Current Progress of Phytomedicine in Glioblastoma Therapy

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Summary: Glioblastoma multiforme, an intrusive brain cancer, has the lowest survival rate of all brain cancers. The chemotherapy utilized to prevent their proliferation and propagation is limited due to modulation of complex cancer signalling pathways. These complex pathways provide infiltrative and drug evading properties leading to the development of chemotherapy resistance. Therefore, the development and discovery of such interventions or therapies that can bypass all these resistive barriers to ameliorate glioma prognosis and survival is of profound importance. Medicinal plants are comprised of an exorbitant range of phytochemicals that have the broad-spectrum capability to target intrusive brain cancers, modulate anti-cancer pathways and immunological responses to facilitate their eradication, and induce apoptosis. These phytocompounds also interfere with several oncogenic proteins that promote cancer invasiveness and metastasis, chemotherapy resistance and angiogenesis. These plants are extremely vital for promising anti-glioma therapy to avert glioma proliferation and recurrence. In this review, we acquired recent literature on medicinal plants whose extracts/bioactive ingredients are newly exploited in glioma therapeutics, and also highlighted their mode of action and pharmacological profile.
Key words: phytomedicine; phytochemicals; glioblastoma; chemotherapy resistance; bioactive

Human glioblastoma multiforme (GBM) is a pernicious and malignant form of tumor that mainly affects the central nervous system. It is characterized by aberrations in glial cells, causing uncontrollable growth and development[1, 2]. It is the only fatal type of brain cancer that has no ultimate curative therapy to prevent its rapid proliferation and expansion inside the brain. The contemporary intervention for treating GBM consists of surgical removal, radiotherapy or chemotherapy that provides momentary relief and low survival rate (4%–5%)[3, 4]. These numbers suggest that the current therapeutic approach is not as effective as anticipated in *in vitro* studies and requires further discovery and development of new therapies.

There are two main reasons why current chemotherapeutic intervention is failing in preventing tumor development. (1) The GBM has a complex vascularized network that deters the entry of these chemotherapeutic agents, leading to drug resistance whereas the blood-brain barrier (BBB) impedes drug transference resulting in glioma recurrence[5, 6]. (2) Another potential reason for the poor effectiveness of chemotherapeutic agents is enzymatic alteration of drug action via O6-methylguanine-DNA methyltransferase (MGMT)[7]; adverse consequences of these agents include cerebral edema and myelosuppression[8].

Medicinal plants remain promising for procuring different types of anti-cancer compounds such as vincristine from *Catharanthus roseus*, colchicine (*Colchicum autumnale*), combretastatins (*Combretum caffrum*) and so on[9, 10]. These compounds can penetrate BBB without any hindrance, with the potential to target cancerous cells, promote various immunological and anti-proliferation proteins and pathways to prevent further tumor propagation and motivate tumor cell apoptosis[10, 11]. However, recent literature showed glial cells became more aggressive, with the ability to resist the action of these inhibitory compounds[12–14]. In addition, these medicinal-based chemotherapeutic agents also provoke a number of adverse effects including nerve demyelination, neurological impairment and peripheral neuropathy[13, 15]. All these factors hinder their usage in many intrusive brain cancers. Therefore, there is a strong demand for novel cytotoxic compounds that can inflict fewer side effects whilst maintaining the ability to diminish tumor proliferation and progression. It is worth considering new medicinal plant sources

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to identify these new compounds. In this review, we assembled the latest literature on new medicinal plants whose extracts had been utilized in GBM therapy for the first time. Their molecular influence on various anti-cancer related cellular pathways have also been elucidated, and their pharmacological profile has been highlighted.

1 MEDICINAL PLANTS IN GBM THERAPY

1.1 *Viola odorata*

These plants contain various naturally-occurring bioactive constituents that possess cytotoxic activity against different cancer cells, and their inhibitory influence was recently assessed in GBM cell line by Hashemi *et al*[16].

The ethanolic extract of *V. odorata* results in instability in the inner-mitochondrial membrane potential of glioma cells by triggering the release of cytochrome C. This event initiates the expression of pro-apoptotic BAX protein while suppressing the activity of anti-apoptotic BCL-2 protein. The upregulation of pro-apoptotic BAX protein levels causes the augmentation of mitochondrial permeability transition pore (MPT) which promotes the release of cytochrome C in the cytoplasm, incites mitochondrial swelling and reactive oxygen species (ROS) production, thus inflicting outer-mitochondrial membrane rupture. This ultimately leads to the disruption of adequate energy supply for the glioblastoma cells, which is otherwise crucial for proliferation[17]. Through activation of caspase 9/3 signalling, chromatin fragmentation takes place, triggering a DNA damage response and leading to apoptosis.

1.2 *Spondias pinnata*

Most of the anti-cancer studies emphasized an ethyl acetate fraction of *S. pinnata* bark, as it contains a high percentage of gallic acid and methyl gallate. The ethyl acetate fraction of *S. pinnata* shows appreciable activity against U87 glioblastoma cell lines through cytochrome C-dependent apoptosis pathways by upregulating tumor suppressor p53[18, 19].

These extracts also modulate BAX and caspase proteins that facilitate glioma suppression by disrupting mitochondria and Poly (ADP-ribose) polymerase (PARP) functioning to induce cell death. It also has the targeted affinity towards cancer cells, enhances resistant cancer cells susceptibility to chemotherapeutic drugs and inhibits the action of pro-angiogenic factors that lead to angiogenesis. Glioma cells can invade the surrounding healthy tissue which helps them to metastasize to different parts of the brain and body, hence making it challenging to be eradicated through surgical or chemotherapeutic intervention. This proliferation and invasiveness are enabled by ADAM Metallopeptidase Domain 17[20] and Protein kinase B (AKT)[21] which are successfully downregulated by these bioactive compounds. The administration of these compounds also increases extracellular signal-regulated kinase ½ (ERKs) expression levels to induce cell apoptosis[18, 19].

1.3 *Portulaca oleracea*

Among different phytochemical constituents, the flavonoid content of these plants are greatly exploited for anticancer activity. These flavonoids are commonly procured from ethanolic fractions which contain higher quantities of homoisoflavonoids, kaempferol and apigenin[22, 23]. Rahimi *et al* attempted to assess the cytotoxic activity of ethanolic fractions from *P. oleracea* leaves against GBM cancer cell lines (U-87)[24]. Exposure of leaf extract containing kaempferol and apigenin greatly abrogates nuclear factor kappa-B (NF-κB) activity and ROS responsible for assisting glioma proliferation. But the anti-glioma activity of kaempferol and apigenin is dose- and time-dependent, and their cytotoxicity is amplified when combined with vitamin C. The effective dosage recorded for *P. oleracea* extract is 140 μg/mL and 100 μg/mL for vitamin C conjugated extract. Further studies suggested that these two flavonoids (kaempferol and apigenin) suppress AKT/mTOR and MAP kinase intracellular signalling pathway (regulating cell division, cancer proliferation and cellular apoptosis) to encourage cancer cells apoptosis and also increase the activity of T-helper cell-1. This directs the immune system in cytotoxic response towards glioma[25–28]. In addition to that, they halt the cancer cell cycle at G_1 phase and induce DNA fragmentation, therefore leading to apoptosis[26, 27, 29]. Furthermore, elevated neuronal nitric oxide synthase modulates tumor angiogenesis, cancer invasion and metastasis to stimulate glioma proliferation and disrupt cytotoxic and programmed cell death responses[30]. It is observed in these studies that these two compounds, along with other flavonoids, participate in averting tumor growth and development by negatively regulating the expression of nitric oxide synthase and thwarting oxidative stress by decreasing ROS[24, 26, 27, 29].

1.4 *Ruta graveolens*

Several studies have shown that soaking aerial parts of *R. graveolens* in methanolic extract yields a high amount of rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside), which is an active agent against invasive cancers[31–33]. A study was conducted by Gentile *et al* in 2015 to evaluate the antiproliferative activity of *R. graveolens* leaf extract against 4 different glioblastoma cell lines[34]. Instead of adopting the methanolic fraction approach, researchers opted for an aqueous solution to prepare rue extract and increase extract bioavailability. This extract (1 mg/mL) selectively targets glioma cells without harming nearby healthy neurons. ERK ½, AKT and caspase 3 protein levels are promotes by these

extracts, mediating cytotoxic effects on glioma cells and leading to apoptosis. However, literature regarding the pharmacological mechanism of rue extract on glioma cells is limited and further molecular studies were required to confirm their action. On the other hand, rutin alone shows no promising anti-proliferative activity against glioma cells, hence indicating that this compound may require other phytochemicals to induce anti-cancer activity.

1.5 *Pavetta crassipes*

The phytochemicals contained in these plant extracts are of great interest due to their broad pharmacological application, ranging from anti-microbial to anti-tumor activity, but their cytotoxic influence on malignant brain tumors remains unknown[35, 36]. From a study by Wilcox[37], it is observed that 20 μg/mL ethyl acetate leaf fraction stunted the proliferation of glioma cells at quiescent G_0 phase, induced cleavage of PARP and reduced EGF-mediated activation of AKT and ERK ½ levels, proteins that facilitate apoptosis evasion and resistance to chemotherapy.

1.6 *Caesalpinia sappan*

In the majority of anti-cancer studies, aromatic haematoxylin, especially brazilin, are more commonly exploited due to their competent cytotoxic activity in various cancer cell lines[38, 39] except for glioma U87 cell lines, as observed by Lee *et al* in 2013[40]. The administration of 15 μmol/L brazilin sequestered glioma cells in G_1 phase, thus averting further proliferation. The compound also increases the expression of caspases 3 and 9, facilitating PARP fragmentation, and substantially controlling the progression of glioma cells.

1.7 *Abutilon indicum*

The cytotoxic activity of *A. indicum* extract against human glioblastoma cells was explored for the first time by Khan *et al*[41]. Isolated extracts were tested against U87MG glioma cell lines. Of these, chloroform fractions exhibited appreciable anti-glioma activity and were further subjected to chromatographic purification, which yielded 4 different compounds: methyl trans-p-coumarate, methyl caffeate, syringic acid, and pinellic acid. In particular, methyl caffeate at an approximate concentration of 8.5 mg/mL shows promising inhibition of glioma cells. However, the mechanism of glioma inhibition remains elusive.

1.8 *Passiflora edulis*

Various bioactive extracts have been procured from different parts of *P. edulis* plants. However, Kuete *et al* adopted a newer approach by utilizing methanolic fruit extract (3.5 μg/mL fruit pericarp and 1 μg/mL fruit). These extracts successfully inhibits U87MG Human Glioblastoma cells by G_0/G_1 cell cycle arrest and depolarises mitochondrial membrane potential, resulting in apoptosis[42]. But their influence and mode of action on different important cancer pathway and proteins are not known yet.

1.9 *Euphorbia tirucalli*

Among different phytochemicals contained in this plant, triterpenoids are extensively explored in several cytotoxic studies. Of these tripernoids, tetracyclic triterpene euphol is highly effective against solid tumors; its activity in glioma cells was first characterised by Reis *et al* in 2013 by employing a range of both commercial and clinically extracted glioma cell lines[43]. Euphol remarkably inhibits all glioma cell lines in a dose- and time-dependent manner in comparison to anti-cancer drug temozolomide. In 2018, Silva *et al* determined the molecular anti-proliferative mechanism of euphol on glioma cells[44]. It was observed that euphol suppressed the concentration of pro-apoptotic proteins (TNRI/TNFRSF1A, BAX, BAD, caspases 3 and FASTNFR6/CD95), and promoted cyclin-dependent kinase inhibitors p21/CIP1/CDKN1A and p27, which prevented glioma proliferation and induced autophagy by upregulating associated protein LC3-Ⅱ levels and reducing BCL-2 and NF-κB expression. From this reduction, it can be assumed that euphol mediates cytochrome c-dependent apoptosis and subsequent mitochondrial dysfunction. Euphol also activates the cell stress pathway by increasing the expression of superoxide dismutase 2 (SOD2), which in turn modulates reactive oxygen species formation and imposes oxidative stress on glioma cells, leading to their death. Their study also highlighted the inhibitory effect of euphol on protein kinases PKD1 and PKDμ that instigate oncogenesis.

1.10 *Liriodendron tulipifera L.*

Quassinti *et al* isolated new medicinal and bioactive metabolites from *L. tulipifera* and identified seventy novel compounds that were categorized into sesquiterpenes, monoterpenoids and other essential oils[45]. These compounds were further assessed for anti-glioblastoma activity. It was observed that leaf oil fractions were capable of inducing dose-dependent cytotoxicity by activating caspase-3 activity to a higher degree than with other fractions and compounds. Further studies are required to confirm whether the apoptosis was caspase-dependent or if any protein factor was activated prior to caspase 3 activation that may have rendered cytotoxicity.

1.11 *Chelidonium majus L.*

Crude phytometabolite extract procured from *C. majus* is renowned for inducing immunomodulatory responses to promote cytotoxicity in different resistant cancer types[91, 92]. Extracts such as ukrain are composed of different alkaloids, including derivatives of chelidonine. These have shown promising anti-glioma activity based on cell lines studies[46, 47]. It has been observed that ukrain downregulates secreted protein acidic and rich in cysteine (SPARC) levels, which are

partially responsible for the breakdown of extracellular matrix in order to provide a favorable environment for the growth and penetration of glioma cells in visceral brain regions[46].

In a similar study, it was confirmed that glial fibrillary acidic protein (GFAP) activity was modulated by ukrain, which truncated the proliferation and invasiveness of glioma cells[47]. Recently, Lee *et al*[48] showed that other phytometabolic component chelidonine adversely intervenes with T98G glioblastoma cell division by upregulating cyclin B1 levels, leading to aberrant phosphorylation/dephosphorylation of CDK1 and extension of the transition of glioma cells from G_2 to M phase of the cell cycle. It also inactivates the anaphase promoting complex responsible for enabling the transition from metaphase to anaphase, resulting in multipolar spindle assembly and activation of apoptosis via mitotic catastrophe (abnormal cell division). On the other hand, chelidonine negates antiapoptotic protein MCL-1 activity and stimulates the expression of proapoptotic proteins BAK and BAX, causing mitochondrial outer membrane permeabilization (MOMP). This incident destabilizes mitochondrial integrity, releasing cytochrome c, endonuclease G, and AIF into the cytoplasm, thus forming the apoptosome via interaction with APAF-1 to facilitate caspase 3 and 9 cleavage, PARP cleavage, and chromatin fragmentation via endonuclease G and AIF. All events caused by chelidonine are dose- and time-dependent and causes imminent glioma cell death.

1.12 *Anisomeles indica*

These plants are promising anti-cancerous metabolites known for alleviating inflammation and other oncogenic hallmarks of resistant cancer types. Among these metabolites, Su *et al* investigated the therapeutic effect of ovatodiolide, a diterpenoid, on two different glioblastoma cell lines (U-87MG and GBM8401 cells)[49]. It was observed that 2.5 μmol/L of ovatodiolide instigated cytochrome c-mediated apoptosis through promotion of BAX and BAK proteins and disrupted JAK2/STAT3 signaling pathways that would have otherwise modulated glioma proliferation, self-renewal and metastatic potential. It also attenuates glioma invasiveness and migration by deregulating epithelial-to-mesenchymal (EMT) oncogenic β-catenin, N-cadherin, Slug and Vimentin protein expression and makes glioma cells susceptible to the anti-cancer drug temozolomide.

2 CONCLUDION AND FUTURE RECOMMENDATIONS

This review covers recent literature about medicinal plants whose extracts or bioactive compounds have not been previously exploited in glioma therapeutics. These studies showed some medicinal plants have got promising anti-glioma activity capable of inducing apoptosis and cytotoxicity, activating different anti-proliferation pathways, inhibiting notable protein targets that are involved in angiogenesis, and preventing glioma rapid proliferation, invasiveness and metastasis (table 1). These constituents incite mitochondrial and chromatin-associated DNA fragmentation, sensitize chemo-resistant glioma cells to chemotherapy, modulate immunological response towards these cancerous cells and tumor suppressor proteins (p53, p27) activity to stunt G_1 phase proliferation and encourage apoptosis.

Most of these studies highlighted the inhibitory effect of medicinal plants on different oncogenic proteins whereas others highlighted their cytotoxicity. Therefore, it is necessary to elucidate their pharmacological influence on oncogenic proteins and avoid off-target effects that might arise with their administration in animal and clinical studies.

Another aspect of this review was to analyze the EC_{50}/IC_{50} concentration of these bioactive constituents on glioma cells (table 2). It is observed that the majority of the compounds show appreciable activity at 100 μg/mL or higher, whereas some show promising influence at around 10–30 μg/mL. To qualify for pre-clinical studies or clinical trials, these bioactive compounds have to show satisfactory anti-glioma activity less or equivalent to 20 μg/mL as indicated by the National Cancer Institute (NCI)[50]. Some studies suggest that glioma cells form an intricate vascular network and employ different complex drug detoxification pathways to attenuate the drug influence and penetration[51], whereas some medicinal extracts have reduced bioavailability[52] or hindered delivery[53] into the cancer cells, thus leading to chemotherapy resistance. Therefore, increasing the concentration of these extracts can yield appreciable activity but also provoke numerous adverse effects and toxicity[54].

This problem could be efficiently addressed by employing different nanodrug delivery systems[53] or next generation proteolysis-targeting chimeras (PROTACs)[55] to facilitate targeted oncogenic protein degradation and inhibition, and increase the bioavailability of these compounds. On the other hand, an alternative approach is to synergistically utilize two different plant extracts in low concentration[56] or to extract a single bioactive compound from the entire plant. Single phytoconstituents may not demonstrate sufficient anticancer effects as their activity may be dependent on the coordinated activity of other phytochemicals contained within the plant, as evidenced from these reported studies[34, 42, 44, 45, 49]. Introducing these bioactive compounds as adjuvants alongside conventional chemotherapeutic agents might improve cancer prognosis and counteract off-target effects usually incited by cancer medications[57, 58]. These

Table 1 Therapeutic anti-glioma influence conferred by medicinal plants

Medicinal plant	Pathway activated	Target protein inhibition/activation	Therapeutic mechanism	Ref
Viola odorata	Cytochrome c and Caspases 9/3 mediated apoptosis,	BCL-2↓, BAX ↑, caspase 9, 3 proteins↑	DNA fragmentation, mitochondrial rupture, apoptosis	[16]
Spondias pinnata	Cytochrome c, Caspases 9/3 mediated apoptosis, Extracellular signal regulated kinase (ERK1/2)	p53↑, BAX ↑, caspase 9, 3 proteins↑, Poly (ADP-ribose) polymerase↓, Bcl-2↓, ADAM 17 ↓, AKT ↓, p-ERK1/2↑, p-ERK↓	DNA fragmentation, mitochondrial rupture, anti-neoangiogenic, apoptosis	[18, 19]
Portulaca oleracea	–	NF-κB↓, ROS↓, NO↓	Cytotoxicity, apoptosis	[24]
Ruta graveolens	Caspases 9/3 mediated apoptosis, extracellular signal regulated kinase (ERK1/2) and PI3K/AKT pathway	Caspase 9, 3 proteins↑, ERK1/2↑, AKT↑,	Cytotoxicity, apoptosis	[34]
Pavetta crassipes	Caspases 3/7 mediated apoptosis	Poly (ADP-ribose) polymerase↓, p-ERK1/2↓, AKT↓	Cell cycle suppression at G_1 phase, cytotoxicity, apoptosis	[35, 37]
Caesalpinia sappan	Caspases 9/3 mediated apoptosis,	Poly (ADP-ribose) polymerase↓, caspase 9, 3 proteins↑	Cell cycle suppression at G_1 phase	[40]
Abutilon indicum	–	–	Cytotoxicity	[41]
Passiflora edulis	Caspases 9/7/3 mediated apoptosis	Caspase 9, 7, 3 proteins↑	Cell cycle suppression at G_1 phase, instability of mitochondrial membrane potential, apoptosis	[42]
Euphorbia tirucalli	Autophagy associated apoptosis, cytochrome c dependent apoptosis	TNRI/TNFRSF1A↓, BAX↓, BAD↓, caspases 3↓, FASTNFR6/CD95↓, P21/CIP1/CDKN1A↑, P27↑, LC3-II↑, Bcl-2↓, NF- κB ↓, SOD2↑, PKD1↓, PKDM↓	Mitochondrial rupture, apoptosis, cytotoxicity	[43, 44]
Liriodendron tulipifera L	Caspases 3 dependent apoptosis	Caspase 3↑	Cytotoxicity, apoptosis	[45]
Chelidonium majus	Caspase dependent and independent mediated apoptosis	Glial fibrillary acidic protein↑, Cyclin B1 ↑, SPARC↓, Mcl-1↓, caspase 3, 9 ↑, BAK↑, BAX↑,	DNA fragmentation, mitochondrial rupture, cell cycle suppression at G_1 phase	[46–48]
Anisomeles indica	Cytochrome c mediated apoptosis,	BAK↑, BAX↑, p-STAT3↓, Cyclin D1↓, p-AKT↓, p-JAK2↓, c-Myc↓, β-catenin↓ Vimentin↓, slug↓, N-cadherin↓, BCL-2↓, BCL-xL	Mitochondrial rupture, disrupted JAK/STAT3 pathway	[49]

Ref: reference

Table 2 Pharmacological profile of medicinal plants averting glioma proliferation

Medicinal plant	Plant organ	Extract/bioactive constituent	Concentration/exposure time	EC50, IC50	Cell line	Ref
Viola odorata	Flowers, leaves	Methanolic extract	10–500 μg/mL for 24, 48, 72 h	400 μg/mL (48 h)	Clinical cell line	[16]
Spondias pinnata	Bark	Gallic acid and methyl gallate	10–75 μg/mL	10 μg/mL (48 h)	U87MG, U251n	[18, 19]
Portulaca oleracea	Leaves	Kaempferol and apigenin	100–800 μg/mL for 72 h	133.3 μg/mL (48 h)*	U87MG	[24]
Ruta graveolens	Leaves	Aqueous extract (rue extract)	1 mg/mL for 72, 48, 24 h	1 mg/mL (72 h)	C6, U138, U87MG, A1 mes c-myc cells (A1)	[34]
Pavetta crassipes	Leaves	Methanolic extract, methanolic/dichloromethane	20 μg/mL for 72, 48, 24 h, 100 μg/mL for 72 h	5.0 μg/mL (48 h), 3.7 μg/mL (72 h)	U251 MG, U1242 MG, U373	[35, 37]
Caesalpinia sappan	Heart wood	Brazilin	0–40 μg/mL for 24 h	7.5 μg/mL (72 h)	U87MG	[40]
Abutilon indicum	Leaves	Chloroform fraction extract	0–10 μg/mL for 72 h	8.2 μg/mL (72 h)	U87MG	[41]
Passiflora edulis	Fruit, pericarp (FP), fruit (F)	Methanolic extract	0–10 μg/mL for 72, 48, 24 h	3.4 μg/mL (FP), 1 μg/mL (F)	U87MG	[42]
Euphorbia tirucalli	–	Euphol	10–40 μmol/L for 72, 48, 24 hours	19* μmol/L	U87MG, U251, U373, SW1088, GAMG, NB19, SW1783, RES186, RES259, UW479, KNS42, HCB2, SF188, HCB149	[43, 44]
Liriodendron tulipifera L	Leaves, flower and fruits	Leaf oil fraction	1–10 μg/mL for 72 hours	7.4* μg/mL (72 h)	T98G	[45]
Chelidonium majus	–	Ukrain, chelidonine	1–10 μmol/L, 0.1–10 μmol/L for 24, 48, 72 h	1 μmol/L, 10 μmol/L (72 h)	T98G, T60, T63	[46–48]
Anisomeles indica	–	Ovatodiolide	0.6–1.0 μmol/L 24, 48, and 72 h	≥ 5 μmol/L (48 h)	U87MG, GBM8401	[49]

*Average mean value. Ref: reference

medicinal plants also hold other potentially active compounds that have not been exploited and therefore are recommended for further studies prior to clinical trials and onco-therapeutic development.

Conflict of Interest Statement

The author declares that there is no conflict of interest.

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