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ELLAGITANNIN EXTRACTED FROM PLANT *EUPHORBIA PROSTRATA* CLAIM MEMORY ENHANCING ACTIVITY

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ABSTRACT: Human memory can store and recall previously learned information to be applied for the routine purpose. Memory disorders caused by diseases can have an impact on an individual's quality of life as well as their overall cognitive abilities. Memory disorders are associated with the alteration in cholinergic neurotransmission. The herbal extract of *Euphorbia prostrata* was found to be used traditionally for the maintenance of memory-related disorders, but the active constituent and its mechanism were still unrevealed. Thus, in the current study, a ligand library was prepared with some potential lead molecules from the plant *Euphorbia prostrata* and was computationally screened to identify the most potent ligand responsible for the memory enhancement effect as establishing the probable mechanism of action involved in it. Ellagitannin was found to be the most potent ingredient of the Euphorbia prostrata plant, which is supposed to have a memory enhancement effect. Ellagitannin is supposed to exert its therapeutic effect via an agonistic effect on the muscarinic receptor, muscarinic acetylcholine G-protein coupled receptor, N-methyl-D-aspartate receptor, as well as antagonizing acetylcholinesterase enzyme.

INTRODUCTION: Alterations in the normal physiological process leading to the management and maintenance of memory-related functions may cause dementia leading to mental issues like amnesic syndrome and Korsakoff syndrome [1-2]. Dementia is a group of symptomatic observations associated with cognitive deterioration causing partial or complete loss of memory.

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Chronic dementia is associated with more serious neuronal issues like *amnesic syndrome* characterized by an altered neuronal state affecting the process related to memory and learning. When symptoms similar to the *amnesic syndrome* were observed in a patient because of the nutritional deficiency of thiamine is called as *Korsakoff syndrome* [3-4].

Neuropathological alterations responsible for disturbing the neural network connecting diverse parts of the brain may be the main reason for memory dysfunction. Pathophysiological changes in various neurological diseases like epilepsy, Parkinson's, stroke, *etc.*, are also associated with

memory-related issues leading to mental retardness [5-6]. The loss of memory is commonly observed in the elderly aged population claiming the association of loss of memory with aging [7]. The neurological problems associated with anxiety, like panic disorder, obstructive compulsive disorder, social phobia, generalized anxiety disorder, *etc*. among healthy adult individuals is also found to have memory-related issues [8-9]. So, we can conclude that there are diverse reasons for causing memory-related dysfunctions that must be addressed to control the issue.

A wide range of traditional plants has been used for ages for the maintenance and management of mental health. The plants of *Euphorbia* species are commonly used plant for the management of mental health. *Euphorbia prostrata* is a very popular plant and the herbal extract of this plant is reported to have anti-hemorrhoidal and antioxidant properties [10].

Various herbal or synthetic molecules possessing antioxidant activity are also reported for the management of diverse neurobiological disorders leading to mental retardness. Having very good antioxidant property, the Euphorbia prostrata is also supposed to be useful in treating memory-related dysfunctions. The human brain is considered as one of the most complex biological systems of the body. Despite tremendous scientific advancements, we're still unable to resolve the functioning of the human brain as a whole.

Various neurological drugs and herbal extracts have very good pharmacological activity and are used clinically, but their exact mechanism of action is still not completely resolved. Therefore, there is an urgent need to resolve the mechanism of action of the drugs and the herbal extracts having clinical applications. Molecular docking simulation is a computational technique used to predict the strength of association between the ligand and a specific macromolecular target at the molecular level. The docking analysis can be highly useful for establishing the most probable mechanism of action of any drug or lead compound with an unknown mechanism of action [11-12]. Based on our hypothesis, a study focused on the unexplored anti-Alzheimer activity of *Euphorbia prostrata* through computational technique.

MATERIAL AND METHODS:

Design of Ligand Library: A ligand library of 15 herbal leads from *Euphorbia prostrata* plant was prepared by exploring the literature from various sources. The *E. prostrate* plant has been reported for the presence of Alkaloids, Terpenoids, Saponins, Tannins, Steroids and Glycosides, Carbohydrates, Monosaccharide's, combined reducing sugars, and soluble starch [13-16]. Thus, 15 ligands of the plant belonging to the diverse chemical classes were included in the ligand library with the intent to identify the most prominent lead molecule responsible for the generation of memory-enhancing effect in humans as well as establishing the most probable mechanism of action involved in the memory enhancing activity of that particular active constituent of the plant *E. prostrate* [17-18].

Target Identification: The macromolecular target molecules involved in pathophysiological management and maintenance of memory in humans were explored through the available literature. It has been observed that certain macromolecular targets were actively involved in the physiological maintenance as well as pathophysiological deterioration of the human memory during certain neurological diseased conditions like Alzheimer's, Parkinson's syndrome, epilepsy, *etc*. The existing pharmacological data confirm the involvement of both nicotinic and muscarinic acetylcholine receptors in enhancing human memory. Therefore we can target the acetylcholinesterase enzyme responsible for acetylcholine's metabolic termination. By targeting the acetylcholinesterase enzyme, the systemic concentration of acetylcholine can be elevated, which can further enhance memory *via* the concerned receptors [19-21].

Cholinergic stimulation within the prefrontal cortex is associated with human memory and the impairment of muscarinic receptors is responsible for the mental retardation [22-23]. Muscarinic m1 receptors are the postsynaptic cholinergic fibers, and the muscarinic m2 receptors are presynaptic cholinergic receptors mainly distributed in the cerebral cortex region and involved in the excitatory neurotransmission associated with the generation of memory [24-25].

Muscarinic receptors are G protein-coupled receptors (GPCR) which stimulates in the presence of acetylcholine and have a crucial role in neurotransmissions [25]. The regulation of cholinergic neurotransmission *via* muscarinic acetylcholine receptors was found to be concerned with elevated intellect due to enhanced learning and memory. Their termination may lead to various neuronal diseases like Alzheimer's [26-27]. The N-methyl-D-aspartate (NMDA) receptor is the main synaptic plasticity and memory, function regulator. Therefore, the regulatory control of the central synapses by the NMDA receptor was supposed to be a key therapeutic target for the treatment of memory-related disorders [28]. Overall, acetylcholine plays a key neurotransmitter related to maintaining and managing memory-related biochemical processes in the human body. Its elevated systemic concentration may enhance human memory.

Molecular Docking Studies: The macromolecular drug targets which were having active involvement in the maintenance or enhancement of human memory were shortlisted to proceed further with molecular docking studies [29-31]. The three-dimensional structural models of all the shortlisted macromolecular drug targets were procured from protein databank and prepared for molecular docking simulation studies [32-36]. The complexed ligand was separated from the downloaded macromolecular and both the nascent target protein as well as the separated ligand was saved in default Autodock format to proceed further with their redocking for validating the utilized docking parameters. After successful validation of the docking protocol for each of the drug target, the similar parameters were further utilized for computational screening of the ligand library against each of the macromolecular targets used in the current study [37-41].

RESULTS:

Design of Ligand Library: Based upon the available literature ligands like aesculetin, apigenin, apigenin-7-glucoside, astragalin, β-sitosterol, daucosterol, ellagitannin, gallotannin, ingenol-3-angelate, luteolin, luteolin-7-glucoside, quercetin, scopoletin, and vanillic acid were shortlisted for generating a ligand library. The two-dimensional structure of these ligands was generated by obtaining isomeric SMILES from PubChem and converting them into two-dimensional structures using ChemDraw8.0. These two-dimensional structures of all the shortlisted ligands were utilized to generate their three-dimensional structure, followed by the energy minimization process.

Target Identification: Acetylcholinesterase is a metabolic enzyme responsible for acetylcholine's metabolic degradation. Its systemic inhibition may lead to elevated acetylcholine concentration leading to a pronounced cholinergic effect in the human body. The three-dimensional structure model of acetylcholinesterase for executing the docking studies was procured from protein databank (pdb id: 2HA2). M1 and M2 isoforms of muscarinic receptor profoundly impact cholinergic transmission in the human body.

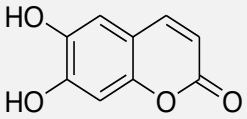
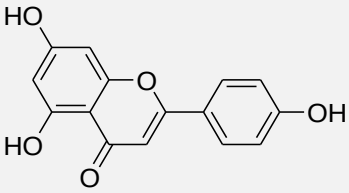
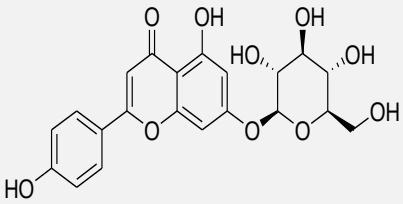
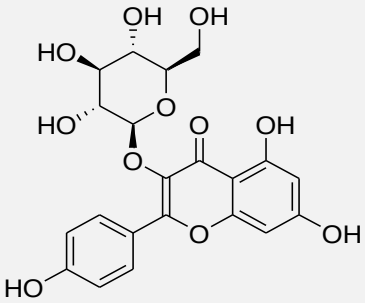
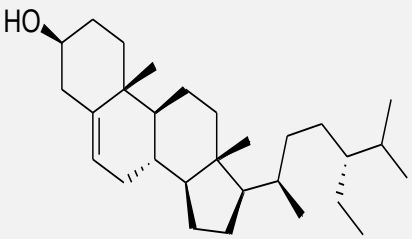
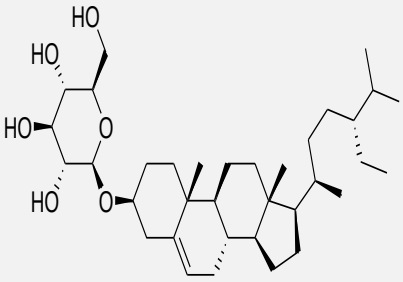
The agonistic effect on both M1 and M2 receptors may lead to increase cholinergic neurotransmission which is supposed to be associated with the enhanced memory in humans. The three-dimensional structure model of M1 and M2 isoforms of muscarinic receptors for executing the docking studies was procured from the protein databank (pdb id: 6OIK). The muscarinic receptor is complexed with G-Protein Coupled Receptors (GPCR) which plays a crucial role in the process of cholinergic neurotransmission. Therefore, the agonistic effect of muscarinic receptor GPCR complex may result in smooth and fast transmission of cholinergic neurotransmission. The three-dimensional structure model of M1 isoforms of muscarinic receptor complexed with GPCR for executing the docking studies was procured from the protein databank (pdb id: 6OIJ).

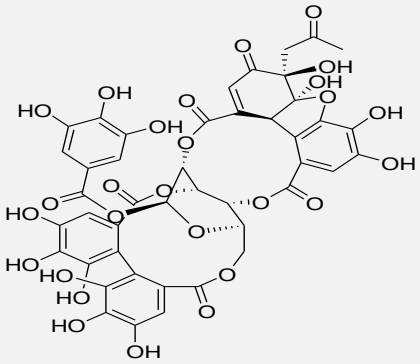
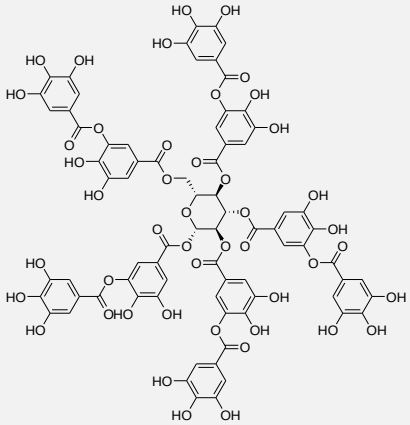
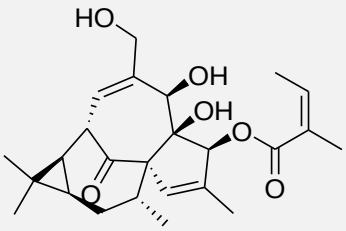
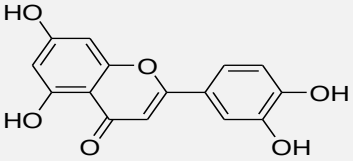
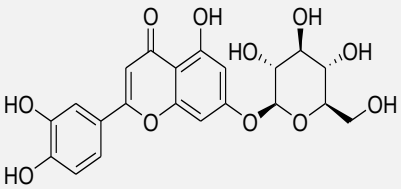
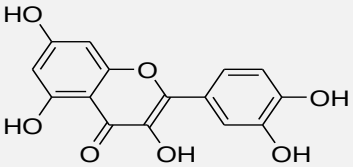
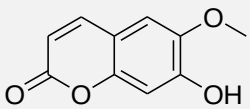
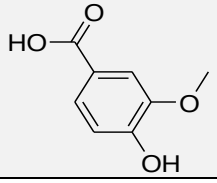
The regulation of the synaptic plasticity by NMDA receptor leads to enhanced memory functions. Thus, the agonistic effect on NMDA receptor is supposed to enhance synaptic plasticity leading to the enhanced memory in humans. The three-dimensional structure model of NMDA receptor for executing the docking studies was procured from the protein databank (pdb id: 7EOT).

Molecular Docking Studies: The three-dimensional structural model of all the shortlisted macromolecular targets were redocked against the complexed reference ligand leading to the

validation of the utilized docking protocol [42-44]. After successful validation the prepared molecular ligand library was computationally screened against each of the shortlisted macromolecular targets involved in the maintenance and management of memory related functions in the humans. After the ligand library's virtual screening is completed, the best lead molecule is selected based on the minimum binding energy within the predefined range of -5 to -15 kcal/mole. The binding score of each ligand library against each macromolecular target is tabulated in **Table 1.**

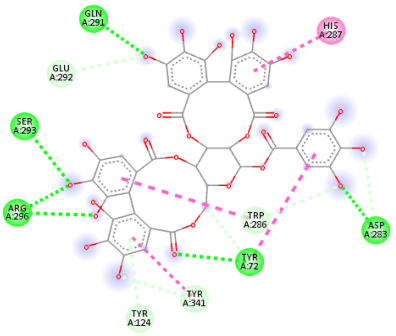
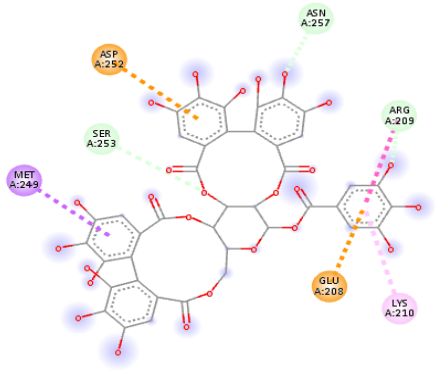
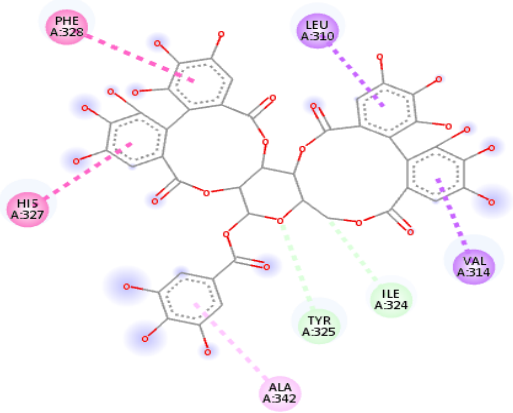
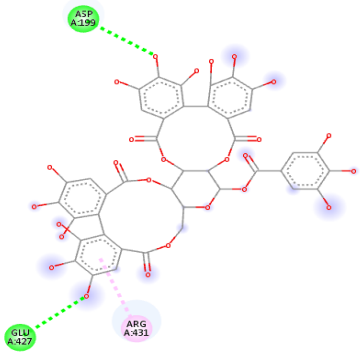
TABLE 1: BINDING SCORE OBTAINED FOR EACH OF THE LIGANDS OF THE DESIGNED LIGAND LIBRARY AGAINST EACH OF THE SHORTLISTED MACROMOLECULAR TARGETS INVOLVED IN THE MAINTENANCE AND MANAGEMENT OF MEMORY-RELATED FUNCTION

S. no.	Compounds	Structure	Docking Score			
			AChE	Muscarinic	Muscarinic acetylcholine receptor	NMDA
			(2HA2)	(6OIK)	(6OIJ)	(7EOT)
1	Aesculetin		-7.3	-5.2	-5.4	-5.9
2	Apigenin		-9.4	-6.2	-6.3	-7.5
3	Apigenin-7-glucoside		-10.1	-7.0	-7.0	-8.7
4	Astragalin		-8.8	-6.2	-6.8	-8.4
5	β-Sitosterol		-10.3	-6.8	-6.8	-8.5
6	Daucosterol		-10.6	-7.2	-7.5	-8.6

7	Ellagitannin		-11.8	-9.2	-10.2	-11.4
8	Gallotannin		-9.3	-7.4	-7.4	-9.6
9	Ingenol-3-angelate		-8.2	-6.2	-6.8	-8.4
10	Luteolin		-9.4	-6.3	-6.4	-7.7
11	Luteolin-7-glucoside		-9.6	-7.2	-6.0	-8.5
12	Quercetin		-8.8	-6.2	-6.1	-7.8
13	Scopoletin		-7.3	-5.3	-5.2	-5.8
14	Vanillic acid		-6.1	-4.5	-4.2	-4.9

Analyzing the docking score obtained after the computational screening of the designed library clearly shows that ellagitannin shows the best binding affinity against all the macromolecular targets used in the current study. The detailed analysis of the obtained results for ellagitannin after docking-based computational screening is tabulated in **Table 2.**

TABLE 2: DOCKING SCORE OBTAINED FOR EACH TARGET RECEPTOR AND 2D-STRUCTURE OF LIGAND AND RECEPTOR INTERACTION

S. no.	Target Receptor	Docking score (Standard)	Docking score (Ellagitannin)	2D-structure
1	AChE (2HA2)	-5.5	-11.8	
2	Muscarinic receptors (M1, M2) (6OIK)	-4.1	-9.7	
3	Muscarinic Acetylcholine receptor (6OIJ)	-3.8	-10.3	
4	NMDA Receptor (7EOT)	-4.7	-11.4	

DISCUSSION: The maintenance and management of the memory-related functions were supposed to be controlled through cholinergic neuro-transmission. In various neurological disorders like Alzheimer, epilepsy, Parkinson's, *etc*., the cholinergic neurotransmission is impacted, leading to impaired memory functions. So, it has been supposed that depression in the cholinergic neurotransmission is responsible for the retardation of memory-related functions.

Cholinergic neurotransmission in humans is regulated through various macromolecular enzymes and biomolecular receptors. Some of the important biomolecules regulating cholinergic transmission are muscarinic M1 and M2 receptors, muscarinic acetylcholine GPCR receptors, and NMDA receptors. Agonistic cum synergistic impact on these receptors influences the impact of cholinergic transmission *via* acetylcholine at an elevated rate. Conversely, the AChE is a metabolic enzyme responsible for the metabolic degradation of acetylcholine leading to the depression of cholinergic transmission. The antagonistic impact on the AChE enzyme will inhibit the acetylcholine's metabolic degradation, resulting in its elevated systemic concentration.

Euphorbia prostrata is a traditional plant that has been used for managing various neurological diseases in humans and maintaining memory-related functions. The herbal extract of *Euphorbia prostrata* has already been reported to maintain memory-related functions in neurologically impaired patients and memory-enhancing activity in normal individuals. But the exact mechanism of action for this pharmacological action by the herbal extract of the *Euphorbia prostrata* plant was not completely known.

The herbal extract of *Euphorbia prostrata* is supposed to improve memory by modulating cholinergic neurotransmission. Therefore, in the current research a ligand library containing the potential leads from *Euphorbia prostrata* plant was developed for computational screening against various biomolecular drug targets which are actively involved in the cholinergic neurotransmission with the intent to identify the most potential molecule in the extract based upon their interaction against the used drug targets. Also, it has been tried to establish the most probable mechanism of action involved in the generation of memory-enhancing effect of the *Euphorbia prostrata* extract.

CONCLUSION: Human memory-related functions were greatly regulated through cholinergic neurotransmission. Cholinergic neurotransmission is greatly affected by neurological as well as neurodegenerative disorders like Alzheimer's, Parkinson's, epilepsy, etc. leading to impaired memory functioning. The elevated systemic concentration of acetylcholine, agonistic impact on the cholinergic receptors, and the inhibition of the AChE are supposed to enhance memory-related functioning in the normal individual and management of memory in neurologically impaired patients. In the current research, we have tried to identify the most potent active ingredient of the plant *Euphorbia prostrata* responsible of generating memory enhancement and the most probable mechanism of action for the same. It has been concluded based on molecular docking analysis that ellagitannin is the most active ingredient present in the *Euphorbia prostrata* plant that is responsible for the memory enhancement effect and its is supposed to exert its therapeutic effect *via* agonistic effect onmuscarinic receptor, muscarinic acetylcholine GPCR, NMDA receptor as well as antagonizing AChE enzyme.

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