Pertanika Proceedings 1 (6): 13 - 17 (2025)

PERTANIKA JOURNALS

PERTANIKA PROCEEDINGS

Journal homepage: http://www.pertanika.upm.edu.my/

Comparative Study of Antioxidant and Anticholinesterase Activities in *Moringa oleifera* Leaves across Different Growth Stages

Faidruz Azura Jam[1]*, Hamizah Shahirah Hamezah[2], Ahmed Mediani[2], Emelda Rosseleena Rohani[2], Liew Jia Yi[3], Chua Wan Yi[3], Tharani Rajendran[3], Pearlyne Willie Wong[3], April Tang Yee Ying[3], Ummi Kalthum Azlan[2], and Hadi Azamuddin Abdul Hakim[4]

[1]*Department of Biochemistry, Faculty of Medicine, Manipal University College Malaysia, Jalan Batu Hampar, 75150 Bukit Baru, Malacca, Malaysia*
[2]*Institute of Systems Biology, Universiti Kebangsaan Malaysia, UKM, 43600 Bangi, Selangor, Malaysia*
[3]*Faculty of Medicine, Manipal University College Malaysia, Jalan Batu Hampar, 75150 Bukit Baru, Malacca, Malaysia*
[4]*Faculty of Science and Technology, Universiti Kebangsaan Malaysia, UKM, 43600 Bangi, Selangor, Malaysia*

ABSTRACT

Alzheimer's disease (AD) treatment often targets increasing acetylcholine levels in the brain by inhibiting acetylcholinesterase (AChE) using AChE inhibitors (AChEIs). *Moringa oleifera* leaves (MOL) possess neuroprotective, antioxidant and anticholinesterase activities. However, the variation in bioactivity of MOL at different growth stages is unclear. This study aims to compare the antioxidant and anticholinesterase activities of MOL harvested at 30, 45, and 60 days. The DPPH assay was used to measure antioxidant activity and the acetylcholinesterase assay assessed AChE inhibition. MOL were harvested at the specified stages, processed, and extracted with ethanol for the DPPH assay, while acetylcholinesterase activity was measured using lysate from homogenized MOL. Results showed that MOL harvested at 60 days exhibited the highest antioxidant activity compared to those harvested at 45 days ($p < 0.05$) and 30 days ($p < 0.001$). However, MOL harvested at 30 days demonstrated the most significant inhibition of acetylcholinesterase activity compared to the other stages ($p < 0.001$). These findings reveal the varying bioactivities of MOL at different growth stages and highlight the importance of timing in harvesting for therapeutic purposes.

ARTICLE INFO

Article history:
Received: 30 September 2025
Published: 28 November 2025

DOI: https://doi.org/10.47836/pp.1.6.003

E-mail addresses:
faidruz.jam@manipal.edu.my (Faidruz Azura Jam)
hamizahshahirah@ukm.edu.my (Hamizah Shahirah Hamezah)
medianiahmed47@gmail.com (Ahmed Mediani)
emelda@ukm.edu.my (Emelda Rosseleena Rohani)
jiayi.liew9@gmai.com (Liew Jia Yi)
wanyi9428@gmail.com (Chua Wan Yi)
tharani2127@gmail.com (Tharani Rajendran)
pearl01231@gmail.com (Pearlyne Willie Wong)
apriltangyy@gmail.com (April Tang Yee Ying)
kalthumazlan96@gmail.com (Ummi Kalthum Azlan)
hadiazam2510@gmail.com (Hadi Azamuddin Abdul Hakim)
* Corresponding author

e-ISSN: 3083-9475

This study provides valuable insights into the potential use of MOL in AD treatment, emphasizing stage-specific bioactivity.

Keywords: Anticholinesterase, Alzheimer's disease, antioxidant, *Moringa oleifera*, neuroprotective

INTRODUCTION

The aging population is rapidly increasing in Malaysia with the country projected to become an aged society by 2050 (Department of Statistics, Malaysia). This demographic shift is expected to contribute significantly to the escalating prevalence of Alzheimer's disease (AD), a neurodegenerative disorder that affects millions of people globally each year and is known for its significant impairment of sufferers' cognition and behaviour. The neuropathological hallmark of AD includes the abnormal accumulation of misfolded protein (Ashraf et al., 2014), which disrupts neuronal function and contributes to a decline in acetylcholine. One of the main approaches to AD treatment has been to increase the level of acetylcholine (ACh) in central synapses by inhibiting acetylcholinesterase via the use of AChE inhibitors (AChEIs). The USFDA has approved many AChE inhibitors for the treatment of mild-to-moderate AD patients, including galantamine, donepezil and rivastigmine. However, these drugs have several side effects, including gastrointestinal disorders and depression (Yiannopoulou et al.,2020). Natural AChEIs are gaining attention as alternative treatments for AD due to their efficacy and fewer side effects. The World Health Organization (WHO; 2013) stated that herbal medicines serve the health needs of approximately 80 percent of the world's population, particularly in rural areas. *Moringa oleifera*, known as the 'miracle tree' has various pharmacological properties such as antioxidant, anti-inflammatory, immunomodulatory, and anticancer (Dubey et al., 2013). *Moringa oleifera* leaves (MOL) also exhibit a neuroprotective effect, a vital role as antioxidants and anticholinesterases (Omodanisi et al., 2017). However, the different growth stages of MOL that possess different potent bioactivities remain unclear. This study aimed to compare the antioxidant and anticholinesterase activities of MOL harvested at 30-, 45-, and 60-days using DPPH and acetylcholinesterase assays respectively.

METHODS

Moringa oleifera leaves were provided by MR Moringa Sdn. Bhd. and harvested on days 30, 45, and 60. The antioxidant activity of MOL was assessed using the DPPH assay while the inhibition of acetylcholinesterase activity of MOL was assessed using acetylcholinesterase assay. In the DPPH assay, MOL extracts were prepared using ethanol at a solid-to-liquid ratio of 1:10. Next, DPPH solutions were added to the MOL extracts followed by incubation in the dark at room temperature for 30 minutes. Absorbance readings were taken at 515 nm with ascorbic acid serving as the positive control. For

the acetylcholinesterase assay, MOL lysates were obtained through homogenization in a lysis buffer. Next, acetylthiocholine-reaction mixtures were added to MOL lysates, followed by incubation in the dark at room temperature for 30 minutes. Absorbance measurements were conducted at 410 nm, using donepezil as a positive control. The experiments were carried out in triplicate, and data were analysed with ANOVA followed by Tukey's post-hoc test using SPSS software (version 28).

RESULTS

From the DPPH assay, we found MOL harvested at day 60 exhibited significantly higher antioxidant activity compared to those harvested at day 45 ($p < 0.05$) and day 30 ($p < 0.001$) (Figure 1). The IC50 values for MOL harvested at day 30, 45, and 60 were 611.4 µg/ml, 535.2 µg/ml, and 256.6 µg/ml, respectively. In contrast, based on the acetylcholinesterase assay, the inhibition of acetylcholinesterase activity was highest in MOL harvested at day 30, followed by those harvested at day 45 and day 60 ($p < 0.001$) (Figure 2).

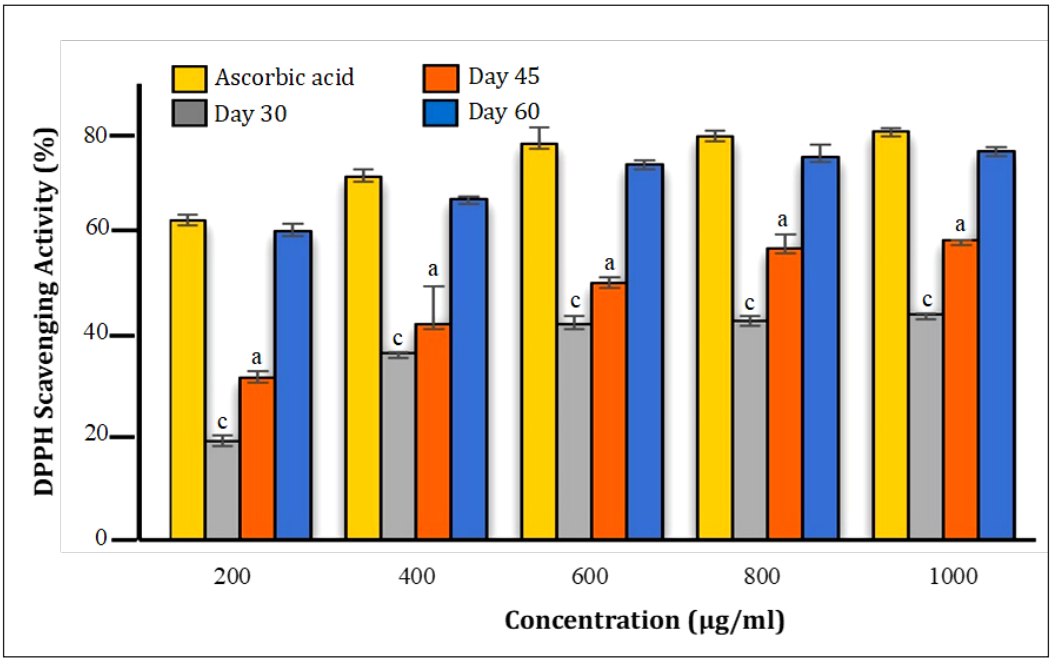

Figure 1. Antioxidant activity of MOL. Data is shown as mean ± SD (n=3). a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ compared to Day 60

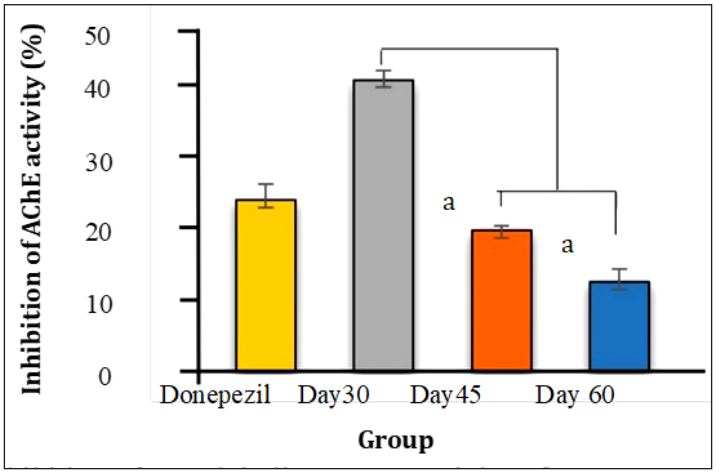

Figure 2. Inhibition of acetylcholinesterase activity of MOL. Data is shown as mean ± SD (n=3).
a: $p < 0.001$ compared to Day 30

DISCUSSION

Moringa oleifera leaves (MOL) contain approximately 35 important phytochemical constituents, among which tannin, saponin, carotenoids, alkaloids, flavonoids and phenols play significant roles in antioxidant and anticholinesterase activity. We hypothesized that *Moringa oleifera* leaves (MOL) harvested at different growth stages may exhibit variations in their phytochemical constituents, contributing to their pharmacological properties. Our findings indicate that MOL harvested at day 60 exhibited the highest antioxidant activity, while those harvested at day 30 showed the most significant inhibition of acetylcholinesterase activity. Previous research has highlighted the antioxidant properties of MOL, attributing these effects to their rich content of polyphenols and flavonoids. Nwidu et al. (2018) reported an inverse correlation between acetylcholinesterase inhibition activity and the total phenolic and flavonoid contents, further supporting the notion that the bioactive compounds present in MOL may vary significantly with growth stage and influence their pharmacological potential.

CONCLUSION

Our data reveal that MOL harvested at different growth stages exhibit varying bioactivities. This study provides a valuable reference for future research in related fields, emphasizing the importance of identifying and correlating specific phytochemical constituents across the various growth stages of *Moringa oleifera,* particularly in the context of Alzheimer's disease. By elucidating these phytochemicals and their associated bioactivities, targeted approaches can be developed, potentially advancing treatment strategies for Alzheimer's

disease. Further investigations are warranted to fully realize the therapeutic potential of *Moringa oleifera* in combating this condition.

ACKNOWLEDGEMENT

This work was supported by Manipal University College Malaysia Research Grant Scheme 2022 (MUCM/001/2022).

REFERENCES

Ashraf, G. M., Greig, N. H., Khan, T. A., Hassan, I., Tabrez, S., Shakil, S., Sheikh, I. A., Zaidi, S. K., Wali, M. A., Jabir, N. R., Firoz, C. K., Naeem, A., Alhazza, I. M., Damanhouri, G. A., & Kamal, M. A. (2014). Protein misfolding and aggregation in Alzheimer's disease and type 2 diabetes mellitus. *CNS & Neurological Disorders-Drug Targets*, *13*(7), 1280-1293. https://doi.org/10.2174/1871527313666141130210507

Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A multipurpose tree—*Moringa oleifera*. *International Journal of Pharmaceutical and Chemical Sciences*, *2*(1), 415-423.

Nwidu, L. L., Elmorsy, E., Aprioku, J. S., Siminialayi, I., & Carter, W. G. (2018). In vitro anti-cholinesterase and antioxidant activity of extracts of *Moringa oleifera* plants from Rivers State, Niger Delta, Nigeria. *Medicines*, *5*(3), Article 71. https://doi.org/10.3390/medicines5030071

Omodanisi, E. I., Aboua, Y. G., & Oguntibeju, O. O. (2017). Assessment of the anti-hyperglycaemic, anti-inflammatory, and antioxidant activities of the methanol extract of *Moringa oleifera* in diabetes-induced nephrotoxic male Wistar rats. *Molecules*, *22*(4), Article 439. https://doi.org/10.3390/molecules22040439

WHO. (2013). *Catalysing ancient wisdom and modern science for the health of people and the planet*. World Health Organisation. https://www.who.int/publications/i/item/9789241507450

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and future treatments in Alzheimer disease: An update. *Journal of Central Nervous System Disease*, *12*, Article 1179573520907397. https://doi.org/10.1177/1179573520907397