharmacological and phytochemical analyses are now elucidating the bioactive constituents of the different plant species and herbal formulations, including ashwagandha, curcumin, guduchi, triphala, and others.

To provide an overview of: 1) the ethnopharmacology of Ayurveda and several of its most important plant species and formulations, including pharmacological and molecular mechanisms of its anti-cancer effects; 2) review the literature applying Ayurvedic herbs and formulations to brain tumors.

A detailed PubMed search was performed that included publications involving Ayurveda, cancer, ethnopharmacology, phytochemical analysis, molecular analysis, and brain tumors.

In recent decades, significant research has begun to elucidate the bioactive compounds of ashwagandha, tumeric, guduchi, and triphala, such as withaferin A, withanolides, curcumin, palmatine, and many others. These compounds and extracts are now being applied to brain tumor cells *in vitro* and in animal models, with positive signs of anti-cancer activity including reduced cell growth, increased apoptosis, cell cycle arrest, increased differentiation, and inhibition of important internal signal transduction pathways.

Several Ayurvedic herbs (ashwagandha, curcumin) have bioactive compounds with significant anti-cancer activity, and are effective in early pre-clinical testing against brain tumor cells *in vitro* and in animal models. Further pre-clinical testing is warranted, along with advancement into phase I and phase II clinical trials of patients with glioblastoma and other brain tumors.

1. Introduction

Medicinal plants and herbs have been used for the treatment of human disease for thousands of years, especially in the Chinese and Indian forms of traditional medicine. Ayurveda is the traditional medical system of India, and has been practiced for over 5000 years [1,2]. Within the Ayurvedic texts are descriptions of more than four hundred herbs, as well as thousands of herbal formulations for specific ailments. Some of the plants/herbs that are commonly prescribed in Ayurvedic applications include ashwagandha (*Withania somnifera*), curcumin (*Curcuma longa*), fenugreek (*Trigonella foenum-graecum*), pipalli (*Piper longum*), guduchi (*Tinospora cordifolia*), amalaki (*Embilica officinalis*), bramhi (*Bacopa monnien*), and many others. In most cases the herbs are not used as individual compounds, but instead are administered as part of a recipe, mixture, or formulation called a "*Rasayanas*" [2]. These recipes of multiple herbs contain numerous potentially active and synergistic compounds that can target various biochemical and cellular pathways in the tissues simultaneously. The Ayurvedic approach is holistic and personalized, and takes into account an individual's body type, strength of digestion, immunity, and mental health.

Since the advent in the 19th century of the new discipline of Ethnopharmacology, there has been a keen interest to study and analyze the plants, recipes, and treatment approaches of traditional and ethnic medicine [3]. Ethnopharmacological research of medicinal plants and phytochemicals has been very important for drug discovery programs in many areas, including anti-infectives, central nervous system disorders (e.g., epilepsy, depression, dementia), anti-inflammatory agents, metabolic disorders and, most importantly, anti-cancer drugs [4,5]. As of 2017, of the more than 120 anti-cancer prescription drugs available, 90 were plant based, and many of them were discovered from "folklore"

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* University Hospitals of Cleveland, Seidman Cancer Center, 11100 Euclid Avenue, Cleveland, OH, 44106, USA.
E-mail address: Herbert.Newton@UHhospitals.org.

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claims of activity. Once a plant has been identified as "medicinally active", it is subjected to a "western medicine" style phytochemical analysis to search for the active component or components. The plant is processed – including leaves, flowers, fruit, seeds, branches, stem, and roots – and then an initial extraction is performed of all the compounds in the plant tissue, using various procedures: e.g., methanol extraction, ethanol extraction, organic extraction; the aqueous phase contains water-soluble molecules, including nucleic acids, while the organic phase contains the proteins and lipids. Further separation/characterization and/or standardization of the extracted chemicals is then performed using techniques such as Thin Layer Chromatography, High-Performance Liquid Chromatography, and Capillary Electrophoresis. Once the compounds and molecules of interest have been separated and isolated, they are subjected to further analysis depending on their classification: Northern blotting, Western blotting, proteomics analysis, Nuclear Magnetic Spectroscopy, RNA and DNA sequencing, and *in vitro* testing against various cell cultures, including a panel of cancer cells (lung, breast, colorectal, brain, etc). In recent years, it is also important for the compound to be tested at the molecular level, to determine how it is affecting the cancer cell in terms of specific pathways, including various receptors (e.g., epidermal growth factor receptor), internal signal transduction pathways (e.g., Raf-Mek-Erk, PI3K, mTOR), apoptotic proteins, angiogenesis, DNA methylation, extracellular matrix invasion, and transcription. If the chemicals have activity against brain cancer cells in culture, then they are subjected to testing in animal models, most often mouse or rat models with implanted tumors into the subcutaneous tissues or the brain.

2. Ethnopharmacological analysis of ayurvedic herbal compounds

There are roughly 2000 plants found in India that are used in the Ayurvedic medical system; of this group, 45 have been specified to have anti-cancer activity – the highest number reported by any country [4,5]. The most dominant anti-cancer plant families were Asteraceae, Euphorbiaceae, Apocynaceae, and Fabaceae [5]. Most of the herbal formulations consisted of leaves (28%), roots (15%), and bark (13%); only 10% of formulations were made using the whole plant [5]. Other plant parts that were used in some formulations included seeds, fruits, flowers, stem, rhizome, shoots, resins, and tuber [5]. Ayurvedic herbs and herbal formulations have been undergoing ethnopharmacological analysis for many years, and the various compounds have become a very fertile area for drug discovery as applied to cancer treatment [4–6]. Many different types of phytochemicals have been isolated from Ayurvedic herbs, including flavonoids, terpenoids, tannins, saponins, alkaloids, hemagglutinins, and phenolic compounds. Research publications on the topic of Ayurveda have increased dramatically since the early 2000's, with a peak in 2020 [2]. Most of these published studies were from India, with a few publications from researchers in the USA, United Kingdom, Germany, Australia, and Asia. In general, the studies were focused on isolating chemical components from Ayurvedic herbs such as ashwagandha and curcumin, and analyzing their mechanisms of action for drug discovery potential. The majority of the studies were reductionist, and did not take into account the fact that the majority of Ayurvedic treatment paradigms prescribe multiple herbs in various combinations and concentrations, fine-tuned over 1000's of years. This has lead to a new, broader pharma approach called "*Network Pharmacology*" – first described in 2007–2008 by Hopkins – that does not adhere to the assumption of "one drug for one target for one disease" [6,7]. Instead, it suggests that many drugs could act on multiple targets simultaneously, rather than on a single intended target. Network pharmacology integrates systems biology, proteomics and other "-omics" technologies, and computational biology to study multi-component and multi-targeted formulations. This approach has been applied to Ayurvedic herbs such as triphala and ashwagandha by Chandran and Patwardhan, with intriguing results (see below) [8,9].

In the following sections, several Ayurvedic herbs and herbal formulations that have undergone comprehensive ehthnopharmacological evaluation will be reviewed with a focus on their anti-cancer properties and mechanism(s) of action:

Ashwagandha (Withania somnifera): Since its initial discovery and description in 6000 BCE, Ashwagandha has been one of the most commonly used herbal medicines (see Table 1) [6,10,11]. It is an evergreen woody shrub of the Solanaceae family that is distributed widely in the drier parts of India; however, it is also grown specifically as a medicinal crop. Within the Ayurvedic medical system, all of the different parts of the ashwagandha plant have medicinal applications and are used in various formulations, but the roots are the most extensively prescribed. Over the centuries, ashwagandha has been reported to have numerous applications, including benefits for inflammation, arthritis, stress, cancer, depression, cardiac disease, epilepsy and other neurological disorders, GI health, hepatic disease, and pain syndromes. Several different classes of bioactive phytochemicals have been isolated from ashwagandha, including steroidal lactones (e.g., withanolides, withaferin A), alkaloids (e.g., withanine), flavonoids (e.g., quercetin), steroids, salts, and nitrogen-containing compounds (e.g., withanol) [10–12]. Aqueous root extracts of the plant have demonstrated the ability to modulate peripheral blood mononuclear cell and leukemic THP-1 cell viability, as well as increase oxidant scavenging and caspase (−8, −9, −3/-7) activities, while also decreasing tumor necrosis factor-alpha (TNF-α), interleukin-10, and glutathione levels. Crude water extracts from ashwagandha were also noted to alter pro-apoptotic and tumor-promoting proteins, thereby suppressing tumor growth, including nuclear factor-kappa B (NF-κB), phospho-Akt, Bcl-xl, heat shock protein 70 (HSP70), cyclin D1, vascular endothelial growth factor (VEGF), matrix metalloproteinases, and others.

Table 1
Molecular mechanisms and anti-cancer effects of Ayurvedic herbs and preparations.

Ayurvedic Herb/ Preparation	Signaling Pathway(s)	Anti-cancer Effect
Ashwagandha aqueous root extract	increase caspase (−8, −9, −3/-7) activity, reduce TNF-α, IL-10, glutathione	increase apoptosis & oxidant scavenging
Ashwagandha crude water extract	reduce expression NF-κB, phospho-Akt, HSP70, cyclin D1, & VEGF	promote apoptosis suppress growth reduce angiogenesis
Withaferin-A	inhibits proteasome pathway, arrest cell cycle at G_2/M, increase Wee-1, p21, reduce expression EMT markers, modify cancer stem cells (CSC), inhibit activation of NF-κB by TNF-α, bind annexin II, aggregation of actin, bind HSP90; disrupt HSP90-Cdc37 complex	inhibit proliferation reduce cell motility differentiate CSC enhance apoptosis disrupt cytoskeleton degradation of HSP90
Curcumin	inhibit activation NF-κB, inhibit activity of TNF-α, downregulate AP-1, reduce phosphorylation STAT3, suppress activity cyclin D1, inhibit activity EGFR, downregulate Akt protein, reduce survivin expression	enhance apoptosis reduce angiogenesis inhibit cell cycle reduce EGFR activity reduce Akt activity reduce proliferation
Guduchi (palmatine)	reduce Bcl-2 expression, inhibit activity NF-κB, downregulate VEGF, increase superoxide dismutase, reduce glutathione activity	induce apoptosis reduce angiogenesis antioxidant activation
Triphala (aqueous & ethanolic extracts)	reduce pAkt & pNF-κB, suppress c-myc & cyclin D1, modulate MAPK/ERK, PI3K/ Akt/mTOR, & NF-κB/p53 pathways, suppress VEGF/ VEGFR2	reduce proliferation enhance apoptosis increase ROS inhibit cell cycle reduce angiogenesis

The ethnopharmacological and phytochemical analyses of aswhagandha have suggested that the most bioactive constituents are the steroidal lactones – in particular the withanolides, along with withanone and withaferin A [12–16]. There are numerous withanolide varieties (A, E, F, G, H I, J, K, L, M), many of which are bioactive. However, the most bioactive compound overall, especially in terms of its anti-cancer effects, is withaferin A (see Fig. 1). Structural analysis of withaferin A (WFA) has revealed three likely sites that are the most reactive and would be susceptible to nucleophilic attack: the unsaturated A-ring at C3, the epoxide structure at position 5, and the C24 in the E ring [12–16]. These sites could covalently bind to cysteine residues of proteins through alkylation reactions, inducing loss of activity of the target proteins. Withaferin A has been tested against numerous cancer cell lines *in vitro* and has demonstrated significant activity, including cell cultures of neuroblastoma, leukemia, hepatocellular, prostate, breast, colon, ovarian, multiple myeloma, glioblastoma, and head and neck cancer. In addition, when WFA was used in combination regimens (e.g., TRAIL, cisplatin, Doxorubicin, Etoposide), it was able to sensitize tumor cells and enhance growth suppression and apoptosis [12–16]. The molecular mechanisms underlying the anti-cancer activity of WFA are incompletely understood, but appear to involve polypharmaceutical effects that target numerous proteins and pathways simultaneously. Withaferin A appears to be a potent inhibitor of the ubiquitin-mediated proteasome pathway, leading to accumulation of ubiquinated proteins within cancer cells such as Bax, p27, and IκBα. It is also able to inhibit cell proliferation by arresting the cell cycle at the G_2/M phase, and can prevent mitosis by upregulating phosphorylated Wee-1, phosphorylated histone H3, p21, and Aurora B targets [12–16]. Micromolar concentrations of WFA have also been shown to modify and increase the redox potential inside of tumor cells, enhancing oxidative stress and triggering cell death in many cell types. Withaferin A can inhibit the tendency for metastasis by decreasing the expression of epithelial mesenchymal transition (EMT) markers, thereby reducing cellular motility, as well as by inhibiting the activity of the urokinase-type plasminogen activator (uPA) protease [12–16]. Cancer stem cells (CSCs) are typically very refractory to treatment and have the capacity for self-renewal. However, WFA has been shown to modify CSCs so that they become more differentiated, and sometimes can enter a senescent state. *In vitro* studies reveal that WFA can induce apoptosis in cancer cells through several mechanisms, including inhibiting the activation of NF-κB by preventing the TNF-induced activation of IκB kinase β via a redox mechanism, as well as by activating tumor suppressor genes such as p53 and pRb [12–16]. In addition, WFA has been shown to upregulate death receptor-5 and transduces apoptosis signals, which results in apoptotic death of cancer cells. Another mechanism for WFA to induce apoptosis is to increase Par-4 induction and p38 MAP kinase activation. In an immune competent tumor-bearing mouse model, treatment with WFA leads to a modulation of anti-tumor immunity through selective inhibition of Treg proliferation and induction of apoptosis in Treg cells [12]. Treatment with WFA also appears to disrupt the cytoskeleton of cancer cells by covalent binding to annexin II, thereby reducing the ability of annexin II to bind with actin, with subsequent aggregation of actin. This disruption of the cytoskeleton is also linked to disassembly of vimentin, vimentin depolymerization, and filament aggregation [12–16]. Cancer cells have high expression of heat shock proteins (e.g., HSP90), which act as molecular chaperones in proliferation, differentiation, invasion, and metastasis. Treatment with WFA leads to binding to HSP90 and disruption of the HSP90-Cdc37 complex, with inhibition of complex activity and degradation of HSP90 target proteins [12].

The pharmacokinetic and pharmacodynamic profile of WFA has been investigated in mouse models [12,14]. After oral administration of WFA (1000 mg/kg), there is rapid oral absorption and the peak plasma concentration is reached within 10 min. After an intraperitoneal injection of a single dose of WFA (4 mg/kg), a maximum plasma concentration up to 2 μM was reached, with a half-life of approximately 1.4 h. WFA has not been specifically evaluated for the potential for drug-drug interactions. However, there has been some preliminary evaluation of ashwagandha and other commonly used herbs [17]. Haron and colleagues screened the 30 top-selling herbs in the US market for their potential to upregulate the activity of the drug metabolizing cytochrome p450 enzymes (i.e., CPY3A4, CYP1A2). Ashwagandha was one of eight herbs that were noted to induce a >50% increase in activity of CYP3A4 and CYP1A2. The increase of activity of these enzymes suggests there is definite potential for ashwagandha (WFA?) to interact adversely with other drugs, including cardiac drugs and chemotherapy. However, not all of the data supports interactions of ashwagandha with CPY3A4 liver enzymes. A report from Patil and co-workers did not demonstrate any significant inhibition of CPY3A4 activity after exposure to an ashwagandha root aqueous extract [18].

Curcumin (Curcuma longa): The *Curcuma longa* plant belongs to the Zingiberaceae family, genus Curcuma, and is a crop cultivated in the tropical and subtropical regions around the world [6,19]. It is mainly found in India and south east Asian countries, and has been used in the Ayurvedic and Traditional Chinese medical systems for millennia in the treatment of inflammation, arthritis, metabolic syndromes, liver disease, obesity, neurodegenerative diseases, and cancer [6,19]. Phytochemical and ethnopharmacological analysis of the rhizome of the plant has revealed the presence of numerous components, including curcuminoids, volatile oils, proteins, fibers, carbohydrates, and minerals. The most representative polyphenol compound extracted from the rhizome is curcumin, which was isolated for the first time in 1815 and then extracted in a pure crystalline form in 1870, and is also the main phytochemical in Haldi/turmeric [19]. The chemical structure of the compound consists of two phenol rings substituted with hydroxyl and methoxyl groups, and connected by a seven carbon keto-enol linker (see Fig. 2). In cell cultures and other *in vitro* systems, curcumin has demonstrated activity against numerous cancer cell types, including prostate, colorectal, head and neck, breast, glioblastoma, pancreatic, lung, gastric, leukemia, multiple myeloma, hepatic, and lymphoma cell

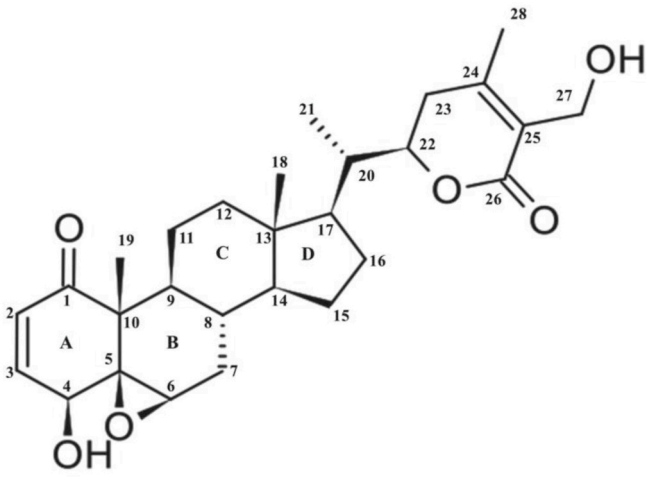

Fig. 1. Structure of withaferin A.

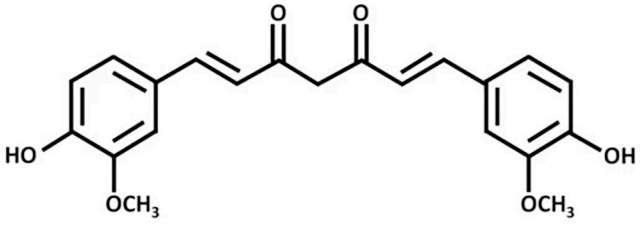

Fig. 2. Structure of curcumin.

lines [20–22]. Similar to WFA, curcumin appears to modulate the activity of multiple proteins, receptors, and signaling pathways simultaneously in tumor cells. Curcumin is able to enhance apoptosis by several mechanisms, including inhibiting the activation of NF-κB by preventing the TNF-induced activation of IκB kinase β via a redox mechanism, as well as by impeding the nuclear translocation of the NF-κB p65 subunit [20–22]. Curcumin is also able to directly reduce the transcription of TNF-α. In addition, the transcriptional factor Activator Protein-1 (AP-1), which is known to be associated with anti-apoptotic, mitogenic, and pro-angiogenic genes, is downregulated by curcumin. Signal transducer and activator of transcription 3 (STAT3) is known to regulate oncogenes and modulate the transduction of pro-inflammatory cytokines, growth factors, and anti-apoptotic proteins such as Bcl-2 and Bcl-xL. Curcumin has been demonstrated to regulate and reduce the phosphorylation of STAT3 directly and via inhibition of Interleukin-6 (IL-6) [20–22]. The cell cycle is also directly inhibited by curcumin, since it is able to suppress the activity of cyclin D1 through its ability to impede the activation of NF-κB. In breast cancer cells, curcumin was able to inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase activity, as well as downregulate the Akt protein, thereby blocking the PI3K/Akt signaling pathway. In addition, curcumin was able to modulate the expression of oncogenic (e.g., miR-19a, miR-19b) and tumor suppressor (e.g., miR-15a, miR-34a) Micro-RNA's in breast cancer cells – suppressing tumorigenesis and proliferation, while also inducing apoptosis [20–22]. In gastric tumor cells, curcumin downregulated pSTAT3 levels, reduced survivin expression, and increased tumor cell death in a dose-dependent manner. Treatment of gastric cells with 5-fluorouracil in combination with curcumin resulted in a synergistic effect on reducing STAT3 and survivin levels.

Pharmacokinetic and pharmacodynamic studies of curcumin have noted that the drug has low water solubility and low chemical stability, which results in poor oral bioavailability and low levels of free curcumin in plasma [20,21]. There is also poor cellular uptake of curcumin, as the hydrophobic molecule tends to penetrate into the cell membrane and not the cytoplasm of tumor cells. In order to circumvent these issues and allow for improved bioavailability, various delivery systems are under study, using different forms of nanotechnology, including polymeric nanoparticles, liposomes, nanogels, peptide and protein formulations, and cyclodextrin complexes. In addition, preliminary studies of the potential for drug-drug interactions with curcumin are limited since the oral bioavailability of the drug is typically so poor. However, several studies suggest that curcumin has the potential for drug interactions (e.g., cardiovascular, antidepressants, anticoagulants, antibiotics, chemotherapeutics) – with alterations in C_{max} and AUC due to interactions with hepatic cytochrome isoenzymes (e.g., inhibition) and p-glycoprotein [23,24]. However, herb-drug interactions involving curcumin/Curcuma longa can be variable and may or may not involve drug metabolizing enzymes and transporters. For example, in a diabetic animal model, the administration of curcumin in combination with Metformin resulted in pharmacokinetic and pharmacodynamic interactions that lead to beneficial antihyperglycemic and antihyperlipidemic effects [25].

Guduchi (Tinospora cordifolia): *Tinospora cordifolia* is a herbaceous climbing vine from the family Menispermaceae – commonly called "Guduchi" – that is indigenous to the tropical regions of the Indian subcontinent [6,26]. It has been used in the Ayurvedic medical system for centuries to treat various disorders, including fever, jaundice, chronic diarrhea, cancer, dysentery, urinary infections, pain, skin diseases, poisoning, asthma, gout, diabetes, and eye disorders. The leaves, stems, and roots of the plant are used in various preparations in Ayurveda. Preliminary ethnopharmacological and phytochemical analyses have noted various chemical constituents in the plant, including Alkaloids, Flavonoids, Terpenoids, Lignans, Sterols, Phenols, Saponins, Tannins, and others [26,27]. Guduchi has now undergone enough evaluation to clarify several distinct pharmacological activities, such as antioxidant activity, antimicrobial activity, anti-toxic effects, anti-diabetic activity, anti-stress activity, hypolipidemic effects, hepatic protective effects, anti-HIV potential, anti-cancer activity, immunomodulating effects, and neuro-protective activity [26–30]. Extracts of Guduchi – including the active constituent palmatine (see Fig. 3) – are able to induce apoptosis through a mechanism of reducing Bcl-2 expression. Palmatine has also been shown to have angio-inhibitory effects through initial inhibition of NF-κB, with subsequent down-regulation of VEGF expression. The aqueous extract of Guduchi has significant antioxidant activity, mainly due to the presence of phenolic, flavonoid, and tannin constituents, and is able to increase the levels of superoxide dismutase while reducing the levels of glutathione, glutathione-S-transferase, glutathione peroxidase, and glutathione reductase. Aqueous and ethanolic extracts of guduchi have demonstrated anti-cancer activity against neuroblastoma, Ehrlich ascites carcinoma, glioma cells, and cervical carcinoma, as well as in a mouse model of skin cancer [26–30].

There is very limited data on the pharmacokinetics and bioavailability of guduchi. However, there have been some preliminary reports on potential interactions of guduchi with the hepatic cytochrome p450 system [18]. An *in vitro* assay of CYP34A was used to analyze potential inhibition from Asparagus racemosus, Withania somnifera, and Tinospora cordifoli (guduchi). Guduchi demonstrated mild overall inhibition of CYP34A. However, when using only the non-polar fraction (berberine, jatrorrhizine, palmatine), the degree of CYP34A inhibition was significant. The authors concluded that the potential for drug-drug interactions between guduchi – especially the protoberberine alkaloid derivatives – and chemotherapeutic agents was significant and should be monitored carefully.

Triphala: *Triphala* is a very popular Ayurvedic formulation that has been used for centuries, and consists of the pitted fruits – known as myrobalans – of three botanicals including *Emblica officinalis* (EO), *Terminalia chebula* (TC), and *Terminalia bellerica* (TB) that are all native to the Indian subcontinent [6,31]. In *Triphala*, the myrobalans are used as a mixture consisting of equal proportions of all three components. Other minor compounds can be added in, depending on the Ayurvedic application, such as sugar, honey, rock-salt, black ash, and various oils. Traditional uses for *Triphala* include digestion and overall bowel health, improving skin and hair quality, longevity, immune boosting, asthma, peptic ulcers, liver diseases, degenerative disorders, metabolic disorders, and cancer. Ethnopharmacological and phytochemical analyses have clarified the major active constituents of the *Triphala* formulation, which includes tannins, gallic acid, ellagic acid, and chebulinic acid, all of which are potent antioxidants and immunomodulatory agents. In addition, other bioactive compounds are present, such as flavonoids (e.g., quercetin), saponins, anthraquinones, amino acids, and fatty acids. *In vitro* studies with aqueous and ethanolic extracts of *Triphala* have demonstrated significant antineoplastic activity against breast, lung, prostate, colon, pancreatic, gastric, cervical, and endometrial cell lines [31–35]. The cell lines demonstrated reduced proliferative activity and an increase in Reactive Oxygen Species (ROS), as well as more frequent apoptotic cell death. *Triphala*-induced apoptosis occurred through the intrinsic mitochondrial apoptotic signaling pathway. Treatment with *Triphala* was able to reduce the expression levels of pAkt, phospho-p44/42, and pNF-κB, as well as suppress expression of the oncogenes, c-Myc and Cyclin D1. In addition, *Triphala* was demonstrated

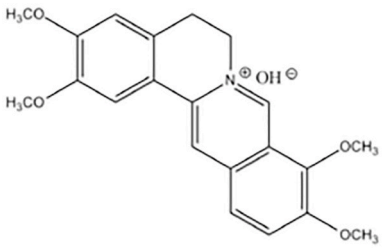

Fig. 3. Structure of palmatine.

to modulate the MAPK/ERK, PI3K/Akt/mTOR, and NF-κB/p53 signaling pathways [31–35]. *Triphala* has also been shown to have immunomodulatory effects, including stimulating neutrophil function, cytotoxic T cells, and natural killer cells. Lastly, *Triphala* and its major constituent, chebulinic acid, have demonstrated the ability to inhibit tumor angiogenesis by blocking multiple components of the VEGF/-VEGFR2 signaling pathway – mainly through suppression of VEGFR2 phosphorylation [36,37].

There is very limited data on the pharmacokinetics and bioavailability of *Triphala* in human subjects. However, preliminary investigations into the possibility of drug-drug interactions have begun. Nontakham and co-workers recently reported on the inhibitory effects of *Triphala* on CYP isoforms *in vitro* and in a rat model [38]. *In vitro* testing revealed that *Triphala* induced non-competitive inhibition of CYP1A2 and competitive inhibition of CYP3A4. In rats oral dosing of *Triphala* resulted in increased bioavailability of phenacetin and midazolam by 61.2% and 40.7%, respectively.

For more detailed information on the various herbs and other selected drugs used in the Ayurvedic medical system, the *Ayurvedic Pharmacopoeia of India* is a very detailed and excellent reference [39,40]. The book describes the quality, purity, strength, and other aspects of the many herbs and drugs that are manufactured, distributed, and sold by licensed manufacturers in India. Part 1, volume 1 of the pharmacopoeia was published in 1986, with subsequent volumes published through 2016.

3. Application of ashwagandha/withaferin A to brain tumors

The first few dedicated applications of ashwagandha to brain tumor cells used extracts of the leaves of the plant (see Table 2) [41,42]. In 2009, Shah and colleagues applied an alcoholic extract of ashwagandha leaves to C6 (rat glioma) and YKG1 (human glioma) cell lines [41]. In addition to the extract itself (termed i-Extract), the cell lines were also treated with several of the components of the extract, including WFA, withanolide A, and withanone. All four of the treatment arms were noted to cause a significant growth arrest of both cell lines in a dose dependent manner, with a maximum effect between 48 and 72 h. The IC_{50} values were lower for the human glioma cells in comparison to the rat glioma cells; WFA had the lowest IC_{50} for both cell lines: 0.1 μM and 0.2 μM, respectively. High doses of i-Extract and its constituents were noted to induce apoptosis in the glioma cell lines. However, low to moderate doses were associated with cell cycle arrest in the S and G_2/M phases, as well as reduced motility and a change in phenotype to a more senescent state. In combination studies of the four compounds, WFA (0.01 μM) plus withanone (5 μg/mL) was the most active in inducing a senescent and more differentiated state. The authors considered ashwagandha and its constituents as potential treatment options for differentiation therapy in glioblastoma. Another study from the same group used the water extract from ashwagandha leaves and applied it to the rat C6 glioma cell line, as well as several human glioma cell lines – YKG1, U118MG, and A172 [42]. Preliminary phytochemical analysis of the aqueous extract noted the presence of flavonoids, steroids, tannins, amino acids, saponins, reducing sugars, and alkaloids. Similar to the ethanol extract data noted above, the aqueous extract was also able to induce apoptosis in all of the cell lines at higher concentrations (>1.0%). At lower concentrations (≤0.5%) there was a reduction of cell proliferation and all of the cell lines became growth arrested, with morphological changes consistent with a more senescent state (e.g., increased glial fibrillary acidic protein [GFAP] expression, enlarged cell size, increased number of processes). Treatment with the aqueous extract also reduced cell motility and altered the adhesion molecule expression, including neural cell adhesion molecule (NCAM) isoforms, NCAM-120 and NCAM-140. The authors concluded that the aqueous extract of Ashwagandha also had potential to be used as a differentiation form of treatment for glioblastoma.

After the two initial studies noted above, the subsequent applications of ashwagandha to brain tumors were focused on treatment with the major active constituent – WFA (see Fig. 1). After some initial

Table 2
Summary of Application of Ayurvedic herbs to Brain Tumors

ASHWAGANDHA/WITHAFERIN-A (WFA)		
Herb Preparation	Tumor Type	Anti-cancer Effect
alcoholic extract leaves, WFA, Withanolide A, Withanone Ref [33]	C6 rat glioma & YKG1 human glioma cell lines	induce growth arrest induce apoptosis cell cycle arrest WFA lowest IC_{50}
aqueous extract leaves Ref [34]	C6 rat glioma & YKG1, U118MG, A172 human glioma cell lines	reduce proliferation, induce apoptosis, inhibit cell cycle induce differentiation
WFA Ref [37]	GL26 murine & U87, U251 human glioma cell lines	dose-dependent shift cell cycle; G_2/M arrest WFA: 0.5–1.5 μM, induce apoptosis reduce Akt & mTOR, reduce EGFR, cMET
WFA Ref [38]	TMZ-resistant U251, U87 human glioma cell lines	reduce proliferation, cell cycle arrest G_2/M, induce apoptosis reduce EGFR & cMET, reduce MGMT
WFA Ref [39]	C6 rat glioma cell lines	induce apoptosis induce caspase-3, -9, increase Bax reduce Bcl2, reduce TNF-α, NF-κB
WFA & AshwaMAX, Ref [40]	U87, GBM2, GBM39 human glioma cell lines mouse xenograft model	reduce proliferation, reduce neurospheres; WFA 0.25–0.31 μM, AshwaMAX 2.1–14.8 μg/ml, xenografts inhibited x 3–4 weeks
WFA & AshwaMAX tumor treating fields (TTFs) Ref [41]	U87, GBM2, GBM39 human glioma cell lines	synergistic effect of WFA & TTFs; reduce growth, neurospheres
WFA Ref [44]	U251, U87 human glioma cell lines, nude mouse xenografts	inhibit cell growth, increase apoptosis, increase Bim, Bad cell cycle arrest G_2/M, shrink xenografts
CURCUMIN		
Curcumin Ref [46]	glioma cell lines	reduce MMP-9 action suppress NF-κB, AP-1, suppress pERK, pJNK
Curcumin Ref [47]	U87 glioma cell lines xenografts nude mice	reduce proliferation, inhibit MMP-9, IC_{50} 11.6 μM, shrink xenografts
Curcumin Ref [48]	MED-4, MED-5, DAOY human medulloblastoma cell lines	inhibit proliferation, cell cycle arrest G_2/M, downregulate SHH, reduce pAkt, pNF-κB induce apoptosis
Curcumin Ref [49]	U251 human glioma cell lines	increase DAPK1, reduce pSTAT3, reduce pNF-κB enhance apoptosis
Curcumin & RT Ref [50]	mouse GL261 & human U251 glioma cell lines with RT	enhance apoptosis, increased apoptosis, enhance ICD
Curcumin encased in RDP-nanoliposomes Ref [51]	U251 human glioma cell lines; xenografts	inhibit proliferation, enhance apoptosis, shrink xenografts
GUDUCHI		
50% ethanolic extract from the stem Ref [55]	C6 rat, human U87 glioma cell lines	arrest growth, enhance apoptosis, induce differentiation and cell cycle arrest in G_0/G_1, G_2/M, reduce cyclin D1, IC_{50} 200 μg/ml
chloroform and hexane extracts Ref [56]	U87 human glioma cell lines and IMR-32 neuroblastoma cell lines	reduce proliferation, induce differentiation, reduce migratory capacity

preliminary investigations using WFA isolated from the *Vassobia breviflora* plant, which is similar to ashwagandha and also in the Solanaceae family [43,44], Grogan and co-workers reported the results of their more complete investigation into treatment of glioblastoma cell lines with WFA [45]. They used two human glioblastoma cell lines (U87, U251) and one murine cell line (GL26), and exposed them to varying concentrations of WFA. All of the cell lines were noted to have a dose-dependent shift in the cell cycle, with arrest in G_2/M, after exposure to WFA. The concentrations of WFA to attain maximal G_2/M arrest were variable between the three cell lines, and ranged from 0.5 μM to 1.5 μM. With increasing concentrations of WFA, there was enhanced apoptosis in all cell lines, with a reduction in the levels of uncleaved caspases, likely mediated via activation of the extrinsic and intrinsic apoptotic pathways. Similarly, with increasing concentrations of WFA, there was a reduction in the total levels of Akt and mTOR, with reduced levels of phosphorylation as well in some cell lines. Treatment with WFA also resulted in a decrease in the total levels of EGFR, Her2/ErbB2, and c-MET. In the GL26 and U87 cell lines, treatment with WFA induced an elevation of oxidation status, including peroxides and mitochondrial superoxide. The same group has reported a follow-up study using WFA in glioblastoma cell lines that have become resistant to Temozolomide (TMZ), the "standard of care" chemotherapy drug for this tumor type [46]. U251 and U87 TMZ-resistant sub-lines were generated by exposing the parental lines to increasing concentrations of TMZ (30–300 μM) over 8 weeks. WFA treatment of the resistant cell lines resulted in reduced cell proliferation and viability, with cell cycle arrest in G_2/M. At higher concentrations of WFA, there was evidence of an increase in apoptotic cell death through the intrinsic and extrinsic systems. Similar to TMZ non-resistant cells, treatment with WFA induced decreased total levels of Akt and mTOR, as well as their phosphorylated counterparts. In addition, the total levels of EGFR, Her2/ErbB2, and c-MET were reduced after treatment. Methylguanine methyltransferase (MGMT) is one of the most important TMZ resistance enzymes in glioma cells. In the TMZ resistant cell lines, treatment with WFA resulted in significant depletion of MGMT, with complete elimination at doses of 10 μM. When TMZ and WFA were used in combination, there was a synergistic effect on reducing MGMT, which resulted in TMZ regaining efficacy against the cell lines. The authors suggested that future research was indicated *in vitro* and in animal models investigating the use of WFA in combination with TMZ.

A report by Hou et al. corroborated many of the findings noted in the studies reviewed above, using rat C6 glioma cell lines [47]. Increasing concentrations of WFA induced a dose-dependent increase in the number of cells undergoing an apoptotic cell death. This was correlated with an upregulation in the activity of caspase-3 and caspase-9. In addition, there was a dose-dependent increase in the expression of Bax, while the expression of Bcl2 was reduced. Expression of the inflammatory marker TNF-α was reduced after WFA treatment, along with inhibition of nuclear translocation of the NF-p65 subunit from the cytosol into the nucleus, thereby lowering NF-κB activation.

Chang and colleagues have reported several studies investigating the use of WFA, AshwaMAX, and tumor treating fields (TTF's) on glioma cell lines and murine orthotopic brain tumor models [48–50]. AshwaMAX is a specialized ashwagandha root extract that is standardized to contain no less than 4.3% of WFA, as well as 8.4% of total withanolides. In the first study, they used pure WFA and AshwaMAX for treatment of patient-derived cell lines (GBM2, GBM39) and the commercial cell line U87 [48]. Increasing concentrations of WFA and AshwaMAX induced reductions in growth and cell proliferation in all three cell lines. The IC_{50} values for WFA were in the submicromolar range – from 0.25 to 0.31 μM, while the IC_{50} values for AshwaMAX were higher and ranged from 2.1 to 14.8 μg/ml. These inhibitory effects were associated with a reduced ability of glioma cells to form neurospheres; any neurospheres that were present prior to WFA and AshwaMAX exposure tended to collapse. GBM2 and GBM39 mouse xenografts were developed using Luciferase bioluminescent modified cells, and then treated with AshwaMAX by oral gavage [48]. After treatment, the bioluminescent signal was significantly reduced after one week, and maintained for another 3–4 weeks. However, soon after the bioluminescent signal recovered and increased over the next month – suggesting the outgrowth of an AshwaMAX resistant cell clone. Overall, AshwaMAX mice lived an extra 4 weeks longer than control treated mice.

In the second study, Chang et al. also used GBM2, GBM39, and U87 glioma cells and treated the cell lines with WFA, TTF's, or a combination of both therapies [49]. Tumor treating fields are an approved form of therapy for GBM that induces electrical fields in the tumor tissue, thereby disrupting the activity of polarized proteins in the cells involved in mitosis (e.g., actin, septin), leading to metaphase and telophase arrest and apoptosis [51]. Initial exposure of all three glioma cell lines to TTF's (4.0 V/cm) resulted in significant and progressive inhibition of growth. When WFA was applied at low doses (0.1 μM) to U87 cells, there was no significant reduction of growth. However, when TTF's were applied following WFA, further growth was inhibited, with a significant reduction in cell numbers. When glioma cells were exposed to WFA and TTF's in combination, there was a synergistic effect on inhibition of tumor growth, along with reduced size of neurospheres and less adherent connectivity between the cells. In an overview of WFA and its potential as a treatment modality for GBM and other cancers, the authors concluded that significant positive evidence was accumulating [50]. However, further pre-clinical, animal model, and human clinical trials were necessary for validation.

In a more recent report, Tang and co-workers evaluated the response of human glioma cell lines (U251, U87) and nude mouse xenografts to treatment with WFA [52]. Similar to data presented above, they noted a concentration and time-dependent inhibition of cell growth, along with an increase in cell death due to apoptosis, with significant increases in cleaved PARP1 and caspases −3, −7, and −9, without an increase in cleaved caspase-8. These results suggest that the WFA-mediated increase in apoptosis were mediated mainly through the intrinsic pathway. In addition, the intrinsic pathway was activated through an upregulation in the expression of Bim and Bad. They also corroborated the finding of an effect on the cell cycle, with arrest in G_2/M induced by an upregulation of p21. The WFA-mediated effects on apoptosis and the cell cycle were further explored using mRNA transcriptional analysis, which demonstrated that the ATF3-ATF4-CHOP axis may be playing a pivotal role. In U87 nude mouse xenografts, WFA treatment induced a significant reduction in tumor growth and size in comparison to control animals.

A recent overview of the pre-clinical activity and mechanisms of action of WFA, along with a discussion of its potential in the treatment of adult and pediatric brain tumors, was reported by Marlow and colleagues [53]. They note that WFA seems likely to penetrate the blood-brain barrier (BBB), since it has a relatively low molecular weight, has a favorable blood/brain partition coefficient, has only 2 hydrogen bond donors, and less than 10 hydrogen bond acceptors (i.e., it passes Lipinski's Rule of Five). Withaferin A also targets many pathways that are known to be dysregulated in high-grade gliomas, including the p53 signaling pathway, MAPK/ERK signaling pathway, PI3K/Akt/mTOR signaling pathway, NF-κB signaling pathway, and the angiogenesis pathways (i.e., reducing VEGF and vimentin, endothelial cell inhibition). The authors are very supportive of considering WFA for further *in vitro* and animal studies, and to start early clinical trials in humans with GBM and other high-grade gliomas.

4. Application of curcumin to brain tumors

The first report of curcumin being applied to brain tumors was by Woo et al., in 2005, when they studied its ability to effect the activity of MMP-9 in glioma cells, which is involved in glioma invasion and angiogenesis (see Table 2) [54]. They were able to demonstrate that curcumin was able to significantly inhibit MMP-9 enzymatic activity and protein expression. This effect was mediated through suppression of DNA binding of NF-κB and AP-1. In addition, curcumin was noted to

suppress the phosphorylation of ERK, JNK, and p38 MAP kinase.

Perry and co-workers then reported on the effect of curcumin when used to treat U87 glioma cultures and xenografts in athymic mice [55]. In U87 glioma cultures, exposure to increasing concentrations of curcumin resulted in a significant antiproliferative effect, with an IC_{50} of 11.6 μM. In the flank xenograft animals, treatment with curcumin at 60 mg/kg/day was able to significantly reduce tumor growth. In other experiments, pre-treatment with curcumin had a preventive effect on tumor implantation, with consistently smaller tumors. Curcumin was also noted to have anti-angiogenesis activity, by reducing the migratory capacity of endothelial cells, as well as their ability to form proper tubular structures. They were also able to demonstrate the ability of curcumin to reduce the activity of MMP-9. Curcumin was also tested for its ability to cross the BBB, which it was able to do at a high level in an *in vitro* assay. In an intracerebral xenograft model, treatment with curcumin 120 mg/kg/day resulted in a significant increase in animal survival time of 12% (23.4 days versus 20.9 days; $p < 0.05$).

Around the same time, Elamin and colleagues applied curcumin to cultures of medulloblastoma (MED-4, MED-5, DAOY), which is a common malignant tumor of the cerebellum in childhood [56]. Exposure of medulloblastoma cells to curcumin resulted in inhibition of proliferation and triggered cell cycle arrest in the G_2/M phase. Curcumin was also noted to inhibit the sonic hedgehog (SHH) signaling pathway by downregulating the SHH protein, as well as its most important downstream targets – GLI1 and PTCH1. Curcumin also reduced levels of beta-catenin, the active phosphorylated form of Akt, and NF-κB, which lead to downregulating their key effectors – C-myc, N-myc, and Cyclin D1. These changes induced apoptosis through the mitochondrial pathway, along with downregulation of Bcl-2. Treatment with curcumin was also noted to enhance the killing efficiency of nontoxic doses of cisplatin and irradiation.

Wu et al. analyzed the response of U251 GBM cells to treatment with curcumin, focusing on its ability to regulate the activity of STAT3, NF-κB, caspase-3, and the tumor suppressor – death-associated protein kinase 1 (DAPK1) [57]. Curcumin was demonstrated to cause a dose-dependent and time-dependent increase in DAPK1 mRNA and protein expression in U251 cells. Curcumin was also noted to reduce the phosphorylation of STAT3 and NF-κB, thereby reducing their activity. In addition, the exposure to curcumin reduced the DNA binding activity of STAT3 and NF-κB. The increased expression of DAPK1 enhanced the ability of curcumin to regulate the inhibition of STAT3 and NF-κB. Caspase-3 activation was also demonstrated after exposure to curcumin, and was associated with an increase in apoptotic activity. Curcumin also affected the cell cycle of U251 cells, inducing a G_2/M arrest.

Xiu et al. evaluated the effects of combination treatment with curcumin and irradiation on inducing immunogenic cell death (ICD) in glioma cell lines [58]. ICD is a special form of apoptosis mediated through endoplasmic reticulum (ER) stress via ROS, with the release of damage-associated molecular patterns (DAMPs) from cells, subsequently inducing an immune cell death through phagocytosis. Escalating doses of curcumin reduced the viability of glioma cells in culture (human U251, mouse GL261), while increasing the rate of apoptosis. Adding curcumin prior to irradiation improved the ability to induce apoptosis in hypoxic glioma cells. The addition of curcumin was shown to enhance the ability of irradiation to induce ICD in glioma cells, through increasing ER stress via activation of protein kinase RNA-like ER kinase (PERK) and inositol-requiring protein 1-α (IRE1α) signaling pathways, thereby upregulating the pro-apoptotic transcription factor CHOP. The combination of curcumin and irradiation accelerated the recognition and phagocytosis of irradiated glioma cells by dendritic immune cells.

As noted above in Section II, curcumin is very hydrophobic and therefore is difficult to use clinically due to poor water solubility and low bioavailability. In order to improve its clinical utility and to test efficacy in cell cultures (human glioma U251 cells) and xenograft models, Zhao and co-workers used curcumin encased in nanoliposomes [59]. The nanoliposomes were modified by the addition of a brain-targeting peptide – RDP. When escalating doses of the curcumin nanoliposomes were added to the glioma cultures, there was a dose-dependent inhibition of cell growth and proliferation. The internalization of the nanoliposomes was determined to be an energy-dependent, microtubule- and microfilament-involved endocytic process. Curcumin induced an increase in apoptosis in the cells, mediated by a cell cycle block and arrest in S-phase. Using an intracranial xenograft model, the curcumin nanoliposomes were noted to target within the brain and glioma tissue of the animals. The curcumin treated animals had tumors that exhibited reduced cell mass and smaller volume, and were associated with an increase in mean survival time.

In recent years there have been several in depth reviews of the mechanisms of anticancer activity of curcumin and its potential for treatment of brain tumors, and glioblastoma in particular [60–62]. Curcumin has activity against many of the pathways that are dysregulated in GBM, as noted above, including the ability to induce G_2/M cell cycle arrest, activating the apoptotic pathways, inducing autophagy, disrupting and downregulating multiple signaling pathways (e.g., NF-κB, AP-1, STAT3, SHH), reducing the capacity for invasion and metastasis (including inhibiting MMP expression), modulating protein ubiquitination, inducing oxidative stress, and increasing the efficacy of traditional chemotherapy agents and irradiation. There are numerous active clinical trials using curcumin and applying it to many different disease states, including cancer. Many of the trials are using large oral doses to overcome the poor bioavailability issues, while others are using more advanced delivery systems such as liposomes, nanotechnology, microparticles, and antibody conjugates. Unfortunately, none of the more than 100 clinical trials investigating curcumin are involving brain tumor patients. The authors conclude that additional pre-clinical research is needed – including further *in vitro* and in vivo studies – and that clinical trials should be undertaken to corroborate the benefits of curcumin in brain tumor patients.

5. Application of guduchi to brain tumors

Several papers applying Guduchi to brain tumors have been written by Kaur and colleagues (see Table 2). The first report used a 50% ethanolic extract from the stem of *Tinospora cordifolia*, and applied it to rat C6 glioma cells, human U87 glioma cells, and PC3 prostate cells [63]. After escalating concentrations of the extract (10 μg/ml to 1000 μg/ml), the tumor cells were growth arrested and had morphological changes consistent with more differentiated cells, with multiple and elongated processes. In the C6 cells, this was further confirmed by increased expression of GFAP. The IC_{50} for the C6 and U87 cells was 200 μg/ml. Treatment resulted in an increase in apoptotic cell death in tumor cells, with a reduction of expression of the anti-apoptotic gene Bcl-xl. The extract also induced cell cycle arrest in G_0/G_1 and G_2/M, with a reduction of expression of the cell cycle protein cyclin D1. Migratory ability of C6 cells was reduced after treatment with the extract, along with downregulation of NCAM.

In a follow-up report from the same group, they applied chloroform and hexane extracts from *Tinospora cordifolia* to human U87 glioma cells and IMR-32 neuroblastoma cells [64]. The chloroform and hexane extracts were derived from the initial ethanolic extract, and had more pronounced anti-proliferative activity at very low doses (i.e., 10–15 μg/ml). Increasing concentrations of both extracts were able to inhibit growth and altered morphology consistent with more differentiated cells, with multiple and elongated processes. Treated cells also had increased expression of GFAP and MAP-2, both markers of more differentiated and senescent cells. Other markers of differentiation, including HSP70 and Mortalin, also had upregulation of expression in both cell types. After treatment with both fractions, the migration capacity of both cell types was significantly reduced. When the chloroform and hexane extracts were applied to primary astrocyte cultures, no toxicity was observed. The authors concluded that the chloroform and

hexane extracts had more pronounced induction potential for differentiation and anti-migratory effects than the initial ethanolic extract.

6. Conclusions and future considerations

It is obvious from the overview of Ayurveda in Section II, that many of the plants, herbs, and herbal formulations that have been used for millennia by Ayurvedic physicians have significant efficacy for numerous human ailments, such as inflammation, digestion, diabetes, depression, and many others, including cancer. More recent ethnopharmacological and phytochemical analyses have now been able to shed some light on the likely bioactive compounds that are mediating these beneficial effects – WFA, withanones, curcumin (Haldi/turmeric), berberine, palmatine, etc. Further analysis of these plants and herbal formulations will be important to delineate all of the active compound or compounds, and if they could be more specifically applied to cancer treatment. In addition, it may be helpful to also consider a "Network Pharmacology" approach in these studies, since many of the herbals and/or formulations seem to be modulating multiple targets simultaneously.

Clinical trials have begun to apply Ayurvedic herbs and preparations to human subjects for treatment of various diseases, as well as for pharmacokinetic analyses. For example, Ashwagandha, Withanoloides, Curcumin, and *Triphala* have all been applied to treat psychiatric diseases, including depression, anxiety, bipolar disorder, obsessive-compulsive disorder, and others [65–68]. Much of the preliminary evidence supports that these herbs and preparations can have a positive effect on affective disorders, and have been well-tolerated. In addition, pharmacokinetic studies in healthy volunteers have been performed using an Ashwagandha root extract after ingesting a 500 mg capsule [69]. The results demonstrated a C_{max} for Withanolide A and WFA of 2.926 ± 1.317 and 2.833 ± 0.981, respectively, along with a T_{max} of 1.361 ± 0.850 and 0.903 ± 0.273, respectively. Other studies have evaluated the activity of *Triphala* against gingivitis and scalp seborrhea in randomized clinical trials – with significantly positive results [70,71]. Additional studies will be necessary to explore the efficacy and tolerability of Ayurvedic herbs and preparations in many other medical diseases and conditions.

The current worldwide standard of care for treatment of GBM and other malignant gliomas is to perform an extensive surgical resection (if possible), followed by 6000 cGy of RT in 30 fractions, along with concomitant daily oral TMZ chemotherapy (75 mg/m^2/day x 42 days) [72,73]. After the completion of chemo-RT, the patient then undergoes adjuvant chemotherapy with TMZ (150–200 mg/m^2/day) for 5 days every 28 days, for at least 6 cycles. Other than this approach, there are very few FDA approved treatment options for GBM patients, and include the use of TTF's and Bevacizumab – a monoclonal antibody targeted to VEGF [51,72,74].

There are 100's of clinical trials for patients with GBM and high-grade gliomas, most of which are trying to find a drug or compound that will add progression-free and overall survival time to what has been achieved with TMZ-based chemo-RT and adjuvant TMZ ± TTF's. Based on the review above of Ayurvedic medicine and the ethnopharmacological and phytochemical analyses of several of the most active plants, herbs, and herbal formulations, it is clear that several of these compounds and extracts have significant anti-cancer potential (e.g., WFA, curcumin, Guduchi). In addition, they are natural products that have been used for millennia and have proved to be well tolerated. It will be important to apply these more specifically to treatment of GBM and other malignant brain tumors. Additional *in vitro* studies will be needed, especially in more sophisticated model systems, such as GBM organoid cultures, which more closely resemble the 3-dimensional structure of the human tumor [75]. Thus far, there have been very few phase I or II clinical trials with an Ayurvedic-related compound or herbal extract in patients with GBM, medulloblastoma, or any other type of malignant brain tumor. A bioavailability trial of Curcumin in GBM patients that require surgical resection has been launched in Germany (NCT01712542). In addition, a liposomal form of Curcumin will be used in addition to standard chemo-radiotherapy in another trial of newly diagnosed patients with GBM in the USA (NCT05768919). There are no current clinical trials listed for Ashwagandha, WFA, Guduchi, or Triphala for treatment of brain tumors on ClinicalTrials.gov. It will be important to bring some of these Ayurvedic compounds into clinical trials of GBM patients, since the pre-clinical and animal studies for several of them have been so compelling – in particular WFA and curcumin – including reports suggesting synergistic interactions with TMZ and TTF's. The addition of one of these products to TMZ during chemo-RT and adjuvant monthly chemotherapy would be a reasonable first step in a small Pilot Study or a larger Phase I trial. In addition to the hope for an anti-cancer effect that might be synergistic with RT/TMZ and monthly TMZ, there is also the possibility that the addition of these formulations or compounds might have other clinical benefits as well, such as less fatigue, reduced GI upset, better digestion, improved immune strength, and less RT-induced inflammation. During the adjuvant phase of TMZ treatment, which can last 6–12 months in many patients, the use of Ayurvedic formulations and compounds could add significantly to patient tolerability of therapy, with an overall improved quality of life.

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