ORIGINAL ARTICLE

In vitro anti-Alzheimer's potential of *Pennisetum purpureum*: Phytochemical profiling, AChE inhibition, and molecular docking analysis

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Abstract

Aim and Objectives: The present study aimed to evaluate the phytochemical composition and *in vitro* anti-Alzheimer's potential of the Hydroalcoholic extract of *Pennisetum purpureum* (HAEPP), focusing on its acetylcholinesterase (AChE) inhibitory activity and identification of key bioactive compounds for possible therapeutic development. **Materials and Methods:** HAEPP was subjected to preliminary phytochemical screening to identify major secondary metabolites. *In vitro* AChE inhibition was assessed using Ellman's colorimetric method, and the IC_{50} value was determined. Gas chromatography-mass spectrometry (GC-MS) was employed to analyze the chemical constituents of the extract. Molecular docking studies were conducted to evaluate the binding interaction between glaucine (identified from GC-MS) and AChE. **Results and Discussion**: Phytochemical screening of the HAEPP confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. HAEPP showed dose-dependent AChE inhibitory activity with an IC_{50} of 32.35 µg/mL, compared to Donepezil (12.43 µg/mL). GC-MS analysis identified 22 bioactive compounds, with glaucine as a major alkaloid. Molecular docking revealed that glaucine binds effectively to AChE (binding energy: −9.08 kcal/mol; Ki: 220.04 nM), interacting with key residues, such as TRP86, TYR337, SER293, and ASP74. These findings highlight the neuroprotective potential of HAEPP, with glaucine emerging as a promising candidate for Alzheimer's therapy.

Key words: AchE inhibition, alzheimer's disease, gas chromatography-mass spectrometry, molecular docking, *Pennisetum purpureum*

INTRODUCTION

Dementia is a broad clinical term that describes a significant decline in cognitive abilities severe enough to interfere with a person's daily life and functional independence.[1] It is not considered a normal aspect of aging but rather results from damage to brain cells, impairing their communication and affecting memory, thinking, behavior, and overall cognitive function[2] Among the various types of dementia, Alzheimer's disease (AD) is the most prevalent, accounting for approximately 60–80% of cases globally. According to the World Health Organization, more than 55 million people are currently living with dementia worldwide, and this number is projected to rise to 78 million by 2030 and 139 million by 2050 due to the aging population.[3] AD is thus recognized as the leading cause of dementia, contributing significantly to the global health burden. It is characterized as a chronic, irreversible, and progressive neurodegenerative (ND) disorder.[4] Pathological hallmarks of AD include the accumulation of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. While these features receive significant attention, studies suggest that the loss of neuronal synapses correlates more directly with the degree of cognitive decline observed in patients.[5,6]

Acetylcholine (ACh), a key neurotransmitter involved in learning, memory, and attention, is markedly reduced in AD, supporting the "cholinergic hypothesis" of the disease.

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This hypothesis posits that the dysfunction of the cholinergic system, due to neuronal loss and decreased ACh levels, plays a pivotal role in the cognitive symptoms of AD.[7] Both acetylcholinesterase (AChE) and butyrylcholinesterase are enzymes involved in the hydrolysis of ACh, and their overactivity is associated with AD pathology. Currently, AChE inhibitors, such as donepezil, galantamine, and rivastigmine, as well as the NMDA receptor antagonist memantine, are approved for AD management.[8,9] These drugs primarily provide symptomatic relief by enhancing cholinergic transmission or modulating glutamatergic activity. However, their efficacy is limited, and they are often associated with adverse effects, particularly gastrointestinal disturbances. Therefore, there is a pressing need to identify safer and more effective therapeutic agents that can slow or halt disease progression.[6] Oxidative stress and neuroinflammation are central to the pathogenesis of AD, contributing to Aβ aggregation, tau phosphorylation, synaptic dysfunction, and neuronal death.[10] These processes disrupt the balance between Aβ production and clearance, exacerbating neurodegeneration and cognitive decline.

In recent years, there has been growing interest in medicinal plants and herbal remedies as potential sources of neuroprotective agents. Numerous studies have documented the anti-Alzheimer potential of plants, such as Curcuma longa, *Ginkgo biloba*, *Withania somnifera*, *Convolvulus pluricaulis*, *Tinospora cordifolia*, *Allium sativum Linn*, and *Azadirachta indica*, attributed largely to their antioxidant, anti-inflammatory, and cholinesterase-inhibitory properties.[11,12] *Pennisetum purpureum*, commonly known as Napier grass, elephant grass, or Uganda grass, is a tall perennial plant of the Poaceae family native to tropical and subtropical Africa. Traditionally, its extracts have been used in folk medicine for the treatment of various conditions, including fever, wounds, rheumatism, and as a diuretic.[13] Phytochemical studies reveal that *P. purpureum* is rich in flavonoids, alkaloids, and phenolic compounds, which are known for their antioxidant and neuroprotective activities. Pharmacological evaluations have demonstrated that *P. purpureum* possesses anti-oxidant, anti-inflammatory, anti-diabetic, anti-hypertensive, antimicrobial, and cytotoxic properties.[14] Despite these promising attributes, no scientific reports have been published to date validating its efficacy against AD. Hence, the present study was undertaken to evaluate the *in vitro* anti-Alzheimer activity of *P. purpureum*, with a particular focus on its cholinesterase-inhibitory potential. In addition, molecular docking studies were conducted to investigate the binding interactions between key phytoconstituents of *P. purpureum* and Alzheimer's-related targets, aiming to elucidate the possible mechanisms underlying its neuroprotective effects.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Acetylthiocholine iodide (ATChI), Ellman's reagent (DTNB), Tris-HCl buffer, and AChE (AChE, 0.02 U/mL) were procured from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India.

Plant Collection, Authentication, and Extraction

Whole plants of *P. purpureum* were collected in December and authenticated by Dr. M.U. Sharief, Director, Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2022/Tech/641). The plant material was shade-dried, coarsely powdered, and stored in an airtight container. Extraction was performed using a Soxhlet apparatus with a Hydroalcoholic solvent system (ethanol: Water, 50:50 v/v). The extract was filtered and concentrated by evaporation, and the dried Hydroalcoholic extract of *P. purpureum* (HAEPP) was used for further studies.

Phytochemical Screening

Preliminary qualitative screening for major secondary metabolites, such as alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and carbohydrates was performed using standard procedures.[15]

AChE Inhibitory Assay

The AChE inhibitory activity was assessed using a modified Ellman's colorimetric method.[16] The reaction mixture consisted of 50 µL of 50 mM Tris-HCl buffer (pH 8.0), 50 µL of AChE (0.02 U/mL), and 40 µL of test extract at various concentrations, incubated for 30 min at 4°C. Subsequently, 30 µL of DTNB (10 mM) and 30 µL of ATChI (12 mM) were added to initiate the reaction. Absorbance was recorded at 412 nm for 10 min at 25°C using a microplate reader. Donepezil was used as a standard. The percentage inhibition was calculated using the formula: % Inhibition = [(OD_control – OD_sample)/OD_control] × 100. All experiments were performed in triplicate.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of HAEPP was carried out using a Shimadzu GCMS-QP 2020 system equipped with an EI source at 70 eV and a capillary column (VF-5ms, 30 m × 0.25 mm × 0.25 µm).[17] Helium (99.99%) served as the carrier gas at a constant flow rate of 1 mL/min. The injection temperature was 280°C, and the oven program ranged from 60°C to 280°C. The sample was injected with a 10:1 split ratio, and the scan range was 45–1000 m/z.

Molecular Docking Analysis

Preparation of protein structure

The crystal structure of AChE (PDB ID: As per relevant database) was retrieved from the RCSB Protein Data Bank.

The protein structure was prepared by removing water molecules and ligands using PyMOL and AutoDock Tools.[18]

Ligand preparation

Phytoconstituents identified through GC-MS were drawn and optimized using UCSF Chimera and saved in PDB format for docking.

Docking protocol

Molecular docking was performed using iGEMDOCK (v2.1) to assess interactions between AChE and phytoconstituents from HAEPP.[18] Docking was executed on a Windows 10 system (Intel Core i5, 2.5 GHz). Binding affinities and interactions were analysed, and docked complexes were visualized using BIOVIA Discovery Studio Visualizer.

RESULTS

Phytochemical Screening

The HAEPP appeared as a dark greenish-brown semi-solid with a percentage yield of 14.8% w/w. Qualitative phytochemical screening revealed the presence of major bioactive constituents, such as alkaloids, saponins, tannins, flavonoids, phenols, and terpenoids, while carbohydrates were absent. These results are summarized in Table 1.

AChE Inhibition Activity

The AChE inhibition activity was determined by Ellman's colorimetric method. The standard drug donepezil exhibited potent AChE inhibition with an IC_{50} value of 12.43 µM/mL, whereas HAEPP demonstrated moderate inhibitory activity with an IC_{50} of 32.35 µM/mL. The percentage inhibition values at varying concentrations are presented in Table 2 and illustrated in Figure 1.

GC-MS

The GC-MS analysis spectrum of HAEPP [Figure 2] revealed the major phytochemical constituents, identified based on their retention times, molecular formulae, molecular weights, and peak area percentages. The chromatographic profile and compound details are summarized in Table 3.

Table 1: Phytochemical constituents present in the Hydroalcoholic extract of *Pennisetum purpureum*

S. No.	Phytochemical constituents	Result
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Carbohydrates	–
5	Terpenoids	+
6	Alkaloids	+

Molecular Docking Analysis

Molecular docking of the active GC-MS-derived compound *Glaucine* with the human AChE enzyme (PDB ID: 4EY7) showed strong binding affinity. The docking results revealed a binding energy of –9.08 kcal/mol and a predicted inhibition constant (Ki) of 220.04 nM. The interaction was characterized by multiple hydrophobic and hydrophilic contacts [Table 4]. The 3D interaction pattern is shown in Figure 3.

DISCUSSION

AD is characterized by degenerate of neurons in brain, loss of memory, deterioration in cognitive ability and senile plaque, abnormal accumulation of Aβ protein finally leading to dementia.[19] ND disease is the becoming serious disorders in aged people. Oxidative stress is the common cause for ND disorders[20] Brain tissues are highly capable for oxygen consumption, low antioxidant capacity, high iron and polyunsaturated fatty acids,[21] and these tissues are highly sensitive to oxidative stress. Excessive oxidative stress[22] on this brain tissue leads to memory deficits due to impairing hippocampal synaptic plasticity. Around worldwide, ND affects the millions of people. The AD and Parkinson disease are the most common type of ND. Aβ is important protein for memory and cognition present in soluble form in brain CSF, but in AD, Aβ is fibrillated to get insoluble Aβ, leading to nerve cell death, eventually loss of memory and cognition, major symptoms of AD.

The popular remedies are not capable either prevention or treating AD, where there is need for defibrillate Aβ, hence the identification of new compound is essential.[23] There are still enormous research giving on for the treatment of AD, either it can prevent or at least efficiently change the course of disease -also known as 'Disease Modifying Therapies.[24] Medicinal plants are substantial source of phytochemical constituents, and researchers believed that

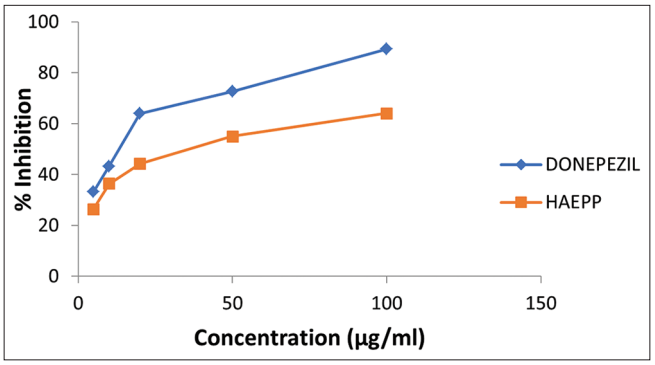


Figure 1: Acetylcholinesterase inhibition activity of the Hydroalcoholic extract of *Pennisetum purpureum*

Table 2: Acetylcholinesterase inhibition activity of HAEPP

Sample	Concentration (µM/mL)	Percentage inhibition (Mean±SD)	IC_{50} (µM/mL)
STD (DONEPEZIL)	5	33.15±0.87	12.43 (µM/mL)
	10	43.30±0.45	
	20	63.96±0.83	
	50	72.73±0.54	
	100	89.24±0.68	
HAEPP	5	26.41±2.05	32.35 (µM/mL)
	10	36.33±1.57	
	20	44.17±1.91	
	50	55.00±1.09	
	100	64.05±0.84	

HAEPP: Hydroalcoholic extract of *Pennisetum purpureum*, SD: Standard deviation

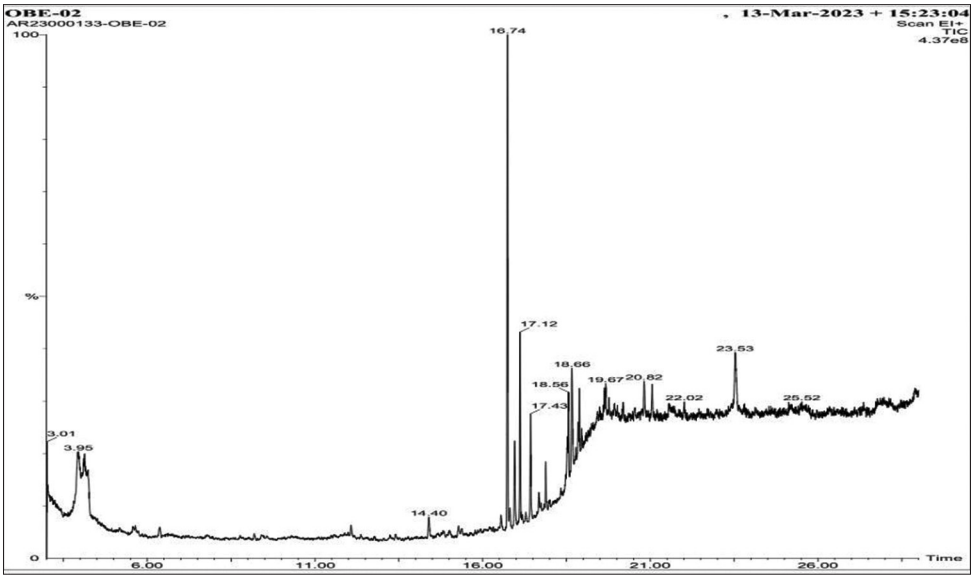


Figure 2: Gas chromatography-mass spectrometry spectrum of the Hydroalcoholic extract of *Pennisetum purpureum*

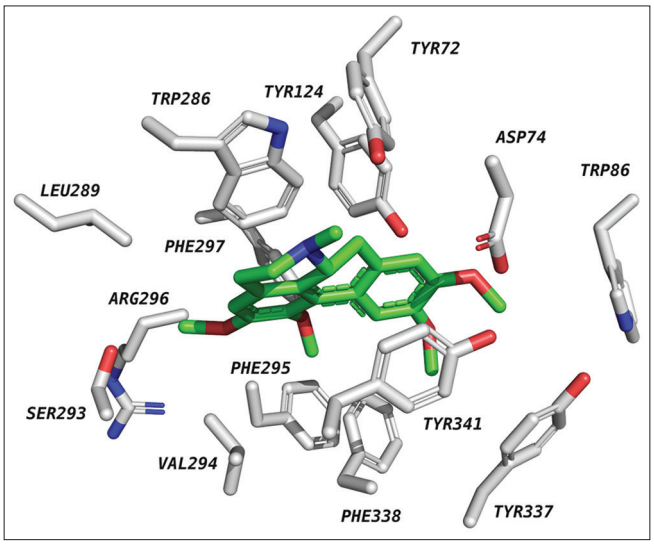


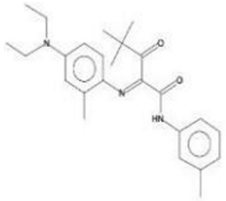
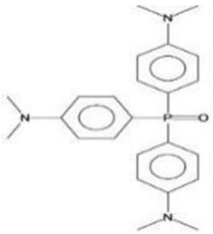
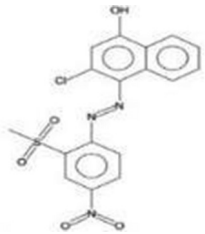
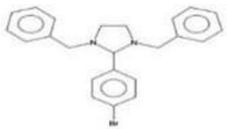
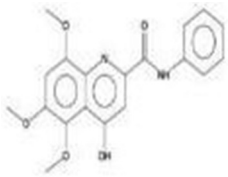
Figure 3: 3D interaction of human acetylcholinesterase with Glaucine

these phytochemicals from medicinal plants were used to treat chronic disease with safe and effective and less side effects.[25] For example: Galantamine is alkaloid obtained from the natural plant (*Galanthus and Narcissus*) used to treat the dementia and AD. The *P. purpureum* belongs to Poaceae family. Hence in the present study an attempt has to made to the anti-Alzheimer's activity of *P. purpureum* by AchE, physiochemical analysis and phytochemicals present in HAEPP which determined by GC-MS analysis.

Preliminary Phytochemical Screening

Phytochemical analysis not only helps to reveal the secondary metabolites present in the medicinal plant extracts, but it also helpful to identify the new bioactive compound,[26] which can be used for synthesis of new molecule. As in our study, the phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins

Table 3: Phytoconstituents identified from Hydroalcoholic extract of *Pennisetum purpureum*

S. No.	Name of the component	Molecular formula	Mol. Wt	RT	Structure	Peak area (%)
1	Pentanamide, 2-[[4-diethylamino)-2-methylphenyl] imino]-4,4-dimethyl-N-(3-methylphenyl)-3-oxo-	$C_{25}H_{33}N_3O_2$	407.25	23.5		20.4
2	Benzenamine, 4,4',4"-phosphinyldynetris[N, N-dimethyl-	$C_{24}H_{30}N_3OP$	407.21	23.5		7.94
3	1-Naphthalenol, 3-chloro-4-[2-[2-(methylsulphonyl)-4-nitrophenyl] diazinyl]-	$C_{17}H_{12}ClN_3O_5S$	405.01	23.5		3.43
4	1,3-Dibenzyl-2-(4-bromophenyl) imidazolidine)	$C_{23}H_{23}BrN_2$	406.10	23.5		2.76
5	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306.25	20.8		4.36
6	4H-Benzopyran-4-one-,2-(3,4-dimethoxyphenyl)-3,5,6,7-tetramethoxy-	$C_{21}H_{22}O_8$	402.13	18.8		2.59

(*Contd...*)

Table 3: (*Continued*)

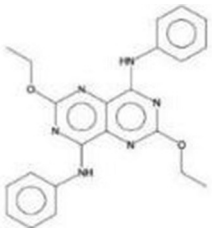
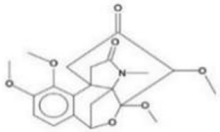
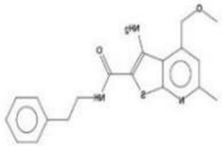
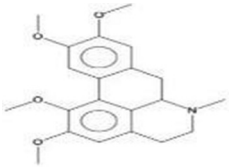
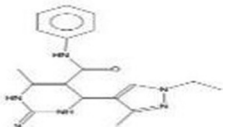
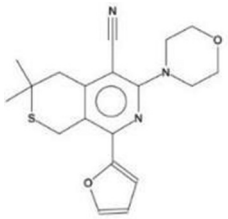
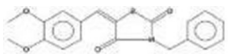
S. No.	Name of the component	Molecular formula	Mol. Wt	RT	Structure	Peak area (%)
7	Pyrimido[5,4-d] pyrimidine, 4,8-dianilino-2,6-diethoxy-	$C_{22}H_{22}N_6O_2$	402.18	18.8		2.59
8	Oxostephamiersine	$C_{21}H_{25}NO_7$	403.16	18.8		2.59
9	Thieno[2,3-b] pyridine-2-carboxylic acid, 3-amino-4-methoxymethyl-6-methyl, phenethylamine	$C_{19}H_{21}N_3O_2S$	355.13	18.5		2.83
10	Glaucine	$C_{21}H_{25}NO_4$	355.17	18.5		2.31
11	4-(1-Ethyl-3-methyl-1H-pyrazol-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid	$C_{18}H_{21}N_5OS$	355.14	18.5		2.01
12	8-Furan-2-yl-3,3-dimethyl-6-morpholin-4-yl-3,4-dihydro-1H-thiopyrano[3,4-c] pyridine-5-carbonitrile	$C_{19}H_{21}N_3O_2S$	355.13	18.5		2.04
13	2,4 (3H,5H)-Thiazoladione, 5-[(3,4-dimethoxyphenyl) methylidene]-3-(phenylmethyl)-	$C_{19}H_{17}NO_4S$	355.08	18.5		1.96

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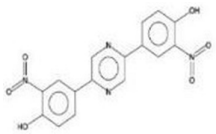
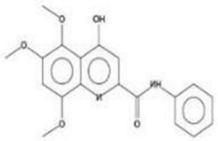
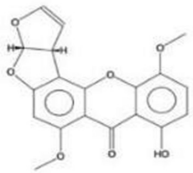
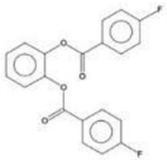
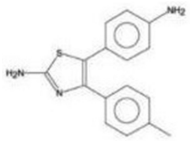
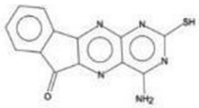
Table 3: (*Continued*)

S. No.	Name of the component	Molecular formula	Mol. Wt	RT	Structure	Peak area (%)
14	Ethyl-14-methylhexadecanoate	$C_{19}H_{38}O_2$	298.26	17.8		10.6
15	2,5-Bis (4-hydroxy-3-nitrophenyl) pyrazine	$C_{16}H_{10}N_4O_6$	354.06	17.4		4.26
16	2-Quinolinecarboxamide, 4-hydroxy-5,6,8-trimethoxy-N-phenyl	$C_{19}H_{18}N_2O_5$	354.12	17.4		4.26
17	7H-Furo[3',2',4,5]furo[2,3-c] xanthen-7-one, 3a, 12c-dihydro-8-hydroxy-6,11-dimethoxy-,(3aR-cis)-	$C_{19}H_{14}O_7$	354.07	17.4		4.26
18	1,2-Benzenediol, O, O'-di (4-fluorobenzoyl)-	$C_{20}H_{12}F_2O_4$	354.07	17.4		4.26
19	Neophytadiene	$C_{20}H_{36}$	278.39	17.1		8.52
20	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	$C_{16}H_{15}N_3S$	281.09	16.9		3.97
21	9-Amino-7-mercapto-5,6,8,10-tetraazabenzo[b] fluoren-11-one	$C_{13}H_7N_5OS$	281.03	16.9		3.10
22	9-Eicosyne	$C_{20}H_{38}$	278.29	16.7		3.82

RT: Retention times

Table 4: Intermolecular interaction of the best docked complex

PDB ID	Binding energy (kcal/mol)	Predicted Ki	Hydrophobic contacts	Hydrophilic contacts
4EY7	–9.08	220.04 nM	TYR72, TRP86, TYR124, TRP286, LEU289, VAL294, PHE297, TYR337, PHE338, TYR341	ASP74, SER293, ARG296

and glycosides. Our results are in good agreement with the previous phytochemical screening of *P. purpureum*.[27,28] The Alkaloids are considered as promising candidate for the treatment of AD, due to their complex nitrogen structure which interact with positively charged AchE binding site.[28] For example: Galantamine and Rivastigmine belong to the alkaloid in which galantamine is a natural alkaloid and rivastigmine is the synthetic alkaloid, used as anticholinesterase inhibitors for treating AD.[29,30] Donepezil and other drugs are synthesized based on the physostigmine structure. Based on the several studies, polyphenols have protective effect against inflammation association with AD, many other chronic diseases, such as metabolic syndrome, diabetes and atherosclerosis. Phenolic acid exerts the neuroprotective and cognition enhancing effects. Flavonoids constitute the most ubiquitously polyphenols group, which have enormous effects on memory and cognition. Flavonoids act by reduce the neuronal inflammation and oxidative stress, thereby it reverses the symptoms of AD. Furthermore, flavonoids enhance the cognitive function. Electro physiologically flavonoids promote long-term potentiation in the hippocampus region and modify the efficacy of synaptic transmission.[31] For example: Quercetin flavonoid present in food and coffee, which enhances learning and memory by neuronal protective from Aβ toxicity.[32] Myricetin, catechins and gossypetin are another natural flavonoid has a role in the inhibition of Aβ aggregation, free radical scavenging property and inhibition of vital enzyme implicated in AD. Terpenoids have been shown to be useful for the treatment of several disease, such as anticancer, antimicrobial, anti-cholinesterase activities due to their diverse pharmaceutical activities.[33] Ginseng and its active metabolites have shown to be useful in the pathogenesis of AD. Ginsenoside is the dammarane-type triterpene isolated from *Panax ginseng*. Ginsenoside Rg3 is effective against AD by promoting Aβ degeneration and enhancing neprilysin gene expression, rate limiting enzyme in Aβ degradation.[34] Saponins consists of an aglycone and a carbohydrate protein in which a glycone part can be a steroid or triterpene. Saponins have diverse biologic activities, including antioxidant, anti- neuroinflammation and neurocognitive benefit. Furthermore, it has potential neuroprotective property against various central nervous system (CNS) disorders, such as stroke, Alzheimer disease, Parkinson disease and Huntington's disease.[35] In the pharma industry, it is used as a precursor for the semi synthetic steroidal compound. Tannin is a type of polyphenol[36,37] (plant polyphenol), it has shown to be strong anticancer, antioxidant/radical scavenging activity, antibacterial and antiviral and also it possesses neuroprotective activity.[38] Glycosides are organic molecules, classified as triterpene glycoside, flavonoid glycoside, iridoid phenyl propanoid glycoside, β sitosterol, anthraquinone glycosides, saponin and kaempferol according to glycoside bond. The various class of glycosides reported possess analgesic,[39] anticancer, cardiac failure, anti-inflammatory,[40] neuroinflammation, Alzheimer activity.[41,42]

AChE Inhibition

The neuropathological theory of AD is Aβ hypothesis, cholinergic hypothesis,[43] oxidative stress hypothesis and inflammatory hypothesis. The over production or decreased clearance of Aβ peptide in brain, leads to amyloid aggregation and develop neuronal degeneration are the significant indicator of AD.[44,45] Moreover Aβ peptide in brain leads to inhibition of cholinergic transmission. Studies shown that decrease the level of Ach neurotransmitter leads to AD. Indicating that, the cholinergic hypothesis is one of the major theories of AD, and AChE is the main therapeutic treatment target for AD. Previous studies have shown that administration of AChE inhibitors leads to improve the cognitive, behavioral symptoms in AD, moreover inhibition of AChE is also considered as promising treatment strategy for CNS disorders, such as dementia, myasthenia gravis and Parkinson's disease. The FDA approved various AChE inhibitors are galantamine, tacrine, donepezil and rivastigmine,[46] it acts by inhibiting breakage of AChE, thereby increase the cholinergic neurotransmission.

Nowadays, the ease availability of more efficacy, lower-price with less side effects of natural plant compared with synthetic drugs, making them to the development of simple, newer, and excellent choices in the treatment of AD. Globally, the discovery of new drugs from plant-derived compounds is still an important strategy finding new biologically active compounds.[47] Natural plant source of AChE inhibitors already proven promising candidate for AD. In our study, various concentration of HAEPP extracts on AChE inhibiting action increased in dose dependent manner. Our results are in agreement with previous reported studies.[48] Donepezil was the standard AChE inhibitor, and IC_{50} value of 12.43 μg/mL while HAEPPshowed an IC_{50} value of 32.35 μg/mL for AChE inhibition. Efficacy of HAEPP extract on enhancing AchE inhibitors may be due to presence of high content of tannins.[49] Our results are agreement to the previous studies, where tannins showed improved learning behavior functions through the AchE inhibiting activities; moreover, tannins exhibit the powerful antioxidant property the AchE inhibiting activity,[18] not only due to tannins but also the presence of natural

AchE inhibitors, such as alkaloid, saponins, and terpenoid, our results are in agreement with previous results.[50] The presence of various phytoconstituent in HAEPP may act on the multi factorial pathogenesis of AD. Thus, HAEPP acts as a promising candidate of AChE inhibition.

GC-MS

The GC-MS analysis of HAEPP showed the presence of 300+ different compounds in it. The most prevailing compounds had been recognized basis of the peak area % the present compounds were like, Pentanamide,2-[[4-diethylamino)-2-methylphenyl]imino]-4,4-dimethyl-N-(3-methylphenyl)-3-oxo- (20.4%), followed by Ethyl-14-methylhexadecanoate (10.6%), Neophytadiene (8.52%), and Benzenamine,4,4',4"-phosphinyldynetris[N,N-dimethyl- (7.94%). Other constituents present in moderate amounts included 2,5-Bis(4-hydroxy-3-nitrophenyl)pyrazine (4.26%), 2-Quinolinecarboxamide, 4-hydroxy-5,6,8-trimethoxy-N-phenyl (4.26%), 7H-Furo[3',2',4,5]furo[2,3-c]xanthen-7-one, 3a,12c-dihydro-8-hydroxy-6,11-dimethoxy-,(3aR-cis)- (4.26%), and 1,2-Benzenediol, O,O'-di(4-fluorobenzoyl)- (4.26%). Minor compounds included 8,11,14-Eicosatrienoic acid (4.36%), 5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine (3.97%), 9-Eicosyne (3.82%), 1-Naphthalenol,3-chloro-4-[2-[2-(methylsulphonyl)-4-nitrophenyl] diazinyl]- (3.43%), and 1,3-Dibenzyl-2-(4-bromophenyl)imidazolidine(2.76%).Several other constituents, such as Thieno[2,3-b]pyridine-2-carboxylic acid, 3-amino-4-methoxymethyl-6-methyl, phenethylamine (2.83%), 4H-Benzopyran-4-one-,2-(3,4-dimethoxyphenyl)-3,5,6,7-tetramethoxy- (2.59%), Pyrimido[5,4-d]pyrimidine,4,8-dianilino-2,6-diethoxy- (2.59%), Oxostephamiersine (2.59%), Glaucine (2.31%), 8-Furan-2-yl-3,3-dimethyl-6-morpholin-4-yl-3,4-dihydro-1H-thiopyrano[3,4-c]pyridine-5-carbonitrile (2.04%),and4-(1-Ethyl-3-methyl-1H-pyrazol-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (2.01%) were detected in trace quantities. The least abundant component was 2,4(3H,5H)-Thiazolidine, 5-[(3,4-dimethoxyphenyl) methylidene]-3-(phenylmethyl)- (1.96%).

The most abundant compound in the extract was Pentanamide,2-[[4-(diethylamino)-2-methylphenyl]imino]-4,4-dimethyl-N-(3-methylphenyl)-3-oxo-, contributing 20.4% of the total peak area, followed by Ethyl-14-methylhexadecanoate (10.6%), Neophytadiene (8.52%), Benzenamine, 4,4',4"-phosphinyldynetris[N,N-dimethyl-] (7.94%), and 8,11,14-Eicosatrienoic acid (4.36%). Several compounds were found in moderate to low quantities, including Glaucine (2.31%), an aporphine alkaloid known for its neuroactive properties.

Molecular Docking

To understand the mechanism of Acetyl cholinesterase inhibition activity of HAEPP molecular docking of active constituents in the extract with AchE was performed. The major component, which is found in the plant glaucine, a major compound characterized in HAEPP by GC-MS analysis, used in molecular docking studies. Notably, Glaucine, an isoquinoline alkaloid with a relative peak area of 2.31%, was of particular interest due to its previously reported neuroactive and anticholinergic effects.[51,52] To explore the therapeutic potential of Glaucine against AD, *in silico* molecular docking was performed targeting AchE. AchE is an enzyme plays a crucial role in degradation process of ACh, a neurotransmitter involved in learning and memory functions, which is responsible in AD pathology.[53] Therefore, AchE is an important target in AD. Hence, the molecular docking with AchE and major compound was investigated. The binding energy represents, estimated free energy change, when ligand binds to the target. In molecular docking, binding energy are used to predict the strength of interaction between the ligand and target. A widely accepted threshold for good binding energy in molecular docking is typically −6 to −10 kcal/mol,[18] more the negative binding energy indicates a favorable binding interaction. In our study, the major compound glaucine binds with AchE with −9.08 kcal/mol indicating that our major compound glaucine is capable to bind target AchE and the negative value suggest a strong and more Table interaction between glaucine and AchE. Nguyen *et al*.,[54] studied that, flavonoids isolated from root bark of *Pinus krempfii* has binding energy of −9.329 kcal/mol with the AChE enzyme, indicating these flavonoids has strong interaction with AchE even at the low and negative energy.[55]

Inhibition constant (Ki) is a measure of how strongly a ligand binds to a target, Ki value represents the concentration required to reduce enzyme activity by 50%, lower Ki values indicating stronger binding and higher inhibitory potency.[56]

A range between 100 and 1,000 nM inhibition constant indicates moderate inhibitor against AchE.[57] Similar studies show that the concentration of genestein derivative's (G1) Ki value 264.24nM, indicating G1 has moderate inhibition toward AchE target enzyme.[58] In our study, glaucine has inhibition constant (Ki) is 220.04nM against AchE, indicates that major compound glaucine has moderate inhibition against AchE.

Hydrophobic interactions are essential for stabilizing ligand-receptor complexes, enhancing binding affinity,[18] and hydrophilic interactions contribute to improved solubility and binding specificity,[51] glaucine interacts with several hydrophobic and hydrophilic residues of target AchE are TYR 72, TRP 86, TYR 124, TRP 286, LEU 289, VAL 294, PHE 297, TYR 337, PHE 338, TYR 341, ASP 74, SER 293 and ARG 296, respectively. Johnson and Moore *et al*., Mahmood *et al*. and Kumar *et al*., reported these hydrophobic and hydrophilic interactions are the key contributors to the determine the stability of the ligand.[59,60]

Glaucine demonstrateds Table binding with AChE through strong hydrophobic and polar interactions, accompanied by

low binding energy and no Table inhibitory potential. These findings suggest its suitability as a promising lead molecule. Our results are consistent with earlier studies reporting glaucine's neuroprotective and cognitive-enhancing effects through CNS modulation.[61]

CONCLUSION

The present findings highlight the potential of *P. purpureum* extract as a source of neuroprotective agents against AD. The presence of key phytochemicals with known antioxidant and neuroprotective properties, along with significant AChE inhibition ($IC_{50:}$ 32.35 µg/mL), supports its cholinergic modulatory role. Among the identified compounds, glaucine showed the highest binding affinity (−9.08 kcal/mol; Ki: 220.04 nM), engaging in strong interactions with active site residues of AChE. These results suggest glaucine as a promising lead compound and validate the traditional use of *P. purpureum* in cognitive disorders. Further *in vivo* and clinical studies are needed to confirm its therapeutic potential in AD.

FUTURE ASPECTS

Glaucine will be isolated from *P. purpureum* extract and formulated into a suiTable dosage form. The formulation will undergo *in vitro* and *in vivo* AChE inhibition studies, and upon successful preclinical results, may progress to clinical trials for potential use in AD treatment.

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