In Silico Evaluation of the compounds of Siddha herbal formulation Panchakarpa Mathirai against Gaba (A) Receptor in the management of Anxiety and Seizure

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ABSTRACT:

Central nervous system (CNS) depressants are drugs that calms down the CNS. These drugs are used for the treatment of anxiety and seizure. GABA is the principal inhibitory neurotransmitter of the central nervous system. CNS depressants brings about their effects by acting on the $GABA_A$ receptors of the Central nervous system. In Siddha system of medicine, mental illness is mentioned as "Brammai". Pancha karpa mathirai is the Siddha herbal formulation widely used for mana noigal (psychiatric illness). Thus, the present study was undertaken to evaluate the Pancha karpa mathirai against the $GABA_A$ receptor through in-silico approaches. Docking calculations were carried out for retrieved phytocomponents against target GABA- A receptor by auto dock program. Total of 10 bioactive lead compounds were retrieved from the herbs, from the reported data of the herb, the phytochemical Chebuloside possess 3 interactions out of 4 target core active amino acid residues present on the $GABA_A$ receptor which signifies 75% of the binding efficacy. Thereby it was concluded that these compounds may exert promising anxiolytic and anti-convulsant activity.

Keywords: Anxiolytic, anti-anxiety, panchakarpa mathirai, docking study, Siddha.

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Introduction:

Mental disorder is characterized by a clinically significant disturbance in an individual's cognition, emotional regulation, or behaviour. [1] Anxiety disorders are the highly prevalent psychiatric disorders and are associated with a high burden of illness. [2] In 2019, 301 million people were living with an anxiety disorder including 58 million children and adolescents.[3] Central Nervous System (CNS) depressants are medicines that include sedatives, tranquilizers, and hypnotics. These drugs can slow brain activity, making them useful for treating anxiety, seizure disorders, panic, acute stress reactions, and sleep disorders. [4] GABA is the principal inhibitory neurotransmitter of the central nervous system. Agonist may bind to the different sites of GABA receptor which has the two subtypes. $GABA_A$ receptor is a ligand-gated chloride channel while $GABA_B$ receptors are the G-protein coupled receptors. [5] CNS depressants brings about their effects by acting on the $GABA_A$ receptors of the Central nervous system while $GABA_B$ receptor is responsible for

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skeletal muscle relaxant activity. [6] Sedative or hypnotic drugs reduce the synaptic transmission through binding with the $GABA_A$ receptor which increases the chloride ion conductance, thus brings about their calming effect. [5,7]

Benzodiazepines (BZDs) are a class of psychoactive drugs most widely prescribed for their anxiolytic, anti-epileptic and sedative effect on the central nervous system (CNS). [8] Diazepam, lorazepam, alprazolam, and lorazepam are commonly used sedatives comes under the group of benzodiazepines. [9] Regular consumption of BZDs has been shown to cause severe, harmful physical and psychological dependence, leading to withdrawal symptoms similar to that of alcohol withdrawal. Some of these withdrawal symptoms can be life threatening. [10] So, it is the time to explore the alternative drug for benzodiazepines in contemporary Medicine to proven the anxiolytic effect.

Recent days most of the people has been rely on the herbal medicines for the treatment. In *Siddha* system of medicine, mental illness is mentioned as "*Brammai*". [11] *Pancha karpa mathirai* is the Siddha herbal formulation widely used for *Mana noigal* (psychiatric illness) and *Valippu noi* (seizure disorder). [12] Hence, the present study was undertaken to evaluate the *Pancha karpa mathirai* against the $GABA_A$ receptor through in-silico approaches.

Materials & Methods:

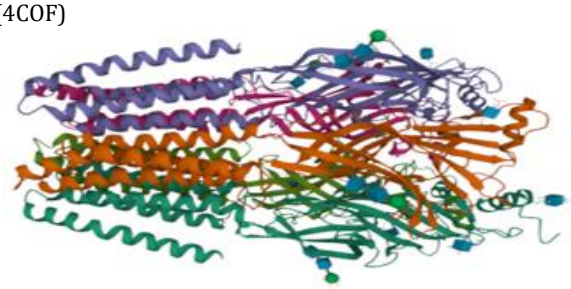
Docking simulation software:

A conventional docking tool was used to predict the potential of the leads under investigation over specified enzyme target (Auto Dock version 4). Different orientation of the lead molecules with respect to the target GABA- A receptor with PDB- 4COF was evaluated by Autodock version 4 program and the best dock pose was selected based on the interaction study analysis. [13]

Ligand Preparation:

Crystalline three-dimensional structure of the target protein GABA- A receptor with PDB-4COF (Fig. 1) was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Protein construct was optimized by eliminating preloaded lead candidate by cleaving adjoining water molecules. AutoDock 4 were calculated Gasteiger charges with extra polar hydrogen atoms, combining non-polar and rotatable links. [14-16]

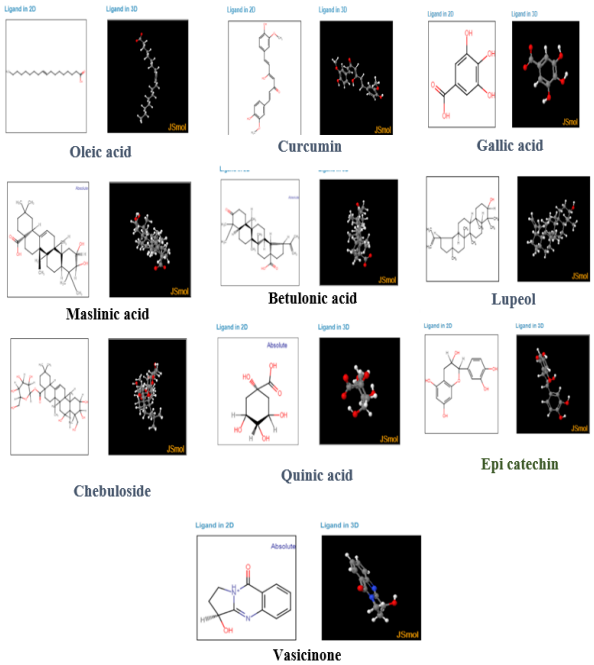
Figure 1: Crystalline Structure of GABA- A receptor (4COF)



Phytocomponents structure:

The herbal ingredients *Santalum album, Curcuma longa L, Terminalia chebula, Phyllanthus emblica, Terminalia bellarica and Justica aadathoda* has the Phyto therapeutics such as Oleic acid, Curcumin, Gallic acid, Maslinic acid, Betulonic acid, Lupeol, Quinic acid, Epi Catechin and Vasicinone. The total of ten phytocomponents (Figure 2) structure were retrieved from the six herbal ingredients present in the siddha formulation *Pancha karpa mathirai* through systematic literature survey against target GABA- A receptor with PDB-4COF. [17-22]

Figure 2: 2D and 3D structure of lead compounds retrieved from database



Methodology of auto docking:

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools.

Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program.[23] AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. [24] Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 2,50,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Results:

Molecular docking studies were performed over 10 phytoconstituents retrieved from the herbs on the binding of target $GABA_A$ receptor to find a potential drug candidate for treating anxiety and epilepsy. All these 10 molecules were docked against the target $GABA_A$ receptor. Binding energy determines whether the desire leads tend to occupy the target amino acid residues that mediates the enzyme function and its relative biological action. [25] Among the docking studies performed on phytocompounds, all the analogs had effective binding interactions with $GABA_A$ receptor (binding energy ranges from -3.23 kcal/mol to -7.66 kcal/mol).

From the results, it reveals that Phytoconstituents with highest docking score were seen for Maslinic acid, Betulonic acid, Chebuloside, and Quinic acid shows -7.66 kcal/mol, -6.63 kcal/mol, -6.46 kcal/mol and -6.07 kcal/mol respectively. (Table 2) Whereas, Epi Catechin, Curcumin, Vasicinone, Gallic acid, and Lupeol with binding energy of -5.52, -4.94, -4.50, -4.44 and -4.39kcal/mol respectively showed moderate binding affinity against the target protein. From the reported data of the herb, the phytochemical Chebuloside possess 3 interactions out of 4 target core active amino acid residues present on the GABA-A receptor which signifies 75% of the binding efficacy. Followed by this other compound such as Oleic acid, Curcumin, Gallic acid, Maslinic acid, Betulonic acid, Lupeol, Quinic acid, Epi Catechin and Vasicinone ranked second with 2 interactions out of 4 target amino acids with the active site of the target protein which contributes 50% of the binding efficacy over the target protein. (Table 3)

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Oleic acid	282.5 g/mol	$C_{18}H_{34}O_2$	1	2	15
Curcumin	368.4 g/mol	$C_{21}H_{20}O_6$	2	6	8
Gallic acid	170.12g/mol	$C_7H_6O_5$	4	5	1
Maslinic acid	472.7 g/mol	$C_{30}H_{48}O_4$	3	4	1
Betulonic acid	454.7 g/mol	$C_{30}H_{46}O_3$	1	3	2
Lupeol	426.7g/mol	$C_{30}H_{50}O$	1	1	1
Chebuloside	666.8 g/mol	$C_{36}H_{50}O_{11}$	8	11	5
Quinic acid	192.17 g/mol	$C_7H_{12}O_6$	5	6	1
Epi Catechin	290.271 g/mol	$C_{15}H_{14}O_6$	5	6	1

Table 2: Summary of the molecular docking studies of compounds against Crystalline Structure of GABA- A receptor (PDB) - 4COF

Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Oleic acid	-3.23 kcal/mol	4.31 mM	-0.20 kcal/mol	-3.72 kcal/mol	308.844
Curcumin	-4.94 kcal/mol	240.41 uM	-0.24 kcal/mol	-5.65 kcal/mol	621.959
Gallic acid	-4.44 kcal/mol	559.69 uM	-0.20 kcal/mol	-3.86 kcal/mol	357.49
Maslinic acid	-7.66 kcal/mol	2.41 uM	-0.01 kcal/mol	-7.49 kcal/mol	626.79
Betulonic acid	-6.63 kcal/mol	13.84 uM	-0.03 kcal/mol	-7.23 kcal/mol	632.974
Lupeol	-4.39 kcal/mol	603.67 uM	-0.11 kcal/mol	-4.69 kcal/mol	398.895
Chebuloside	-6.46 kcal/mol	18.27 uM	-0.04 kcal/mol	-5.88 kcal/mol	660.96
Quinic acid	-6.07 kcal/mol	35.49 uM	-0.05 kcal/mol	-4.89 kcal/mol	351.921
Epi Catechin	-5.52 kcal/mol	89.19 uM	-0.30 kcal/mol	-5.73 kcal/mol	495.78
Vasicinone	-4.50 kcal/mol	502.08 uM	-0.15 kcal/mol	-4.80 kcal/mol	395.574

Table 3: Amino acid Residue Interaction of phytocomponents against GABA- A receptor (PDB) -4COF

Molecule	Interactions	Amino Acid Residue- Binding										
Oleic acid	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE	202 THR					
Curcumin	2	97 TYR	99 LEU	155 GLU	157 TYR	196 ARG	205 TYR	207 ARG				
Gallic acid	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE	205 TYR					
Maslinic acid	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE						
Betulonic acid	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE	202 THR	205 TYR				
Lupeol	2	97 TYR	99 LEU	155 GLU	200 PHE	202 THR	205 TYR					
Chebuloside	2	40 MET	63 PHE	96 THR	97 TYR	98 PHE	99 LEU	154 ILE	155 GLU	157 TYR	166 PHE	168 TRP
Quinic acid	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE	202 THR					
Epi Catechin	2	97 TYR	99 LEU	155 GLU	157 TYR	200 PHE	202 THR	205 TYR				
Vasicinone	2	97 TYR	99 LEU	155 GLU	200 PHE	202 THR	205 TYR					

Discussion:

Anxiety disorders are common and disabling conditions that mostly begin during childhood, adolescence, and early adulthood. Most anxiety disorders affect almost twice as many women than men.[26] GABA receptors are the key mediators of quick inhibitory synaptic transmission in the midpoint of the nervous system. Dysfunction of GABA receptors contributes the neurological disorders include anxiety, Alzheimer, parkinsonism, and seizures.[27] So the drugs act through GABA receptors are responsible in the management of many neurological illness. Siddha system of medicine is one of the ancient traditional medicine systems in the world which treats not only the body but also the mind and the soul.[28] In this work, we have chosen Siddha Formulation *Pancha karpa mathirai* to evaluate the action of anxiolytic and anti-convulsant activity through *in-silico* approaches against the $GABA_A$ receptor.

This study finds the efficacy of the lead molecules to bind with these core bio active amino acid residues, Leu99, Ile154, Glu155 and Asp163 which mediates the chlorine channel opening results in execution of inhibitory post synaptic potential (IPSP) followed by hyperpolarisation. Lead molecules/ Phytochemicals that occupies these significant amino acids synergise the action of GABA will considerably have higher therapeutic potential in management of anxiety and epilepsy. This neurological illness caused due to the imbalance of *pitham* and *kabam* dosham. [29]

Santalum album is one of the ingredients in the test drug which diminishes the *pitham* and dominates *kabam*. During, post digestive transformation, it turns into cold potency and helps in reinstating *kabam* to normalcy and eliminates *pitham* slowly out of the body. [30] Oleic acid present in this herbal produced sedative-like effects through $GABA_A$ receptors. [31] *Curcuma longa* by its bitter taste and hot potency it restores the *pitham*.[30] Study suggests that extracts of this herb such as curcumin have anti-convulsant activity [32,33] and anxiolytic activity. [34,35]

Many studies have proven the anxiolytic activity of gallic acid at low doses, while at the highest dose it has sedative effect. [36] Maslinic acid in *Terminalia chebula* has the highest binding energy (-7.66kcal/mol) which reinstating the *pitham*. [37,38] *Phyllanthus emblica* is the herb which diminishes the *pitham* and dominances the *kabam* due to its sweet taste in post digestive transformation it turns into cold potency. [30] Betulin and lupeol in amla shows the anti-convulsant activity [39] through $GABA_A$ receptors. Chebuloside present in *Terminalia bellarica* binds with the three amino acid residues (Leu99, Ile154, Glu155) which shows highest binding efficacy. Compounds other than chebuloside ranked moderate by offering prominent interactions with the two residual bioactive amino acids (Leu99, Glu155)

The docking studies of bioactive compounds from *Pancha karpa Mathirai* was strongly evident that therapeutics such as Maslinic acid, Betulonic acid, Chebuloside, and Quinic acid

ranked first by revealing potential binding score with target $GABA_A$ receptors. Followed by other leads including Epi Catechin, Curcumin, Vasicinone, Gallic acid, and Lupeol ranked second by offering the significant binding score.

Conclusion:

Based on the results of the computational analysis it was concluded that the bio-active compound's like Chebuloside, Oleic acid, Curcumin, Gallic acid, Maslinic acid, Betulonic acid, Lupeol, Quinic acid, Epi Catechin and Vasicinone present in the *Pancha karpa Mathirai* reveals significant binding against the target protein GABA-A receptor by interacting with active amino acid present on the active site of the protein. Thereby it was concluded that these compounds may prove the anxiolytic and anti-convulsant activity. In future, further *in-vivo* studies and clinical trial on *pancha karpa mathirai* will exerts the promising efficacy.

Conflict of interest:

None.

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None.

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