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SHORT COMMUNICATION

# An *In Silico* Computational Screening of Bioactive Compounds Retrieved from Raw Drugs of *Kuruvaer Kudineer*, against Autism Target – Akt Serine/Threonine Kinase 1

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## Abstract

**Background:** Autism spectrum disorder (ASD) refers to a range of early-onset social communication deficits and repetitive sensory-motor behaviors. ASD is a broad category of disorders of brain development. According to the WHO, about 1 in 100 children has autism. The estimated prevalence of autism in India is around 1 in 68 children. The rising prevalence of ASD, coupled with limited services and the side effects of available treatments, underscores the urgent need for effective management approaches. This study aims to ascertain the binding affinity of the bioactive compounds of the raw. **Objective:** drugs of *Kuruvaer Kudineer* against Autism Target AKT serine/threonine kinase 1 by *in silico* molecular docking method. **Materials and Methods:** From the literature survey, eight major bioactive compounds having a higher therapeutic value from the raw drugs of *Kuruvaer Kudineer* had been selected as ligands and AKT serine/threonine kinase 1 (PDB ID: 4GV1) as a potential protein drug target for *in silico* molecular docking analysis against ASD using Autodock 4 software. **Results:** The results showed that estimated binding energy appeared to be in the magnitude of kcal/mol, as follows: Rutin-12.11, β-sitosterol-9.45, Geniposide-8.86, Betulinic acid-8.79, Gingerenone-A-7.74, β-vetivenene-6.73, 6 Gingerol-5.93, and Thymol-4.66 towards the target (PDB ID: 4GV1). Betulinic acid has seven interactions, β-Sitosterol, Gingerenone-A, and Geniposide have 5, Rutin and β-vetivenene have 3, and Thymol has 2 interactions with the target. Conclusion: The examined results indicate that certain bioactive drugs can suppress the advancement of AKT1 phosphorylation of the PI3K-AKT/mTOR signaling pathway, hence reducing apoptosis in individuals with autism. Thus, it provides a new avenue in ASD management using *Kuruvaer Kudineer,* a *Siddha* polyherbal formulation.

**Major Findings:** The study found that bioactive compounds from *Kuruvaer Kudineer* bind effectively to the autism-related AKT1 protein. Rutin showed the highest binding affinity, while Betulinic acid had the most interactions. These compounds may help reduce neuronal damage in autism, supporting the use of *Kuruvaer Kudineer* for ASD management.

**Keywords:** Autism, Ayush, *Kuruvaer Kudineer,* Molecular Docking, Network Pharmacology, *Siddha* Medicine

**Abbreviations:** ASD = Autism Spectrum Disorder; WHO = World Health Organization; LEU = Leucine; GLY = Glycine; GLU = Glutamic acid; ASP = Aspartic acid; MET = Methionine; ASN = Asparagine; LYS = Lysine; THR = Threonine; PHE = Phenylalanine; PDB ID = Protein Data Bank Identifier; PDBQT = Protein Data Bank, Partial Charge (Q), and Torsion (T)

## 1. Introduction

ASD is a term used to describe a group of early-onset social communication impairments and repetitive sensory-motor behaviors that are impacted by important hereditary and non-genetic factors[1]. ASD constitutes a diverse group of conditions related to the abnormal development of the brain. Genetic heritability

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estimates for ASD vary between 40% and 90%, with recent studies suggesting about 50%[2]. According to the WHO, about 1 in 100 children has autism[3]. Over 2 million people may be affected by ASD in India, a variety of nearly 1.3 billion people, with prevalence ranging from 0.09% to 1.07% among children aged 0 to 17 years. The estimated prevalence of autism in India is around 1 in 68 children[4].

Phosphatidylinositol 3-kinase, protein kinase B, and the mammalian target of rapamycin (PI3K-AKT/mTOR) signaling pathway play a pivotal role in managing crucial cellular functions, such as growth, differentiation, cell survival, and metabolism[5]. Dysregulation of this pathway has been implicated in various brain disorders, including autism and other neurological conditions[6]. Disruptions in this signaling pathway leads to genetic anomalies, neuronal death, mitochondrial impairment, and neuroinflammation and cause autism, which is identified by distinctive behavioural and psychiatric symptoms[7,8].

Current interventions for ASD include a mix of behavioural and communication strategies alongside drug treatments like occupational therapy, music therapy, speech therapy, and physical therapy. These treatments target severe symptoms like hyperactivity, aggression, self-harm, and temper tantrums using drugs such as dopamine antagonists (haloperidol), atypical neuroleptics (olanzapine, risperidone), Divalproex, secretin (peptide hormone, selective), serotonin reuptake inhibitors (fluvoxamine), and famotidine[9,10].

Management combines educational and behavioural strategies with medications, some of which may cause irreversible side effects, necessitating long-term care. The rising prevalence of ASD, coupled with limited services and the side effects of available treatments, underscores the urgent need for effective management approaches[11,12].

This molecular docking of bioactive components retrieved from raw drugs of *Kuruvaer Kudineer* against AKT serine/threonine kinase 1 explores the potential of *Kuruvaer Kudineer* in inhibiting the PI3K-AKT/mTOR signaling pathway. This potentiality is the key to the management of autism.

The amino acids such as $LEU^{156}$, $LEU^{295}$, $GLY^{157}$, $GLY^{294}$, $GLU^{234}$, $GLU^{278}$, $GLU^{298}$ $ASP^{274}$, $ASP^{292}$, $MET^{281}$, $ASN^{279}$, $LYS^{276}$, $THR^{291}$, and $PHE^{293}$which are responsible for mediating the activity of the enzyme AKT1. Hence, the binding of phytochemicals with these bioactive amino acids is expected to halt the progression of AKT1 phosphorylation and reduce apoptosis in autistic subjects.

The raw drugs of *Kuruvaer Kudineer* are dried roots of *Vettiver* (*Vetiveria zizanioides* (L.) Nash), *Vilamichu ver* (*Plectranthus vettiveroides* (Jacob)), *Parpadagam* (*Hedyotis corymbosa* (L.)), and *Siruthaeku* (*Clerodendrum serratum* (L.)) and dried rhizome of *Inji* (*Zingiber officinale* Roscoe) which is mentioned in *Siddha* classical book Baalavagadam[13].

## 2. Materials and Methods

Docking analysis was accomplished using Auto Dock Version 4.2.6. A common molecular screening method for determining the binding energy between each ligand's three-dimensional structure and the target protein was used to undertake *in silico* molecular docking analysis for all active components found in the herbal formulation of *Kuruvaer Kudineer* with potential drug target AKT serine/threonine kinase 1 (PDB ID: 4GV1) for ASD.

### 2.1 Selection and Preparation of Ligands

Eight bioactive compounds from raw drugs of *Kuruvaer Kudineer* such as Geniposide, β-sitosterol, Betulinic acid, Gingerenone-A, 6 Gingerol, Rutin, β-vetivenene, and Thymol were collated from published research papers and a public database. Next, they were obtained in SDF format from https://pubchem.ncbi.nlm.nih.gov. By converting these ligands to PDB format using the open babel program (http://openbabel.org/wiki/Main_Page), they are now ready for docking analysis. The torsion requirements needed for proper binding were then defined using the Autodock 4.2 6 application. The vernacular and botanical names of the selected ligands for docking from the raw drugs of the *Kuruvaer Kudineer* with their PubChem ID is shown in (Table 1). The ligands that were chosen for docking analysis are shown in (Table 2) along with their molar weight (g/mol), molecular formula, H bond donor, H bond acceptor, and rotatable bonds.

### 2.2. Protein Preparation

The three-dimensional crystal structure of AKT serine/threonine kinase 1 (PDB ID: 4GV1) is shown in Figure

1. It was extracted from the Protein Data Bank, energy-minimized, and converted to the appropriate PDBQT formats. The ligand molecules were first positioned, oriented, and torsored randomly. When the device docked, all spinning torsions were relieved. Every docking experiment came from two distinct runs, each of that was intended to end after a maximum of 2,50,000 energy assessments. The population size was set to 150. A translational step of 0.2 Å and quaternion and torsion steps of 5 were used in the search. Protein structures were cleaned by eliminating the lead components that were already there; water molecules were broken; polar hydrogens were included in the computation of Kollman's charges[19] and Auto Dock 4.2.6 software was used to describe the merging of non-polar and rotatable bonds.

## 2.3 Molecular Docking Methodology

Following the preparation of the protein target and ligand selection, Autodock 4.2.6 was used to establish molecular docking. The dimensions of x, y, and z were set at 60×60×60 with a resolution of 0.375 Å to generate binding. To obtain the best docking configurations, the grid box was centered. Once the grid file has been saved as a (.gpf) file, launch autogrid. Using a preset run count of 10, the Lamarckian genetic technique was used to compute the docking. Following the execution of auto-dock, the dock file was saved as dpf. A (.dlg) file included the final docked findings, which included details on the inhibition constant, binding energy, and binding residues. The structures showing how ligands and proteins interact were seen using Discovery Studio[20,21].

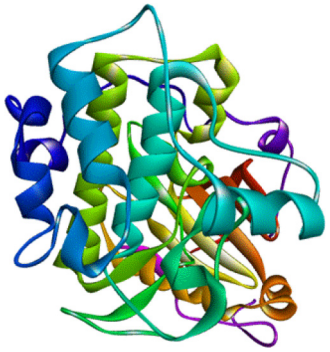


**Figure 1.** Receptor structure in 3D (AKT 1- AKT serine/threonine kinase 1) – (PDB: 4GV1).

**Table 1.** Selected ligands for docking from the raw drugs of the *Kuruvaer Kudineer*

| Vernacular Name (Tamil) | Botanical name of the herbs | Selected ligands | PUBCHEM ID |
|---|---|---|---|
| *Vettiver* | *Vetiveria zizanioides* (L.) Nash[14] | β-vetivenene | 14475467 |
| *Vilamichuver* | *Plectranthus vettiveroides* (Jacob)[15] | Thymol | 6989 |
| *Chukku* | *Zingiber officinale* Roscoe[16] | Gingerenone-A | 5281775 |
| | *Zingiber officinale* Roscoe | 6 Gingerol | 442793 |
| *Parpadagam* | *Hedyotis corymbosa* (L.)[17] | Rutin | 5280805 |
| | | Geniposide | 107848 |
| *Siruthaeku* | *Clerodendrum serratum* (L.)[18] | β-sitosterol | 222284 |
| | | Betulinic acid | 64971 |

**Table 2.** Properties of ligands selected for docking analysis

| Compound | Molar weight (g/mol) | Molecular Formula | H Bond Donor | H Bond Acceptor | Rotatable bonds |
|---|---|---|---|---|---|
| β-vetivenene | 202.340 | $C_{15}H_{24}$ | 0 | 0 | 0 |
| Thymol | 150.201 | $C_{10}H_{14}O$ | 1 | 1 | 1 |
| Gingerenone-A | 356.203 | $C_{21}H_{24}O_5$ | 2 | 5 | 9 |
| 6 Gingerol | 294.391 | $C_{17}H_{26}O_4$ | 2 | 4 | 10 |
| Rutin | 610.50 | $C_{27}H_{30}O_{16}$ | 10 | 16 | 6 |
| Geniposide | 388.402 | $C_{17}H_{24}O_{10}$ | 5 | 10 | 6 |
| β-sitosterol | 414.701 | $C_{29}H_{50}O$ | 1 | 1 | 6 |
| Betulinic acid | 456.703 | $C_{30}H_{48}O_3$ | 2 | 3 | 2 |

## 3. Results and Discussion

The molecular docking studies of compounds against AKT 1 (PDB ID: 4GV1), detailing the estimated free energy of binding, estimated inhibition constant (Ki), electrostatic energy, total intermolecular energy, and interaction surface are summarized in (Table 3).

The amino acid residue interactions of the lead compounds with AKT 1 (PDB ID: 4GV1) are outlined in (Table 4).

The docking poses of the ligands with the target receptor, AKT 1(PDB ID: 4GV1) are illustrated in Figure 2. The 2D interaction plot analysis of the ligands with the target receptor AKT 1 (PDB ID: 4GV1) is presented in Figure 3. The hydrogen bond interactions along with core amino acid analysis of the ligands with the target receptor AKT 1 (PDB ID: 4GV1) are illustrated in Figure 4.

Eight bioactive compounds in total were extracted from the raw drugs of the *Kuruvaer Kudineer Siddha*

**Table 3.** Summary of the molecular docking studies of compounds against AKT 1- AKT serine/threonine kinase 1 (PDB)- 4GV1

| Compounds | Est. Free Energy of Binding (kcal/mol) | Est. Inhibition Constant, Ki | Electrostatic Energy (kcal/mol) | Total Intermolec. Energy (kcal/mol) | Interact. Surface |
|---|---|---|---|---|---|
| Rutin | -12.11 | 1.33 nM | -0.32 | -6.95 | 1121.347 |
| β-sitosterol | -9.45 | 117.88 nM | -0.22 | -10.73 | 1010.916 |
| Geniposide | -8.86 | 321.54 nM | -0.41 | -8.96 | 743.425 |
| Betulinic acid | -8.79 | 361.55 nM | -0.01 | -11.52 | 1307.956 |
| Gingerenone-A | -7.74 | 2.12 uM | -0.31 | -6.41 | 795.363 |
| β-vetivenene | -6.73 | 11.74 uM | -0.02 | -7.02 | 535.498 |
| 6 Gingerol | -5.93 | 44.67 uM | -0.07 | -8.07 | 837.418 |
| Thymol | -4.66 | 382.73 uM | -0.07 | -5.26 | 475.984 |

**Table 4.** Amino acid Residue Interaction of Lead AKT 1- AKT serine/threonine kinase 1 (PDB)- 4GV1

| Com pounds | Inter actions | Amino acid Residues | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| β-veti venene | 3 | 179 LYS | 181 LEU | 191 GLU | 194 HIS | 195 THR | 198 GLU | **274 ASP** | **292 ASP** | **295 LEU** | | | | | | |
| Thymol | 2 | **156 LEU** | 164 VAL | 177 ALA | 211 THR | 229 TYR | 281 MET | **291 THR** | 438 PHE | | | | | | | |
| Ginge renone-A | 5 | **156 LEU** | **234 GLU** | **278 GLU** | 279 ASN | **281 MET** | **292 ASP** | 442 PHE | | | | | | | | |
| 6 Gingerol | 2 | **156 LEU** | 161 PHE | 164 VAL | 177 ALA | 181 LEU | 229 TYR | **234 GLU** | 281 MET | 438 PHE | | | | | | |
| Rutin | 3 | 158 LYS | 161 PHE | 164 VAL | 179 LYS | 191 GLU | 227 MET | **274 ASP** | **279 ASN** | 281 MET | **292 ASP** | 442 PHE | | | | |
| Geniposide | 5 | 161 PHE | 164 VAL | 179 LYS | 181 LEU | 186 ILE | 191 GLU | 194 HIS | 195 THR | **274 ASP** | **276 LYS** | **279 ASN** | **292 ASP** | **295 LEU** | 312 THR | |
| β-sitosterol | 5 | **156 LEU** | 161 PHE | 179 LYS | 181 LEU | 191 GLU | 194 HIS | 195 THR | **234 GLU** | **281 MET** | **291 THR** | **292 ASP** | | | | |
| Betulinic acid | 7 | **156 LEU** | 161 PHE | 164 VAL | 179 LYS | 191 GLU | 194 HIS | 198 GLU | **234 GLU** | **274 ASP** | **278 GLU** | **281 MET** | **292 ASP** | **295 LEU** | 438 PHE | 442 PHE |

formulation and were then examined *in silico* for potential interactions with the core active amino acid residues present on the target receptor AKT 1. The binding energy of selected 8 bioactive compounds involved in *Kuruvaer Kudineer* ranked in order of their interaction energy.

β-vetivenene primarily interacts with acidic residues (274 ASP, 292 ASP) and hydrophobic residues (295 LEU). The presence of multiple ASP residues suggests potential ionic or hydrogen

bonding interactions, while LEU indicates hydrophobic interactions. This profile may influence β-vetivenene's binding affinity and specificity to certain proteins or enzymes. Thymol interacts with hydrophobic (156 LEU) and polar (291 THR) residues. The interaction with LEU suggests a preference for hydrophobic environments, while THR indicates possible involvement in hydrogen bonding. This combination might affect thymol's binding to proteins with both hydrophobic and polar regions. Thymol helps to reduce inflammation and neuronal development in rat models. This indicates that this may play a role in autism[22].

Gingerenone-A interacts with a combination of hydrophobic (156 LEU), acidic (234GLU, 278 GLU, 292 ASP), and polar (281 MET) residues. The presence of multiple GLU residues suggests significant ionic interactions, while LEU and MET contribute to

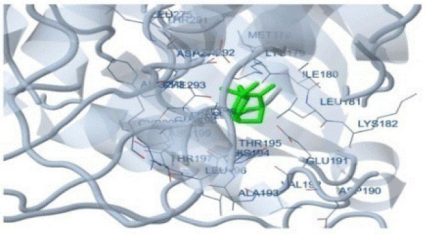


Figure 2.1. β-vetivenene with Target

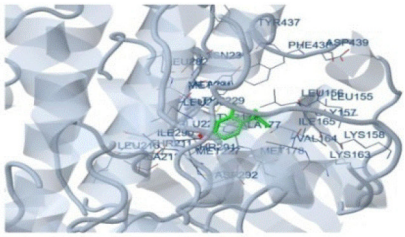


Figure.2.2. Thymol -A with Target

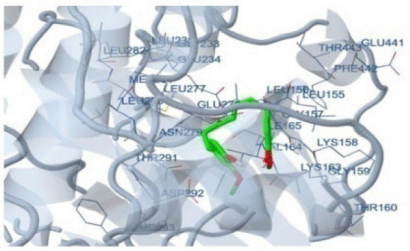


Figure 2.3. Gingerenone-A with Target

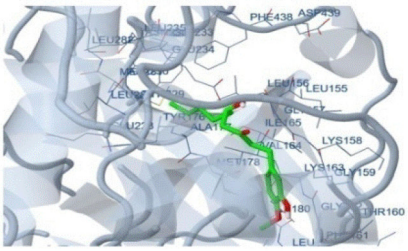


Figure 2.4. 6 Gingerol with Target

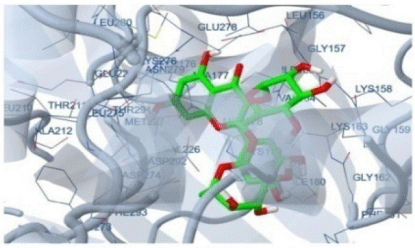


Figure.2.5. Rutin with Target

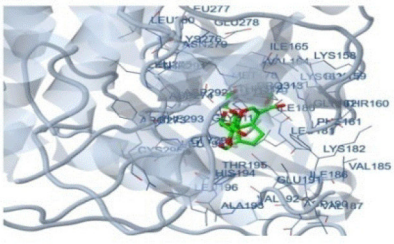


Figure.2.6. Genioposide with Target

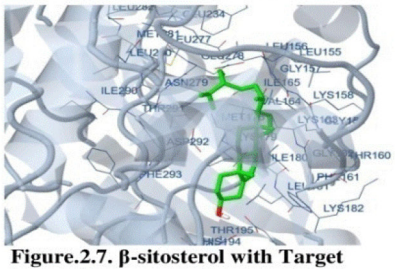


Figure.2.7. β-sitosterol with Target

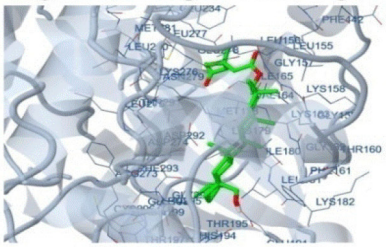


Figure.2.8. Betulinic acid with Target

**Figure 2.** Docking pose of the ligands with the target receptor AKT serine/threonine kinase 1 (AKT 1).

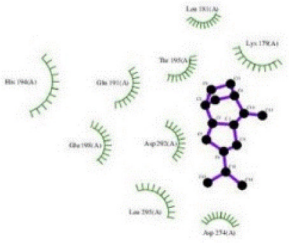


Figure 3.1. β-vetivenene with Target

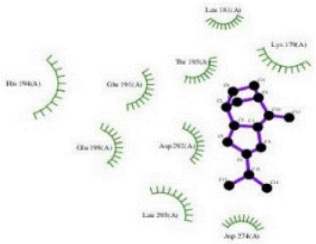


Figure 3.2. Thymol -A with Target

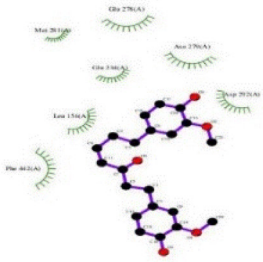


Figure 3.3. Gingerenone-A with Target

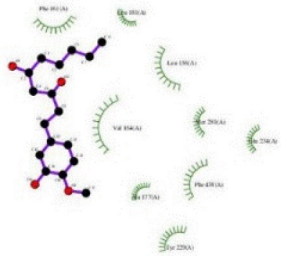


Figure 3.4. 6 Gingerol with Target

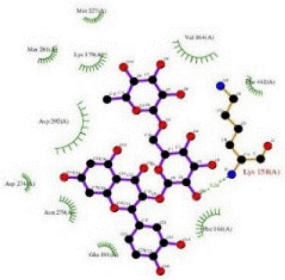


Figure 3.5. Rutin with Target

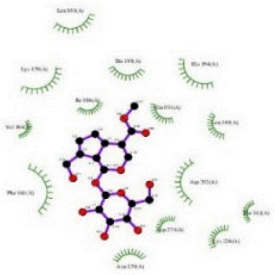


Figure 3.6. Genioposide with Target

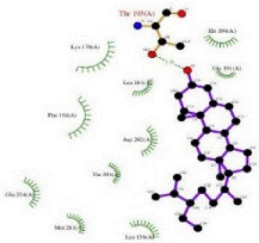


Figure 3.7. β-sitosterol with Target

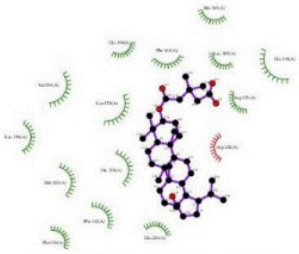


Figure 3.8. Betulinic acid with Target

**Figure 3.** 2D interaction plot analysis of the ligands with the target receptor AKT serine/threonine kinase 1 (AKT 1) - PDB ID: 4GV1).

hydrophobic interactions. This diverse interaction profile may indicate strong and varied binding capabilities. 6-Gingerol interacts with hydrophobic (156 LEU) and acidic (234 GLU) residues. The interaction with LEU suggests a preference for hydrophobic environments, while GLU indicates potential ionic or hydrogen bonding interactions. This profile might suggest 6-gingerol's role in stabilizing interactions within hydrophobic and acidic regions. Research findings revealed that 6-gingerol possesses high gastrointestinal absorption and good permeation of blood-brain barrier[23].

Rutin interacts predominantly with acidic (274 ASP, 292 ASP) and polar (279 ASN) residues. The multiple ASP interactions suggest significant ionic or hydrogen bonding capabilities, while ASN may contribute to polar interactions. This profile could indicate that rutin has a strong affinity for proteins with acidic and polar residues. Studies have shown that polyphenols like Rutin improve concentration, reduces abnormal

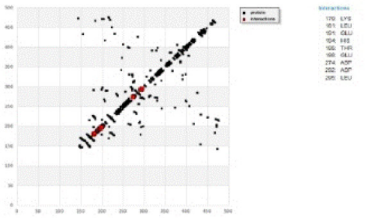


Figure 4.1. β-vetivenene with Target

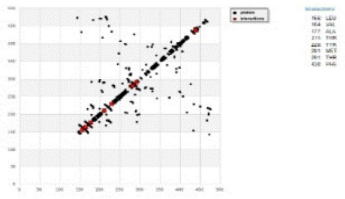


Figure 4.2. Thymol -A with Target

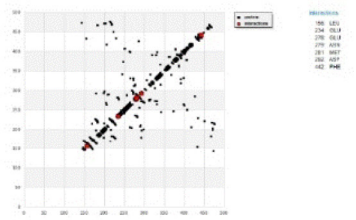


Figure 4.3. Gingerenone-A with Target

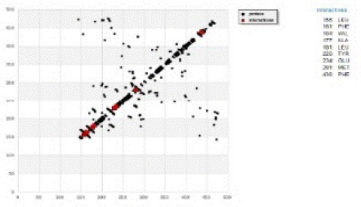


Figure 4.4. 6 Gingerol with Target

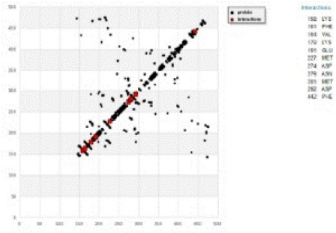


Figure 4.5. Rutin with Target

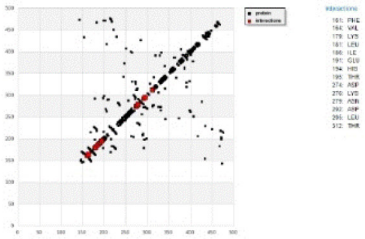


Figure 4.6. Genioposide with Target

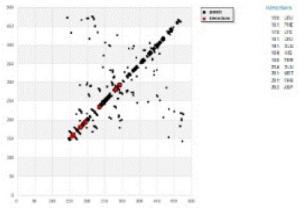


Figure 4.7. β-sitosterol with Target

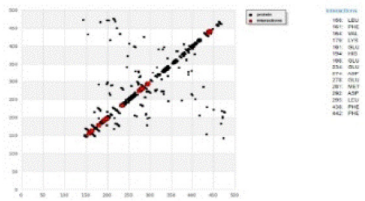


Figure 4.8. Betulinic acid with Target

**Figure 4.** Hydrogen bond interactions along with core amino acid analysis of the ligands with the target receptor AKT serine/threonine kinase 1 (AKT 1) - PDB ID: 4GV1).

behaviour and decreases proinflammatory cytokines in children with ASD[24].

Geniposide shows a broad range of interactions with acidic (274 ASP, 292 ASP), basic (276 LYS), and polar (279 ASN) residues, along with hydrophobic (295 LEU) interactions. This diverse interaction profile suggests that geniposide may bind effectively to a variety of protein sites, potentially influencing its biological activity. Pro-inflammatory cytokine levels in peripheral blood are markedly elevated in ASD patients. Proinflammatory cytokines produced by Th17 have the potential to induce brain inflammation. Patients diagnosed with autism exhibited considerably higher levels of IL-17A[25]. In ASD, geniposide lowers proinflammatory cytokine levels by inhibiting Th17 development through modulating GSK3β[26].

β-sitosterol interacts with hydrophobic (156 LEU), acidic (234 GLU, 292 ASP), and polar (281 MET, 291 THR) residues. The combination of hydrophobic and polar interactions, along with acidic residues, suggests

a balanced binding profile that may affect β-sitosterol's ability to interact with various proteins or cellular targets. People with autism have high triglyceride levels. Research finding reveals that β-sitosterol helps to reduce cholesterol effectively by reducing adipogenesis and lipid damage[27].

Betulinic acid has a broad interaction profile, engaging with hydrophobic (156 LEU, 295 LEU), acidic (234 GLU, 278 GLU, 274 ASP, 292 ASP), and polar (281 MET) residues. The extensive involvement of both hydrophobic and acidic residues, along with some polar interactions, suggests that betulinic acid might bind to diverse protein sites with a strong and complex interaction profile. Betunilic Acid provides improved cognitive function with a higher antioxidant effect and thereby inhibit the secretion of proinflammatory cytokines in the brain. So, this biomolecule helps to improve the cognitive function in the brain[28].

Compounds like Thymol, 6-Gingerol, and β-sitosterol interact with hydrophobic residues (e.g., LEU), indicating a preference for hydrophobic binding sites. β-vetivenene, Rutin, and Betulinic Acid show interactions with multiple acidic residues (e.g., ASP, GLU), suggesting significant ionic or hydrogen bonding interactions. Compounds such as Gingerenone-A and Geniposide have interactions with a wide range of residues, indicating complex binding mechanisms.

## 4. Conclusion

This analysis of data concluded that the selected bioactive compounds like Rutin, β-sitosterol, Geniposide, Betulinic acid, Gingerenone-A, β-vetivenene, 6 Gingerol and Thymol present in the raw drugs of *Kuruvaer Kudineer* have the inhibitory potential in the progression of AKT1 phosphorylation of the PI3K-AKT/mTOR signalling pathway by having high binding energy between target protein and ligands, more interactions with amino acid residues of the target with ligands. Being the PI3K-AKT/mTOR signalling pathway gets inhibited by these bioactive compounds present in the raw drugs of *Kuruvaer Kudineer* through suppressing the AKT-1, the neuronal damage does not occur in the brain which is helpful in the management of ASD.

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## 6. Author Contribution

S. Karthik Nagarajan - Conceptualization, Methodology, Study plan, Data Collection, Writing the original draft. C. B. S. Bharath Christian. and K. Rajamaheswari. - Data analysis, Validation, Formal analysis. A. Lavanya and K. Arunachalam - Data interpretation, Final article drafting, grammar and spelling correction. All authors have read and agreed to the published version of the manuscript.

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