Original Research Article

Molecular Docking Analysis of Bacoside A with selected Signalling factors Involved in Glioblastoma

Abstract

Glioblastoma is the malignant tumor affecting the central nervous system. Despite the advancement in treatment modalities, presence of blood-brain barrier, recurrence after surgical removal, resistance to radiotherapy and chemotherapy remain the major obstacle for long term survival of the patients. In this context, finding suitable therapeutics which have anticancer potential and are nontoxic might be useful to improve the overall survival of GBM. Plant products are safer, nontoxic and cheaper when compared to the chemotherapeutic drugs in trend which are expensive and highly toxic, owing to their systemic effects. Bacoside A (BA) is one such plant constituent isolated from *Bacopa monnieri* which offers neuroprotection and possesses anticancer potential. The present investigation elucidates the specific interaction of BAA with various cell surface receptors, signal transduction proteins, effector proteins and transcription factors involved in glioblastoma signalling such as EGFR/Ras/Raf/MAPK pathway, Notch signalling and wnt-beta catenin signalling through molecular docking studies. The interaction between BA and the target proteins of glioblastoma were analysed through Glide module (Version 6.5) of Schrodinger Suite (2015) software. According to the results of molecular docking, jagged-1 ligand interact with BA with stronger affinity and Frizzled receptor interact with least affinity in terms of glide score. The results indicate that BA interacts well with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr, Gln, Asp, Lys and Glu. The ligand also showed interactions with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied. The in vitro cytotoxicity studies reveal the cytotoxic potential of BA on U87MG glioblastoma cell line. This study warrants further research studies on elucidation of the modulatory effect of the cell signalling pathways resulting from the specific interaction of molecular targets with BA in glioblastoma.

Keywords: Glioblastoma, *Bacopa monnieri*, Bacoside A, molecular docking, EGFR signalling, Notch signalling, Wnt-beta catenin signaling

1. Introduction

Glioblastoma (GBM) is one of the aggressive tumors of the central nervous system which accounts for 57.3% of all gliomas and 48.3% of all malignant brain tumors (Ostrom *et al.*, 2016). Also, the prevalence of GBM increases with age and normally diagnosed at an age of 65 years (Ostrom *et al.*, 2019; Ostrom *et al.*, 2018). Although the treatment modalities are significantly advanced, the prognosis of the affected individuals remains poor with a mean survival rate of less than 2 years (Paszat *et al.*, 2001; Stupp et al., 2005). The primary treatment option for glioblastoma includes surgery involving the complete removal of the tumor, followed by treatment with temozolomide (TMZ) and radiotherapy (Krex et al., 2007; Burton et al., 2002). However, current treatment regimens are not satisfactory as they do not seem to improve patient's survival significantly. Further, the major concerns in the treatment of GBM include the presence of blood-brain barrier, recurrence after surgical removal, and resistance to radiotherapy and chemotherapy (Saito et al., 2014). Hence, the current study involves identification of suitable phyto compounds with anticancer potential which improve the overall survival of GBM and to deal with the afore mentioned issues.

Previous literatures document the usefulness of several plants and their products as medhyarasayana (which improve mental health. Brahmi isolated from *Bacopa monnieri* which is traditionally known as "medhya rasayana," because it is found to enhance the cognitive properties of brain and hence is prevalent among the practitioners of Ayurveda, where it is utilised to treat multiple ailments like loss of memory, epilepsy, inflammation, fever, asthma, etc. Also, there exists evidence for the wide spectrum of its activity in treating insomnia, insanity, depression, psychosis, epilepsy, parkinson's disease, alzheimer's disease and stress (Russo., 2005). Several reports suggest the anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid peroxidative activities of Brahmi (Kishore et al., 2005; Anbarasi et al., 2005; Vishnupriya et al., 2017). Interestingly, certain recent reports highlight the potential of the individual components of *Bacopa monnieri* and crude extract for the treatment of cancer. The chemotherapeutic agents in prevalence these days are systemic in nature and hence entail harmful side effects. Plant products are safer, nontoxic and cheaper when compared to the chemotherapeutic drugs. The *in vitro* and *in vivo* studies in the recent past have unveiled the significant antitumor and cytotoxic activity of Bacopa extract (Kumar et al., 1998; Peng et al., 2010) and Bacoside A in the cells of many human cancers, including hepatocellular carcinoma (Janani et al., 2010; Kalachaveedu et al., 2014), sarcoma (Rohini and Devi, 2008), mammary carcinoma (Kalyani et al., 2013), breast

cancer (Mallick et al., 2015). Aithal and Rajeswari (2019) reported BA induced cell cycle arrest and apoptosis via notch signaling in glioblastoma. The majority of the pharmacological properties of Bacopa *monnieri* can be attributed to its major phyto constituent BA which was first reported by Chatterji et al. in 1965 (Singh et al., 1997). However, the exact mechanism of action of BA in glioblastoma has not been evaluated so far.

In view of this, we have selected several proteins which play key role in GBM proliferation and survival. The interaction of the selected proteins with BA, was analysed through molecular docking studies. The various parameters considered for evaluating the interaction between the protein and the ligand include: the docking score, docking energy, the interacting amino acids and hydrogen bonding.

2. METHODOLOGY

2.1 Chemicals and Reagents

Bacoside A was purchased from Natural remedies Pvt Ltd, Bangalore, India. DMEM media, Fetal Bovine Serum (FBS), antibiotics and other fine chemicals were obtained from HiMedia Laboratories (Mumbai, India).

2.2 Animal Cell Culture Maintenance

Human GBM cancer cell line (U87MG) was procured from National Centre for Cell Science (NCCS), Pune, India. Cell line was maintained by culturing in DMEM media containing 10% FBS and Penicillin (100 Units/ml), Streptomycin (30 µg/ml) and Gentamycin (20 µg/ml).

2.3 Cell proliferation Assay - Sulforhodamine B Assay (SRB assay)

The cell density on the basis of protein content was assessed by SRB assay (Vichai and Kirtikara, 2006). Microtitre plate having 96-wells were inoculated with 1 x 10^4 U87MG cells/ml. The used media was removed after 16 hours of attachment to the plate and different concentrations of BA (5, 10, 15, 20, and 25 µM) diluted in incomplete media was added and kept for 24 hours to determine "the effect of the compound on the cell viability" in a dose dependent manner. Once the treatment period was over, 10% (wt/vol) trichloroacetic acid was added to the plate and placed in in 4^0C for 1 hr to fix the cells. Further, the plate was washed well by immersing in tap water in a tray and dried at room temperature. Then, SRB staining solution prepared in 1 % acetic acid was added to the wells and kept at room temperature for 1hr.Used SRB stain was removed by washing the wells four times with 1%

(vol/vol) acetic acid. The solubilization of the protein-bound dye was done by the addition of 10 mM Tris base solution followed by shaking the plate for 10 minutes. The absorbance (OD) was determined at 510nm by using a microplate reader (BioTek, USA). The results were shown as percentage viability.

2.4 Preparation of Protein

The 15 proteins considered for the study are EGFR (PDB ID: 1IVO), Ras (PDB ID:1CTQ), Raf (PDB ID:3IQJ), VEGFR(PDB ID:1YWN), DLL-1(PDB ID:4XBM), Jagged-1(PDB ID:4X17), Notch-2(PDB ID:2OO4) , Gamma secretase (PDB ID:5A63), Hes (PDB ID:2MH3), Hey (PDB ID:2DB7), Beta Catenin(PDB ID: 2Z6H), GSK 3β (PDB ID:1GNG), Frizzled Receptor (PDB ID:6BD4),Wnt-3 (PDB ID: 2YZQ), p53(PDB ID:1YC5). The structure of the selected target proteins used for the molecular docking study was obtained from the PDB (Protein Data Bank, hyperlink: http://www.rcsb.org/pdb/-home/home.do).

The protein structure is refined in order to solve the geometrical inconsistencies and hydrogen bonds were optimized using protein preparation wizard of Glide module Version 6.5) of Schrodinger suite. The selected chains of the target proteins have undergone corrections for missing hydrogen for assigning proper bond orders and searching overlaps. Water molecules which are not going to be involved in ligand binding within a distance of of 5 Å were removed Finally the proteins were minimised to 0.30 Root Mean Square Deviation (RMSD) value . Both RMSD and OLPS-2005 (optimized potential for liquid simulation) was used to perform the minimization (Anonymous).

2.5 Ligand Preparation

The structure of Bacoside A (CID_53398644) was retrieved from PubChem. (https://pubchem.ncbi.nlm.nih.gov/) (**Fig.1**). The structure of the ligand was optimized by the LigPrep wizard Schrodinger Maestro 11.9 (Glide). Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. Preparation of the ligands before docking is strongly recommended. Corrected Lewis structure was generated for a ligand; it is skipped by the docking job. Glide also automatically skips ligands containing un parametrized elements, such as arsenic, or atom types not supported by the OPLS force fields, such as explicit lone pair “atoms”. Corrections such as 2D to 3D conversion, addition of hydrogen, stereochemistry, low energy state, corrections of bond

lengths and bond angles, ring conformations along with minimization and optimizations were done using OPLS3 force field (Anonymous).

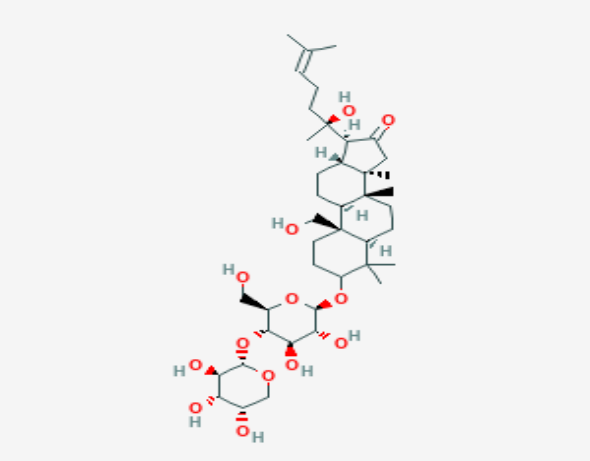


Figure1: Structure of Bacoside A retrieved from PubChem.

2.6. Grid generation

The receptor grid was prepared using the grid generation tool of Maestro 11.9 of the Schrödinger suite. The receptor grid can be set up and generated from the Receptor Grid Generation panel. The grid box is generated on the center of the macromolecule. The active site of the receptor is represented by a box at the centre of the ligand of interest. Then using the default Glide settings, a grid box centered on the ligand was generated. Ligand docking jobs cannot be performed until the receptor grid has been generated. Receptor grid generation requires a "prepared" structure: an all-atom structure with appropriate bond orders and formal charges. The atoms were of size equal to Van der Waals radii of 1.0 (scaling factor) > while the partial atomic charge was less than 0.25 (van der waals radius) defaults. The ligand is able to bind forming the achievable conformation using this receptor grid which also highlights the active site of the protein as with the co-crystallized ligand molecule. This co-crystallized ligand will then be removed from the active site to be occupied by our ligand of interest (Anonymous; 2018).

2.7. Molecular Docking

The Docking analysis and characterization of biding pocket was performed by using the accomplished using Maestro-GLIDE module of the Schrödinger suite (Artese et al. 2013; Friesner 2006; Schrodinger Inc. 2018). The optimized ligand was then docked in a flexible manner to the active site of the protein drawn within the grid box using the extra precision (XP) feature of Glide module, version 5.6, 2010 (Glide 2010). It also helps in generating the 2D ligand interaction diagram corresponding to analysis of each protein-ligand interaction. A post docking energy minimization was applied in the analysis. The characterization of binding pockets of the ligand was done by generating Different molecular interactions were represented by varying colour patterns and shapes.

2.8. Selection of the Best-Scored Pose.

The best docking poses for the selected protein-ligand were primarily scrutinized by the docking scores but values of different energies, number of H bonds, and visual inspection of all docking poses in Maestro (Schrodinger, USA) were also taken into account. Interaction energy between protein and ligand can be related to binding affinities. Different criteria were laid down to select best docked structure for each ligand. Then, rankings were derived by directly using the Glide G Score.

3. RESULTS AND DISCUSSION

3.1. Bacoside A Induced Cytotoxicity Glioblastoma U87MG Cell Lines

In order to ascertain the toxicity of BA in GBM cell line U87MG, cell viability was measured by SRB assay after exposure to BA at various concentrations for 24 hours of treatment. Treatment with BA inhibited GBM cell growth in a dose-dependent manner. The concentration required by the U87MG cells to cause 50% inhibition (IC_{50}) was found to be 22μM (**Fig 2**). Previous literatures extensively worked on the effects of *Bacopa monnieri* and its constituents against different cancer cell lines such as MCF-7 and MDA-MB 231 cell line (Mallick etal., 2015) mammary carcinoma and oral cancer cell lines (Krishna et al., 2016). Previous studies suggest that BA inhibits the cell proliferation in Hep G2 cells and the IC_{50} value was found to be 0.625 µg/ml (Kalachaveedu et al., 2016). BA induced Sub G0 arrest in theGBM cell line through notch signaling pathway and they had found out the IC_{50} was found to be (83.01 µg/ml) (Aithal et al., 2018).

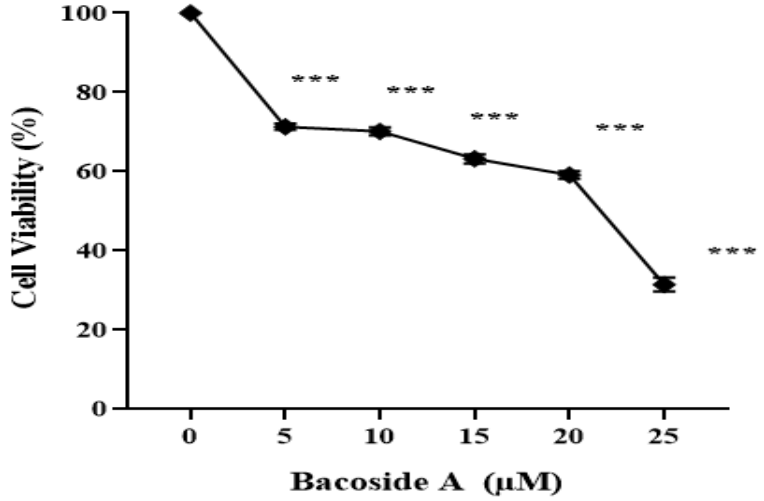


Figure 2: To determine the cytotoxic effect of Bacoside A, U87MG cells were treated with different concentrations (1-25µM) for 24hrs. Cell viability of U87MG cells checked after the treatment schedule through SRB assay. The Bacoside A induced cytotoxic effects on U87MG cells in a dose depended manner (Figure - 1). Data were obtained & analyzed from experiments carried out in triplicates and expressed as Mean ± S.D. ***$p<0.001$ when compared to control (One-way ANOVA followed by Tukey's multiple comparison test). The IC25 & IC50 values were determined as 12µM &22µM respectively.

3.2. Target protein selection

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase which is overexpressed and its aberrant signalling has been observed in GBM. Though several mutations to EGFR are identified, EGFRvIII (EGFR type III, EGFRvIII, del2-7, ΔEGFR) is the most common type among them which is unable to bind to the ligand (Ekstrand et al, 1990; Wong et al., 1992). Hence, its signalling occurs in a constitutive manner. Usually, it is found in association with wild type EGFR (Biernat et al., 2004). EGFR can regulate various signalling pathways such as PI-3 K/AKT, RAS/MAPK, and JAK2/STAT. Therefore, EGFR functions as a hub for regulating various cellular processes (Hervieu et al., 2018; Eskilsson et al., 2018; An et al., 2018). Small molecule receptor tyrosine kinase inhibitors such as Gefitinib, Erlotinib, Lapatinib, Monoclonal Antibodies like Cetuximab, Nimotuzumab, CAR-T Cells Targeting EGFRvIII are some of the treatment modalities targeting EGFR (Oprita et al., 2021).

GBM tissues express high levels of VEGF, an important angiogenic protein with an upregulation of VEGFR2. VEGF binding to VEGFR leads to an increased vascular permeability (Steiner et al., 2004; Joensuu et al., 2005). VEGF binding to VEGFRs on tumor

blood vessels also activates endothelial cell proliferation, survival, and migration (Abounader et al., 2005).

Ras is a small GTP-binding protein, which is the common upstream activator molecule of several signaling pathways including Raf/MEK/ERK, PI3K/Akt and Ral/EGF/Ral (Peyssonnaux et al., 2000). Constitutive expression of Ras proteins gave been observed in 30% of cancers which arise as a result of whether the amplification of *ras* proto-oncogenes or due to activating mutations (Flotho et al., 1999; Tirewalt et al., 2001). Experimental results done on a transgenic mouse model suggest that activated Ras alone is sufficient to transform normal astrocytes and neuronal precursor cells into malignant gliomas (Abel et al., 2009; Shannon et al., 2005). Nevertheless, it contributes an indispensable role in gliomagenesis, activated Ras-Raf signaling axis may also be crucial in the maintenance of malignant gliomas. Moreover, increased Ras-RAF-ERK activity is a common characteristic of malignant gliomas with deregulated EGFR and PDGFR pathways (Lo et al., 2008; Lo et al., 2006). In contrast, the transcriptional down-regulation of Ras-RAF-ERK and the small molecular inhibitors reduce growth of glioma.

Another vital component of Ras/Raf/MEK/ERK signaling pathway is Raf which can also be targeted for GBM treatment. The RAF family consists of three kinases namely A-, B-, and C-RAF/RAF-1. These serine/threonine kinases have a common structure with an N-terminal regulatory region and a C-terminal catalytic domain (ebisch et al., 2007). Levels of RAF-1, BRAF proteins and RAF kinase activity are increased in human GBM samples and constitutive activation of Raf-1 Induces Glioma Formation in Mice (Lyustikman et al., 2008). Though Sorafenib, a Raf kinase inhibitor, has been tested in combination with Erlotinib (an EGFR tyrosine kinase inhibitor) and in a phase II trial for patients with recurrent GBM, their combinational therapy have not manifested any desired impact due to its failure to reach the goal of a 30% improved survival time. Seemingly, this owes to the pharmacokinetic interaction between the drugs that lessens their efficacy.

Notch signaling often gets triggered in human gliomas and assures the self-renewal ability of glioma stem cells (Hu et al., 2011). It follows that expression of Notch-1 predicts poor patient survival in proneural and classic glioblastomas (Hai et al., 2018). Notch is a cytoplasmic receptor and is able to bind to two types of ligands Delta-like (Dll1-3 and -4) and Jagged (Jagged1 and -2) on itself (cis-activation) or a neighboring cell (transactivation) (Hori

etal., 2013). In comparison with the normal brain cells, mRNA and protein levels of Notch1, Notch4, Dll1, Dll4, Jagged1, CBF1, Hey1, Hey2, and Hes1 would be higher in brain tumor cells which correlates with an elevated expression of VEGF and pAKT, and reduced levels of PTEN (Hulleman et al., 2009; Zhang et al., 2011). Similarly, differentiated cells within the tumor express higher levels of Dll1 compared to Glioblastoma Stem cells (GSCs), contributing to Notch signaling activation in GSCs (Zhu et al., 2011). Generally, the Notch activity is measured by the expression levels of its direct target genes. Hey is one main downstream effector of notch signalling and there are reports linking the expression of Hes/Hey transcriptional repressors to cancer prognosis. Recurrently, Hey-1 expresses in GBM among other astrocytomas. Patients expressing higher levels of Hey-1 are related to two fold shorter disease free survival in comparison with the patients carrying Hey-1 negative tumors (Hulleman et al., 2009). Notch also activates Hes-1, which is a transcriptional repressor that arrests cell cycle (Sang et al., 2008). Receptor-ligand binding triggers sequential cleavage events by a disintegrin and metalloprotease (ADAM) and Gamma secretase that culminate in release of the Notch intracellular domain (NICD) and translocation to the nucleus. Interaction with the RBP-J (or CSL) transcription factor and recruitment of a transcriptional coactivator, mastermind-like family (MAML), then enact changes in gene expression (Kovall t al., 2010). At present, there is a growing interest in developing therapies targeting Notch signaling pathway at various cascade levels. Monoclonal antibodies, antisense or RNA interference, receptor, and glycosylation/protease inhibitor strategies have been developed targeting NOTCH receptors and ligands. Out of these, γ-secretase inhibitors (GSIs) targeting receptor activation have been examined in different preclinical models and numerous clinical trials as anticancer agents that inhibits active NICD's release from the receptor by the γ-secretase complex (Ran, et al., 2017).

Wnt/*β*-catenin signalling plays a notable role in the proliferation of glioma tumor cells and tumor progression and also promotes growth and invasion through the maintenance of stem cell properties (Zhang et al., 2012; Gong et al., 2012). Binding of Wnt ligands to the cell surface recptors like Frizzled and LRP families triggers the signalling which subsequently causes the disassembly of the complex consisting of AXIN, adenomatous polyposis coli (APC), and GSK3*β*, thereby stabilizing *β*-catenin. By virtue of this, *β*-catenin is translocated from the cytoplasm into the nucleus where it forms a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) and thereby promoting transcription of multiple target genes including c-MYC and cyclin D1(Lee et al., 2016). *β*-catenin is a prognostic marker and its

increased levels of mRNA and protein in GBM and astrocytomas of high grade indicates its role in malignant transformation (*Sareddy et al,, 2009)*. In addition, constitutive activation of *β*-catenin increased the proliferation of mouse neural progenitor cells *in vivo*, whereas deletion of *β*-catenin decreased their proliferation and cellular invasion in U87MG and LN229 GBM cells (Yue et al., 2010). An increased expression of positive regulators of WNT signalling also found to regulate EMT-associated genes, such as ZEB1, SNAIL, TWIST, SLUG, and N-cadherin, indicating the role of WNT in EMT (Han et al., 2011; Yang et al., 2010). Hence, targeting Wnt signalling is a novel therapeutic method to kill GBM. For instance, inhibition of GSK3β activity also induces tumor cell differentiation and enhances apoptosis in glioblastoma (Kotliarova, et al., 2008). Moreover, GSK3β inhibition elevated the level of tumor suppressors p53 and p21 in the cells carrying wild type *TP53* along with downregulation of cyclin-dependent kinase 6 (CDK6) and decreased RB phosphorylation despite the cell genotype (Miyashita et al., 2009).

P53 is a known tumor suppressor which primarily mediates its effect by regulating genes in cell cycle arrest, apoptosis, stem-cell differentiation and cellular senescence. The mutational status of *TP53* is associated with GBM progression and p53 inactivation is correlated with a more invasive, less apoptotic, more proliferative and more stem-like phenotype (Zhang et al., 2018). Moreover, stress signals, such as DNA damage, hypoxia, heat shock and cold shock elicit a p53 response. The p53 protein further activates p21 that inhibits Cdk4/Cyclin D, Cdk2/Cyclin E complexes and cyclin B which helps to stop cell cycle progression. Because of the vitality of p53 in GBM pathogenesis, a gene therapy approach is also suggested to restore p53 expression (Pearson et al., 2017).

3.2 2 Docking Profile of Bacoside A with Target Proteins

Anticancer potential of phytochemicals or drugs was assessed by means of different in vitro, in vivo, and computational methods. However, molecular docking has been considered as an attractive method for drug designing in any disease (Tabassum, et al., 2014; Zahra et al., 2013).

Molecular interaction between protein and ligand predicts the binding conformation or pose of the ligand bounded to the protein, which can be quantified, based on the shape and electrostatic interaction between the ligand and protein 1 (Pagadala .,2016). The totality of interaction observed is approximated to be the docking score of the ligand into the binding

pocket of the protein (Pagadala., 2016). Docking score is expressed in negative value of energy in Kcal/mol where the lower the negative total energy E, the stronger the interaction between the ligands and the protein 1 (Ashwini, 2017). Docking approach predicts the best binding conformation of the compounds at the binding pocket of the protein.

Molecular docking studies were carried out for 15 target proteins from EGFR/Ras/Raf/MAPK pathway, Notch signalling and Wnt-beta catenin pathway with Bacoside A. The docked complexes were further subjected to post docking analysis i.e, Energy comparison between Current Complex Energy and Minimised Complex Energy. Both glide score and Glide energy were computed for all 15 proteins complexes (**Table 1**). Conformers are generated for Bacoside A and docked with all target proteins.

Table 1: Glide SP docking score of Bacoside A with the cancer target proteins

S.No	Target Proteins	Glide Score	Glide Energy (kJ/mol)
1	Jagged-1	-11.2	-82407.026
2	VEGFR	-10.4	-10933.9
3	Gamma secretase	-10	-181166.03
4	EGFR	-10	-45420.8789
5	GSK3- Beta	-9.9	-18164.5469
6	WNT-3	-9.2	-4406.6562
7	P53	-8.72	-48349.47
8	Raf	-8.3	-10627.63
9	Notch-2	-8.2	-7247.2
10	Beta catenin	-8.1	-25205.3046
11	Dll-1	-7.8	-19467.57
12	Hes	-7.3	-4328.17
13	Ras	-5.7	-9904.05
14	Hey	-5.6	0
15	Frizzled Receptor	-4.8	-9675.0586

Energies were calculated as current energy and minimised energy because, the entire protein complex has undergone minimization process by macromodel Module from Schrodinger. It was found that invariably the entire protein complex have minimized energy which confirms the stability of protein after its domain interaction. Out of the 15 proteins that

are taken for the present study, interaction of BA with jagged 1 showed the least Glide score and hence maximum interaction. The highest Glide score was found when frizzled receptor was allowed to interact with BA. Schrodinger Glide calculated the total docking score between Jagged-1 and BA. Total docking score of the complex, bond energy and model energy depicts the efficacy of BA in the surface solvent area and the energy it taken to occupy is -82407.026 KJ/mol. Oxygen as a protein atom of Jagged-1 from CYS88 and SER300 were interacted with Hydrogen from ligand (BA) atom with the bond distance of 2.028 Å, 2.231 Å respectively. The intermolecular interaction of perceived in –OH group as functional which is as side chains of CYS88 and SER300.

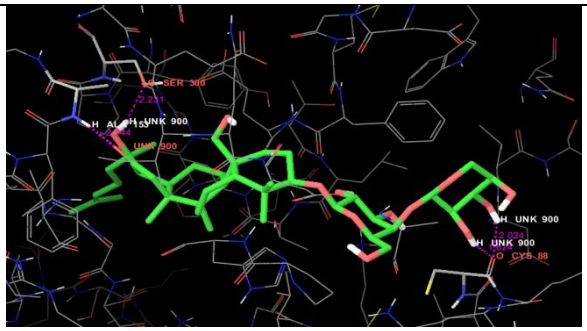
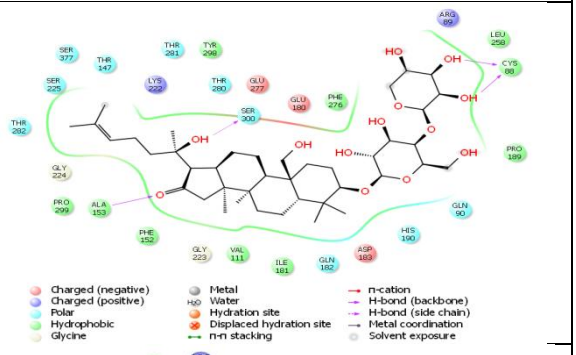
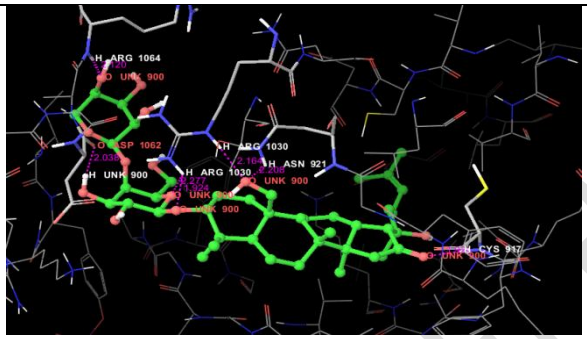
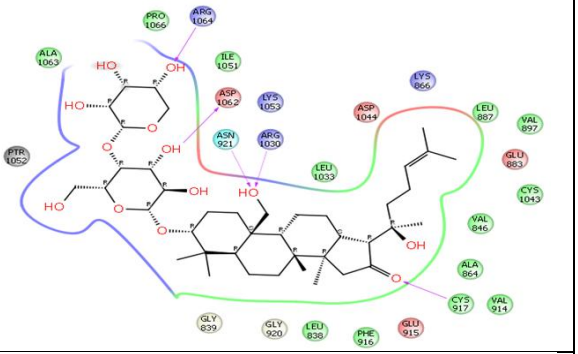
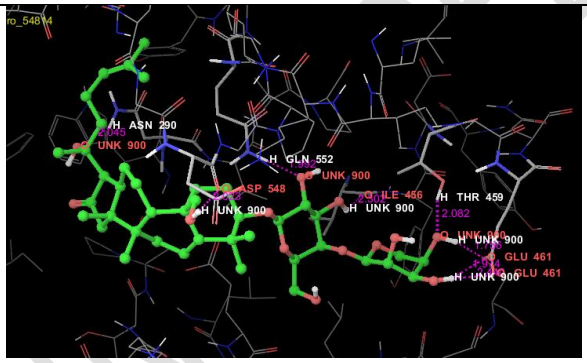
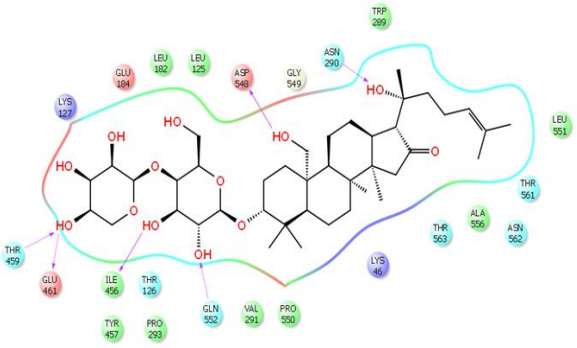
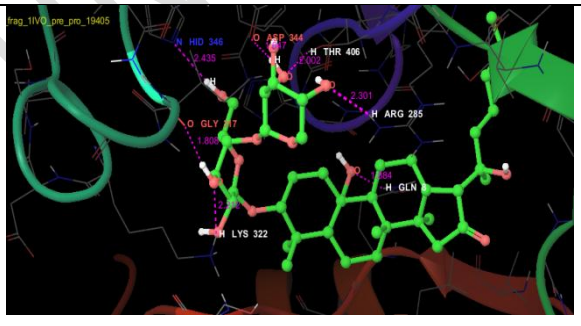
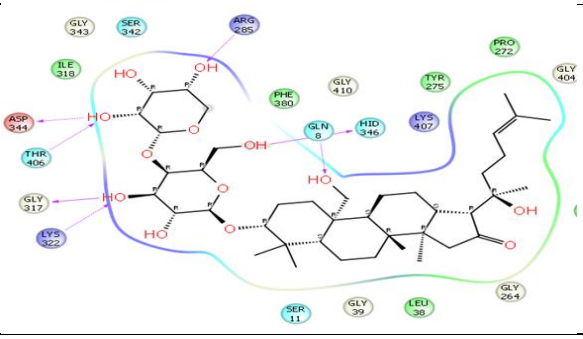
Hydrogen as a protein atom of Jagged-1from ALA153 interacted with Oxygen from ligand (BA) atom with the bond distance of 2.144 Å. The intermolecular interaction of perceived in –OH group as functional which is as side chains of ALA153.

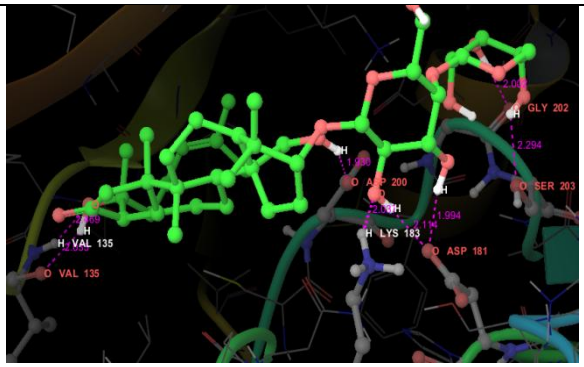
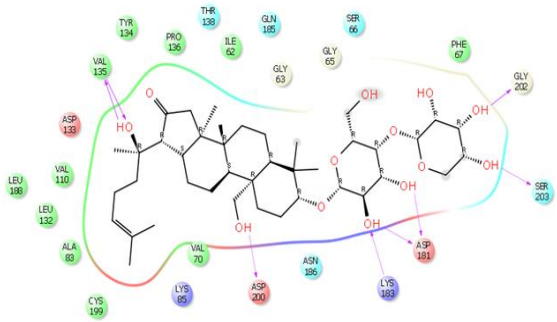
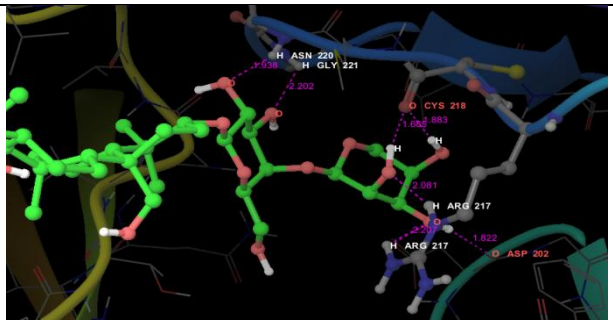
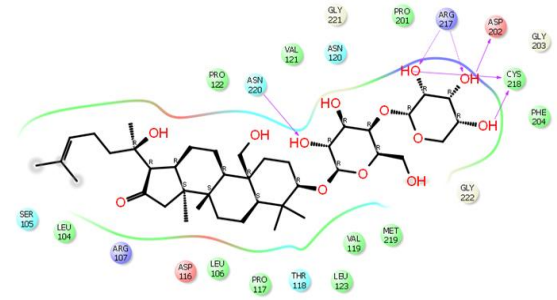
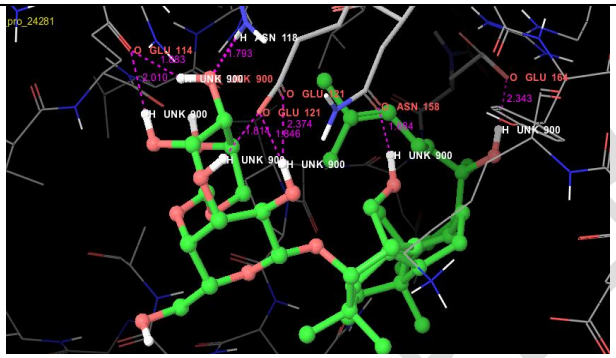
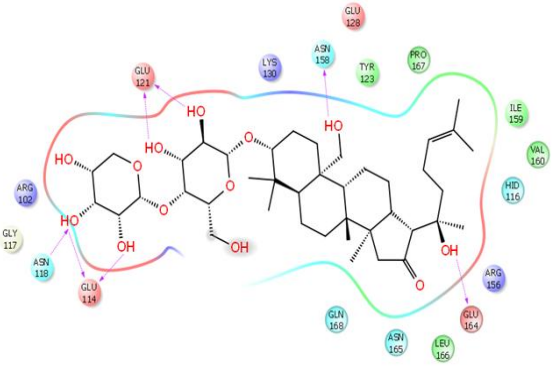
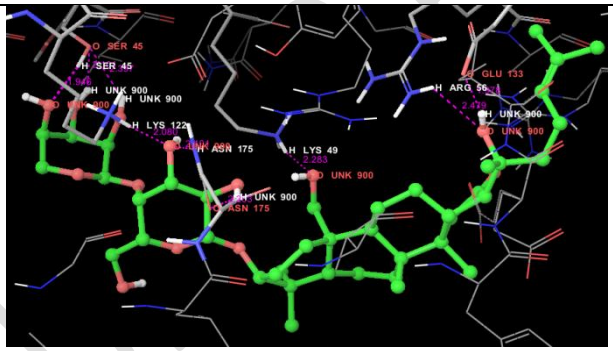
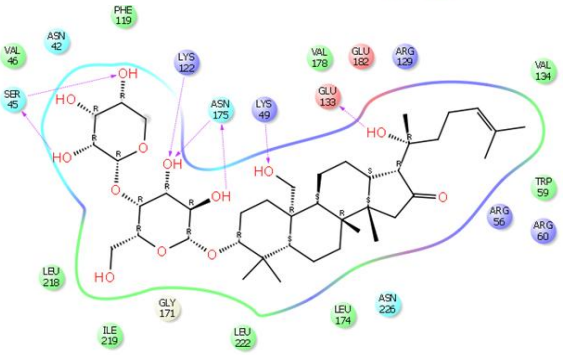
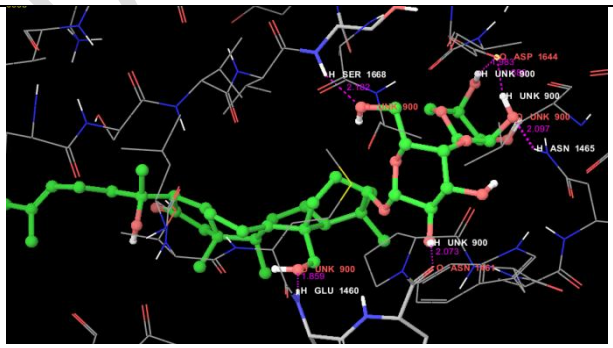
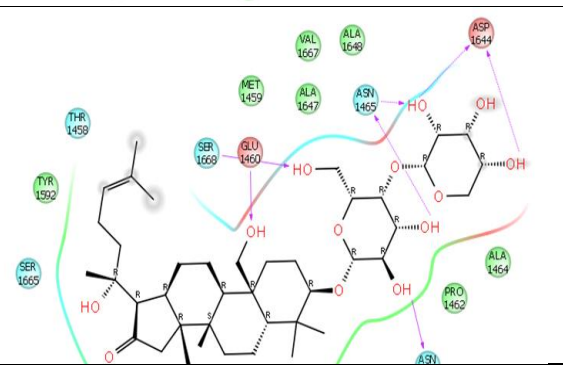
Out of the 15 proteins docked with BA from 3 different pathways, at least three of the components from each pathway have a glide score less than -8.0. They are EGFR, VEGFR, Raf, p53 from EGFR/Ras/Raf/MAPK pathway, Jagged-1, Gamma secretase and Notch-2 from notch signalling and Gsk-3 beta, wnt-3 and beta-catenin from wnt-beta catenin signalling. The only receptor that is showing the least interaction out of the 15 protein ligand interaction I frizzled receptor and the glide score is -4.8 having glide energy of -9675.0586 KJ/mol. Several frizzled receptor inhibitors have been identified and their docking results were also published. The two most potent compounds, SRI35959 and SRI37892, were docked separately into the putative binding site of the Fzd7-TMD model. The docked models suggested that both hits SRI35959 and SRI37892 accommodates into the Fzd7 active site. However, SRI37892 bound relatively tighter than SRI35959 with a docking score (which mimics the binding affinity) of -12.0 kcal/mol as compared to -10.3 kcal/mol of SRI35959, which is consistent with the observed experimental results that SRI37892 is a more potent inhibitor (Zhang et al., 2017).

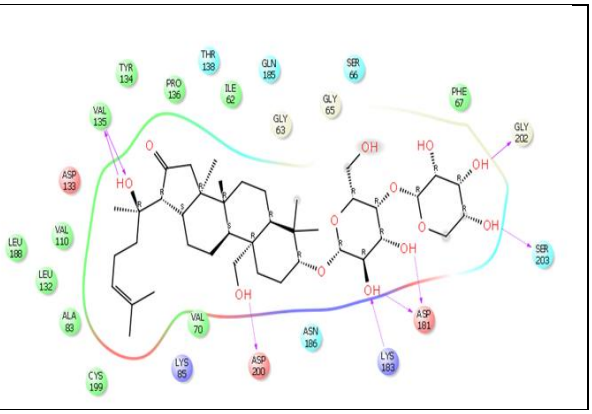
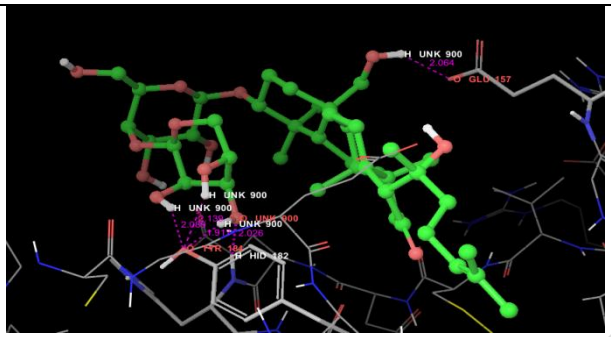
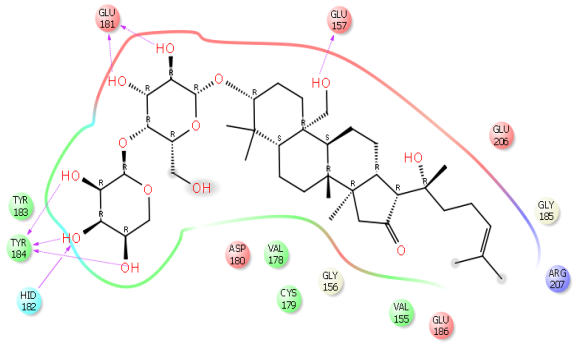
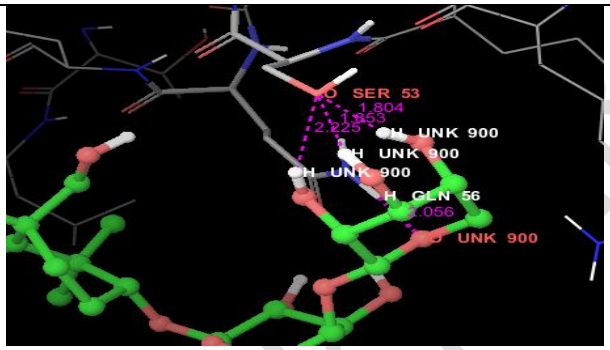
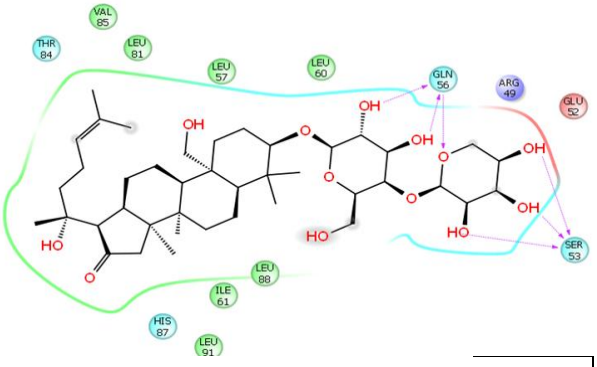
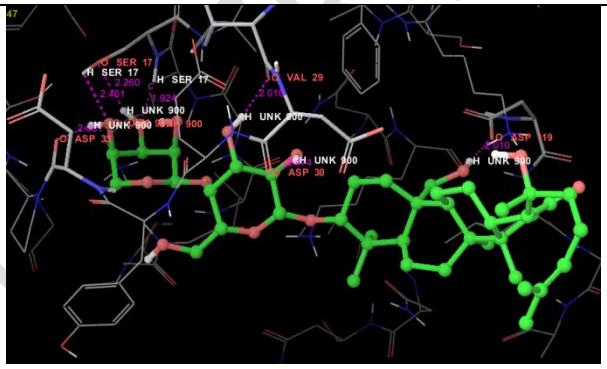
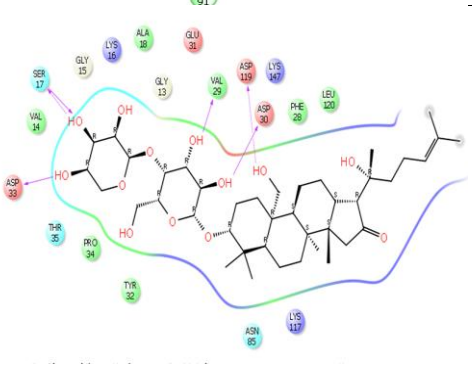
Hydrogen bonds play an essential role in stabilizing the protein-ligand interactions (Chou et al., 2004). The transmembrane receptors EGFR and VEGFR have 7 hydrogen bonds with the ligand and the cytoplasmic receptor notch-2 has 6 hydrogen bonds **(Fig.3 and Table-2).** However, the frizzled receptor interacts with BA via only two hydrogen bonds. From the amino acid interactions of all the target proteins with BA, it was clear that the interaction were stronger with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr,

Gln, Asp, Lys and Glu. The ligand also showed interactions with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied.

Figure- 3

Protein Name	3D image	2D image
Jagged-1		
VEGFR		
Gamma secretase		
EGFR		

GSK 3 beta		
Wnt-3		
p53		
Raf-1		
Notch-2		

β-catenin		
Dll-1		
Hes		
Ras		

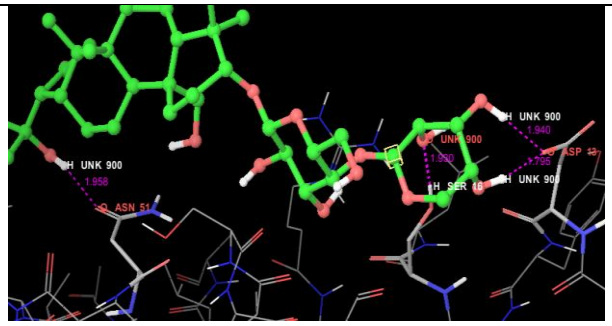
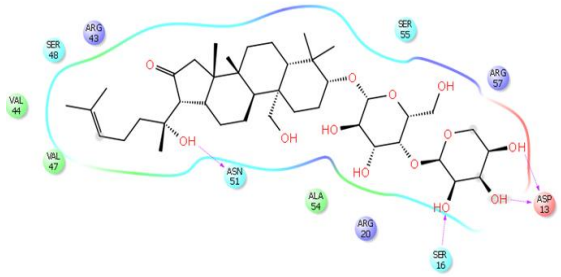
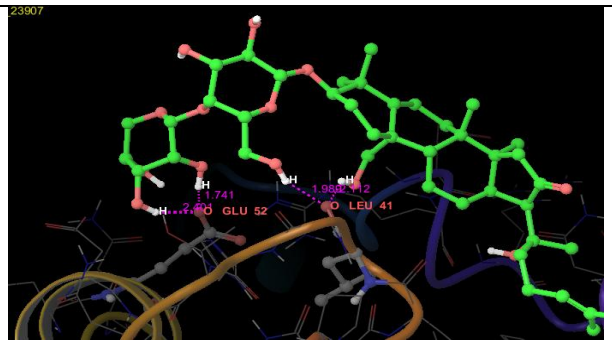
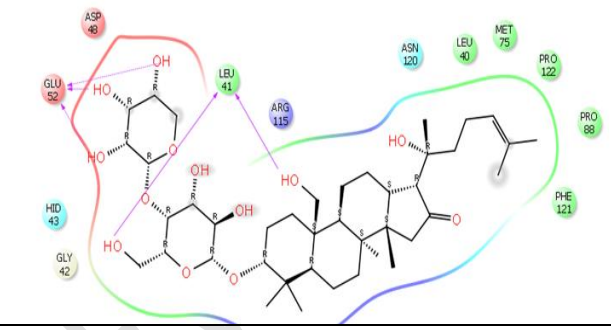
Hey		
Frizzled Receptor		

Figure. 3. Molecular docking studies of Bacoside A with Jagged-1, VEGFR, Gamma secretase, EGFR, GSK-3 beta, Wnt-3,p53,Raf,Notch-2, Beta catenin, Dll-1,Hes,Ras, Hey, Frizzled receptor arranged according to the increasing order of their glide score. The figure on left side represents 3D image whereas second image represents 2D view of the docked complex in which hydrogen bonding has been shown.

4. Conclusion

The present study evaluates the anti-glioma activity of BA through in silico studies. The docking studies revealed that BA obtained interacts with the proteins EGFR, VEGFR, Ras, Raf, DLL-1, Notch-2, Jagged-1, Gamma secretase, Hey, HIF 1-α, p53, Wnt-3, Frizzled Receptor, Beta catenin, GSK3- Beta. Out of the 20 proteins which seem to interact with BA, its interaction with jagged 1(a ligand of Notch receptor) and TGF 1α protein have the best glide score. While, frizzled receptor showed minimum glide score. The amino acid interactions of all the target proteins with BA showed that the interaction is comparatively stronger with the polar amino acids such as Asn, Trp, Arg, Ser, Thr, Tyr, Gln, Asp, Lys and Glu. BA also interacts with specific hydrophobic amino acids such as Val, Ala and Leu in all the protein targets studied. The results of these molecular docking studies indicate the therapeutic potential of BA against glioblastoma and suggest its potential for use as a molecular therapeutic. It warrants further validation of these results through in vitro and in vivo studies.

DISCLAIMER

Commonly used products in research in our area are exploited in this study. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 2: Hydrogen bonding interaction between the amino acid residues of Bacoside A and target protein after molecular docking

Ligand	Signalling	Protein name	PDB ID	No of hydrogen bonds	Interacting amino acid residues
	EGFR/Ras/Raf/MAPK pathway	EGFR	1IVO	7	GLN8,ARG285,THR406,LYS322, ASP344,GLY317, HIS346.
		Ras	1CTQ	7	ASP119, ASP30, VAL29,

Bacoside A					SER17, ASP33, CYS917
		Raf	3IQJ	9	SER45, GLU133, and ASN175, ARG56, LYS49, LYS122,
		VEGFR	1YWN	7	ARG1030,ARG1064, ASN921,CYS917, ASP1062.
		p53	1YC5	8	GLU121 ,GLU114 ,GLU164,ASN158 , ASN118
	Notch Signalling	DLL-1	4XBM	5	TYR184 ,GLU157, HIS182.
		Jagged-1	4X17	4	CYS88,SER300, ALA153
		Notch-2	2OO4	6	ASP1644,ASN1461, GLY1460, SER1668, ASN1465
		Gamma-secretase	5A63	8	GLU461, ASP548, ILE456, ASN290, GLN552,THR459.
		Hes	2MH3	4	SER53, GLN56
		Hey	2DB7	4	ASP13,ASN51, SER16
	Canonical Wnt-Beta catenin signaling	β-catenin	2Z6H	6	LYS354, CYS466, SER425, ASP390, SER351,VAL349
		GSK-3β	1GNG	7	ASP181, VAL135, ASP200 , SER203, GLY202,LYS182
		Frizzled Receptor	6BD4	2	GLU52,LEU41
		Wnt-3	2YZQ	5	ARG217, GLY221,ASN220, CYS218, ASP202, GLY221,