

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer's Disease *Drosophila* Model

Ashwini D.G.¹ and Harini B.P.^{2*}

¹Research Scholar, Department of Zoology, Bangalore University, Bengaluru, Karnataka, India

²Professor, Department of Zoology, Bangalore University, Bengaluru, Karnataka, India

*Corresponding Author

Email Id: dr.harinibp@bub.ernet.in; dr.harinibp@gmail.com

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the degeneration of cholinergic neurons and a consequent reduction in acetylcholine levels within the brain, resulting in cognitive deficits and memory loss. Among the available therapeutic strategies, the inhibition of acetylcholinesterase (AChE) remains one of the most effective approaches for managing AD symptoms. Acetylcholinesterase inhibitors (AChEIs) act by blocking the enzymatic degradation of acetylcholine, thereby enhancing cholinergic neurotransmission and improving cognitive function. The present study aimed to investigate the phytochemical constituents of the 70% ethanolic leaf extract of *Plectranthus amboinicus* through Gas Chromatography–Mass Spectrometry (GC–MS) analysis and to evaluate its *in vitro* and *in vivo* acetylcholinesterase inhibitory potential using Alzheimer's disease model of *Drosophila*. *In vitro* AChE inhibition was determined using Ellman's colorimetric assay, while *in vivo* evaluation was carried out in transgenic *Drosophila* expressing the human amyloid-beta (A β ₄₂) peptide in the nervous system, which mimics the neuropathological features of AD. Flies were supplemented with varying concentrations of the plant extract through their diet, and AChE activity was subsequently quantified in homogenized samples. The findings demonstrated a significant, concentration-dependent inhibition of AChE activity in both *in vitro* and *in vivo* studies. The strong inhibitory and neuroprotective effects observed can be attributed to the presence of bioactive compounds such as flavonoids, terpenoids, and phenolics, known for their antioxidant and cholinergic-modulating properties. The results suggest that the 70% ethanolic leaf extract of *Plectranthus amboinicus* possesses potent acetylcholinesterase inhibitory activity, indicating its promise as a natural therapeutic candidate for the prevention and management of Alzheimer's disease...

Keywords: Acetylcholinesterase inhibitors, Alzheimer's disease, Ellman's colorimetric assay, Gal4/Cyo-UAS-A β ₄₂, GC-MS, *Plectranthus amboinicus*.

How to cite this article: Ashwini D.G. and Harini B.P., GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer's Disease *Drosophila* Model. *Int J Drug Deliv Technol.* 2026;16(18s): 645-652. DOI: 10.25258/ijddt.16.18s.69

Source of support: Nil.

Conflict of interest: Nil.

INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder and one of the leading causes of dementia among the elderly¹. Its key pathological features include the formation of extracellular amyloid plaques composed of insoluble amyloid- β (A β) peptides and intracellular neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau proteins. These abnormal protein aggregates exert neurotoxic effects, leading to neuronal loss and pronounced cerebral atrophy^{2,3}. The etiology of AD is multifactorial, involving mitochondrial dysfunction, oxidative and inflammatory stress, and dysregulation of neurotransmitter systems, particularly the cholinergic pathway⁴. Under normal conditions, acetylcholine is rapidly hydrolysed in the synaptic cleft by

acetylcholinesterase (AChE). Therefore, therapeutic strategies have focused on cholinomimetics and AChE inhibitors that enhance cholinergic transmission by preventing acetylcholine degradation⁵. Currently, four AChE inhibitors donepezil, galantamine, rivastigmine, and tacrine are clinically approved for AD management. These drugs improve cholinergic signaling, with rivastigmine demonstrating comparatively stronger effects^{6,7}. Clinical trials have documented several side effects associated with currently approved acetylcholinesterase inhibitors, including nausea, vomiting, diarrhoea, syncope, and bradycardia⁸. These adverse reactions highlight the pressing need for safer therapeutic alternatives. As a result, increasing attention is being directed toward medicinal plants as potential sources of new anti-cholinesterase agents

with improved tolerability and fewer side effects⁹. Natural products continue to serve as an important source of therapeutic agents, especially due to their rich content of phytochemicals such as flavonoids and polyphenols, which possess antioxidant, anti-inflammatory, and neuroprotective properties. Many medicinal plants exert potential benefits in AD by mitigating oxidative stress and modulating key molecular targets associated with neurodegeneration. The World Health Organization (WHO) acknowledges the therapeutic value of traditional medicine, provided its safety and efficacy are scientifically verified (WHO, 1985). This highlights the need to evaluate ethnomedicinal practices and to isolate and characterize bioactive compounds that may serve as potential drug candidates¹⁰. In recent years, herbal medicines have gained prominence due to their accessibility, therapeutic effectiveness, minimal side effects, and affordability. Nearly 80% of the global population depends on plant-derived remedies for primary healthcare needs¹¹.

Gas Chromatography–Mass Spectrometry (GC-MS) is widely regarded as a powerful analytical tool for detecting and identifying bioactive constituents such as long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino compounds, and nitro compounds. The combined use of gas chromatography and mass spectrometry, supported by specialized detection systems, offers a highly sophisticated approach for the analysis and characterization of diverse chemical compounds¹².

Plectranthus amboinicus, commonly known as Indian borage, belongs to the *Lamiaceae* family and has long been used in traditional Indian medicine¹³. The plant grows widely across tropical regions of Africa, Asia, and Australia. Phytochemical investigations have identified nearly 30 non-volatile and 76 volatile compounds responsible for its broad pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, analgesic, antitumor, antiepileptic, and wound healing properties¹⁴. Traditionally, the plant is used to treat ailments such as asthma, cold, cough, bronchitis, malaria, and epilepsy. Its leaves contain flavonoids like quercetin, luteolin, apigenin, salvigenin, and genkwanin¹⁵. Herbal preparations, such as leaf juice mixed with honey or sugar, are commonly used to alleviate respiratory conditions and digestive disturbances¹⁶.

Drosophila melanogaster serves as a valuable model for studying AD, particularly through genetic manipulation of amyloid-related pathways. Many *Drosophila* AD models rely on the expression of human APP or A β peptides, since endogenous A β sequences and β -secretase are not conserved in flies^{17,18}. Combined expression of human APP (Amyloid precursor protein) and BACE (Beta-site APP-cleaving enzyme) leads to age-dependent neuronal loss, A β accumulation, and decreased survival^{19,20}. However, studies also indicate that certain APP-associated phenotypes may arise independently of A β , involving APP intracellular domains in synaptic and axonal functions^{21,12}. Thus, several models have been developed that directly express human A β 42 in the fly nervous system, enabling focused investigation of amyloid-mediated toxicity^{23–26}. Differences in construct design such as signal peptides,

poly-A sequences, or copy number can influence A β accumulation and the severity of AD-like symptoms²¹.

Considering the therapeutic relevance of plant-derived compounds, the present study aimed to characterize the phytochemical constituents of the 70% ethanolic leaf extract of *Plectranthus amboinicus* using Gas Chromatography-Mass Spectrometry (GC-MS) and to assess its acetylcholinesterase inhibitory potential through both *in vitro* and *in vivo* experimental models.

Materials and methods

Plant collection

The *Plectranthus* leaves were procured from Medicinal and aromatic herbs nursery, Department of Horticulture, GKVK, UAS, Bangalore. The sample was authenticated by Dr. V. Rama Rao, Research Officer at CARI Bangalore (Acc. no.: RRCBI-mus214). They were washed thoroughly under running water and were dried by shade drying technique. Initial weight was approximately 20 kg which was reduced to 1 kg after drying. The dried leaves were stored in an airtight bag at room temperature until further processing.

Preparation of leaf extract

Plectranthus leaves extract was prepared using soxhlation technology. Dried, grinded, and finely powdered plant material is placed inside thimble and tightly closed. The extraction solvent Ethanol of concentration 70%, was poured into the round bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated from the round bottom flask, evaporates, and passes through the condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind.

GC-MS 70% ethanol leaf extract of *Plectranthus*

Separation and identification were performed on a GC-MS. The GC-MS analysis was performed using Shimadzu GC-2010 connected to AOC-20i injector and GCMS-QP2020 gas chromatograph - mass spectrometer. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used and Helium gas was used as the carrier gas at a constant flow rate of 1.20 mL/min. An injection volume of 1 μ L of sample is taken in the analysis with a split ratio of 20:1. The injector and ion source temperature was set at 220 °C. The oven temperature was programmed at an initial temperature of 60 °C to 240 °C as a final temperature at an increasing rate of 3 °C/min. The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST and Wiley library data of the GC-MS system.

Fly stock

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer’s Disease *Drosophila* Model

For the preparation of media, 70g of jaggery is added to 1 litre of boiling water till it completely dissolves, then 80g of corn starch, 15g of yeast and 9g of agar agar are added and mixed without forming lumps. The mixture is boiled. After taking out from heating, 1.25g of TEGO (methyl parahydroxy benzoate) powder mixed in 6.25ml of ethanol is added to the mixture along with 4.4ml of propionic acid. Medium is poured into culture bottles and cooled. Bottles are plugged with cotton wool to avoid contamination. The fly stocks are maintained in the above-mentioned media at 25 +/- 5 OC, 70 - 80% relative humidity and 12-hour light/ 12-hour dark cycle.

UAS-Aβ42/Cyo strains were used to express human amyloid beta42(Aβ42) protein. Elav-Gal4/Cyo driver strains were used for expressing Aβ42 protein in the neuronal cells to generate the AD *Drosophila* model. The generated AD model flies (Elav-Gal4, UAS-Aβ42) were used for *in vivo* experiments. All the flies were cultured on the above-mentioned media, supplemented with *Plectranthus* at concentrations 0.001% (P1), 0.005% (P2), 0.01% (P3) and 0.05% (P4) along with media without supplement (S).

***In vitro* Acetylcholinesterase Inhibition Assay**

Acetylcholinesterase (AChE) inhibition assay was performed based on an improved Ellman method. The reaction mixture composed of enzyme, 100mM acetylcholine chloride (substrate), 0.4mg/mL 5,5 dithiobis (2 nitro benzoic acid) (DTNB), 0.1mM ethylenediaminetetraacetic acid (EDTA) phosphate buffer saline (PBS- pH 7.5) and various concentrations of test samples(100-500µg). The sample was incubated at 37°C for 10 minutes. Thiocholine produced by action of acetylcholinesterase forms yellow color with DTNB. The intensity of color is proportional to enzyme activity which was measured by the absorbance at 412 nm.

Percentage inhibition was calculated using the formula.

$$\text{Percentage inhibition} = \left[\frac{\text{Abs of control} - \text{Abs of sample}}{\text{abs of control}} \right] * 100$$

***In vivo* Acetylcholinesterase Inhibition Assay**

Briefly 50 fly heads were homogenized in 1 mL 0.1M sodium phosphate buffer with pH 8.0. Homogenate was centrifuged at 3000 rpm for 15 mins and homogenate was collected as sample. 100µl of each of the test samples (P1, P2, P3, P4 and S) was added to test tubes. 100µl of 0.05% BSA was taken in a test tube as blank. The reaction mixtures are allowed to equilibrate at 300C for 10 minutes. 100µl of Acetylcholinesterase Membrane-Bound Enzyme Solution (AChE) is added to each of the test tubes. The contents are mixed using a vortex mixer and incubated at 300C for 10 minutes. 200µl of 200mM sodium acetate solution is added to the test tubes and mixed well. The mixtures are incubated at 4°C for 15 minutes and centrifuged for 3 minutes at 3000rpm. The supernatant is taken in fresh tubes and 1450µl of 1mM acetylthiocholine iodide with 0.2 mM 5,5'-dithio-bis (2-nitrobenzoic acid) solution (ATI/DTNB) is

added. The solutions are incubated for 10 minutes at 300C. 50µl of the supernatant is collected into fresh tubes and the reaction mixture is equilibrated at 300C for 10 minutes. 100µl of 0.001% phenylmethylsulfonyl fluoride solution (PMSF) is added to the supernatant and mixed using a vortex mixer for 10 minutes at 300C. The absorbance is measured at 412nm. Acetylcholinesterase activity is calculated using the following equation.

$$\text{Enzyme activity} = \frac{(A_{412\text{nm Test}} - A_{412\text{nm Blank}}) (0.4) (1.6) (df)}{(0.05) (10) (13.6) (0.1)}$$

where,

1.6 = Total volume (in milliliters) of assay in Step 2

0.4 = Total Volume in (in milliliters) of assay in Step 1

df = Dilution factor 0.05 = Volume (in milliliter) of Step 1 used in Step 2

10 = Time of assay (in minutes) as per the Unit Definition

13.6 = Millimolar extinction coefficient³ of DTNB at 412 nm

0.1 = Volume (in milliliter) of enzyme used

2.7 Statistical Analysis

All experiments were carried out in triplicate, and the results are expressed as mean ± SD. Statistical analysis was performed using ANOVA to evaluate significant differences among different groups. Tukey’s Honestly Significant Difference (HSD) test was conducted for post hoc comparison of means with p-value of less than 0.05 considered as statistically significant. Microsoft Excel and Jamovi 2.7.18 statistical software was used to perform statistical calculation and graphical representation.

Results

GC-MS 70% ethanol leaf extract of *Plectranthus*.

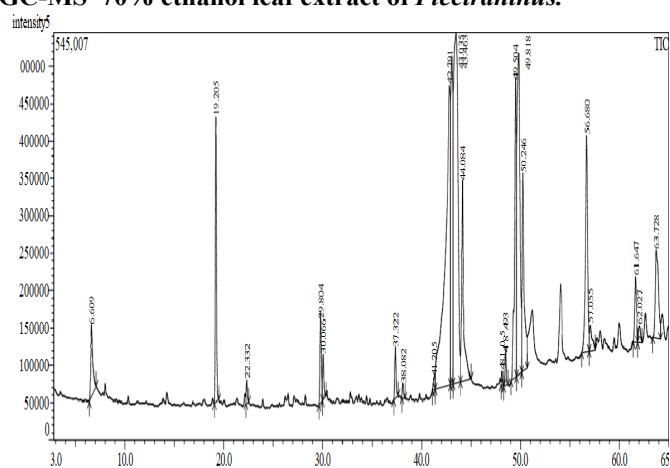


Figure 1: Chromatogram of 70% ethanolic leaf extract of *Plectranthus*.

Table1: GC-MS analysis of 70% ethanolic leaf extract of *Plectranthus*.

Peak #	R. Time	I. Time	F. Time	Area	Area %	Height%	Name
--------	---------	---------	---------	------	--------	---------	------

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer's Disease *Drosophila* Model

1.	6.609	6.405	7.075	1480025	2.01	2.36	Glycerin
2.	19.205	19.035	19.485	2868704	3.89	9.35	Phenol,2-methyl-5-(1- ethylethyl)-
3.	22.332	22.245	22.535	248516	0.34	0.76	2-Allyl-4-methylphenol
4.	29.804	29.615	29.955	1151095	1.56	2.95	p-Cymene-2,5-diol
5.	30.066	29.955	30.325	593442	0.80	1.49	Dodecanoicacid
6.	37.322	37.135	37.575	656355	0.89	1.69	Tetradecanoicacid
7.	38.082	37.935	38.285	193150	0.26	0.51	(S, E)-4-Hydroxy-3,5,5-trimethyl-4-(3- oxobut-1-en-1-yl) cyclohex-2-enon
8.	41.205	41.075	41.355	240723	0.33	0.38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9.	42.791	41.355	42.885	14133882	19.16	9.82	1-(4-Hydroxy-3-methoxyphenyl) tetradec-4-en-3-one
10.	43.035	42.885	43.165	6913300	9.37	10.75	1-(4-Hydroxy-3-methoxyphenyl) tetradec-4-en-3-one
11.	43.463	43.165	43.905	13787997	18.69	11.49	1-(4-Hydroxy-3-methoxyphenyl) tetradec- 4-en-3-one
12.	44.084	43.905	44.985	3782703	5.13	6.55	l-(+)-Ascorbicacid2,6-dihexadecanoate
13.	48.105	48.025	48.285	136623	0.19	0.45	9,12,15- Octadecatrienoicacid, methylester, (Z, Z, Z)-
14.	48.493	48.285	48.745	393208	0.53	1.20	Phytol
15.	49.504	49.025	49.585	5047170	6.84	9.78	Oleic Acid
16.	49.818	49.585	50.105	9265436	12.56	10.52	1-(4-Hydroxy-3- methoxyphenyl) tetradecane-3,5-dione
17.	50.246	50.105	50.665	3786224	5.13	6.46	Octadecanoicacid
18.	56.680	56.185	56.945	4409218	5.98	7.07	Gingerol
19.	57.055	56.945	57.545	616506	0.84	0.83	5-Isopropyl-2-methylphenyl3-methylbutanoate
20.	61.647	61.405	61.845	1035437	1.40	2.14	(9Z,12Z)-3,7-Dimethyloct-6-en-1-yloctadeca-9,12-dienoate
21.	62.027	61.845	62.305	336113	0.46	0.55	Citronellylpalmitoleate

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer’s Disease *Drosophila* Model

22.	63.728	63.385	64.165	2705773	3.67	2.88	(E)-1-(4-Hydroxy-3-methoxyphenyl) hexadec-4-en-3-one
				73781600	100.00	100.00	

(R.Time=Retentiontime,I.Time=Initialtime,F.Time=Final time)

The Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the 70% ethanolic leaf extract of *Plectranthus amboinicus* revealed a total of 22 distinct phytochemical constituents. The identification of these compounds was accomplished through comparison of their retention times, molecular ion fragments, and spectral data (Table1). The chromatogram exhibited multiple well-resolved peaks, each corresponding to an individual chemical component, thereby illustrating the complex and diverse chemical composition of the extract.

The predominant constituents detected were 1-(4-hydroxy-3-methoxyphenyl) tetradecane-3-one, 1-(4-hydroxy-3-methoxyphenyl) tetradecane-3,5-dione, and 1-(4-hydroxy-3-methoxyphenyl) hexadec-4-en-3-one, which together represented a major proportion of the total chromatographic area. Among these, 1-(4-hydroxy-3-methoxyphenyl) tetradec-4-en-3-one was identified as the dominant compound, appearing at retention times 42.79, 43.03, and 43.46 minutes, contributing a combined peak area of 47.22%. Other notable constituents included L-(+)-ascorbic acid 2,6-dihexadecanoate (5.13%), gingerol (5.98%), oleic acid (6.84%), and octadecanoic acid (5.13%). Several fatty acids, such as dodecanoic acid (0.80%), tetradecanoic acid (0.89%), and 9,12,15-octadecatrienoic acid methyl ester (0.19%), were also detected, reflecting the presence of lipid-derived constituents in the extract. The chromatographic profile further revealed the occurrence of phytol (0.53%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.33%), identified as diterpenoid alcohols. Minor peaks corresponding to phenol, 2-methyl-5-(1-methylethyl)- (3.89%), p-cymene-2,5-diol (1.56%), 2-allyl-4-methylphenol (0.34%), and (S,E)-4-hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone (0.26%) suggested the presence of multiple aromatic phenolic and terpenoid derivatives. In addition, ester compounds such as (9Z,12Z)-3,7-dimethyloct-6-en-1-yl octadeca-9,12-dienoate (1.40%) and citronellyl palmitoleate (0.46%) were detected. Another phenolic derivative, (E)-1-(4-hydroxy-3-methoxyphenyl) hexadec-4-en-3-one (3.67%), was also identified, contributing to the chemical complexity of the extract. The GC–MS chromatogram confirmed the presence of a chemically diverse array of secondary metabolites, predominantly phenolic compounds, fatty acids, terpenoid alcohols, and ester derivatives. This comprehensive chemical profile highlights the intricate composition of the 70% ethanolic leaf extract of *Plectranthus amboinicus*.

In vitro Acetylcholinesterase Inhibition Assay

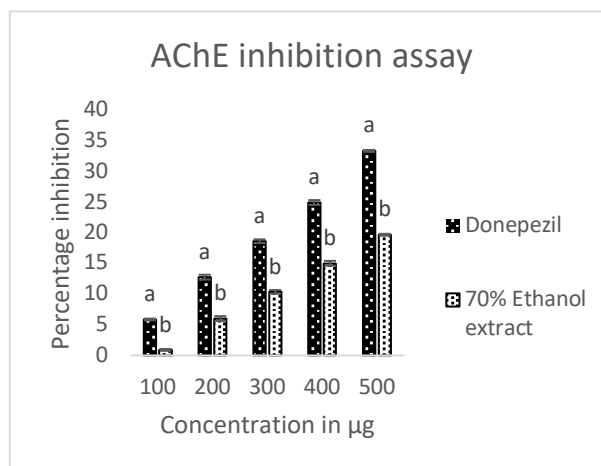


Fig 2: *In vitro* Acetylcholinesterase inhibition activity of Donepezil and 70% Ethanol leaf extract of *Plectranthus amboinicus*.

The *in vitro* acetylcholinesterase (AChE) inhibitory activity of Donepezil and the 70% ethanolic leaf extract of *Plectranthus amboinicus* was evaluated over a concentration range of 100 to 500 µg/mL. Both test samples demonstrated a progressive increase in enzyme inhibition with rising concentration, although the extent of inhibition varied considerably between the standard drug and the plant extract. Donepezil produced a noticeable inhibitory response, beginning with 5.96% inhibition at 100 µg/mL and gradually increasing to 33.08% at 500 µg/mL. Its IC₅₀ value was estimated at 771.47 µg/mL, indicating the concentration required to achieve 50% AChE inhibition. In comparison, the *P. amboinicus* extract showed a relatively weaker activity, starting with only 1.00% inhibition at 100 µg/mL and reaching a maximum of 19.85% at 500 µg/mL. The extract exhibited a higher IC₅₀ value of 1164.57 µg/mL, suggesting substantially lower inhibitory potency compared with the reference drug.

In vivo Acetylcholinesterase Inhibition Assay

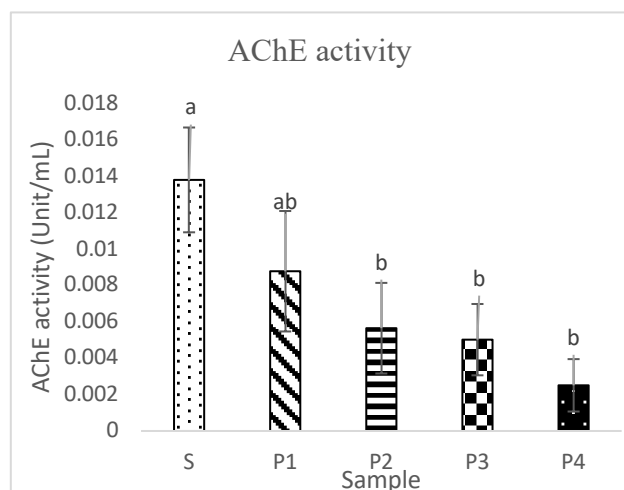


Fig 3: *In vivo* Acetylcholinesterase activity of 70% Ethanol leaf extract of *Plectranthus amboinicus*.

The *in vivo* inhibitory effect of the test samples on acetylcholinesterase (AChE) activity was assessed using the UAS-A β 42/Cyo *Drosophila* model. The results are illustrated in Fig. (3). In the untreated control group (S), AChE activity measured 0.01317 Unit/mL, representing the baseline enzymatic hydrolysis of acetylcholine. Among the treated groups, P4 exhibited the most pronounced reduction in AChE activity, with a value of 0.00188 Unit/mL. This substantial decrease indicates strong enzyme inhibition, suggesting that the P4 treatment effectively limits acetylcholine breakdown and supports enhanced cholinergic signaling. The treatments in P3, P2 and P1 also led to decreased AChE activity, showing values of 0.00470 Unit/mL, 0.00558 Unit/mL and 0.00847 Unit/mL respectively. Although less effective P1, P2, P3 and P4 groups demonstrated a notable reduction in AChE activity relative to the control, indicating moderate inhibitory activity. Overall result showed that, P4 showed the highest level of AChE inhibition, highlighting its therapeutic promise. P3, P2 and P1 produced moderate reductions.

DISCUSSION

Nature has long been recognized as a rich source of medicinal agents, and many modern pharmaceuticals have originated from natural products. Several of these discoveries stem from the traditional use of plants for treating various ailments. Plant-based traditional medicine systems continue to play a significant role in global healthcare, offering valuable knowledge for the treatment of specific medical conditions²⁷. Even today, medicinal plants remain essential to Western medicine and provide a continuous source of new bioactive compounds for drug discovery and development²⁸.

The diverse biological activities of *Plectranthus amboinicus* leaf extracts can be better understood by analyzing their chemical constituents through GC–MS profiling. Earlier studies have reported the presence of nearly 30 non-volatile compounds in methanolic, ethyl acetate, aqueous, and chloroform extracts of the plant’s leaves, stems, and roots from various regions around the world^{14,29,30}. However, to the best of our knowledge, no

previous study has conducted GC–MS-based metabolite profiling specifically on the 70% ethanolic leaf extract of *P. amboinicus* to identify its bioactive constituents. In the present study, the GC–MS chromatogram revealed a wide range of secondary metabolites, including phenolic compounds, fatty acids, terpenoid alcohols, and ester derivatives. This diverse chemical profile highlights the complex phytochemical richness of the 70% ethanolic extract of *P. amboinicus*. The chemically varied spectrum of metabolites identified through GC–MS underpins the extract’s reported antioxidant, anti-inflammatory, and neuroprotective activities. The antioxidant efficacy of *P. amboinicus* is well established and is largely attributed to its abundant polyphenolic content. These compounds effectively neutralize free radicals, reduce lipid peroxidation, and enhance endogenous antioxidant enzyme activity, thereby lowering oxidative stress in biological systems^{29,31,32}. Alongside its antioxidant properties, the extract exhibits notable anti-inflammatory activity. Reports suggest that it can suppress major pro-inflammatory mediators by modulating pathways such as NF- κ B, resulting in decreased expression of cytokines like TNF- α and enzymes such as COX-2, contributing to the reduction of inflammation and associated discomfort^{31,33}. The neuroprotective potential of *P. amboinicus* is closely linked to its ability to reduce oxidative damage and inflammatory responses in neuronal tissues. Experimental studies indicate that the extract enhances memory performance and mitigates neurotoxicity, supporting its potential therapeutic relevance in neurodegenerative diseases, including Alzheimer’s disease. These effects likely arise from the synergistic interactions of its antioxidant and anti-inflammatory constituents³².

The *in vitro* acetylcholinesterase (AChE) inhibition assay, performed using a modified Ellman method, showed a concentration-dependent increase in AChE inhibition for both Donepezil and the 70% ethanolic extract of *P. amboinicus*. Donepezil demonstrated significantly higher inhibitory activity, with inhibition values increasing from 5.96% at 100 μ g/mL to 33.08% at 500 μ g/mL, resulting in an IC₅₀ of 771.47 μ g/mL. In comparison, the plant extract exhibited moderate inhibition, increasing from 1.00% to 19.85% across the same concentration range, with a higher IC₅₀ value of 1164.57 μ g/mL. The lower potency of *P. amboinicus* may be attributed to its phytochemical complexity, as phenolics, flavonoids, and terpenoids typically exert milder cholinesterase inhibition than synthetic AChE inhibitors^{34, 35}. Nonetheless, these compounds contribute substantially to neuroprotection through their complementary antioxidant and anti-inflammatory mechanisms³⁵. The *in vivo* evaluation of AChE activity in the UAS-A β 42/Cyo *Drosophila* model further supported the neuroprotective potential of the extract. A β 42 accumulation is known to disrupt cholinergic signaling by elevating oxidative stress and accelerating acetylcholine degradation; therefore, reducing AChE activity is considered beneficial for restoring cholinergic function in Alzheimer’s like conditions³⁶. In this study, the untreated control group displayed the highest AChE activity, representing normal enzymatic hydrolysis.

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer's Disease *Drosophila* Model

Different concentration treated groups showed reduced AChE activity, confirming the extract *in vivo* inhibitory effect. Among these, P4 concentration exhibited the most pronounced reduction, with an activity level of 0.00188 Unit/mL, indicating strong suppression of acetylcholine breakdown and suggesting substantial protection against A β 42-induced cholinergic deficits. Treatments P3, P2, and P1 concentration also produced reductions in enzyme activity, although to a lesser extent. Overall, P4 concentration emerged as the most effective treatment, consistent with therapeutic strategies aimed at enhancing cholinergic transmission through AChE inhibition.

CONCLUSION

The findings suggest that *P. amboinicus* possesses considerable potential as a supportive therapeutic agent for neurodegenerative conditions such as Alzheimer's disease. Its broad-spectrum antioxidant, anti-inflammatory, and cholinesterase-inhibiting properties make it a promising candidate for further exploration. Future studies focusing on the isolation of active compounds, elucidation of molecular mechanisms, and optimization of dosage will be essential to advance its development as a neuroprotective agent.

REFERENCE

1. Sosa-Ortiz AL, Acosta-Castillo I & Prince MJ. Epidemiology of dementias and Alzheimer's disease. Archives of medical research. 43(8) (2012) 600-608.
2. Parihar MS & Hemnani T. Alzheimer's disease pathogenesis and therapeutic interventions. J Clin Neurosci. 11 (2004) 456-467.
3. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D & Jones E. Alzheimer's disease. Lancet. 377(2011) 1019-1031.
4. Mufson EJ, Counts SE, Perez SE & Ginsberg SD. Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. Expert Rev Neurother. 8(2008) 1703-1718.
5. Seltzer B. Cholinesterase inhibitors in the clinical management of Alzheimer's disease: importance of early and persistent treatment. J Int Med Res. 34 (2006)339-347.
6. Murray AP, Faraoni MB, Castro MJ, Alza NP & Cavallaro V. Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy. Curr Neuropharmacol. 11 (2013) 388-413.
7. Vecchio I, Sorrentino L, Paoletti A, Marra R & Arbitrio M. The State of The Art on Acetylcholinesterase Inhibitors in the Treatment of Alzheimer's Disease. J Cent NervSyst Dis. 13 (2021) 1-13
8. Perry EK, Pickering AT, Wang WW, Houghton PJ & Perry NS. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. J Pharm Pharmacol. 51(5) (1999) 527-34.
9. Curcio CA & Kemper T. Nucleus raphe dorsalis in dementia of the Alzheimer type: Neuro fibrillary changes and neuronal packing density. J Neuropathol Exp Neurol. 43(4) (1984) 359-68.
10. Keservani RK, Shelke SJ, Gawali V, Gaviraj E N, Binorkar SV, Rane SS, Sarvadnya AA. & Patil, SJ. Anti-Alzheimer effect of *Ammannia baccifera* whole plants ethanolic extract. Int J Zoolog Investig. 10(2) (2024) 671-678.
11. Varsha Rani, Patil SJ. & Manjula KR. Pharmacological study on the neuroprotective and antioxidant potential of *Leucas aspera* (Willd.) Link methanolic extract. Plant Sci Today.14(2) (2026) 1-10.
12. Haleshappa R, Patil SJ, Usha T. & Murthy SM. Phytochemicals, Antioxidant Profile and GCMS Analysis of Ethanol Extract of *Simarouba glauca* Seeds. Asian J Biol Life Sci 9(3) (2020) 379-385.
13. K. R. Kirtikar & B. D. Basu, "Indian Medicinal Plants," International Book Distributors, Dehradun 3 (1999) 1970-1971.
14. Arumugam G., Swamy M. K., & Sinniah U. R., *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance, Molecules. 369 (21) (2016) doi:10.3390/molecules21040369
15. Patil SJ, Venkatesh S, Vishwanatha T, Sneha RB, Ravikumar RB, & Patil SB. GCMS Analysis of bioactive constituents from the Petroleum ether extract of *Citrus medica* seeds. World Journal of Pharmacy and Pharmaceutical Sciences, 3(2) (2014) 1239-1249.
16. Prajapati ND, Purohit SS, Sharma AK, & Kumar T. A handbook of medicinal plants section-II: medicinal plants A to Z. India: Agribios; 2003
17. Tanzi, R.E. & Bertram, L. Twenty years of the Alzheimer's disease amyloid hypothesis: A genetic perspective. Cell 120 (2005) 545-555.
18. Greeve I., Kretzschmar D., Tschape J.A., Beyn A., Brellinger C et al. Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic *Drosophila*. J. Neurosci. 24 (2004) 3899-3906.
19. Carmine-Simmen K., Proctor T., Tschape J., Poeck B., Triphan T. et Al. Neurotoxic effects induced by the *Drosophila* amyloid-beta peptide suggest a conserved toxic function. Neurobiol. Dis. 33 (2009) 274-281.
20. Chakraborty R., Vepuri V., Mhatre S.D., Paddock B.E., Miller S., et al. Characterization of a *Drosophila* Alzheimer's disease model: Pharmacological rescue of cognitive defects. PLoS ONE 6 (6) (2011)7 doi:10.1371/journal.pone.0020799
21. Stokin G.B., Almenar-Queralt A., Gunawardena S., Rodrigues E.M., Falzone T., et al. Amyloid precursor protein-induced axonopathies are independent of amyloid-beta peptides. Hum. Mol. Genet. 17 (2008) 3474-3486.

GC–MS-Based Phytochemical Profiling and Acetylcholinesterase Inhibitory Potential of *Plectranthus amboinicus*: *In Vitro* and *In Vivo* Assessment in an Alzheimer's Disease *Drosophila* Model

22. Muller T., Meyer H.E., Egensperger R. & Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog. Neurobiol.* 85 (2008) 393–406.
23. Finelli A., Kelkar A., Song H.J., Yang, H. & Konsolaki, M. A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*. *Mol. Cell. Neurosci.* 26 (2004) 365–375.
24. Crowther D.C., Kinghorn K.J. Miranda E., Page R., Curry J.A. et al. Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience* 132 (2005) 123–135.
25. Iijima K. & Iijima-Ando K. *Drosophila* models of Alzheimer's amyloidosis: The challenge of dissecting the complex mechanisms of toxicity of amyloid- β 42. *J. Alzheimers Dis.* 15 (2008) 523–540.
26. Casas-Tinto S., Zhang Y., Sanchez-Garcia J., Gomez-Velazquez M., Rincon-Limas D.E. et al. The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. *Hum. Mol. Genet.* 20 (2011) 2144–2160.
27. LI Mensoret, Menezes FS, Leitao GG, Reis AS, Dos Santos T, et al. Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. *Phytother.Res.* 15(2001) 127-130.
28. Bowya M, Sivakumar R, Renuka S & Dheebea B. *In Vitro* antioxidant and anti-proliferative activity of *Plectranthus amboinicus* leaves extract on MCF-7 cell line. *Der Pharmacia Lettre.* 8 (12) (2016) 1-9
29. Bhatt P. & Negi P. S. Antioxidant and Antibacterial Activities in the Leaf Extracts of Indian Borage (*Plectranthus amboinicus*) *Food and Nutrition Sciences.* 03(02) (2012) 146–152.
30. El-Hawary S. S., El-Sofany R. H., Abdel-Monem A. R., Ashour R. S. & Sleem A. A. Polyphenolics content and biological activity of *Plectranthus amboinicus* (Lour.) spreng growing in Egypt (Lamiaceae) *Pharmacognosy Journal.* 4 (32) (2012) 45–54.
31. Unnimaya PS, Aiswarya lasksmi AG, & Reshma KV. Neuropharmacological and supporting activities-based review of *Plectranthus amboinicus* (Lour.) spreng. *World J Pharm Res.* 12 (3) (2023) 531-540.
32. Swamy MK, Arumugam G, Kaur R, Ghasemzadeh A, Yusoff MM et al. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. *Evid Based Complement Alternat Med.* 2017(1) (2017) doi: 10.1155/2017/1517683
33. Chiu YungJia CY, Huang TaiHung HT, Chiu ChuanSung CC, Lu TsungChun LT, Chen YaWen CY, et al. Analgesic and antiinflammatory activities of the aqueous extract from *Plectranthus amboinicus* (Lour.) Spreng. both *in vitro* and *in vivo*. *Evid Based Complement Alternat Med.* 2012 (2011) doi: 10.1155/2012/508137
34. Murray AP, Faraoni MB, Castro MJ, Alza NP & Cavallaro V. Natural AChE inhibitors from plants and their contribution to Alzheimer's disease therapy. *Current neuropharmacology.* 11(4) (2023) 388-413
35. Gülcan HO & Orhan IE. The main targets involved in neuroprotection for the treatment of Alzheimer's disease and Parkinson disease. *Current Pharmaceutical Design.* 26 (4) (2020) 509-516.
36. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain.* 141 (7) (2018) 1917-1933