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ORIGINAL ARTICLE

Mechanistic insight the Antiepileptic Potential of Flavonoid of *Sedum lineare* targeting GABA-A receptor: Molecular Docking validation

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ABSTRACT
A subtype of receptors known as GABAA receptors produces persistent inhibition, which in turn regulates the excitability of networks. Recent research has linked these receptors to a wide range of neurological and mental diseases, including as epilepsy, schizophrenia, and Parkinson's disease. Unlike phasic GABAA receptors, they have a different subunit makeup and function, which makes it possible to modify network features selectively. Sedi linearis Herbals the Chinese name for traditional Chinese medicine that uses the entire Sedum lineare Thunb plant. Numerous medical conditions, including hepatitis, throat swelling, dysentery, dermatitis rhus, burns, scalds, traumatic bleeding, etc., were frequently treated with it. Kaempferol was discovered to be an active flavonoid in the hydro-alcoholic whole plant extract based on a review of the literature. Kaempferol may have neuroprotective effects at various doses, according to a prior study. It was discovered to raise Bcl-2 expression while lowering oxidative stress and apoptosis levels. Thus, kaempferol is used as the lead chemical in the current study to target the GABA-A receptor and investigate the antiepileptic effects. "The current study's objective was to assess the anti-epileptic efficacy of kaempferol via in-silico molecular docking." Studies using in-silico molecular modelling were conducted to estimate anti-epileptic potential of kaempferol by designing it to target the GABA -A receptors. The Auto Dock software was utilized to determine the binding through a grid-based docking technique. The results of the lead molecule's molecular modelling with GABA -A receptor demonstrated that the chosen compounds had a high affinity for the chosen target protein. It was discovered that the binding energy of kaempferol to the GABA -A receptor was -4.87 Kcalmol^{-1}.
Keywords: *Anti-epileptic efficacy, kaempferol, GABA -A receptors.*

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INTRODUCTION

Over 50 million individuals globally experience epilepsy. The most prevalent kind of partial onset epilepsy is called temporal lobe epilepsy (TLE). Patients with epilepsy face significant challenges to their quality of life due to two main reasons: first, their seizures can be unexpected and limit their ability to do daily tasks; second, they may also experience other neuropsychiatric comorbidities such depression or cognitive deterioration. There are not enough therapy choices available for epilepsy today. Drug resistance, defined as the inability to achieve seizure independence despite two well-tolerated and well "Applied Antiepileptic Drug" (AED) schedules, affects about 30% of patients. AED adverse effects might also include weight gain, behavioural abnormalities, sleeplessness, and dizziness. More effective AEDs with fewer adverse effects are therefore desperately needed. Being a crucial inhibitory receptor in the brain and a major contributor to the maintenance and growth of epilepsy, the "γ-Aminobutyric Acid (GABA) Type A Receptor" (GABAAR) is a valuable target for AEDs. The ionotropic GABAAR opens when the neurotransmitter GABA binds to it, allowing bicarbonate and chloride ions to permeate into the cell. An inhibitory postsynaptic potential (IPSP), or increased excitation threshold, is the outcome, causing

hyperpolarization [1]. Two distinct forms of inhibition are mediated by GABAARs: phasic inhibition, which is characterised by a short-lasting IPSP and tonic inhibition characterised by a persistent, IPSP (long-lasting). Phasic inhibition as compared to tonic inhibitions mediated by distinct GABAARs. They are called extrasynaptic or perisynaptic receptors because they are found outside of the synapse. Furthermore, tonic GABAARs have a different subunit composition than phasic GABAARs. Tonic inhibition can be viewed as a continuous "brake on the system" that counterbalances excitation because of its persistent hyperpolarization. Consequently, tonic currents are crucial for a variety of vital physiological functions, including information processing, cognition, synaptic plasticity, neurogenesis, neuronal development, and network oscillations and neuronal excitability regulation [2]. The fact that tonic GABA currents carry a larger charge than synaptic currents under physiological settings emphasizes the significance of this kind of inhibition even more. For example, Tonic inhibition accounts for 75% of the overall inhibitory charge that hippocampus neurons receive. [3]. Changes in tonic signalling must contribute to epilepsy, given the significance of tonic GABA signalling in controlling network excitability. The brain can be shielded against oxidative damage by flavonoids. Several flavonoids have anticonvulsive actions in the “Central Nervous System” (CNS) by binding to GABAA-benzodiazepine receptor's site [4]. *Sedi linearis* the term "*Herba*" refers to the usage of the whole *Sedum lineare* Thunb plant in traditional Chinese medicine. It was widely used to cure many different medical diseases, including as hepatitis, dysentery, dermatitis rhus, burns, scalds, traumatic bleeding, etc. [5].Kaempferol is a tetrahydroxyflavone containing 4- hydroxy groups at positions 3, 5, 7, and 4′ and it has a wide spectrum of pharmacological activities.

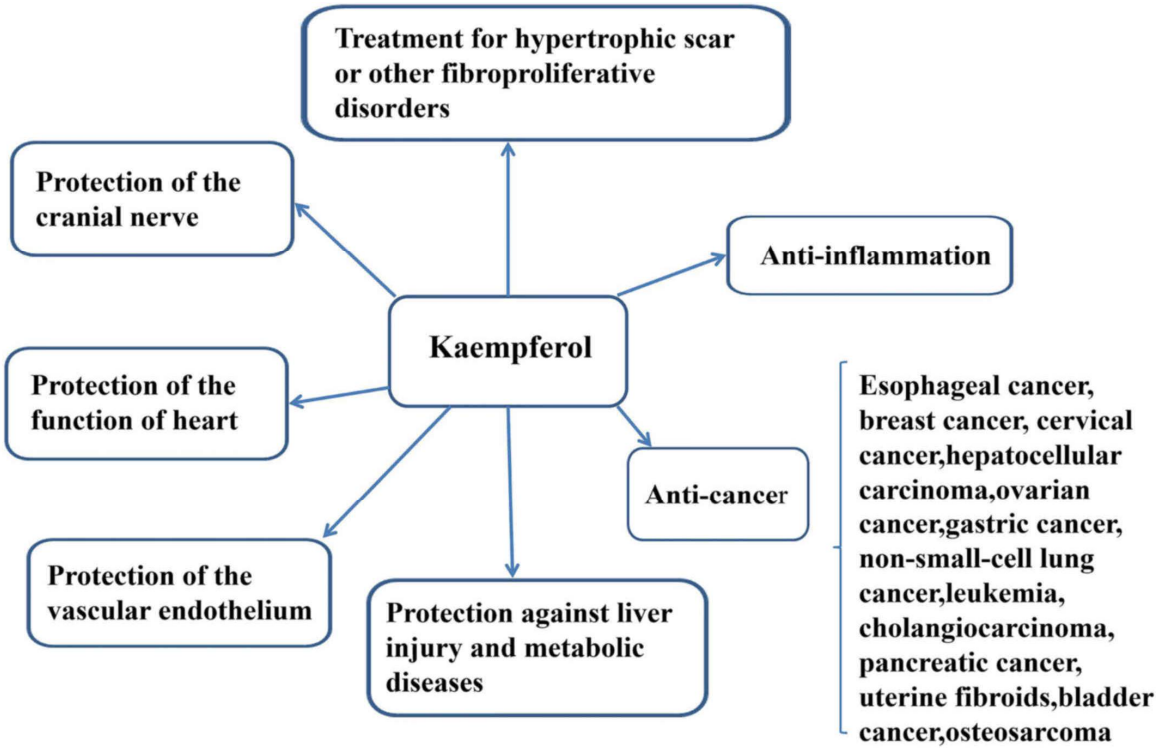


Fig 1: Various Pharmacological Potential of Kaempferol

S.No.	Description of Kaempferol [6-7]	
1.	Molecular formula	$C_{15}H_{10}O_6$
2.	Molecular weight	286.24 g/mol
3.	Source	Antioxidant polyphenol is present in vegetables and fruits.
4.	Category	Flavonol
5.	Pharmacology	It is a crucial part of molecular signal transduction pathways in cells that are linked to angiogenesis, metastasis, apoptosis, and inflammation. Importantly, kaempferol seems to preserve normal cell viability and sometimes has a protective effect in addition to suppressing angiogenesis, proliferation, and death of cancer cells.

EXPERIMENT WORK

Molecular docking studies Kaempferol against $GABA_A$ receptor

Ligand Preparation:

The ligands were optimized using three-dimensional geometry after their two-dimensional structures were converted to three-dimensional ones using ChemSketch [8]. The optimised structure was saved in PDB format to ensure compatibility with AutoDock. The prepared ligand's basic structures are as follows:

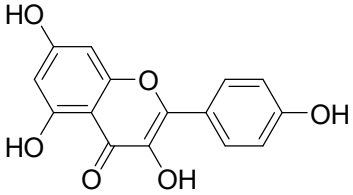


Figure 1: Kaempferol2D structure.

Preparation of the grid file

The active sites are defined by a grid box., Autodock was able to determine its areas of interest. Since the grid box is designed to cover every amino acid found in active sites that is required for binding apart from those found in receptors, it serves a crucial function in the docking process. The grid box has three thumbwheel widgets that let us change the x, y, and z point counts. Table 1 [9] provides the spacing and grid points for every receptor taken into consideration in the present investigation.

Table 1. Grid parameters used to analyse the docking system of GABA-A receptor

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	GABA-A	40	40	40	0.403	-39.77	55.655	42.142

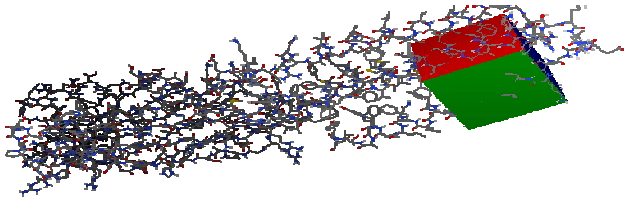


Figure 2: Grid box including each and every GABA-A receptor active site.

Docking file Preparation

Autodock4.2, a docking utility, was used for all calculations. Docking study visualization and other activities were carried out using Chimera, Pymol, MMP Plus, DS visualizer, and other software..[10-12].

Docking Study

Crystal structure

You may download the GABA-A receptor protein's crystal structure from Protein Data Bank website. The Protein Data Bank contains all of the main data pertaining to the structure of the receptors [13–14]. Software named Chimera was used for separating the complex ligand for each of the target receptors.

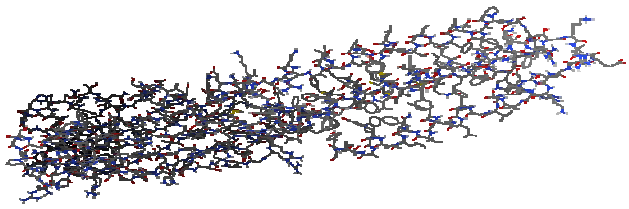


Figure 3: Crystal structure of GABA-A receptor (PDB ID-6cdu)

Protein Processing

All of the receptor proteins only include one chain, chain A, that is chosen for experimentation and had the complex ligand extracted. Chimera software was used to extract the bound ligand from the macromolecular complex [15–16].

Molecular Docking Simulation Studies

Autodock was used for docking the ligand kaempferol against the GABA-A receptor. Every ligand's link was maintained flexible, while the receptor's residues were not [17-19].

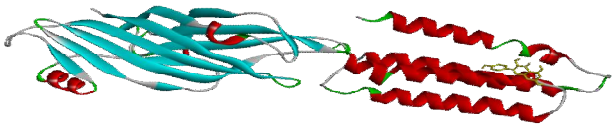


Figure 4: Binding mode of kaempferol at an active site of GABA-A receptor.

ADME-T& Toxicity Studies

The online software OSIRIS examined the ligand molecules, namely kaempferol, in order to anticipate the existence of any hazardous groups as well as ADME-T characteristics [20].

RESULT AND DISCUSSION

With a minimum of three millennia of documented history, epilepsy is among the most ancient neurological conditions. It is the most prevalent neurological ailment, impacting 1-2 percent of people globally and having a significant negative impact on numerous facets of quality of life. Epilepsy affects individuals of all ages, ethnicities, and socioeconomic levels; in industrialized nations, it affects 50 per 100,000 people, but in developing nations, it affects 100 per 100,000 people. The word "epilepsy" refers to a collection of conditions marked by frequent, spontaneous seizures involving hyperexcitable neurons. Neurotransmission mediated by excitatory glutamate and inhibitory GABA are thought to be out of balance. It is frequently linked to brain dysfunctions that cause a variety of behavioural comorbidities. Due to their comparable chemical structures to benzodiazepines, flavonoids have been experimentally shown to have antiepileptic effect via modification of the GABAA-Cl-channel complex [21]. As phenolic compounds, flavonoids have the ability to modify the therapeutic effects of neurodegenerative illnesses by interfering with cellular oxidative processes in the brain. One possible mechanism implicated in the onset and advancement of epileptogenesis is the overproduction of free radicals, which may lead to oxidative stress. The brain can be shielded against oxidative damage by flavonoids. Numerous flavonoids have anticonvulsive actions in the CNS by binding to the GABAA-benzodiazepine receptor's site. Kampferol's *in-silico* molecular modelling against the ***GABA-A*** protein demonstrated the molecule's efficient binding and high bonding affinity. It was discovered that kaempferol was a strong inhibitor of the GABA-A receptor, with a binding energy of -4.87 kcal/mol (table 2). Figure 4 displayed the binding mode. Figures 5 and 6 depict the 2D and 3D binding interactions, respectively. The pharmacokinetic profile of kaempferol shows that while they have a decent pharmacokinetic profile, they also have significant hazardous effects, such as impacts on the reproductive system and mutagenicity. Figures 7, display the pharmacokinetic and toxicity profiling data of ligands such as kaempferol.

Table 2: "Results of docking of ligands like kaempferol against GABA-A receptor"

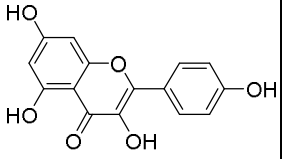
S. No	Compound Name	Structure	GABA-A
1	Kaempferol		-4.87 (ki: 267.22µM)

Table 3: Binding interaction of lead molecule with receptor

Lead molecule & Receptor	CH-bonding	Covalent bonding	Vander Waals interaction	Pi-Alkyl bonding	Pi-Sigma bonding
Kaempferol with GABA A receptor	Ile 239 Trp 246	Gln 242	Val 238 Leu 240 Leu 247	Val 243	---------

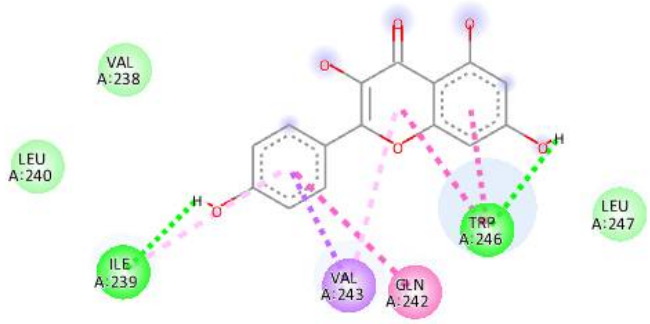


Figure 5: Kaempferol's two-dimensional binding mechanism at the GABA-A receptor's active region

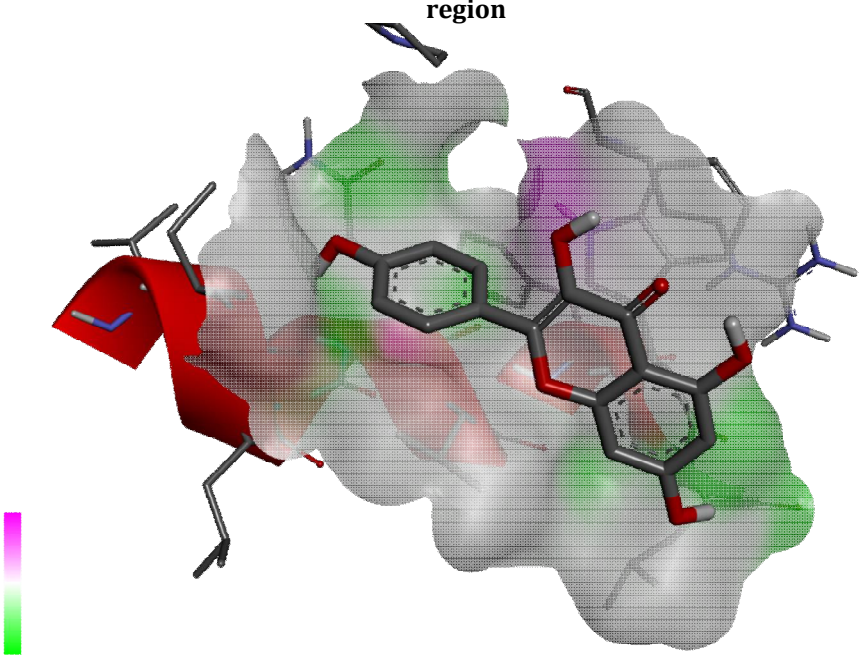


Figure 6: Kaempferol's three-dimensional binding mechanism at the GABA-A receptor's active region.

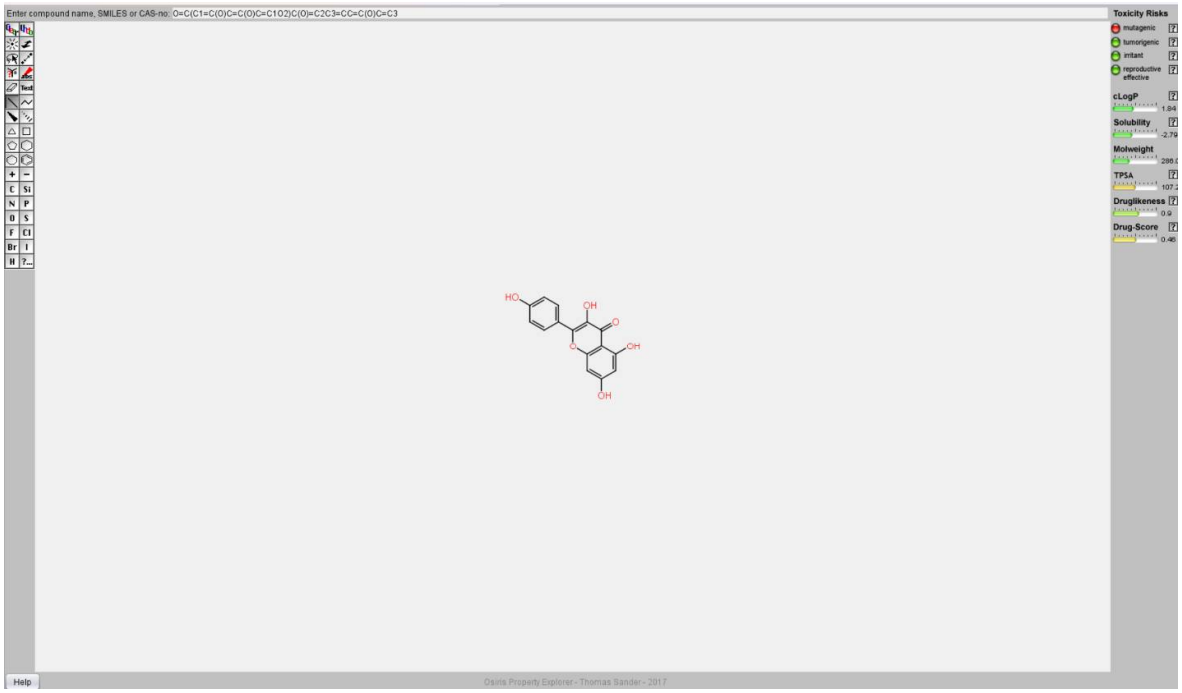
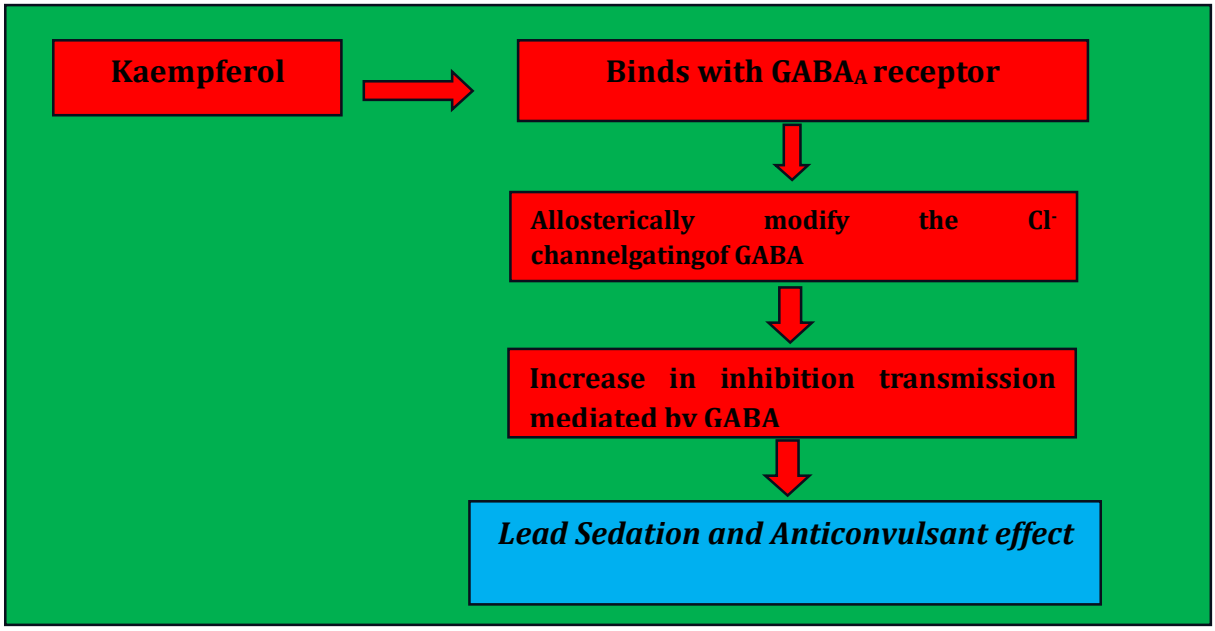


Figure 7: Toxicity and Pharmacokinetic profiling of kaempferol

DIVULGENCE OF INVESTIGATION

Sedum lineare plant was widely recognised for its many therapeutic uses, which included treating hepatitis, diarrhoea, dermatitis rhus, burns, scalds, traumatic bleeding, and throat swelling. Kaempferol's *in-silico* docking has unequivocally shown that flavonoids work to inhibit epilepsy by modifying the GABAA-Cl-channel complex. Thus, flavonoids (kaempferol) found in the *Sedum lineare* might have a modulating role in treating neurodegenerative disorders. As a result of the existence of flavonoid, which possessed neuroprotective activity, the findings of the current inquiry provide evidence that *S. lineare* is effective in terms of their ability to treat epilepsy. The proposed mechanism of action of Kaempferol as antiepileptic agent showed pictorially as:



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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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