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Effect of Natural Compounds on Glioblastoma Multiforme Pathways

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Abstract

Glioblastoma is one of the most debilitating forms of brain tumour. It accounts for 17% of all the brain tumours and has resulted in 251,329 deaths in 2020 itself. Several studies performed on patient profiling have revealed that many genes tend to be overexpressed or mutated in many GBM patients and hamper the signalling and growth pathways. Additionally, there is a requirement to explore supplementary or alternative treatment that can aid in overcoming the resistance caused by standard care treatment (Temozolomide with radiation). The preliminary analysis on inhibitory potential of certain phytocompounds on the crucial genes involved in GBM has been studied in silico. The network of the genes critical for GBM has been analysed through several plugins of Cytoscape software like BiNGO and MCODE based on factors such as degree, closeness and betweenness. The network analysis gave out a cluster of 97 interactions with 16 interacting proteins. ERBB4 emerged as the seed protein indicating its crucial role in pathways. The BiNGO plugin gave out the gene ontology data describing the functions of the genes of the cluster. Additionally, the properties of ligands were studied by the PharmaGist server and 3D patterns of shared features by all or most input ligands of selected phytocompound were analysed and aligned. Upon docking the seed protein (ERBB4) individually with the best combination of aligned ligands yielded bromelain and EGCG as the compounds with best binding affinity of -9.8 and -9.2 kcal/mol respectively. This study has provided an important lead for further in vitro study to analyse the effect of combination of phytocompounds on the critical signalling pathways of GBM.

Keywords Bromelain; Cytoscape; Epigallocatechin 3 Gallate; Phosphatidylinositol-3-kinase; Receptor tyrosine-protein kinase-ERBB4; Signalling pathway; Temozolomide; Tumor Microenvironment;

Introduction

Glioblastoma multiforme is the most devastating form of brain tumour, which happens to be heterogeneous in nature as it includes astrocytoma, oligodendroglioma and oligoastrocytoma. It is a high grade astrocytoma that starts in cerebral glial cells and further leads to malignant tumours possessing excessive proliferation capacity, necrotic and angiogenic activity. Despite therapeutic strategies being available, a robust cure for the disease has not been found yet. One of the prime factors associated with the redundancy of therapy with temozolomide is defective mismatch repair (MMR) and O6-methylguanine DNA methyltransferase (MGMT) [1]. Tumour heterogeneity is also a major factor leading to aggressiveness and invasiveness of GBM tumours attributed to clonal selection. It refers to the development of cells possessing stem cell like properties where each group acquires different sets of genetic alterations and these sub clones can undergo expansion in a variety of environmental conditions [2,3]. The aggressiveness of these sub clonal populations is characterized by deregulation of many key signalling pathways involving growth, proliferation, survival, and apoptosis. This necessitates exploring novel diagnostic and therapeutic strategies that target these pathways to improve the treatment of GBM in the future.

The available literature indicates that overexpression and mutation of certain critical genes of molecular pathways are significantly present in GBM patient profile. Epidermal growth factor receptor (EGFR) is overexpressed in around 60% of the primary GBM and 10% of secondary GBM. Additionally, EGFRΔIII is the most common form of EGFR mutation majorly due to in-frame deletion of DNA sequence leading to formation of a truncated but active receptor contributing to excessive proliferation of GBM [4]. Apart from this, Wnt signaling is one of the most important signals during the developmental embryonic process and maintenance of tissues in the body [5]. During the development of neuronal cells, the Wnt pathway along with others such as NOTCH and fibroblast growth factor mediated signaling result in isolating the cells to the subventricular region. This leads to proliferation of progenitors of neural cells leading to GBM progression.

Additionally, mitogen activated protein kinase (MAPK) signaling pathways are involved in glioblastoma cell migration and proliferation. So, this can also be an important factor to be targeted for anti GBM therapy [6]. Similarly, pathways involving phosphatase and tensin homolog (PTEN), phosphatidylinositol-3-kinase (PI3K), protein kinase B (AKT)/mTOR have also emerged as a crucial player in GBM development and progression [7]. Over activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a critical role in various endpoints of GBM development such as tumor cell growth, survival, migration, angiogenesis and metabolism. Since it has been reported that 50% of GBM tumors possess mutation of PI3K-AKT pathway intermediates, they can be candidates for rational development of therapeutics for GBM [8].

There is a need to explore alternative therapies for GBM. Studying biological networks of these crucial pathway genes can be helpful in understanding their relationship. The present study elucidates an in silico insight on the role of these pathways and potential effect of therapeutic compounds on them.

Materials and Methods

Selection of candidate genes

Literature was extensively curated for genes involved in progression and survival of GBM. Articles were retrieved from NCBI PubMed and Google Scholar using keywords combination: GBM and patient profiling; GBM and genetic profiling; GBM and gene expression; GBM and protein interaction; GBM and gene and mutation; GBM and gene and co-occur; GBM and gene and present together. Shortlisting of candidate genes was done for pathways involved in GBM and their intermediate genes were studied for co-occurrence with genes of different pathways.

Construction of gene network

Gene network was created using a string database (https://string-db.org/). The list of names of genes was uploaded under the tab of multiple proteins and *Homo sapiens* was selected as the organism. The closest match was chosen and the network of the input proteins was generated.

Clustering gene analysis using Cytoscape

The network obtained from the STRING database was used to study the topological parameters through the Cytoscape software version 3.7.2. The network was analyzed considering centrality and neighbourhood connectivity parameters. Following this, clusters of the network was obtained through the MCODE plugin of Cytoscape software [9]. MCODE parameters for the study were set as Node Score Cutoff: 0.2, K-Core: 2, and Threshold: 2 for each sub-network [10]. The protein-protein interaction (PPI) network was analysed based on certain topological parameters such as betweenness, closeness centrality, node degree distribution, and shortest path length distribution.

Functional gene analysis using Biological Networks Gene Ontology

Biological Networks Gene Ontology (BiNGO) plugin of the Cytoscape was utilized to get an insight on the biological role and mechanisms of groups of genes in each cluster. Benjamini and Hochberg false discovery rate correction option was used for multiple testing correction and *Homo sapiens* was selected as the organism [11].

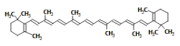
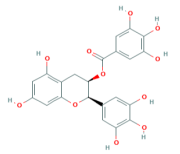
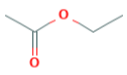
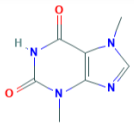
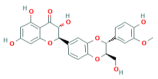
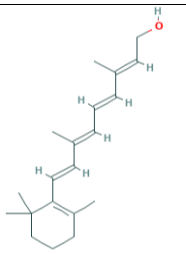
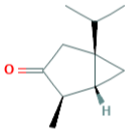
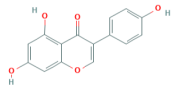
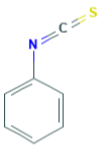
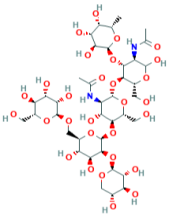
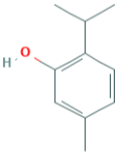
Pharmacophore analysis of phytocompounds

Literature was screened for potential phytocompounds using keywords: GBM and phytocompound; GBM and inhibitory phytocompound; GBM and treatment and phytocompound; Phytocompound and immunomodulatory. Compounds were selected based on their general immunomodulatory or specific anti-GBM effects. The shortlisted compounds are listed in Table 1. PharmaGist, a webserver, was utilized to build ligand-based pharmacophore models on shortlisted compounds (Table 1) [12]. It employs ligand-based pharmacophore detection methods [13]. The detection of pharmacophores was done based on the highest scoring 3D configuration of features common to a group of certain ligands.

Docking of seed protein with phytocompounds

The aligned phytocompounds based on pharmacophoric features were docked with the seed protein (ERBB4) of the network obtained from the Cytoscape through AutoDock Vina. The 3D equivalent structural derivatives of phytocompounds were extracted

Table 1: The ligands shortlisted for study

Name	Molecular formula	Ligand Diagram	SMILE Structure
Beta-carotene	C40H56		CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CCCC2(C)C)C)C)C
Epigallocatechin 3 gallate	C22H18O11		C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O
Ethylacetate	CH3COOC2H5		CCOC(=O)C
Theobromine	C7H8N4O2		CN1C=NC2=C1C(=O)NC(=O)N2C
Silibinin	C25H22O10		COC1=C(C=CC(=C1)C2C(OC3=C(O2)C=C(C=C(C=C5O4)O)O)O)CO)O
Retinol	C20H30O		CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CCO)C)C
Thujone	C10H16O		CC1C2CC2(CC1=O)C(C)C
Genistein	C15H10O5		C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O
Phenyl isothiocyanate	C7H5NS		C1=CC=C(C=C1)N=C=S
Bromelain	C39H66N2O29		CC1C(C(C(C(O1)OC2C(C(OC2OC3C(C(C(C(O3)CO)OC4C(C(C(C(O4)COC5C(C(C(C(O5)CO)O)O)O)O)O)O)OC6C(C(C(CO6)O)O)O)O)O)NC(=O)C)CO)O)NC(=O)C)O)O)O
Thymol	C10H14O		CC1=CC(=C(C=C1)C(C)C)O

from the PubChem (https://pubchem.ncbi.nlm.nih.gov/). Crystal structure of ERBB4 extracellular domain (2AHX) was downloaded and subsequently, the phytocompounds and the receptor were subjected to ligand and protein preparation by AutoDock Tools. The binding energies were calculated through the AutoDock Vina extension using Lamarckian Genetic Algorithm. [14]. The docked structures were visualized through Discovery Studio, where the details of the interactions between the receptor and ligand were also obtained.

Result and Discussion

Selection of candidate genes and construction of network

There are many reasons for increasing complications in GBM such as aggressiveness of growth, increased progression, and resistance. They are attributed to mutations and aberrant expression of the key genes regulating GBM. The literature was extensively studied for such occurrences and a list of 22 genes were selected and subjected to the STRING database (Table 2). The results indicated EGFR, PI3KCA, KRAS, PTEN, TP53, CDKN2A were closely linked into a dense network. Each gene was linked to at least two genes except COL1A2 and COL3A1. The proteins were observed to interact through undirected edges in the Cytoscape software version 3.7.2, which implies that the nodes of proteins affect each other bidirectionally (Figure 1) (Table 3) [15].

Table 2: Receptor proteins chosen as targets (retrieved from: The Human Protein Atlas https://www.proteinatlas.org/)

Protein code	Protein name	Protein Position
TP53	Tumor protein p53	Cytoplasm
KRAS	GTPase KRas	Cell nucleus
EGFR	Epidermal growth factor receptor	Plasma membrane, Cell Junctions
CDK4	Cyclin dependent kinase 4	Cytosol
TOP2A	DNA topoisomerase II alpha	nucleoplasm
CDK6	Cyclin dependent kinase 6	Nucleoplasm, Cytosol
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Mitochondria, Cytosol
PTEN	Phosphatase and tensin homolog	Nucleoplasm, Cytosol
CDKN2A	Cyclin dependent kinase inhibitor 2A	Nucleoli
NOTCH1	NOTCH1	Nucleoplasm
MDM2	MDM2 proto-oncogene	Nucleoplasm
MTOR	Mechanistic target of rapamycin kinase	Cytosol
MGMT	O-6-methylguanine-DNA methyltransferase	Nucleoplasm
RRM2	Ribonucleotide reductase regulatory subunit M2	Cytosol
SMC4	Structural maintenance of chromosomes 4	Nuclear speckles, Cytosol
IDH2	Isocitrate dehydrogenase (NADP(+)) 2	Mitochondria
RAF1	Raf-1 proto-oncogene, serine/threonine kinase	Nuclear speckles
KDR	Kinase insert domain receptor	Plasma membrane
ERBB4	Erb-b2 receptor tyrosine kinase 4	Intracellular, Membrane
COL1A2	Collagen type I alpha 2 chain	Endoplasmic reticulum
COL3A1	Collagen type III alpha 1 chain	Endoplasmic reticulum
RIN1	Ras and Rab interactor 1	Nucleoplasm, Nuclear membrane

Table 3: Simple Parameters of the network through Cytoscape

Name of rece ptor	Avera ge shorte st path length	Cluste ring coeffi cient	Closene ss Centrali ty	Degree	Bet wee nne ss	Neigh bourh ood Conne ctivity
CDK4	1.32	0.68	0.76	13.00	0.06	12.38
KRAS	1.16	0.69	0.86	16.00	0.07	12.13
MGMT	1.47	0.89	0.68	10.00	0.00	14.20
RRM2	1.84	0.83	0.54	4.00	0.00	11.00
SMC4	2.05	0.83	0.49	4.00	0.00	9.50
TOP2A	1.47	0.67	0.68	10.00	0.04	12.20
NOTCH1	1.26	0.81	0.79	14.00	0.01	13.14
MTOR	1.32	0.86	0.76	13.00	0.01	13.54
PIK3CA	1.16	0.74	0.86	16.00	0.03	12.63
PTEN	1.16	0.74	0.86	16.00	0.03	12.63
MDM2	1.26	0.82	0.79	14.00	0.01	13.29
TP53	1.11	0.67	0.90	17.00	0.08	12.06
CDK6	1.42	0.78	0.70	11.00	0.03	13.55
CDKN2A	1.16	0.74	0.86	16.00	0.03	12.63
RAF1	1.63	1.00	0.61	8.00	0.00	15.38
KDR	1.53	1.00	0.66	10.00	0.00	14.70
EGFR	1.21	0.69	0.83	15.00	0.06	12.07
IDH2	1.58	0.97	0.63	9.00	0.00	14.89
ERBB4	1.53	1.00	0.66	10.00	0.00	14.70
COL1A2	1.00	0.00	1.00	1.00	0.00	1.00
COL3A1	1.00	0.00	1.00	1.00	0.00	1.00
RIN1	2.00	1.00	0.50	2.00	0.00	15.50

Molecular profiling of GBM patients support our result since co-occurrence of genetic mutations is elucidated by several studies. Recently, it has been revealed in a clinical study that higher EGFR mutation along with concomitant alterations such as PTEN led to progression of single-foci to multiple lesions GBM and presence of KDR led to worse survival (Dono et al., 2020) [16]. Gene mutation profiling of primary GBM samples by Tang et al. showed frequent detection of EGFR, PIK3CA, PTEN, and TP53 alterations. Similarly, Ceccarelli et al. demonstrated that IDH1, IDH2, PTEN, TP53 and NRAS were mutated together in samples of GBM patients [17].

Many of these mutated genes are related to the PI3K/Akt signalling pathway indicating the importance of its involvement in GBM progression. Recent research supports this suggestive observation since ablation of PI3K isoforms resulted in blockage of GBM progression in PTEN deficient model [18].

Analysis of cluster genes involved in GBM

Upon studying the network through MCODE, betweenness of PPI indicated the proteins' ability to communicate with distant proteins in terms of the number of shortest paths that pass through

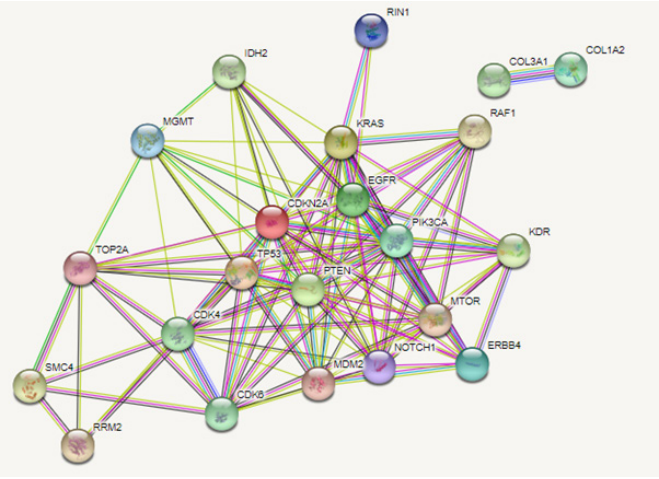


Figure 1: Network of input protein as obtained by STRING database

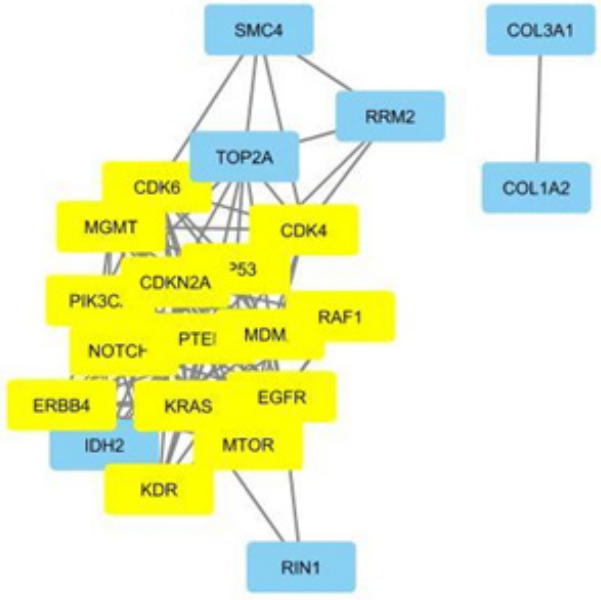


Figure 2: Network Cluster represented in yellow provided by Cytoscape

the node (Figure 2). Out of the protein nodes with high betweenness, TP53; KRAS; EGFR showed high degree, suggesting that they are related to a larger number of neighbourhood nodes (proteins). Wang et al., 2021 have reported the similar observation that genes with high betweenness, closeness centrality and degree performed the major functions in hepatocellular carcinoma [19]. Similarly, genes with higher betweenness, closeness centrality and degree were shortlisted as differentially expressed genes (DEGs) for non-small cell lung cancer [20].

The deregulation of TP53 has elucidated the most prominent role in tumour suppression and is found to be hampered in 30% of GBM patients [21]. TP53 has been found to be associated with EGFR in IDH mutant GBM patients indicating their importance [22]. TP53 gain-of-function mutation resulted in enrichment of genes related to inflammation through upregulated nuclear factor kappa B (NFκB) signalling. Therefore, this is suggestive of the critical role played by TP53 in GBM patients deteriorating their survival. This is in accordance with our result which shows TP53 with the highest betweenness can affect many proteins of the network [23].

Upon using the MCODE plugin of Cytoscape, it was observed that the protein network was grouped into 1 cluster with 15 genes found to be linked with 97 edges as shown in Table 4. The MCODE weighted nodes based on factors such as local neighbourhood density employ an outward traversal from the seed protein to find related networks. The ERBB4 was found to be the seed protein indicating its crucial role in communicating to other proteins in the cluster. In similar work done previously, Maghvan et al., 2017, reported SP1, TUBA1A and HDAC2 to be the seed of a network cluster and have shown that these proteins play key regulatory roles in breast cancer, choline metabolism and GnRH signaling pathway [24].

The ERBB4 is one of the most prominent members of the EGFR family, found to be present in neuron-like elements of the brain. It dimerizes with itself and leads to activation of PI3K and Ras/MAPK pathways eventually leading to GBM survival. This indicates its inter-relationship with other pathways [25, 26]. Cluster analysis is helpful in understanding the connectivity of genes and functions related to a group of proteins so that drugs can be efficiently designed to target the group of proteins together. For instance, Korkut et. al, have shown that clustering helped in identifying the cancer subtypes and found linked expression of multiple genes which collectively regulate gene expression and control tumour development [27]. Therefore, it can be observed from our study that the genes highlighted in Figure 2 play a critical role in GBM progression due to their functional relatedness and inter connectivity.

Functional relationships analysed through the BiNGO plugin

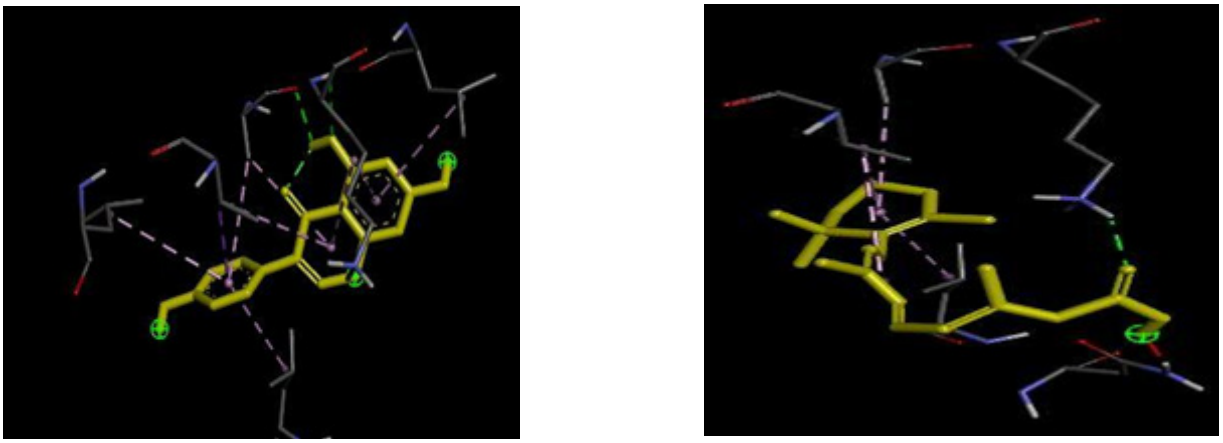


Figure 3 Binding of ERBB4 with (a)Bromelain (b) EGCG (c)Genistein (d) silibinin

Table 4: Cluster Analysis through Cytoscape

Name	Avg shortest path length	Clustering coeff	Closeness centrality	Degree	Betweeness	Neighbourhood connectivity
MTOR	1.32	0.86	0.8	13.54	0.01	13.54
PTEN	1.16	0.74	0.9	12.63	0.03	12.63
ERBB4	1.53	1.00	0.7	14.70	0.00	14.70
KDR	1.53	1.00	0.7	14.70	0.00	14.70
KRAS	1.16	0.69	0.9	12.13	0.07	12.13
NOTCH1	1.26	0.81	0.8	13.14	0.01	13.14
CDK6	1.42	0.78	0.7	13.55	0.03	13.55
EGFR	1.21	0.69	0.8	12.07	0.06	12.07
MGMT	1.47	0.89	0.7	14.20	0.00	14.20
CDK4	1.32	0.68	0.8	12.38	0.06	12.38
TP53	1.11	0.67	0.9	12.06	0.08	12.06
MDM2	1.26	0.82	0.8	13.29	0.01	13.29
PIK3CA	1.16	0.74	0.9	12.63	0.03	12.63
RAF1	1.63	1.00	0.6	15.38	0.00	15.38
CDKN2A	1.16	0.74	0.9	12.63	0.03	12.63

Table 5: Brain related gene ontology through BiNGO plugin of Cytoscape

GO description	GO ID	corrected p-value	cluster frequency	Genes
7420	brain development	1.37E-02	3/15 20.0%	NOTCH1 ERBB4 EGFR
14015	positive regulation of gliogenesis	3.26E-02	1/15 6.6%	NOTCH1
14013	regulation of gliogenesis	4.81E-02	1/15 6.6%	NOTCH1
42063	gliogenesis	6.90E-04	3/15 20.0%	NOTCH1 CDK6 EGFR

of Cytoscape exhibited 614 functions pertaining to one gene or interaction of genes, which involved various cellular and developmental pathways. Out of which, four were found to be directly linked to glioma regulation and brain development, making these genes a great target for GBM therapeutics (Table 5). Similarly, the major functions of the cluster that were confirmed through BiNGO specially related to GBM were gliogenesis, brain development, forebrain development and glial cell differentiation. Two groups of genes from the network, one consisting of NOTCH1, ERBB4 and EGFR; other of NOTCH1 CDK6 EGFR have been found linked to brain development and gliogenesis, respectively. The former happens to be more significant since it shows lower corrected p-value and 20% of cluster frequency indicating weightage of its importance in functional enrichment.

This observation is in accordance with the literature available, which indicates that crosstalk of NOTCH and EGFR pathways have a crucial role in survival and progression of GBM [27]. Simultaneous targeting of both the genes in GBM cells can result in enhanced angiogenesis inhibition [28]. The multi pronged approach by usage of different drugs can also address the resistance issues of a single drug. In a similar study by Jiang et al., 2020, analysis through BiNGO confirmed the direct relation of top ranked hub gene FCER1G to immune response [29]. Menein et al. also exhibited through cluster analysis the functional relevance of KRAS interacting genes which can be collectively targeted for malignancies [30].

Table 6: Spatial features of the ligands considered for pharmacophore scoring

Molecule	Atoms	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
betamol2.mol2	96	51	32	0	51	0	0	0	0
egcgmol2.mol2	51	23	15	3	1	8	11	0	0
ethylacmol2.mol2	14	4	4	0	2	0	2	0	0
theobmol2.mol2	21	9	9	2	2	1	3	0	1
silibininmol2.mol2	57	19	14	3	1	5	10	0	0
retinmol2.mol2	49	26	26	0	23	0	2	1	0
thujonemol2.mol2	27	13	13	0	12	0	1	0	0
genol2.mol2	30	11	8	3	0	3	5	0	0
phenmol2.mol2	16	3	3	1	0	1	1	0	0
brommol2.mol2	83	37	32	6	0	2	29	0	0
thymmol2.mol2	25	10	9	1	7	1	1	0	0

Note: betamol=beta carotene; egcgmol=epigallocatechin 3 gallate; ethylacmol; ethylacetate; theobmol=theobromine; silibinmol=silibin; retinmol=retinol; thujonemol=thujone; genol=genistein; phenmol=phenylisothiocyante; brommol=bromelain; thymmol= thymol

Pharmacophore analysis

Protein drug interaction is an important aspect in developing therapeutics. Therefore, it is necessary to gain an insight on interaction of inhibitory compounds with the shortlisted receptors. For this purpose, alignment of pharmacophoric features was studied with the compounds shortlisted through PharmaGist software. The pharmacophore features of the selected compounds were aligned and arranged based on the selectivity score (Table 6). The webserver gives out output in terms of the name of the participating molecules, the number of common features and their type distribution. Like best hits from libraries were selected by Kumar et al. for further studies, [31] similarly best hits were selected based on highest selectivity score, 36.742, consisting of a set of four compounds (Table 7). However, amongst the same reported values, the best alignment was found to be of compounds namely Silibinin, EGCG, Bromelain and Genistein, owing to the larger number of molecules involved. This combination had 5 spatial features, 1 aromatic feature, and 4 acceptors in common, which were necessary to bind to the receptor of ERBB4. The analysis yielded common chemical characteristics from 3D structures through ligand alignment that aids in finding common steric and electronic features. This improved understanding of ligand protein interaction in terms of structural requirements for bonding [32, 33]. Bommu et al., 2018 studied pharmacophore features to analyze

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Table 7: Features of aligned pharmacophore

Score	# mols	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives	Molecules
36.742	4	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
36.742	3	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
36.742	3	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
36.742	3	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
33.941	5	3	3	1	0	0	2	0	0	brommol2.mol2 egcgmol2.mol2 theobmol2.mol2 silibininmol2.mol2 genol2.mol2
33.068	4	4	4	1	0	0	3	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2 egcgmol2.mol2
33.068	3	5	5	0	0	0	5	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
33.068	3	5	5	0	0	0	5	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
33.068	3	5	5	0	0	0	5	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
33.068	3	6	6	1	0	0	5	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
31.749	5	3	3	1	0	1	1	0	0	brommol2.mol2 egcgmol2.mol2 theobmol2.mol2 genol2.mol2 silibininmol2.mol2
31.749	4	3	3	1	0	0	2	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
29.698	5	4	4	1	0	0	3	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2 egcgmol2.mol2 theobmol2.mol2
29.698	5	3	3	0	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 theobmol2.mol2 silibininmol2.mol2 genol2.mol2
29.394	4	4	4	0	0	0	4	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2 egcgmol2.mol2
29.394	4	4	4	0	0	0	4	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2 egcgmol2.mol2
29.394	3	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
29.394	3	5	5	1	0	0	4	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2
29.394	3	5	5	1	0	0	4	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2
27.78	5	4	4	1	0	0	3	0	0	brommol2.mol2 silibininmol2.mol2 genol2.mol2 theobmol2.mol2 egcgmol2.mol2
27.78	5	3	3	0	0	1	2	0	0	brommol2.mol2 theobmol2.mol2 silibininmol2.mol2 genol2.mol2 egcgmol2.mol2
27.78	4	4	4	1	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
27.78	4	4	4	1	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
27.78	4	4	4	1	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
27.78	4	4	4	1	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
27.78	4	4	4	1	0	0	3	0	0	brommol2.mol2 egcgmol2.mol2 silibininmol2.mol2 genol2.mol2
25.456	5	3	3	1	0	0	2	0	0	brommol2.mol2 egcgmol2.mol2 genol2.mol2 theobmol2.mol2 silibininmol2.mol2
23.812	5	3	3	1	0	1	1	0	0	brommol2.mol2 egcgmol2.mol2 theobmol2.mol2 genol2.mol2 silibininmol2.mol2
19.843	5	4	4	1	0	1	2	0	0	brommol2.mol2 theobmol2.mol2 genol2.mol2 egcgmol2.mol2 silibininmol2.mol2
15.875	5	3	3	1	0	0	2	0	0	brommol2.mol2 egcgmol2.mol2 genol2.mol2 silibininmol2.mol2 theobmol2.mol2

Note: brommol=bromelain; egcgmol=epigallocatechin 3 gallate; genol=genistein; silibinmol=silibin; theobmol=theobromine

strong bond patterns in the ligand receptor binding where, H-bond acceptor, donor and atomic pi stacking interactions with ligands were revealed by the pharmacophore model [34]. Pharmacophore studies can also be used to screen the databases for potential compounds showing similar binding profiles so as to filter the database particularly for certain common features. Insights on the structural and biological profile of pharmacophore candidates can help in developing the compound as an agonist or antagonist [35].

Validation of shortlisted ligands through docking

The aligned compounds based on pharmacophore features were docked with the seed protein ERBB4. The results showed that bromelain and EGCG had better affinity with binding affinity scores of -9.8 and -9.2 respectively (Table 8). Docking after pharmacophore analysis gives a confirmatory insight on the binding pattern of ligands with receptors. This is in accordance with previous studies where common pharmacophoric features for ligands reported in literature

Table 8: Binding Affinity of selected phytocompounds ERBB4

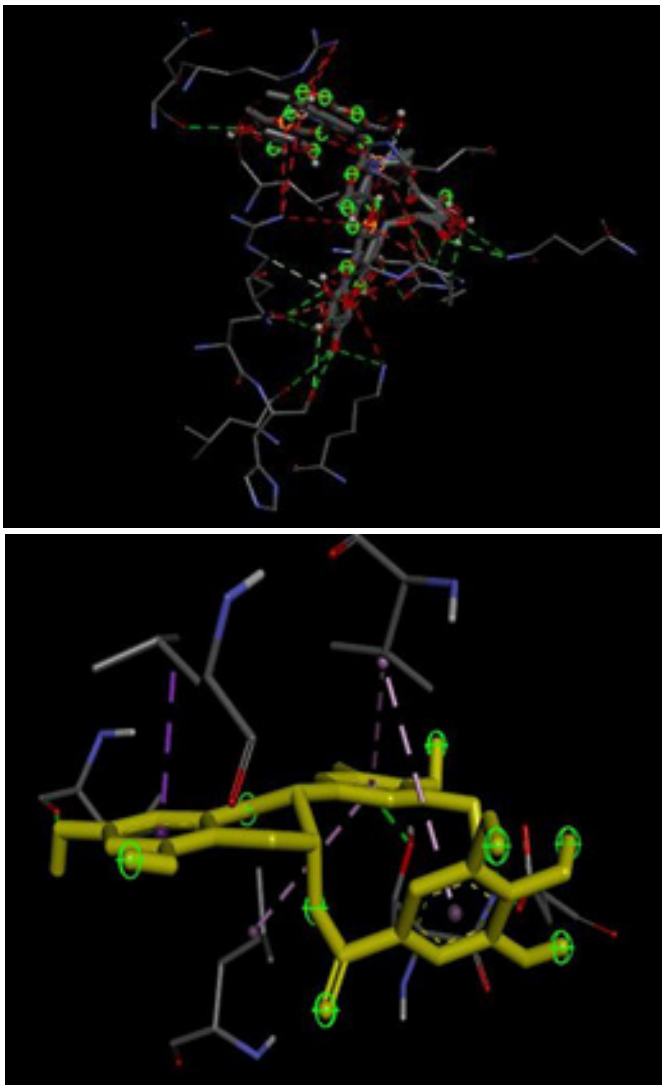
Name of the compound	Binding Affinity (kcal/mol)	H Bond	
		Acceptor	Donor
Bromelain	-9.8	B:GLN772:NE2 B:LYS833:NZ D:GLN772:NE2 D:GLN772:NE2 D:LYS827:NZ D:LYS827:NZ D:SER828:OG A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O B:ARG757:CD	A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O A:UNL1:O B:GLN684:O B:LEU759:O B:GLN772:OE1 B:ASP751:OD1 B:ASP751:OD1 B:HIS752:O B:HIS752:O B:LEU755:O A:UNL1:O
		A:UNL1:C A:UNL1:C A:UNL1:C A:UNL1:O	A:UNL1:O A:UNL1:O A:UNL1:O D:HIS776
EGCG	-9.2	A:UNL1:H A:UNL1:H A:UNL1:H	B:THR835:OG1 B:ASP836:OD2 B:MET774:O

were used to perform inhibitory activities against c-Met and ALK and then subjected to docking to confirm the evidence [36].

The ligands genistein and silibinin gave binding affinity as -8.6 and -7.6 kcal/mol. The interactions between docked structures of the ligands and ERBB4 are as shown in Figure 3. The bonds involved with bromelain involved electrostatic and hydrogen bonds. While EGCG, Genistein and silibinin were found to have hydrophobic and hydrogen bonding. There has been evidence suggesting that hydrogen bonding can form a strong bond as compared to the case of hydrophobic conditions [37]. Thus, the bonding of ERBB4 with EGCG, genistein and silibinin is more stable than that of bromelain. Scoring functions in docking are an efficient way to predict the strength of binding so this study highlights the interaction of ligands with the seed target of the cluster [38]. In previous studies, bromelain has been observed to reduce pro-inflammatory mediators and thus can be a good candidate to elicit antitumor effect [39]. Additionally, EGCG is a well-established compound for anti-tumour effect and therefore can be combined with several drugs to study its synergistic effect [40].

Conclusion

The aggressiveness and heterogeneity of GBM tumour necessitates development of robust therapies for GBM. To make the process of therapy development easy, in silico screening plays a very important role in initial therapy development. The present study aimed to analyse networks of various proteins potentially involved in GBM progression.



The network of critical genes involved in GBM exhibited close linkage of EGFR, PI3KCA, KRAS, PTEN, TP53, CDKN2A genes indicating inter relationship of cell cycle regulation and PI3K/Akt and Ras/MAPK pathways. The study on functional relationship indicated involvement of a set of genes NOTCH1, ERBB4 and EGFR; in gliogenesis. Pharmacophore analysis of phytocompounds identified silibinin, EGCG, bromelain and genistein as best alignment based on pharmacophoric features that can help us gain insight on the potential bonding pattern of these ligands with the seed protein of network, ERBB4. Lastly, docking of seed protein with the shortlisted ligands confirmed the best interaction and potential therapeutic phytocompound to target the seed protein to curtail GBM.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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