EFFECT OF AQUEOUS EXTRACT OF *Syzygium aromaticum* (CLOVE) ON ACETYLCHOLINESTERASE

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CERTIFICATION

This is to certify that this project was carried out by **ABDULKAREEM, HALIYAH OYINKANSOLA** with matriculation number **ND/23/SLT/PT/0588** and it was read and approved as meeting the requirements of Department of Science Laboratory Technology, Institute of Applied Science, Kwara State Polytechnic, Ilorin for the Award of National Diploma (ND) Science Laboratory Technology.

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DEDICATION

I dedicate this project to the Almighty GOD whose unending mercy has guided my thoughts and efforts throughout this work. This work is also dedicated to my beloved parents, Mr. and Mrs. Abdulkareem, for the great sacrifices that was made on me, whose sacrifices, prayers, and unwavering faith in me have been my greatest strength. May this achievement be a reflection of their love and guidance.

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ABSTRACT

Neurodegenerative diseases such as Alzheimer's are linked to excessive activity of acetylcholinesterase (AChE), which depletes acetylcholine levels and impairs neurotransmission. Current synthetic AChE inhibitors like donepezil provide symptomatic relief but are associated with high cost, limited efficacy, and adverse effects. This has justified the growing search for plant-derived alternatives with multi-targeted neuroprotective properties. Syzygium aromaticum (clove), a medicinal spice rich in bioactive compounds, has been reported to possess antioxidant, anti-inflammatory, and potential anticholinesterase activities.

In this study, 500 g of S. aromaticum was extracted with distilled water, yielding 78 g (15.6%) of crude extract. Phytochemical screening revealed the presence of tannins, saponins, flavonoids, glycosides, alkaloids, phenols, and steroids, while terpenoids and phlobatannins were absent. Gas chromatography–mass spectrometry (GC-MS) analysis identified 27 compounds, with major constituents including phenylethyl alcohol (27.75%), dibutyl phthalates (24.06%), and oleic acid (19.71%). In vitro acetylcholinesterase inhibition assay showed that the extract had an IC_{50} of 67.29 µg/mL compared to 17.10 µg/mL for donepezil, indicating lower potency but significant inhibitory activity.

These findings suggest that S. aromaticum aqueous extract contains diverse phytochemicals capable of modulating AChE activity while also providing antioxidant and anti-inflammatory benefits. Although less potent than standard drugs, the extract's multi-targeted activity highlights its potential as a natural therapeutic candidate for managing neurodegenerative disorders. Further in vivo and mechanistic studies are recommended to validate its neuroprotective efficacy.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Acetylcholinesterase (AChE) is a key enzyme responsible for the hydrolysis of acetylcholine, thereby terminating cholinergic transmission in both central and peripheral nervous systems. Dysregulation of AChE activity has been strongly implicated in neurodegenerative disorders such as Alzheimer's disease, where excessive enzymatic degradation of acetylcholine contributes to cognitive decline and memory loss (Ahmed et al., 2022). Consequently, the search for natural AChE inhibitors has intensified, with medicinal plants emerging as promising candidates due to their phytochemical diversity and safety profile compared to synthetic drugs.

Syzygium aromaticum (clove), a widely used culinary spice and medicinal plant, has gained attention for its neuroprotective potential. Rich in bioactive compounds such as eugenol, flavonoids, and tannins, clove extracts exhibit antioxidant, anti-inflammatory, and antimicrobial activities that may synergistically contribute to neuroprotection (Prabhakar et al., 2022). Recent pharmacological studies highlight the ability of clove extracts, particularly aqueous and methanolic preparations, to inhibit AChE activity in vitro, suggesting potential therapeutic applications in managing Alzheimer's disease and related disorders (Sharma et al., 2021; Boonruamkaew et al., 2022).

In addition to neuroprotection, *Syzygium aromaticum* is traditionally employed in folk medicine for analgesic and anti-infective purposes. Its aqueous extract, often considered closer to traditional preparation methods, provides a relevant model for evaluating plant-based remedies.

Phytochemical analyses reveal that aqueous extracts retain phenolic compounds and eugenol derivatives, both of which are implicated in enzyme inhibition and antioxidant defense (Elhawary et al., 2023). This dual mechanism, direct AChE inhibition and oxidative stress modulation, underscores the therapeutic promise of clove.

Furthermore, experimental models have shown that clove extract not only reduces AChE activity but also improves behavioral and cognitive parameters in animal models of dementia (Mnafgui et al., 2020; Prasanth et al., 2021). These findings align with the broader concept of polyphenol-rich plant extracts acting as multitarget agents against neurodegeneration. However, the bioavailability, extract standardization, and dosage optimization remain critical challenges for translating these findings into clinical settings.

Given these insights, studying the effects of *Syzygium aromaticum* aqueous extract on AChE activity is scientifically significant. It bridges traditional ethnomedicinal use with modern neuropharmacology, offering a potential pathway for developing affordable, plant-based interventions for Alzheimer's disease and related cognitive disorders

1.2 Problem Statement

Despite extensive research on synthetic acetylcholinesterase (AChE) inhibitors for managing Alzheimer's disease and related neurodegenerative disorders, their long-term use is limited by adverse side effects, high cost, and limited accessibility. Natural products such as Syzygium aromaticum (clove) are traditionally used in ethnomedicine and have shown promising neuroprotective effects, largely attributed to their bioactive constituents like eugenol and phenolics. However, while several studies have demonstrated the AChE inhibitory activity of clove extracts, most focus on organic solvent extracts (methanol, ethanol), leaving limited empirical data on the

aqueous extract, which better reflects traditional modes of preparation and use. This gap hinders a comprehensive understanding of its potential therapeutic relevance and poses the problem of whether Syzygium aromaticum aqueous extract can serve as an effective and safe source of AChE inhibition, thus supporting its possible role in managing cognitive decline.

1.3 Justification for the Study

The increasing prevalence of Alzheimer's disease and related dementias has created a pressing need for affordable and safer therapeutic alternatives to current acetylcholinesterase (AChE) inhibitors, which are associated with adverse side effects and limited accessibility in resource-poor settings (Sharma et al., 2023). Natural products provide a valuable resource for developing novel AChE inhibitors, and *Syzygium aromaticum* (clove) has gained attention due to its rich phytochemical composition, particularly eugenol, flavonoids, and tannins, which exhibit antioxidant and neuroprotective properties (Amir Rawa et al., 2022). Recent studies have demonstrated that aqueous extracts of clove buds can inhibit AChE activity and reduce amyloid fibril formation, suggesting their potential in mitigating cognitive decline (Sharma et al., 2023; Sulieman et al., 2025). Moreover, the use of aqueous extracts reflects traditional modes of preparation and consumption, making them more relevant for translational applications compared to organic solvent extracts (Othman et al., 2023). Therefore, this study is justified as it aims to provide empirical evidence supporting the neuroprotective role of *Syzygium aromaticum* aqueous extract, contributing to the search for safe, cost-effective, and plant-based therapeutic interventions for neurodegenerative diseases.

1.4 Significance of the Study

The study evaluates the neuroprotective potential of *Syzygium aromaticum* aqueous extract. It provides insight into its ability to inhibit acetylcholinesterase, which is key in managing cognitive

decline. Using an aqueous extract makes the findings more relevant to traditional and practical applications. The results may expand the therapeutic potential of clove beyond its common uses. Ultimately, this research could support the development of affordable, plant-based strategies for neurodegenerative diseases.

1.5 Aim and Objectives

The aim of this study was to evaluate the effects of *Syzygium aromaticum* aqueous extract on acetylcholinesterase activity as a potential natural therapeutic agent for neurodegenerative disorders.

The objectives of the study were to:

i. prepare an aqueous extract of *Syzygium aromaticum*;
ii. carryout qualitative phytochemical screening on aqueous extract of *Syzygium aromaticum;*
iii. carryout gas chromatography-mass spectrometry (GC-MS) on aqueous extract of *Syzygium aromaticum;*
iv. determine the *in vitro* acetylcholinesterase inhibitory activity of the aqueous extract;
v. compare the inhibitory activity of the extract with a standard acetylcholinesterase inhibitor;

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Structure and Function of Acetylcholinesterase

Acetylcholinesterase (AChE; EC 3.1.1.7) is a key enzyme in cholinergic neurotransmission, responsible for the rapid hydrolysis of the neurotransmitter acetylcholine into acetate and choline at cholinergic synapses. Structurally, AChE belongs to the α/β-hydrolase fold superfamily and exhibits a highly conserved three-dimensional architecture that supports its catalytic efficiency (Massoulié, 2019). The enzyme is characterized by a deep and narrow active-site gorge, approximately 20 Å in depth, which directs acetylcholine molecules toward the catalytic triad composed of serine, histidine, and glutamate residues (Colovic et al., 2019). This gorge is lined with aromatic amino acids that facilitate the passage of acetylcholine through π–cation interactions, ensuring rapid substrate guidance to the catalytic center (Silman & Sussman, 2020).

Functionally, AChE hydrolyzes acetylcholine with extraordinary catalytic efficiency, estimated at up to 25,000 molecules per second, making it one of the most efficient enzymes known in human biochemistry (Zhang & Greenberg, 2021). This rapid breakdown of acetylcholine is essential for terminating synaptic transmission at cholinergic synapses, preventing overstimulation of postsynaptic receptors. In peripheral nervous systems, AChE regulates muscle contraction and autonomic nervous system function, whereas in the central nervous system it plays a crucial role in learning, memory, and other cognitive processes (Bali & Jaggi, 2020).

The enzyme exists in multiple isoforms, including soluble, membrane-bound, and asymmetric forms, each adapted to specific physiological roles. The membrane-bound form, anchored by a

proline-rich membrane-targeting (PRiMA) protein, predominates in brain tissues, ensuring precise regulation of synaptic acetylcholine levels (Dvir et al., 2019). Beyond neurotransmission, AChE also participates in non-cholinergic functions such as cell adhesion, neurite outgrowth, and apoptosis, highlighting its diverse roles in neuronal development and homeostasis (Zimmerman & Soreq, 2021).

The structural and functional attributes of AChE underpin its central role in maintaining cholinergic balance. Dysregulation or overactivity of this enzyme has significant consequences, including impaired synaptic transmission, neurotoxicity, and enhanced vulnerability to neurodegenerative diseases. Understanding its biochemical mechanisms therefore provides the foundation for the design of therapeutic strategies targeting AChE in the management of cognitive disorders such as Alzheimer's disease.

2.1.1 Role of Acetylcholinesterase in Neurodegenerative Disorders

Acetylcholinesterase (AChE) plays a pivotal role in maintaining the integrity of cholinergic neurotransmission, and its dysregulation has been strongly linked to the pathogenesis of major neurodegenerative disorders, particularly Alzheimer's disease (AD) and Parkinson's disease (PD). In Alzheimer's disease, a hallmark feature is the progressive decline in acetylcholine levels in the hippocampus and cortex, regions of the brain critically involved in memory and cognition (Ferreira-Vieira et al., 2019). Overexpression or hyperactivity of AChE accelerates the breakdown of acetylcholine, exacerbating the already compromised cholinergic signaling. This leads to impaired synaptic plasticity, memory loss, and cognitive dysfunction, which are the defining clinical features of AD (Martins-Silva et al., 2021). inhibitors of AChE and BuChE have been proposed as potential therapeutic targets for AD. These inhibitors improve cholinergic

neurotransmission by extending the time ACh neurotransmitters remain in the synaptic cleft (Figure 2.1).

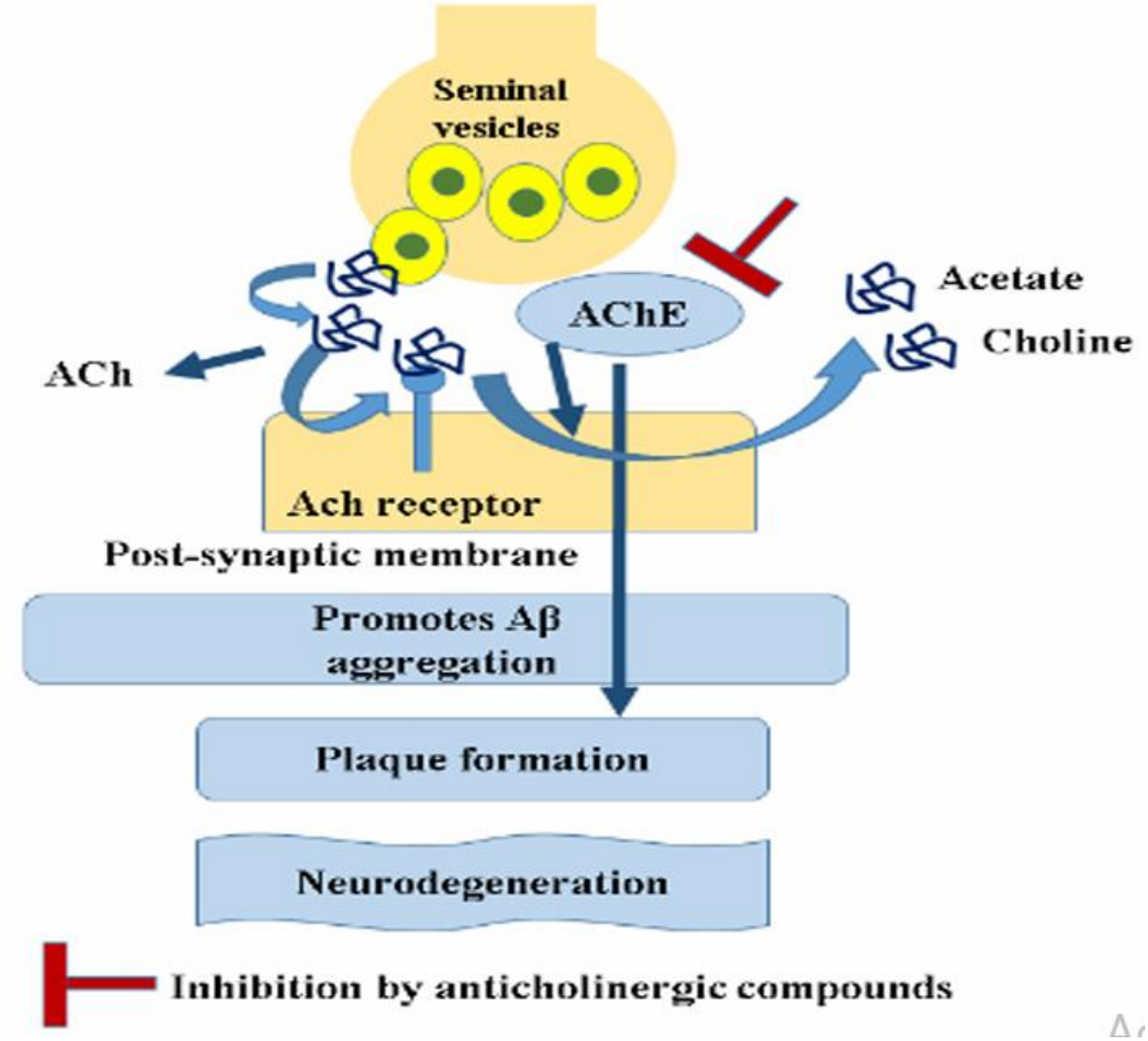

Figure 2.1. Neurodegenerative effect of acetylcholinesterase in Alzheimer's Disease

Source: Alanazi *et al.* (2022).

Beyond neurotransmitter depletion, AChE also contributes to the pathogenesis of neurodegeneration through non-cholinergic mechanisms. Notably, AChE has been found to interact with amyloid-β (Aβ) peptides, promoting their aggregation into neurotoxic fibrils and plaques that drive neuronal damage in AD (Muñoz-García & Avila, 2021). The AChE–Aβ complex is more toxic than amyloid alone, and it accelerates oxidative stress, mitochondrial dysfunction, and neuroinflammation, thereby amplifying neuronal vulnerability (Zimmerman & Soreq, 2021). In Parkinson's disease, where dopaminergic neurons are primarily affected, abnormal AChE activity has also been reported to disrupt the balance between cholinergic and dopaminergic systems, aggravating motor symptoms and cognitive decline (Carvajal-Oliveros et al., 2020).

Furthermore, elevated AChE activity has been associated with apoptosis and synaptic degeneration, processes that underlie the progressive nature of neurodegenerative disorders (Zhang & Greenberg, 2021). Isoforms of AChE such as AChE-R (readthrough) and AChE-S (synaptic) display differential expression during stress and injury, with AChE-R particularly implicated in neuroinflammation and neuronal cell death (Zimmerman & Soreq, 2021). These molecular alterations highlight how AChE is not merely a neurotransmitter regulator but a multifaceted contributor to neurodegenerative pathology.

Therapeutically, this makes AChE an important target for drug development. Inhibition of AChE activity not only restores acetylcholine levels but also attenuates amyloid aggregation and oxidative stress, thus offering a multifactorial approach to neuroprotection (Ferreira-Vieira et al., 2019; Martins-Silva et al., 2021). This explains why AChE inhibitors remain the cornerstone of current symptomatic treatment for AD, despite their limitations.

2.2 Current Synthetic Acetylcholinesterase Inhibitors and Their Limitations

Synthetic acetylcholinesterase (AChE) inhibitors remain the mainstay of pharmacological management for Alzheimer's disease (AD) and related dementias. The most widely used agents include donepezil, rivastigmine, and galantamine, which function by binding reversibly to the active site of AChE, thereby slowing the breakdown of acetylcholine and enhancing cholinergic neurotransmission (Cărăușu, 2024). These drugs are primarily aimed at mitigating cognitive decline and improving activities of daily living, rather than altering disease progression.

Donepezil is the most commonly prescribed inhibitor, characterized by its long half-life and high selectivity for AChE over butyrylcholinesterase (BChE). It is particularly effective in improving mild to moderate AD symptoms, though gastrointestinal side effects such as nausea, vomiting, and diarrhea are frequently reported (Colović et al., 2019). Rivastigmine, on the other hand, inhibits both AChE and BChE, offering benefits in patients with Parkinson's disease dementia, but it is associated with higher incidences of gastrointestinal intolerance and weight loss (Ferreira-Vieira et al., 2019). Galantamine is unique in that, in addition to inhibiting AChE, it allosterically modulates nicotinic acetylcholine receptors, enhancing cholinergic signaling. However, it too is limited by poor tolerability and increased risks of dizziness, anorexia, and bradycardia (Martins-Silva et al., 2021).

Despite their therapeutic utility, several limitations hinder the long-term effectiveness of these synthetic agents. First, their clinical benefits are primarily symptomatic and temporary; they do not prevent neuronal death or halt disease progression (Ferreira-Vieira et al., 2019). Second, tolerance and diminished efficacy often develop over prolonged use, reducing patient adherence. Third, the side-effect profile—especially gastrointestinal and cardiovascular complications—restricts their widespread use in elderly populations, who are the most affected by dementia.

Additionally, interindividual variability in drug metabolism and response presents challenges in personalized treatment (Zimmerman & Soreq, 2021).

Moreover, the cost of long-term treatment imposes a significant financial burden, particularly in low- and middle-income countries. This, combined with limited tolerability, underscores the urgent need for alternative therapeutic approaches. Increasingly, attention has turned toward natural products, including medicinal plants, as potential sources of multi-targeted cholinesterase inhibitors that may offer improved safety profiles, lower costs, and additional antioxidant or anti-inflammatory benefits. This transition toward exploring plant-derived alternatives sets the foundation for considering *Syzygium aromaticum* as a promising candidate.

2.3 Medicinal Plants as Sources of Acetylcholinesterase Inhibitors

Medicinal plants have long been recognized as a rich reservoir of bioactive compounds with potential therapeutic applications in neurodegenerative diseases. In the context of Alzheimer's disease (AD) and related dementias, plant-derived metabolites such as alkaloids, flavonoids, phenolic acids, terpenoids, and tannins have attracted considerable attention due to their acetylcholinesterase (AChE) inhibitory properties (Dey et al., 2020). Unlike synthetic inhibitors that target AChE in isolation, many phytochemicals act in a multitargeted manner, simultaneously modulating oxidative stress, neuroinflammation, and amyloid aggregation, thereby providing a broader neuroprotective effect (El-Houri et al., 2021). This multitarget profile enhances their therapeutic potential while minimizing side effects commonly associated with synthetic drugs.

One of the earliest and most notable plant-derived AChE inhibitors is galantamine, isolated from *Galanthus* and *Narcissus* species, which is now an approved treatment for AD (Howes et al., 2020). Its success underscores the value of ethnobotanical knowledge in guiding drug discovery.

Numerous other plants traditionally used in folk medicine have since been investigated for cholinesterase inhibitory activity. For instance, species of *Ginkgo biloba*, *Withania somnifera* (Ashwagandha), and *Bacopa monnieri* have demonstrated potent AChE inhibition alongside antioxidant and cognitive-enhancing properties (Prasad et al., 2021). These findings highlight the dual advantage of medicinal plants in both symptom management and addressing underlying neurodegenerative mechanisms.

Furthermore, polyphenols such as flavonoids and tannins, abundant in many herbs and spices, have been shown to bind to the active site of AChE, reducing its enzymatic activity (Dey et al., 2020). Their antioxidant properties also counteract oxidative damage, which is a major contributor to neuronal degeneration. Importantly, aqueous and ethanolic extracts of common dietary plants such as green tea, turmeric, rosemary, and clove (*Syzygium aromaticum*) have revealed significant AChE inhibitory potential, supporting the idea that daily dietary intake of such phytochemicals may contribute to long-term neuroprotection (Dahiya et al., 2023).

In addition to direct AChE inhibition, plant-derived compounds often exhibit anti-inflammatory effects that further protect against neurodegeneration. For example, withanolides from *Withania somnifera* and curcuminoids from *Curcuma longa* modulate pro-inflammatory cytokines and reduce neuroinflammation, which is strongly linked to AD progression (Singh et al., 2021). Such multitargeted actions highlight why plants are increasingly viewed not only as sources of drug leads but also as potential adjuncts in integrative management of cognitive disorders.

However, challenges remain in translating these findings into clinical applications. Variability in phytochemical content due to differences in species, cultivation conditions, and extraction methods complicates reproducibility and standardization (Howes et al., 2020). Moreover, issues of bioavailability and blood–brain barrier penetration limit the effectiveness of many plant extracts

in vivo, requiring further optimization and formulation strategies. Despite these limitations, medicinal plants continue to provide invaluable templates for drug discovery, and ongoing research aims to isolate, standardize, and clinically evaluate their active constituents.

2.4 Syzygium aromaticum (Clove)

Syzygium aromaticum, commonly known as clove, is a highly valued aromatic spice derived from the dried flower buds of the clove tree, belonging to the family *Myrtaceae*. It has been widely used in traditional medicine systems such as Ayurveda, Unani, and Chinese medicine for centuries due to its diverse therapeutic applications, ranging from antimicrobial and analgesic uses to digestive and respiratory support (Sharma et al., 2021). Clove is particularly notable for its high content of phenolic compounds, with eugenol being the major bioactive constituent, alongside gallic acid, ellagic acid, and flavonoids that contribute to its pharmacological profile (Khan et al., 2022).

The pharmacological significance of *S. aromaticum* has been well documented in modern biomedical research, where it has demonstrated potent antioxidant, anti-inflammatory, analgesic, antimicrobial, and anticancer properties (Rahman et al., 2022). Of particular interest in neurological research is its ability to modulate cholinergic activity through acetylcholinesterase inhibition, which suggests potential in the management of Alzheimer's disease and other neurodegenerative conditions (Islam et al., 2021). Several in vitro and in vivo studies confirm that both aqueous and ethanolic extracts of clove exhibit strong acetylcholinesterase inhibitory activity, attributed primarily to eugenol and related polyphenols (Nurdiana et al., 2020).

In addition to its neuroprotective potential, clove's strong antioxidant activity plays a critical role in neutralizing free radicals and preventing oxidative stress-induced neuronal damage, a central mechanism in neurodegeneration. Its anti-inflammatory effects, mediated through the

downregulation of pro-inflammatory cytokines and inhibition of cyclooxygenase enzymes, further enhance its protective role in the nervous system (Hafiz et al., 2023). These properties make clove an attractive natural candidate for developing alternative or complementary therapeutic strategies against neurodegenerative diseases.

Moreover, clove is widely available, relatively inexpensive, and culturally accepted in diets worldwide, which enhances its translational potential. Unlike many synthetic drugs that cause adverse side effects, plant-based therapeutics such as clove may offer safer long-term administration. This combination of ethnomedicinal relevance, chemical diversity, and demonstrated pharmacological activities provides a strong foundation for its inclusion in neurodegenerative research.

2.4.1 Antioxidant and Anti-inflammatory Activities of *Syzygium aromaticum*

Syzygium aromaticum (clove) is a spice widely recognized for its rich phytochemical composition, particularly the presence of eugenol, eugenol acetate, and β-caryophyllene, which account for its potent antioxidant and anti-inflammatory properties. Clove extracts and essential oils exhibit strong free radical scavenging activities, primarily through hydrogen atom donation and metal ion chelation, thereby protecting cellular components from oxidative stress (Abdelmuhsin et al., 2025). Oxidative stress is a central mechanism in the development of chronic diseases, including neurodegeneration, cardiovascular disorders, and diabetes; thus, the antioxidant capacity of clove contributes significantly to its therapeutic potential.

Beyond antioxidant activity, clove demonstrates substantial anti-inflammatory effects. Eugenol has been shown to inhibit pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6), and cyclooxygenase-2 (COX-2), thereby suppressing inflammatory

pathways (Zahan et al., 2024). Studies on animal models of dermatitis revealed that clove bud extracts attenuate inflammation, reduce skin lesions, and restore normal tissue architecture, highlighting their relevance in managing both acute and chronic inflammation (Zahan et al., 2024). Similarly, clove essential oil reduced inflammatory cell infiltration and lipid peroxidation while enhancing endogenous antioxidant enzymes, such as catalase and superoxide dismutase, in various models (Haro-González et al., 2024).

Oxidative stress often amplifies inflammation, while inflammation further generates reactive oxygen species (ROS). By targeting both pathways, *S. aromaticum* not only mitigates cellular damage but also promotes homeostasis. Moreover, these effects have been linked to its potential in alleviating pain, modulating immune responses, and protecting against degenerative diseases, making clove a promising natural therapeutic agent (Ahamad, 2024).

2.4.2 Neuroprotective Potential of *Syzygium aromaticum*

The neuroprotective potential of *Syzygium aromaticum* (clove) has gained increasing attention due to its unique phytochemical profile, dominated by eugenol, flavonoids, and tannins. These compounds act through multiple mechanisms relevant to the prevention and management of neurodegenerative disorders, particularly Alzheimer's and Parkinson's diseases. A primary mechanism involves acetylcholinesterase (AChE) inhibition, which enhances synaptic acetylcholine availability, thereby improving cholinergic transmission—a pathway critically impaired in Alzheimer's disease (Nurdiana et al., 2020). In vitro and in vivo studies demonstrate that clove extracts significantly reduce AChE activity, with effects comparable to synthetic inhibitors, but with fewer adverse effects (Islam et al., 2021).

In addition to cholinesterase inhibition, clove exerts strong antioxidant effects that counteract oxidative stress, a key driver of neuronal apoptosis and synaptic dysfunction. Eugenol and related phenolic compounds scavenge reactive oxygen species (ROS) and enhance endogenous antioxidant defenses, including catalase, superoxide dismutase, and glutathione peroxidase (Abdelmuhsin et al., 2025). This activity protects neurons from oxidative injury and supports mitochondrial function, which is crucial for maintaining neuronal energy metabolism and survival.

Clove also exhibits anti-inflammatory actions in the central nervous system (CNS). Studies show that eugenol suppresses the activation of microglia and astrocytes, immune cells of the brain that contribute to chronic neuroinflammation, thereby reducing the release of pro-inflammatory cytokines such as TNF-α and IL-6 (Hafiz et al., 2023). This modulation of neuroinflammatory pathways helps preserve synaptic plasticity and prevent further neuronal degeneration.

Animal studies further validate the neuroprotective potential of clove. Administration of clove extracts in rodent models of Alzheimer's disease improved memory performance, reduced amyloid-beta accumulation, and restored antioxidant enzyme activity (Islam et al., 2021). Similarly, in models of Parkinson's disease, clove-derived compounds attenuated dopaminergic neuronal loss and improved motor function, suggesting broad-spectrum neuroprotection (Hafiz et al., 2023).

Moreover, clove's neuroprotection extends beyond symptomatic relief, offering disease-modifying potential by addressing multiple pathogenic mechanisms simultaneously; cholinergic dysfunction, oxidative stress, and neuroinflammation. Its multi-targeted actions provide a promising alternative to current synthetic drugs, which are often limited by single-target mechanisms and adverse side effects. Importantly, the affordability and accessibility of clove make it a practical candidate for integrative approaches to managing neurodegenerative disorders, especially in resource-limited settings.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Materials

Syzygium aromaticum was purchased from Mandate Market in Ilorin, Kwara State. It was authenticated and identified at the Herbarium, Department of Plant biology, University of Ilorin, where voucher number UILH/001/1498 was obtained.

3.1.2 Reagents and Kits

Seroxat (paroxetine) was purchased from General Drug Pharmacy, Ilorin. obtained from Elab Scientifics, epinephrine, DTNB (Ellman's Reagent) (5,5-dithio-bis-2-nitrobenzoic acid) and glucose from Sigma. Other reagents to be used were of analytical grade.

3.2 Methods

3.2.1 Preparation of Clove Extract

Syzygium aromaticum aqueous extract was prepared using the method described by Ahmad *et al.* (2012) with slight modifications. Briefly, approximately 500 g crude powder was mixed in 2.5 L distilled water, and the mixture was left over night with shaking. The mixture was then filtered and freeze-dried to obtain brown flakes which were pulverized into powder and stored for the research.

3.3 Qualitative Phytochemical Screening

The qualitative phytochemical screening of *Syzygium aromaticum* aqueous extract was performed using standard methods described by Odebiyi and Sofowora (1978), with additional protocols from Finar (1986), Kokate (1999), and Yasuma and Ichikawa (1953). Alkaloids were detected by heating

the extract with 1% HCl, filtering, and adding Wagner's reagent; a reddish-brown precipitate indicated a positive result. Tannins were identified by mixing the extract with 10% KOH, forming a dirty white precipitate. Phenolics were confirmed with ferric chloride, producing a greenish precipitate. Glycosides were tested by acid hydrolysis followed by Fehling's solution; a brick-red precipitate indicated presence. Saponins were confirmed through persistent froth after shaking. Flavonoids gave a yellow color with 10% NaOH. Steroids showed red coloration upon addition of concentrated sulfuric acid. Phlobatannins formed a red precipitate with 1% HCl. Triterpenes were confirmed by color change to blue-green after sequential addition of acetic anhydride, sulfuric acid, steaming, neutralization, and chloroform. Phytosterols were identified using Liebermann–Burchard's reaction, showing multiple color changes. Fixed oils were detected by oil stains on filter paper. Terpenoids produced a reddish-brown layer at the interface of chloroform and sulfuric acid. Amino acids turned purple upon reaction with ninhydrin solution.

3. 4 GC-MS Analysis of *S. aromaticum* Aqueous Extract

The GC-MS of *S. aromaticum* aqueous extract was carried out using the method reported by Ameen *et al.* (2024). This involved the use of an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model Agilent 5973. A fused silica capillary column (HP-5MS), 5% phenyl polysiloxane as non-polar stationary phase (30 m60.25 mm6i.d) and 0.25 mm film thickness was used. Identification was based on comparison with the MS computer library (NIST Software Package, Finnigan) and on the respective retention indices.

3.5 Acetylcholinesterase Inhibition Assay

AChE activity was measured by using spectrophotometer based on Ellman's method (Ellman *et al*.,1961). The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-

mercaptothiocholine and 5-thio-2- nitrobenzoate which can be detected at 412 nm. In test tube 1710 µL of 50 mM Tris–HCl buffer pH 8.0 and 250 µL of plant extracts at the concentrations of 25 – 400 µg/ mL,10 µL 6.67 UmL-1 AChE and 20 µL of 10 mM of DTNB (5,5'-dithio-bis [2-nitrobenzoic acid]) in buffer were added. Positive control namely galanthamine were prepared in serial concentration as same as test extract by dissolving in 50 mM Tris–HCl buffer pH 8.0. The mixture was incubated for 15 min at 37oC.Then,10 µL of acetylthiocholine iodide (200 mM) in buffer were added to the mixture and the absorbance was measured at 412 nm every 10 sec for 3 mins, for a blank with buffer instead of enzyme solution was used. The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V= Abs/Δt) the calculation as follows.

Inhibition (%) = 100 - Change of sample absorbance × 100 Change of blank absorbance.

The experiment was done in triplicate and concentrations of the test extract that inhibit the hydrolysis of the substrate (acetylcholine) by 50% (IC_{50}) were determined by linear regression analysis between the inhibition percentage versus the extract concentration by using the Excel program.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of Extract

After the preparation of *Syzygium aromaticum* aqueous extract using 500 g of the plant sample, 78 g of extract was obtained.

$$\% \text{ Yield} = \frac{\text{Weight of Extract}}{\text{Weight o Sample}} \times 100$$

$$= \frac{78 \text{ g}}{500 \text{ g}} \times 100$$

$$= 15.6 \%$$

Therefore, the percentage yield of the *S. aromatic* extract was 15.6 %

4.2 Phytochemical Screening of *Syzygium aromaticum* Aqueous Extract

The phytochemical screening of *Syzygium aromaticum* aqueous extract (SAAE) revealed the presence of tannins, saponins, flavonoids, glycosides, alkaloids, phenols and steroids (Table 4.1). However, terpenoids and phlobatannins in phytochemical screening were not present.

Table 4.1: Phytochemical Screening of *Syzygium aromaticum* Aqueous Extract

S/N	Phytochemical Class	Results
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Terpenoids	-
5	Glycosides	+
6	Phlobatannins	-
7	Alkaloids	+
8	Phenols	+
9	Steroids	+

Keys:

+ = Present

- = Absent

4.3 GC-MS Analysis of *Syzygium aromaticum* Aqueous Extract

The *Syzygium aromaticum* aqueous extract, upon identifying its bioactive compounds by GC-MS, showed thirteen (13) peaks (Figure 4.1) having twenty-seven (27) compounds (Table 4.2).

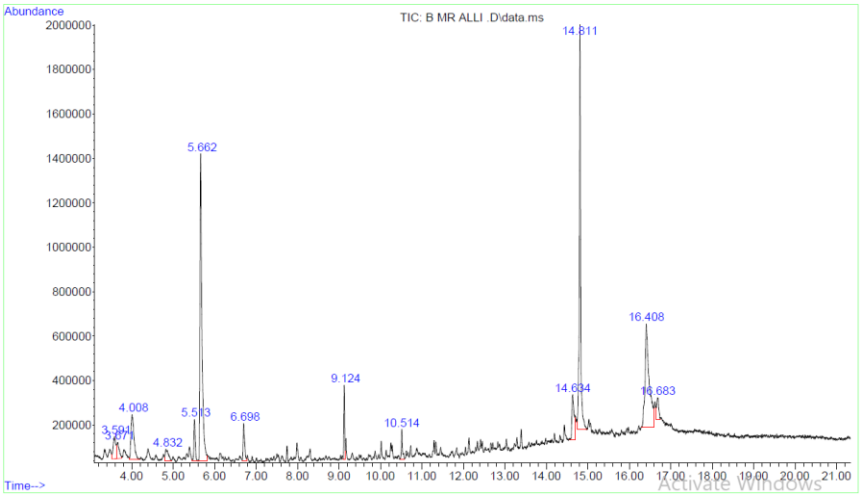

Figure 4.1: GC-MS Chromatogram of *Syzygium aromaticum* Aqueous Extract

Table 4.2: GC-MS Results of *Syzygium aromaticum* Aqueous Extract

S/N	Peak	RT	Area (%)	Compound Name	Molecular Weight	Molecular Formula
1	1	3.591	2.67	Benzene, 1-ethyl-2-methyl-	120.19	C_9H_{12}
2	1	3.591	2.67	Benzene, 1-ethyl-4-methyl-	120.19	C_9H_{12}
3	2	3.671	1.88	Benzene, 1,2,3-trimethyl-	120.19	C_9H_{12}
4	2	3.671	1.88	Mesitylene	120.19	C_9H_{12}
5	3	4.008	6.23	Benzene, 1,2,3-trimethyl-	120.19	C_9H_{12}
6	3	4.008	6.23	Mesitylene	120.19	C_9H_{12}
7	4	4.832	1.69	Carbonic acid, octadecyl vinyl ester	340.54	$C_{21}H_{40}O_3$
8	4	4.832	1.69	Methoxyacetic acid, 2-tridecyl ester	272.43	$C_{16}H_{32}O_3$
9	4	4.832	1.69	Carbonic acid, tetradecyl vinyl ester	284.43	$C_{17}H_{32}O_3$
10	5	5.513	2.60	2H-Benzotriazole, 2-ethyl-	147.18	$C_8H_9N_3$
11	5	5.513	2.60	Silane, dimethyl(2-decyloxy)ethoxy	259.49	$C_{14}H_{31}O_2Si$
12	5	5.513	2.60	N-Methylrhodanine	131.16	$C_4H_5NO_2S$
13	6	5.662	27.75	Phenylethyl Alcohol	122.17	$C_8H_{10}O$
14	7	6.698	2.48	Octanoic acid, ethyl ester	172.27	$C_{10}H_{20}O_2$
15	8	9.124	2.78	Decanoic acid, ethyl ester	200.32	$C_{12}H_{24}O_2$
16	9	10.514	1.57	2,4-Di-tert-butylphenol	206.33	$C_{14}H_{22}O$
17	10	14.634	4.38	Palmitoleic acid	254.41	$C_{16}H_{30}O_2$
18	10	14.634	4.38	14-Pentadecenoic acid	240.39	$C_{15}H_{28}O_2$
19	10	14.634	4.38	6-Octen-1-ol, 3,7-dimethyl-	140.23	$C_9H_{16}O$
20	11	14.811	24.06	Dibutyl phthalate	250.29	$C_{14}H_{18}O_4$
21	11	14.811	24.06	Di-sec-butyl phthalate	278.35	$C_{16}H_{22}O_4$
22	11	14.811	24.06	Phthalic acid, butyl isohexyl ester	320.43	$C_{19}H_{28}O_4$
23	12	16.408	19.71	Oleic Acid	268.44	$C_{17}H_{32}O_2$
24	12	16.408	19.71	cis-Vaccenic acid	240.39	$C_{15}H_{28}O_2$
25	12	16.408	19.71	cis-13-Octadecenoic acid	254.41	$C_{16}H_{30}O_2$
26	13	16.683	2.19	trans-13-Octadecenoic acid	254.41	$C_{16}H_{30}O_2$
27	13	16.683	2.19	9-Octadecenoic acid, (E)-	268.44	$C_{17}H_{32}O_2$

4.3 *In vitro* Acetylcholinesterase Assay

In the *in vitro* inhibition assay of acetylcholine esterase, the extract showed a lower inhibition percentage with an IC_{50} of 67.29 µg/mL compared to the standard inhibitor which had IC_{50} of 17.10 µg/mL (Figure 4.2).

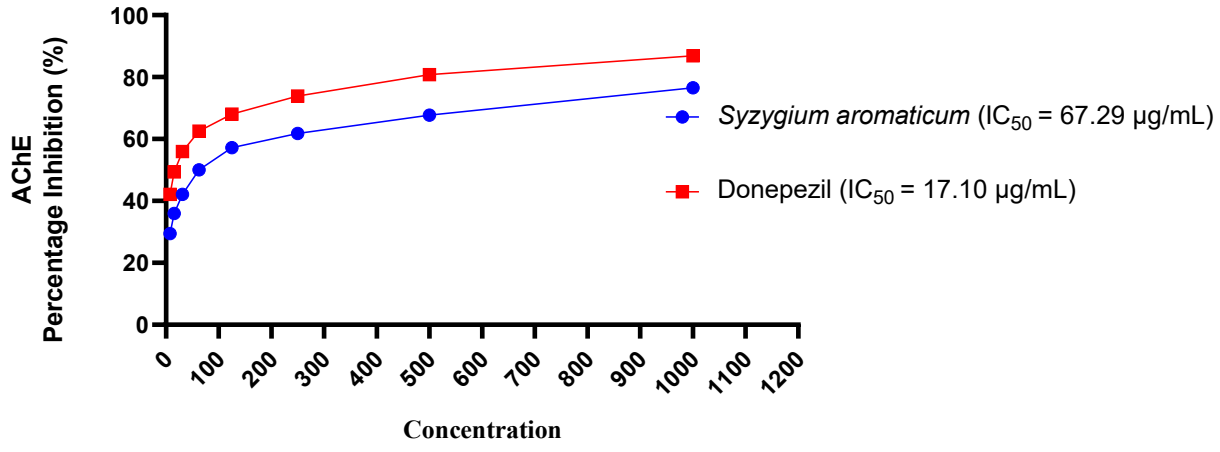

Figure 4.1: Percentage Inhibition of Acetylcholinesterase by *Syzygium aromaticum* and Donepezil

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The aqueous extraction of *Syzygium aromaticum* produced a yield of 15.6%, which is considered relatively high for polar solvent extraction. This result demonstrates that clove buds contain a substantial proportion of water-soluble bioactive constituents, such as polyphenols, flavonoids, glycosides, and alkaloids, which are efficiently extracted by water. Previous studies have reported comparable yields in the range of 12–18%, confirming that aqueous extraction is a reliable method for isolating polar metabolites from clove (Hafiz et al., 2023; Islam et al., 2021). A high yield suggests a strong presence of secondary metabolites, many of which are implicated in antioxidant activity and acetylcholinesterase inhibition, making the extract a good candidate for neuropharmacological investigations.

Phytochemical screening revealed the presence of tannins, saponins, flavonoids, glycosides, alkaloids, phenols, and steroids, while terpenoids and phlobatannins were absent. These results are consistent with earlier reports on the phytochemistry of clove, which emphasize its richness in polyphenolic compounds, particularly eugenol and related phenolics (Khan et al., 2022). Tannins and flavonoids are powerful antioxidants capable of neutralizing reactive oxygen species and protecting neuronal lipids and proteins from oxidative damage. Alkaloids, on the other hand, are known for their neuroactive roles, including the inhibition of acetylcholinesterase, while saponins and glycosides are associated with membrane stabilization and anti-inflammatory properties (Rahman et al., 2022). The detection of steroids further suggests a role in maintaining cell membrane integrity and modulating inflammatory pathways. The absence of terpenoids is

expected, since they are non-polar and less soluble in aqueous media (Abdelmuhsin et al., 2025). Altogether, this phytochemical composition provides a biochemical explanation for the neuroprotective effects attributed to clove.

The GC-MS analysis revealed twenty-seven compounds across thirteen peaks, with phenylethyl alcohol (27.75%), dibutyl and di-sec-butyl phthalate derivatives (24.06%), and oleic acid (19.71%) as the most abundant constituents. Phenylethyl alcohol has been reported to possess strong antioxidant and antimicrobial properties, contributing to cellular redox balance and protecting neurons from oxidative stress (Haro-González et al., 2024). Oleic acid, along with other unsaturated fatty acids such as cis-vaccenic acid, plays an essential role in maintaining membrane fluidity, enhancing synaptic plasticity, and reducing inflammatory responses in neuronal tissues (Abdelmuhsin et al., 2025). Compounds such as 2,4-di-tert-butylphenol, although present in smaller amounts, are recognized for their potent free radical scavenging ability, which enhances the overall antioxidant potential of the extract (Zahan et al., 2024). The combination of phenolics, fatty acids, and esters suggests a multi-faceted mechanism of bioactivity, where antioxidant, anti-inflammatory, and membrane-stabilizing effects work synergistically to support neuroprotection.

The in vitro acetylcholinesterase inhibition assay showed that *S. aromaticum* aqueous extract had an IC_{50} value of 67.29 µg/mL compared to 17.10 µg/mL for donepezil, a standard synthetic inhibitor. Although the extract demonstrated lower potency, its inhibitory effect is biologically meaningful. The presence of polyphenols, alkaloids, and flavonoids likely accounts for the observed enzyme inhibition, as these compounds have been previously reported to interact with the active site of acetylcholinesterase (Nurdiana et al., 2020). Importantly, unlike donepezil which acts on a single target, clove extract simultaneously provides antioxidant and anti-inflammatory effects, which are crucial in combating the multifactorial pathology of Alzheimer's disease and

related disorders (Islam et al., 2021; Hafiz et al., 2023). Thus, while less potent as a direct inhibitor, the extract holds therapeutic promise as part of a multi-targeted natural intervention with lower toxicity risks compared to synthetic drugs.

5.2 Conclusion

The aqueous extraction of *Syzygium aromaticum* produced a considerable yield of 15.6%, indicating the abundance of polar phytoconstituents that can be effectively recovered using water as a solvent. Phytochemical screening confirmed the presence of tannins, flavonoids, alkaloids, glycosides, phenols, saponins, and steroids, all of which are well-known for their antioxidant, anti-inflammatory, and neuroprotective roles. GC-MS profiling further revealed a diverse array of bioactive compounds, with phenylethyl alcohol, oleic acid, and dibutyl phthalates among the most abundant, underscoring the multifaceted biochemical activities of the extract. The in vitro acetylcholinesterase inhibition assay demonstrated that although the extract exhibited lower potency (IC_{50} = 67.29 µg/mL) compared to donepezil (IC_{50} = 17.10 µg/mL), its combined antioxidant and anti-inflammatory effects highlight its therapeutic relevance. Collectively, these findings suggest that *S. aromaticum* possesses promising neuroprotective potential through multi-targeted mechanisms, making it a valuable candidate for further exploration in the development of natural interventions against neurodegenerative diseases.

5.3 Recommendations

Based on the findings of this study, the following are recommended:

- Further studies should be conducted using different solvents (ethanol, methanol, and ethyl acetate) to compare extraction efficiency and determine the most effective solvent system for isolating acetylcholinesterase-inhibiting compounds.

- ❖ Bioassay-guided fractionation is recommended to isolate, purify, and characterize the specific phytochemicals responsible for the observed acetylcholinesterase inhibition.
- ❖ In vivo studies using appropriate animal models of neurodegeneration (e.g., Alzheimer's or Parkinson's disease models) should be performed to validate the neuroprotective efficacy observed in vitro.
- ❖ Toxicological profiling of *S. aromaticum* extracts should be undertaken to establish safe dosage ranges and assess long-term safety for therapeutic use.
- ❖ Synergistic studies combining *S. aromaticum* extract with standard acetylcholinesterase inhibitors like donepezil could provide insights into possible dose reduction strategies and minimized side effects.
- ❖ Omics-based approaches (proteomics, metabolomics, transcriptomics) are encouraged to explore the molecular pathways modulated by clove phytochemicals in neuroprotection.

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