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# Cholinesterase Inhibition and Antioxidant Potential of Syzygium zeylanicum (L.) DC. Leaf Extract: An In-Vitro Comparison with Donepezil

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**ABSTRACT**
Syzygium zeylanicum (L.) DC. is a medicinal plant traditionally valued for its bioactive properties. This study aimed to evaluate the phytochemical composition and in vitro antioxidant potential of its hydro-alcoholic leaf extract. Dried leaves (200 g) were subjected to Soxhlet extraction using 70% ethanol for 72 hours, and the extract was concentrated under reduced pressure. Preliminary phytochemical screening revealed the presence of carbohydrates, phenols, glycosides, flavonoids, lignans, phytosterols, saponins, terpenoids, proteins, and amino acids, while alkaloids and tannins were absent. The antioxidant potential was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which demonstrated dose-dependent activity with an $IC_{50}$ value of 21.79 µg/mL, comparable to ascorbic acid ($IC_{50}$ = 19.34 µg/mL). These findings indicate that the leaf extract possesses significant free radical scavenging activity, likely due to its phenolic and flavonoid content. While the study provides preliminary insights into its bioactive profile and antioxidant efficacy, further investigations involving neuroprotective assays, acetylcholinesterase inhibition studies, and in vivo models are warranted to explore its therapeutic potential, particularly in oxidative stress-related neurodegenerative disorders such as Alzheimer's disease (AD). The findings suggest the leaf extract has strong scavenging properties against free radicals, most likely because of its flavonoid and phenolic content. Although the study offers some initial insights into its bioactive profile and antioxidant efficacy, more research through in vivo models, acetylcholinesterase inhibition studies, and neuroprotective assays is necessary to fully explore its therapeutic potential, especially in neurodegenerative diseases like Alzheimer's disease that are linked to oxidative stress.

**KEY WORDS:** Syzygium zeylanicum; hydro-alcoholic extract; DPPH assay; free radical scavenging; phenolics; flavonoids; neuroprotection

## I. INTRODUCTION

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease of the brain that causes behavioural abnormalities, memory loss, cognitive decline, and ultimately the inability to perform even the most basic tasks. In elderly persons, it is the most frequent cause of dementia. (1) By 2020, more than 55 million individuals worldwide are expected to have dementia, according to recent estimates. Ageing populations in low- and middle-income countries are expected to contribute significantly to the predicted increase in this number, reaching 78 million by 2030 and 139 million by 2050. (2)According to national research, the prevalence of dementia among adults 60 and older in India is estimated to be 7.4%, or 8.8 million people. (3)

Alzheimer's disease is characterized by cholinergic dysfunction, oxidative stress, inflammation, amyloid-β (Aβ) accumulation, and tau hyperphosphorylation (4). Extracellular Aβ plaques form from the aggregation of peptides produced by β- and γ-secretase cleavage of amyloid precursor protein (APP) (5). Intracellular neurofibrillary tangles of hyperphosphorylated tau disrupt microtubule stability and axonal transport, exacerbating neuronal dysfunction (6). Excess reactive oxygen species damage proteins, lipids, and DNA, contributing to mitochondrial dysfunction (7). Loss of basal forebrain cholinergic neurons increases acetylcholinesterase activity and reduces acetylcholine, impairing cognitive function (8).

Acetylcholinesterase inhibitors, which include galantamine, rivastigmine, and donepezil, raise acetylcholine levels in the brain and offer mild to moderate AD patients some cognitive

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advantages. Memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, can enhance behaviour, cognition, and everyday living tasks by modifying glutamatergic neurotransmission in moderate to severe phases. (9)

Donepezil is a reversible, centrally acting acetylcholinesterase (AChE) inhibitor that increases synaptic acetylcholine and enhances cholinergic neurotransmission (10). Clinical studies show that 5–10 mg/day of donepezil improves cognition, global function, and daily living in mild to moderate AD, and provides modest benefits in severe AD (11,12). Long-term treatment (1–2 years) maintains these effects, while discontinuation leads to decline (12,13). Beyond cholinergic enhancement, donepezil may protect neurons from glutamate-induced excitotoxicity, reduce oxidative stress, modulate inflammation, and influence non-amyloidogenic APP processing. Side effects are generally mild to moderate, mostly cholinergic, with occasional bradycardia or dizziness (14,15).

Recent advances include disease-modifying therapies targeting amyloid-β, such as monoclonal antibodies aducanumab and lecanemab, which aim to reduce amyloid plaques and slow cognitive decline, though long-term efficacy remains under investigation (15). Non-pharmacological approaches—lifestyle modification, physical activity, and cognitive training—are increasingly recognized as important adjuncts. Research continues on plant-derived compounds and multi-target agents with antioxidant, anti-inflammatory, and anti-cholinesterase properties for potential neuroprotection in AD (16).

The Myrtaceae family includes Syzygium zeylanicum (L.) DC., also referred to as spicate eugenia, which is native to tropical areas, including parts of India. Its leaves have long been used in folk medicine because of their alleged medicinal properties. (17) Recent phytochemical investigations have revealed a range of bioactive chemicals in S. zeylanicum leaves, including α-humulene and β-elemene, which are well-known for their anti-inflammatory and antioxidant qualities. Both neuroinflammation and oxidative stress are linked to the aetiology of Alzheimer's disease (AD), and these substances have demonstrated promise in reducing both. (18)

The process of hydro-alcoholic extraction uses a solution of water and alcohol (usually methanol or ethanol) as the solvent to separate bioactive chemicals from plant materials. Flavonoids, phenolics, alkaloids, and glycosides are just a few of the phytochemicals that can be effectively extracted with this technique, which combines the polarity of water with the organic solubility of alcohol (19). The polarity of the target chemicals, the plant matrix, and the intended yield can all influence the ideal water to alcohol ratio (20). Because it is easy to use, economical, and effective at preserving thermolabile chemicals, hydro-alcoholic extraction is frequently chosen (21).

The DPPH assay is a widely used method to evaluate antioxidant activity. It relies on the stable free radical DPPH, which changes from violet to yellow when reduced by antioxidants donating hydrogen atoms (22). The decrease in color intensity is measured spectrophotometrically to quantify antioxidant capacity (23). This method is simple, rapid, and cost-effective, but assay conditions—such as solvent, concentration, and reaction time—must be standardized for reliable comparisons (24)

Strong antioxidant activity has been observed for Syzygium zeylanicum (L.) DC. For instance, zeylaniin A, a new macrocyclic ellagitannin found in its leaves, has a strong ability to scavenge radicals in vitro (25). Additionally, S. zeylanicum trunk bark extracts and phenolic fractions have strong antioxidant and associated biofunctional properties in both in vitro and in vivo models; however, there is research that compares these substances to conventional medications in in vitro neuroprotective assays (26).

Thus, this study aims to determine the hydro-alcoholic leaf extract of S. zeylanicum's in vitro antioxidant, acetylcholinesterase inhibitory, and neuroprotective capabilities and compare these effects with donepezil.

**PLANT DESCRIPTION**

Common name:
Myrtus zeylanica L., Syzygium spicatum (Lam.) DC., Poochapazham(In Malayalam)
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: Syzygium zeylanicum (L.) DC.
Traditional use: Joint pain, headache, arthritis, fever,anti-antimicrobial and anti-fungal properties

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## II. MATERIALS AND METHODS

### 1. Plant Material and Authentication

Fresh leaves of Syzygium zeylanicum (L.) DC. were collected from Kottayam,India,and authenticated by Dr.Rojimon.P.Thomas from CMS College, Kottayam.

### 2. Preparation of Hydro-Alcoholic Leaf Extract

Two hundred grams of Syzygium zeylanicum leaves were pulverized and dried. The powdered material was subjected to Soxhlet extraction using 70% ethanol as the solvent. The extraction was carried out continuously for 72 hours. After extraction, the solvent was removed under reduced pressure at 40°C using a rotary evaporator to obtain the crude hydro-alcoholic extract. The concentrated extract was stored at 4°C until further analysis.

### 3. Phytochemical screening of the plant

The hydro-alcoholic leaf extract was subjected to preliminary phytochemical screening, and tests for alkaloids (Mayer's and Dragendorff's), flavonoids (Shinoda and alkaline reagent), phenolics/tannins (ferric chloride and lead acetate), saponins (frothing), glycosides (Keller-Kiliani), and terpenoids/steroids (Salkowski and Liebermann-Burchard) were performed. The test was performed, and the presence or absence of compounds was recorded as described in standard protocols

### 4. Chemicals and Reagents

Donepezil hydrochloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, and ethanol, analytical grade, were purchased.

### 5. In Vitro Antioxidant Assay

**DPPH Radical Scavenging Assay**

- Different concentrations of the leaf extract and standard antioxidant (ascorbic acid) were prepared.
- 1 mL of 0.1 mM DPPH solution was mixed with 1 mL of the test sample and incubated in the dark at room temperature for 30 minutes.
- The absorbance was measured at 517 nm using a UV-Vis spectrophotometer.
- And it is calculated by,

$$\textbf{Percentage inhibition} = \frac{\textbf{control}-\textbf{test}}{\textbf{control}} \times 100$$

## III. RESULTS AND DISCUSSION

### 1. Phytochemical screening of the plant

The hydro-alcoholic leaf extract of Syzygium zeylanicum (L.) DC. was subjected to preliminary phytochemical screening. The extract tested positive for carbohydrates, phenols, glycosides, flavonoids, lignans, phytosterols, saponins, terpenoids, proteins, and amino acids. Other tested phytochemicals, such as alkaloids and tannins, were absent. The results are summarized in **Table 1.**

**Table 1.** Preliminary Phytochemical Screening of Syzygium zeylanicum Leaf Extract

| Phytochemical Group | Result |
|---|---|
| Flavonoids | Present |
| Alkaloids | Absent |
| Glycosides | Present |
| Tannins | Absent |
| Phenols | Present |
| Terpenoids | Present |
| Carbohydrates | Present |
| Proteins | Present |
| Amino acids | Present |
| Saponins | Present |
| Lignans | Present |

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| | |
|---|---|
| Phytosterols | Present |

**2. In Vitro Antioxidant Assay**

DPPH Radical Scavenging Assay

The antioxidant potential of the extract was evaluated using the DPPH assay. The hydro-alcoholic leaf extract demonstrated dose-dependent free radical scavenging activity, with an $IC_{50}$ value of 21.79 µg/mL, which was comparable to the standard antioxidant, ascorbic acid ($IC_{50}$ = 19.34 µg/mL). This indicates the extract has significant radical scavenging ability. The dose-response relationship is illustrated in **Figure 1.**

**Table 1.**Percentage inhibition of DPPH SCAVENGING ASSAY.

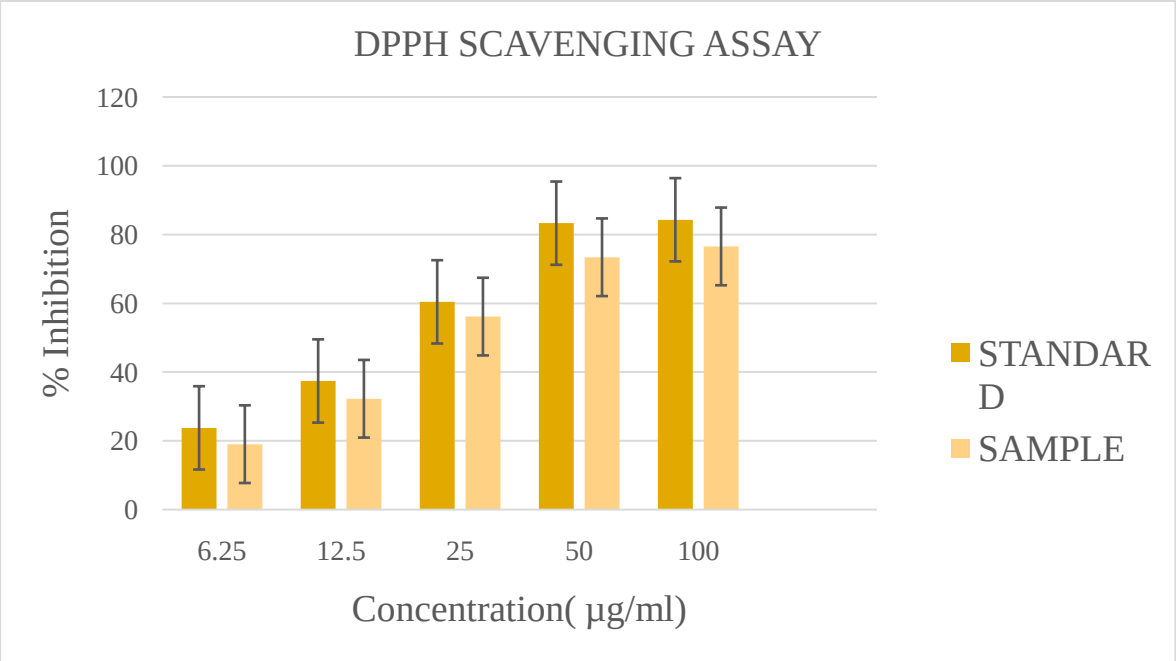


**Fig 1**. Dose-dependent DPPH radical scavenging activity of Syzygium zeylanicum leaf extract compared with ascorbic acid.

**DISCUSSIONS**

The hydro-alcoholic leaf extract of Syzygium zeylanicum (L.) DC. was first evaluated for its phytochemical composition. Preliminary screening revealed the presence of multiple bioactive constituents, including alkaloids, flavonoids, phenolics, saponins, glycosides, terpenoids, and steroids. The diversity of these phytochemicals indicates the potential for multiple biological activities, as many of these compounds are known to contribute to antioxidant, anti-inflammatory, and neuroprotective effects. Notably, flavonoids and phenolics are recognized for their ability to donate electrons or hydrogen atoms to neutralize free radicals, thereby mitigating oxidative stress.

The extract's antioxidant potential was further assessed using the DPPH radical scavenging assay. It demonstrated a strong free radical scavenging activity, with an $IC_{50}$ value of 21.79 µg/mL, which is comparable to the standard antioxidant ascorbic acid ($IC_{50}$ = 19.34 µg/mL). This indicates that the extract possesses electron-donating capacity capable of neutralizing reactive oxygen species. The observed activity may result from the combined effects of its phytochemical constituents, particularly phenolics and flavonoids, which can act as free radical scavengers and metal chelators.

The presence of multiple bioactive compounds along with the observed antioxidant activity suggests potential relevance in mitigating oxidative stress, which is a key factor in neurodegenerative disorders such as Alzheimer's disease. While the current study provides preliminary evidence for antioxidant potential, it is

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limited to in vitro assays. Future studies, including detailed in vitro neuroprotection experiments, acetylcholinesterase inhibition assessments, and in vivo investigations, are needed to better understand the pharmacological and therapeutic potential of S. zeylanicum. Such studies will help clarify the extent to which the observed phytochemical and antioxidant properties can translate into neuroprotective effects.

## IV. CONCLUSION

The present study evaluated the hydro-alcoholic leaf extract of Syzygium zeylanicum (L.) DC. for its phytochemical composition and in vitro antioxidant potential. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, saponins, glycosides, terpenoids, and steroids, indicating a rich profile of bioactive compounds. The extract demonstrated notable antioxidant activity in the DPPH assay, with an $IC_{50}$ value of 21.79 µg/mL, comparable to the standard antioxidant, ascorbic acid ($IC_{50}$ = 19.34 µg/mL).

These findings suggest that the extract possesses compounds capable of scavenging free radicals and potentially mitigating oxidative stress-related damage. However, as the study was limited to preliminary phytochemical evaluation and in vitro antioxidant assessment, further investigations are necessary. Future studies focusing on detailed neuroprotective assays, acetylcholinesterase inhibition, and in vivo models will provide more comprehensive insights into the therapeutic potential of S. zeylanicum and its applicability in neurodegenerative conditions.

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