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Shooting an arrow against convulsion: Novel modified flavonolignan derivatives as carbonic anhydrase II inhibitor

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Abstract
Background: Epilepsy is a prevalent neurological illness in humans, characterised by excessive transient neuronal discharges that lead to uncontrollable seizures, affecting about 60 million individuals globally. If untreated, it is linked to gradually deteriorating cognition and function, cerebral damage, and further neurological abnormalities. While epilepsy may often be well managed with antiepileptic medications (AEDs), around 20%-30% of individuals experience seizures that are refractory to existing pharmacological treatments. Consequently, the pursuit of a novel, more efficacious, and selective agent with less side effects remains a focal point of inquiry for medicinal chemists globally.
Method: The purpose of the current study was to assess the efficiency of bioactive found in S. *marianum* seed for their inhibitory influence on carbonic anhydrase -II(CA-II) enzyme to elicit the anticonvulsant potency. The Auto Dock software used a grid-based docking algorithm to determine the bond.
Result: S. *marianum* seed found to be effective anticonvulsant agent and effectively binds to be target protein carbonic anhydrase -II with binding energy of -7.86 & -6.43 kcal/mol for 7-O-methyl silybin and 2,3, dihydrosilybin respectively.
Conclusion: The outcome of findings revealed that flavanolignan showed potent inhibitory effect on *CA-II* enzyme which reflects the efficacy of S. *marianum* seed as potent anticonvulsant agent.

Keywords: S. *marianum*, anticonvulsant, molecular docking**,** 7-O-methyl silybin and 2, 3, dihydrosilybin**,** CA-II enzyme

Introduction
Epilepsy is a neurological condition characterised by strong, inadequately coordinated, localised, or widely scattered electrical discharges from neurones [1]. An epileptic seizure is a duration of abnormal neuronal discharge that clinically presents as changes in sensory perception, motor coordination, mood, or autonomic function [2]. Various variables, such as congenital, developmental, or hereditary origins, can lead to epileptic seizure disorders. The majority of seizures occur spontaneously, without forewarning, are brief (lasting a few minutes or even seconds), and resolve autonomously [3]. In several human populations, epileptic seizures are considered the most common neurological symptoms and remain the most widespread neurological illness impacting individuals across all age groups. Approximately fifty million individuals globally are afflicted with epilepsy, a persistent noninfectious neurological condition [4]. The first cures known to humanity are plant medicines. India is well acknowledged for its Ayurvedic therapy. India possesses a long history of using many plants for therapeutic purposes. Medicinal plants have a very dynamic role in traditional medicine for the treatment of many diseases [5]. Silybum marianum (Milk Thistle) is a rich source of phytoconstituents such as silybin A, silybin B, isosilybin A, isosilybin B, silychristin, silydianin, and apigenin 7-O-β-(2″-O-α-rhamnosyl). Galacturonide, kaempferol 3-O-α-rhamnoside-7-O-β-galacturonide, apigenin 7-O-β-glucuronide, apigenin 7-O-β-glucoside, apigenin 7-O-β-galactoside, kaempferol 3-O-α-rhamnoside, kaempferol, taxifolin, and quercetin. The plant is solely utilised for its anti-diabetic, hepatoprotective, hypocholesterolemic, anti-hypertensive, anti-inflammatory, anti-cancer, and antioxidant properties. The plant's seeds are utilised for their anti-spasmodic, neuroprotective, antiviral, immunomodulatory, cardioprotective, demulcent, and anti-haemorrhagic properties.

The herb also functions as a galactagogue, an agent that stimulates milk production, and is utilised in the treatment of uterine diseases. The herb is utilised in many traditional treatment methods for the management of diverse ailments [6]. Flavonoids are a varied group of polyphenolic substances found extensively in natural sources such as fruits, vegetables, and plant-based beverages, which may have therapeutic use in treating many life-threatening neurological illnesses. Prior studies emphasise the significance of various flavonoids in several neurodegenerative diseases due to their anti-inflammatory, antioxidant, and neuroprotective properties. They appear to exert both stimulatory and inhibitory actions within the disease process, potentially halting or delaying the advancement of numerous neurological disorders, including Parkinson's, Huntington's, Alzheimer's, and Multiple Sclerosis. Flavonoids play a significant role in regulating oxidative stress, modulating signalling pathways, and enhancing neuroplasticity, positioning them as potential neuroprotective agents in many clinical and experimental contexts. Moreover, their capacity to traverse the blood-brain barrier and provide neuroprotective effects renders them optimal for therapeutic applications in neurodegenerative diseases. Flavonoids have significant pharmacological effects and a broad range of activities. Based on the findings of numerous *in vivo* studies, it is unequivocal that the widespread occurrence of flavonoids in the plant kingdom, coupled with the inclusion of fruits and vegetables in the diet, renders these compounds significantly important in the prevention of various lifestyle diseases and may serve as an adjunct therapy for epilepsy. Consequently, flavonoids may be advantageous in formulating novel therapeutic approaches for the treatment of epilepsy. Despite the confirmed antiepileptic effects of flavonoids in preclinical investigations, there is a significant necessity to incorporate flavonoids as antiepileptic drugs in clinical trials [7-8]. This study aimed to assess the efficacy of modified Flavano-lignan compound present in *S. marianum* seed in inhibiting CA-II enzymes to determine their anticonvulsant potential.

Experiment Work

Selection of Lead molecule

According to the literature review, extracts derived from the seeds of the medicinal plant milk thistle [Silybum marianum (L.) Gaertn. (Asteraceae)] are extensively utilised as dietary supplements owing to their anti-inflammatory, anticancer, and hepatoprotective properties. The principal constituents of lipophilic extracts from milk thistle seeds, known as silymarin, are flavonoids and flavonolignans, including silybin A, silybin B, isosilybin A, isosilybin B, silydianin, silychristin, taxifolin, 7-O-methyl silybin, and 2,3-dehydrosilybins [9]. The modified flavonoids of silybin, specifically 7-O-methyl silybin and 2, 3-dehydrosilybin, were selected as lead molecules for the current work.

Description of lead molecule [10-13]

Description	7-O-Methyl silybin	2, 3-dehydrosilybin
Molecular formula	$C_{26}H_{24}O_{10}$	$C_{25}H_{20}O_{10}$
Synonym	7-O-Methylsilybin B	2-[(2S,3S)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-1,4-benzodioxin-6-yl]-3,5,7-trihydroxy-4H-1-benzopyran-4-one
Molecular weight	496.5 g/mol	480.43
Pharmacology	Anti-diabetic, hepatoprotective, hypocholesterolaemic, anti-hypertensive, anti-inflammatory, anti-cancer, and as an anti-oxidant.	Hepatoprotective agent. Protect against ischemia-reperfusion induced myocardial infarction and oxidative stress in rat cardiac tissues.

Selection of target receptors

Human Carbonic anhydrases II: Recent study has highlighted the significance of carbonic anhydrases (CA) in epilepsy, establishing them as a promising target for the development of novel anticonvulsant medications. Given the established correlation between low CO2 levels, alkalosis, and heightened neuronal excitability that facilitates seizure formation, carbonic anhydrases (CAs) are regarded as significant contributors to the aetiology of epilepsy. Moreover, it is recognised that epileptiform episodes may be induced by excitatory GABA.

GABA serves as the principal inhibitory neurotransmitter in the brain; but, under specific settings and states, such as early developmental phases and epilepsy, GABA may display depolarising effects. Reports indicate that GABAergic excitement is associated with carbonic anhydrase activity, particularly CA II and CA VII. The inhibition of certain isoforms of carbonic anhydrase enzymes has been suggested and utilised as a therapeutic strategy to regulate and manage aberrant epileptic activity. Carbonic anhydrase inhibitors (CAI) demonstrate a favourable anticonvulsant profile [14-19]. In current studies, Human carbonic anhydrases II was chosen as a target molecule involved in the convulsant.

Scientific Validation of *Anticonvulsant Potential* of Bioactive of *Silybum marianum* seed: *In-silico molecular docking*

Molecular docking studies against Human CA II

Ligand Preparation

2D Structure of 7-o-Methyl silybin and 2,3-dehydrosilybin were drawn using ChemSketch [20], the two-dimensional structure of the prepared ligand was converted into their 3-D structures optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligand were given below:

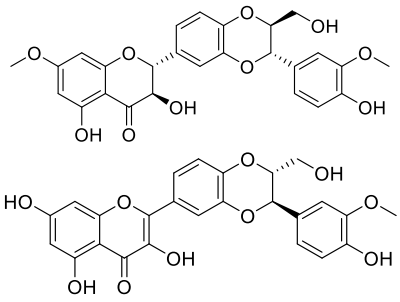


Fig 1: 2D structure of 7-o-Methyl silybin and 2,3-dehydrosilybin.

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points for the considered receptor in the current study are given in table 1 [21-23].

Table 1: Grid parameters used in current docking analysis.

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	CA2	50	50	50	0.403	16.662	5.523	14.085

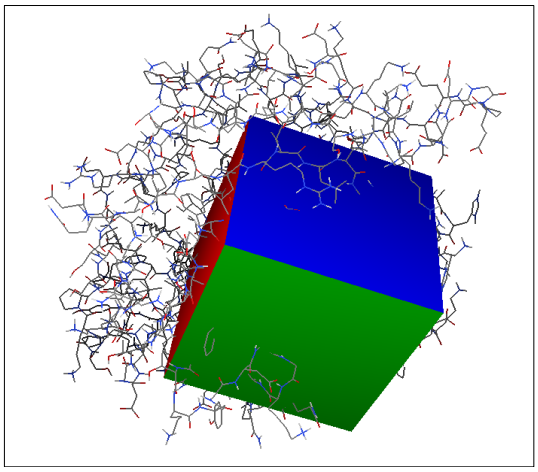


Fig 2: Grid box covering all active sites in CA2 receptor.

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [24-25].

Docking Study

Crystal structure

The crystal structure of the protein consisting of CA2 receptor was downloaded from the Protein Data Bank portal. All the primary information regarding all the receptor's structure was registered in the Protein data bank [26-28]. The complex ligand was separated by using Chimera software for all the target receptors.

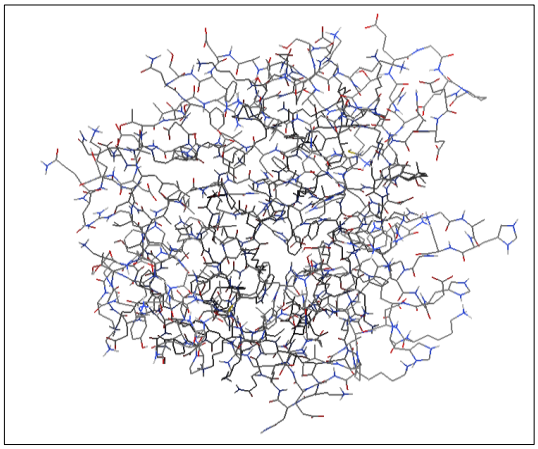


Fig 3: Crystal structure of CA2 receptor (PDB ID-8oo8)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A and B, out of which has chain A has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [29-32].

Molecular Docking Simulation Studies

Docking of ligand 7-o-Methyl silybin and 2,3-dehydrosilybin against CA2 receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [33-34].

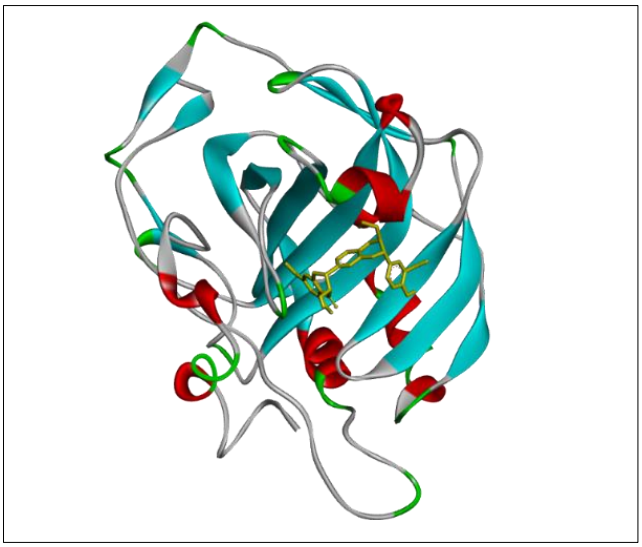


Fig 4: Binding mode of 7-o-Methyl silybin within the active site of CA2 receptor.

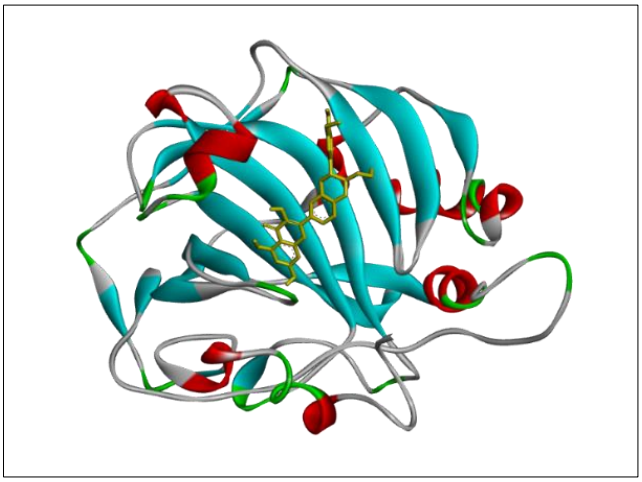


Fig 5: Binding mode of 2,3-dehydrosilybin within the active site of CA2 receptor.

Toxicity & ADME-T Studies

The ligand molecules *viz.* 7-o-Methyl silybin and 2,3-dehydrosilybin was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [35].

Result and Discussion

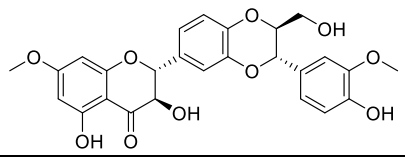
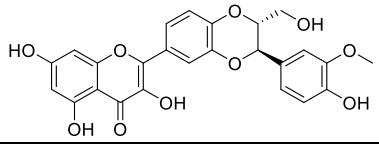
Flavonoids play a significant role in regulating oxidative stress, modulating signalling pathways, and enhancing neuroplasticity, positioning them as potential neuroprotective agents in many clinical and experimental

contexts. Moreover, their ability to traverse the blood-brain barrier and provide neuroprotective effects renders them suitable for therapeutic applications in neurodegenerative diseases. Flavonoids have significant pharmacological effects and a broad range of activities. Based on the findings of various *in vivo* studies, it is unequivocal that the ubiquity of flavonoids in the plant kingdom and the inclusion of fruits and vegetables in the diet render these compounds significant in the prevention of numerous lifestyle diseases and may serve as an adjunctive therapy for epilepsy. Consequently, flavonoids may be advantageous in formulating novel therapeutic approaches for the treatment of epilepsy. Despite the confirmed antiepileptic effects of flavonoids in preclinical investigations, there is a significant necessity to incorporate flavonoids as antiepileptic drugs in clinical trials. This study sought to evaluate the effectiveness of a modified Flavano-lignan molecule derived from S. marianum seeds in inhibiting CA-II enzymes to ascertain its anticonvulsant potential. A docking-based computational study of the CA-II enzyme has been performed to elucidate the most likely mechanism of action for the chosen lead phytoconstituent. The literature analysis indicates that *S.marianus seed* is a rich source of flavonolignan. The scientific confirmation of the bioactive presents in *S.marianus seed*, demonstrating efficacy against convulsant, was examined by *in-silico* molecular docking. As a result, newer modified flavonoid derivatives 7-O-methyl silybin and 2,3, dihydrosilybin were chosen as lead compounds against CA-II.The results of the current investigation indicated that the selected lead molecules serve as effective in treating and preventing convulsant, binding to the target protein of CA-II with binding energies of -7.86 & -6.43 kcal/mol for 7-O-methyl silybin and 2,3,dihydrosilybin respectively. The outcome was recorded in Table 2. The two-dimensional and three-dimensional interactions of the selected chemical are illustrated in Figures 6-9. The affinity of lead compounds for the receptor was determined to be relatively comparable. The interaction of 7-O-methyl silybin and 2,3, dihydrosilybin with the active site of CA-II is illustrated as follows:

Compound	**Binding against CA-II**					
Binding mode	**Conventional Hydrogen bounding**	**π-alkyl**	**Alkyl**	**Week Vander's interaction**	**π-donor H- bond**	**π -σ**
7-O-methyl silybin	His119 His96 His64 Thr64 Thr200 Pro201 ASN67 Glu69	ILE91	Trp209 Val121 His94	Glu106 Thr199 Ala65 ASN62 Trp123 Leu141	Leu60	Phe131
7-O-methyl silybin	Glu106 Thr199 His119 Glu69	Leu198 Val143	-------	Leu141 Val207 Trp209 ASN62 ASN62 Leu60 Arg58 Phe131	-------	Val121 ILE91

The interaction results indicated that lead molecules attach at comparable positions by typical hydrogen, alkyl, π-alkyl, π-σ and vander waal's interactions, demonstrating a synergistic effect of both compounds from *S.marianus seed* in exerting protective action on convulsant. The pharmacokinetic profile indicates a favourable pharmacokinetic profile; however, it also presents significant hazardous consequences, including mutagenicity, tumorigenicity, and reproductive toxicity. The pharmacokinetic and toxicity profiling data of ligands such as 7-O-methyl -silybin and 2,3, dihydro silybin are presented in Figures 10-11 and Tables 3-5. All ligand compounds have demonstrated promising docking scores theoretically.

Table 2: Results of docking of ligands like 7-o-Methyl silybin and 2,3-dehydrosilybin against CA2 receptor.

Sl. No	**Compound Name**	**Structure**	**CA-II**
1	7-o-Methyl silybin		-7.86
2	2,3-dehydrosilybin		-6.43

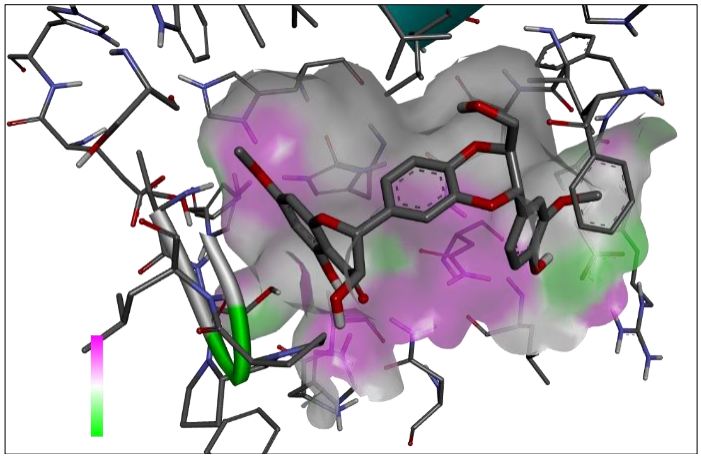


Fig 6: Three-dimensional binding mode of 7-o-Methyl silybin within the active site of CA2 receptor.

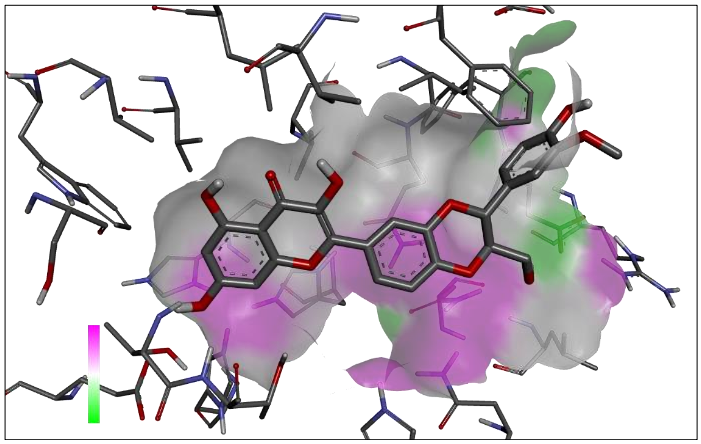


Fig 7: Three-dimensional binding mode of 2,3-dehydrosilybin within the active site of CA2 receptor.

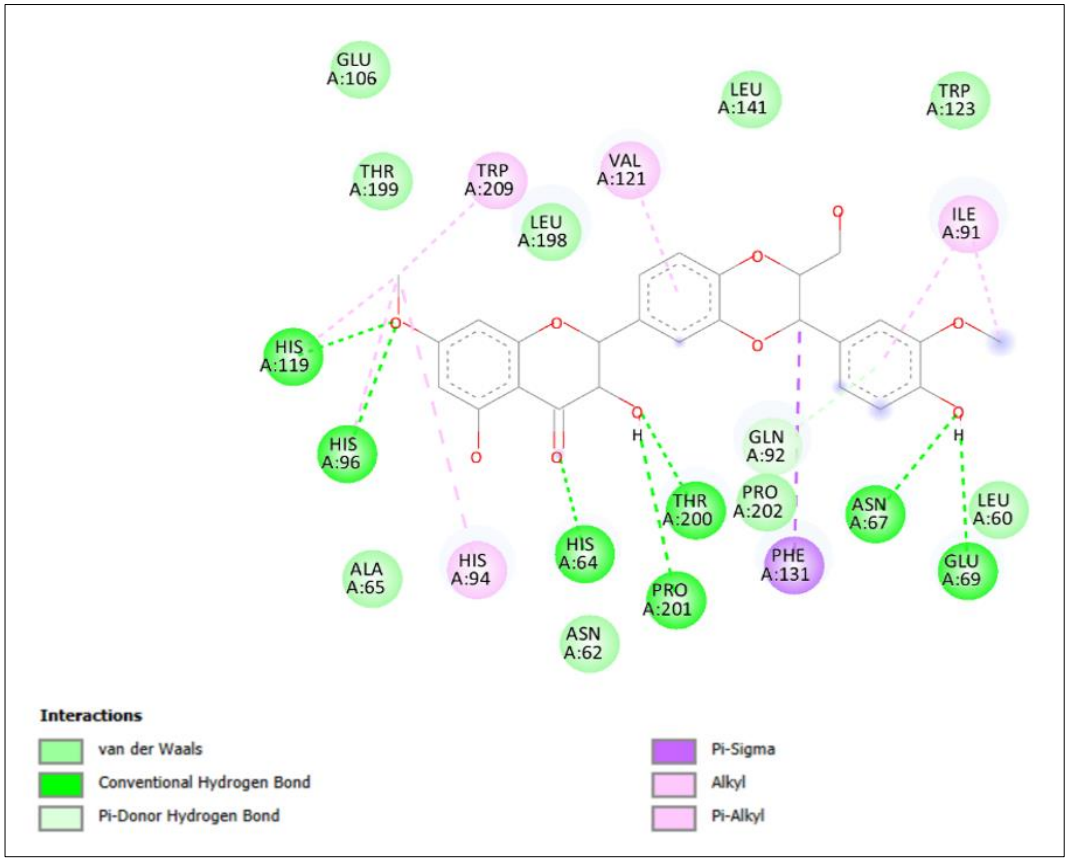


Fig 8: Two-dimensional binding mode of 7-o-Methyl silybin within the active site of CA2 receptor.

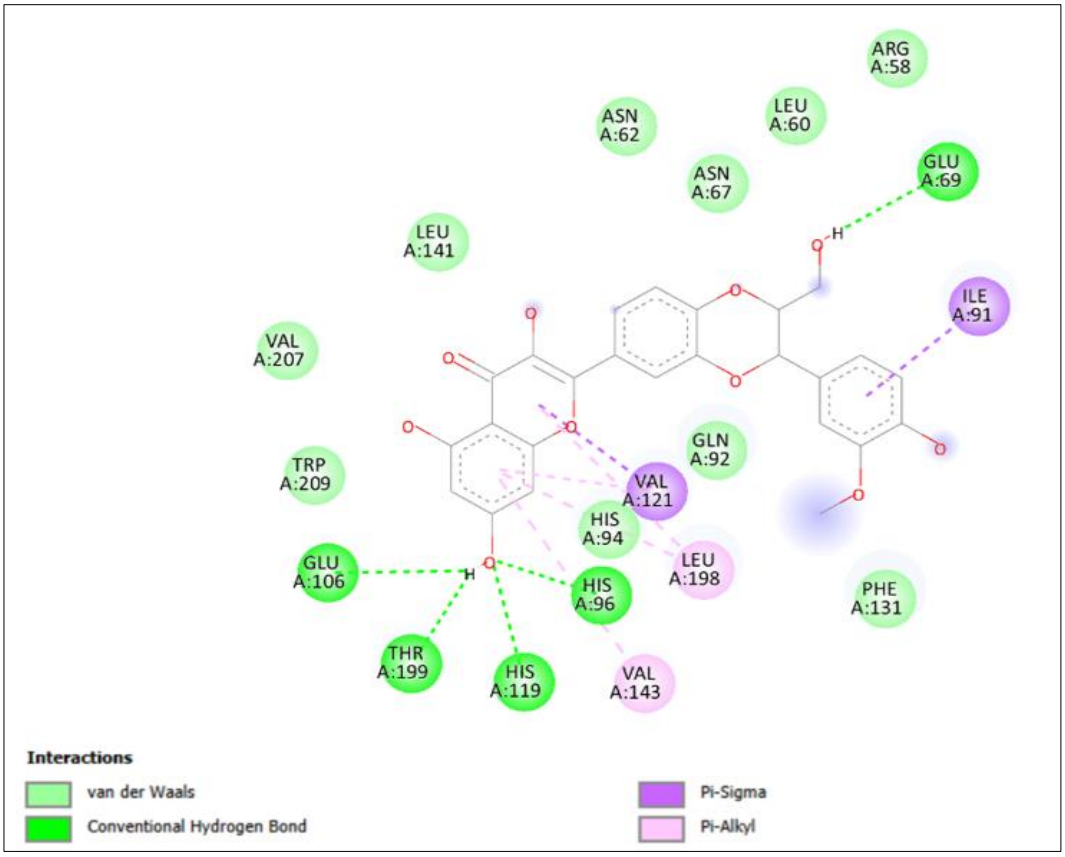


Fig 9: Two-dimensional binding mode of 2,3-dehydrosilybin within the active site of CA2 receptor.

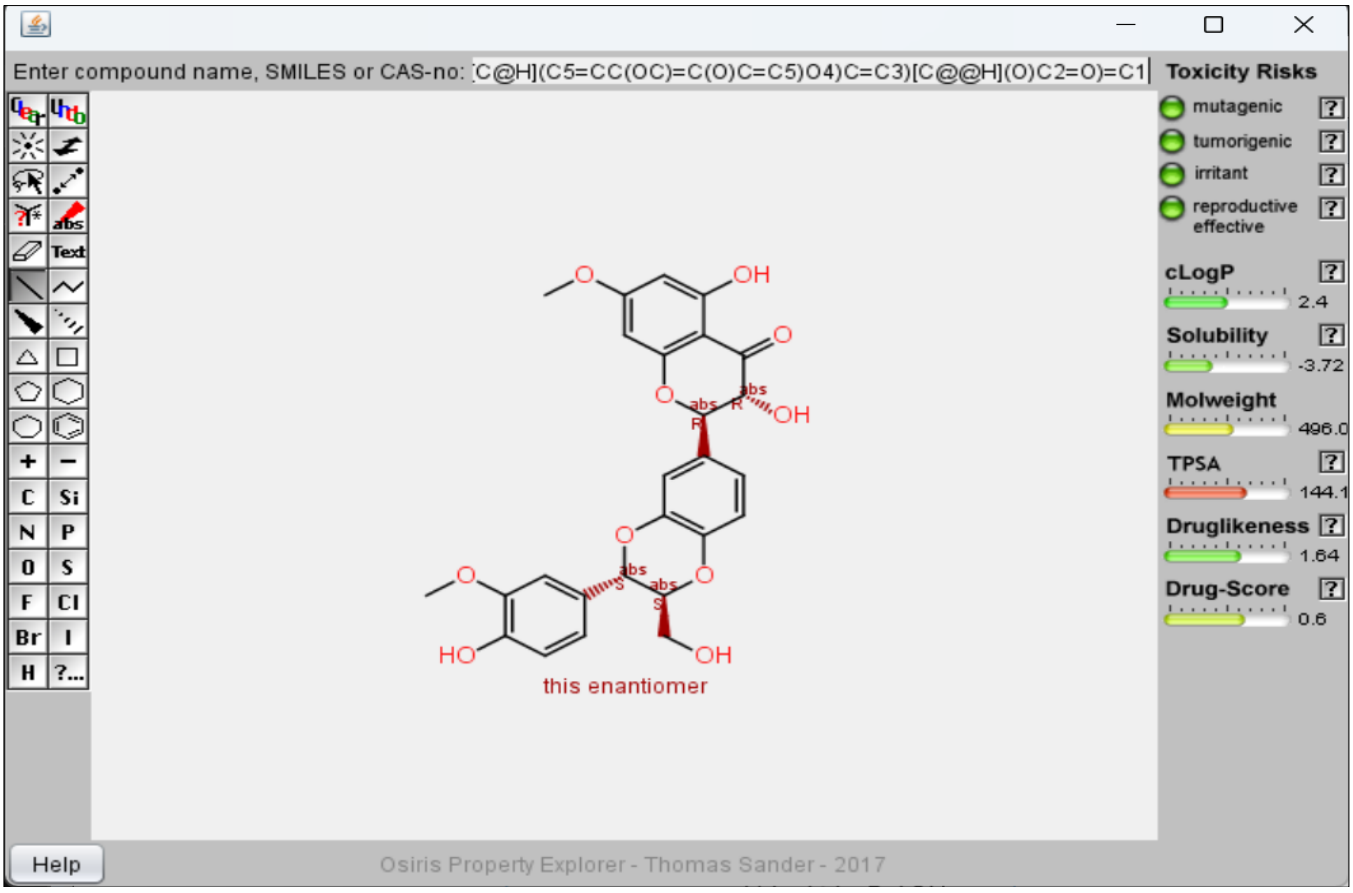


Fig 10: Pharmacokinetic and toxicity profiling of 7-o-Methyl silybin.

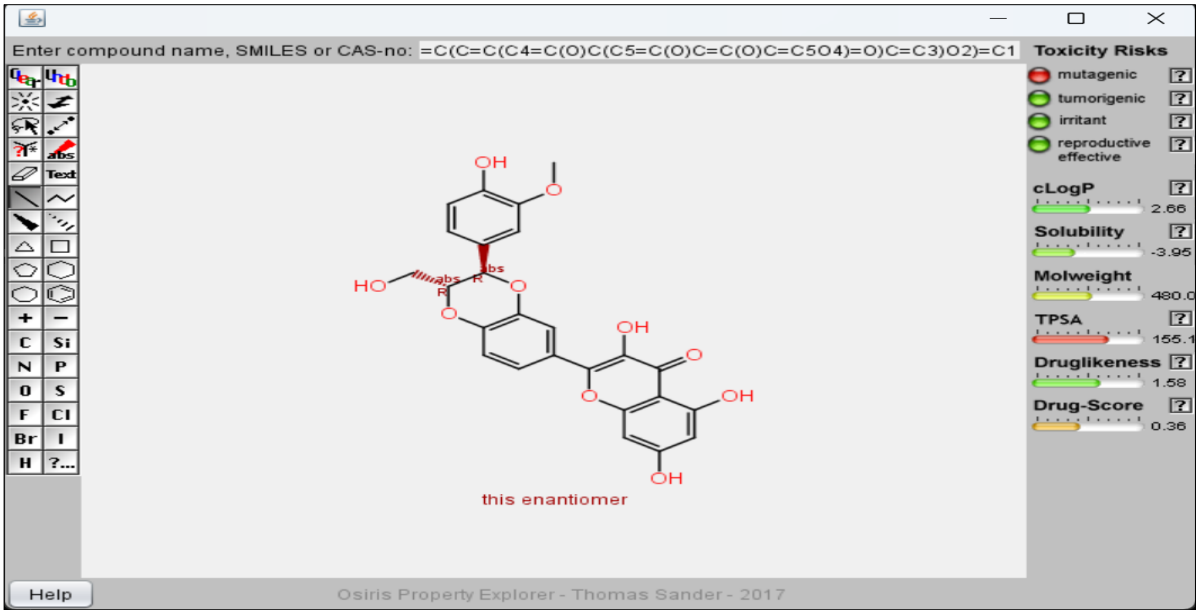


Fig 11: Pharmacokinetic and toxicity profiling of 2,3-dehydrosilybin.

Table 3: Pharmacokinetic Profiling of lead molecules

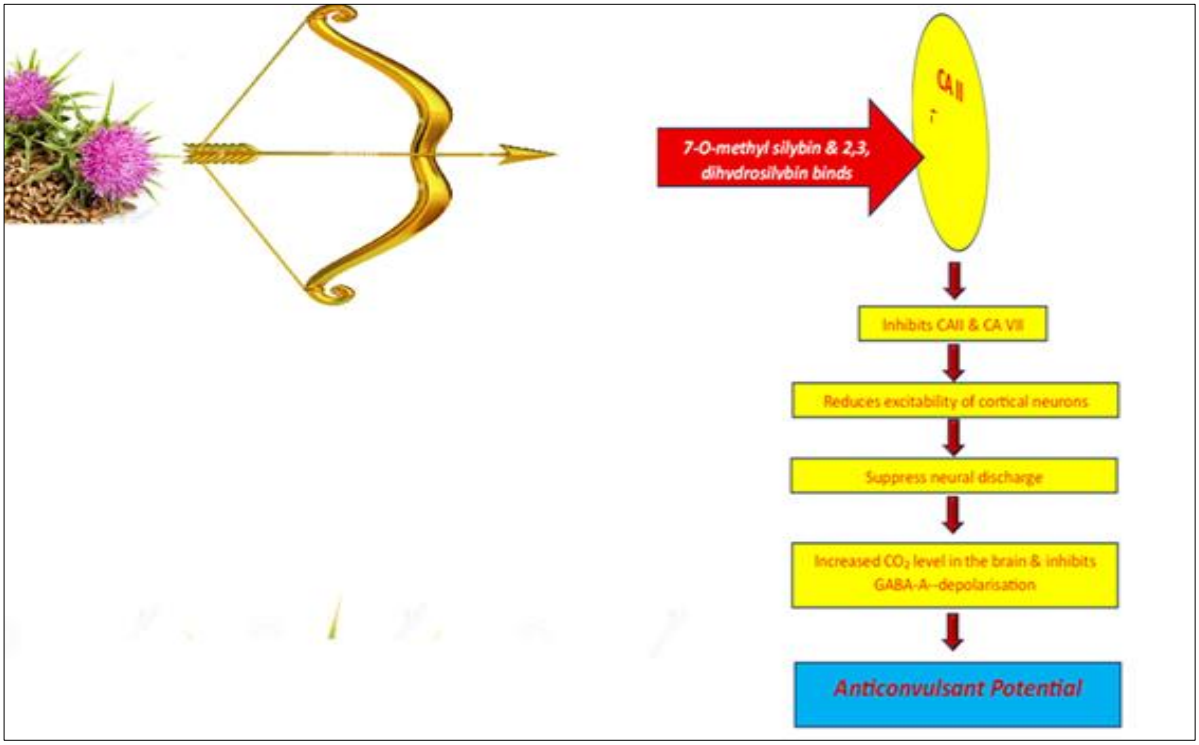
Compound	ADMET			
	Mutagenic	**Tumorigenic**	**Irritant**	**Reproductive effectivity**
Methyl silybin	NO	NO	NO	NO
2,3-dehydrosilybin	NO	NO	NO	NO

Table 4: Lipinski Properties of lead molecules

Compound	*cLogP*	Solubility	Mol.wt.	TPSA	Drug likeness	Drug score
Methyl silybin	2.4	-3.72	496	144.1	1.64	0.6
2,3 dehydrosilybin	2.66	-3.95	480	155.1	1.59	0.36

Table 5: Drug likeness of lead molecules

Compound	Lipinski rule of five	H bond donar (<5)	H bond acceptor (<10)
Methyl silybin	Yes	4	10
2,3-dehydrosilybin	Yes	5	10



Finding Remark

S. marianum contained the richest concentration of flavonoids, prompting an investigation into the suggested mechanism against convulsant by *in-silico* molecular docking.

The results of the current inquiry demonstrated that the selected lead compounds (7-o-methyl-silybin and 2,3-dihydrosilybin) exhibited inhibitory potential against CAII and CA VII and displayed anticonvulsant action.

Divulgence of Investigation

The finding mechanism of action of the chosen modified flavonolignans is presented as follows

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