

RESEARCH PAPER

**Acetylcholinesterase Activity of Crude Extracts and Flavonoids from
Artocarpus anisophyllus Miq. and *Artocarpus lowii* King**

Siti Awani Abdullah, Siti Mariam Abdul Lathiff, Shajarahunnur Jamil*

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,
81310 UTM Johor Bahru, Johor, MALAYSIA

*Corresponding author: shajarah@utm.my

Received: 5 July 2022; **Accepted:** 18 July 2022; **Published:** 21 October 2022

To cite this article (APA): Abdullah, S. A., Abdul Lathiff, S. M., & Jamil, S. (2022). Acetylcholinesterase Activity of crude extracts and flavonoids from *Artocarpus anisophyllus* Miq. and *Artocarpus lowii* King. *Journal of Science and Mathematics Letters*, 10(2), 62-66. <https://doi.org/10.37134/jsml.vol10.2.7.2022>

To link to this article: <https://doi.org/10.37134/jsml.vol10.2.7.2022>

Abstract

A flavonoids-rich extract and seven flavonoids from the leaves and heartwoods of *A. anisophyllus* Miq. and *A. lowii* King were investigated for their inhibitory effects against acetylcholinesterase enzyme. The flavonoids were fully characterized spectroscopically as artocarpin (1), isobavachalcone (2), pyranocycloartobioxanthone A (3), 5,7-dihydroxy-4'-methoxy-6-prenylflavanone (4), 5-hydroxy-7,8-(2,2-dimethylchromano)-4'-methoxyflavanone (5) 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8- γ,γ -dimethylallylflavone (6) and 2',4'-dihydroxy-3,4-(2'',2''-dimethylchromeno)-3'-prenyldihydrochalcone (7). Acetylcholinesterase inhibitory activity of the samples was determined using TLC plate with bioactivity staining based on the Ellman's method. Pyranocycloartobioxanthone A (3) exhibited an excellent inhibitory activity with detection limit at 7.81 $\mu\text{g/mL}$.

Keywords: acetylcholinesterase, plant extracts, flavonoids, *Artocarpus*, TLC

INTRODUCTION

Acetylcholinesterase inhibitors is one of the important approaches to treat Alzheimer's disease. An estimation of 10% from world's population over the age of 65 years are infected by this disease (Giovanni et al., 2008). Alzheimer's disease is a fatal, progressive and degenerative disease that destroys brain cells. Symptoms of having an Alzheimer's disease include having difficulty in remembering things, making decisions and performing everyday activities. These changes can affect the way a person feels and acts. Many researchers tried to find treatment for Alzheimer's disease since there is no way to stop the disease. Research on acetylcholinesterase inhibitor is one way to treat Alzheimer's disease and widely used based on the cholinergic hypothesis (Whitmore et al., 1982).

Acetylcholinesterase (AChE) is an enzyme in the central nervous system that catalyses the hydrolysis of neurotransmitter called acetylcholine to choline and acetic acid. Deficiency of acetylcholine in synapses in cerebral cortex led to the most common type of dementia called Alzheimer's disease (Alzheimer's Association, 2013). Thin-Layer Chromatography (TLC) method and microplate assay can be used to screen for AChE inhibitors. TLC method is a qualitative method which is very simple, easy and no disturbance from solvents dissolving

sample as compared to microplate assay. Previous phytochemical studies of *Artocarpus* species had revealed that this genus is rich source of the isoprenoid-substituted phenolic compounds, including flavonoids (Nomura et al., 1998). Many of the compounds have been reported to show interesting biological properties such as antioxidant, antimicrobial, anti-tyrosinase anti-inflammatory activities. Our previous studies also reported the isolation of active flavonoids from the leaves and heartwoods of *A. anisophyllus* and *A. lowii* with significant biological activities (Jamil et al., 2014; Lathiff et al., 2015; Rosdi et al., 2015; Abdullah et al., 2016; Lathiff et al., 2021).

On continuing our research on *Artocarpus* species, this study focused on the potential of the twelve crude extracts and seven isolated flavonoids from the leaves and heartwoods of *A. anisophyllus* and *A. lowii* for their acetylcholinesterase inhibitory activity using qualitative method (TLC).

MATERIALS AND METHODS

Plant materials

The leaves and heartwoods of *A. anisophyllus* (HTBP1568) and *A. lowii* (AZ7094) were collected from Hutan Madek Kahang, Kluang, Johor and Paka, Terengganu, respectively. Both plants were authenticated by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia. All parts of plants were dried for a few weeks before ground to form fine powdered samples.

Tested samples

The crude extracts and flavonoids isolated from the leaves and heartwoods of *A. anisophyllus* and *A. lowii* were screened for their acetylcholinesterase inhibition activity using TLC analysis. The tested flavonoids isolated from *A. anisophyllus* and *A. lowii* were artocarpin (**1**), isobavachalcone (**2**), pyranocycloartobioxanthone A (**3**), 5,7-dihydroxy-4'-methoxy-6-prenylflavanone (**4**), 5-hydroxy-7,8-(2,2-dimethylchromano)-4'-methoxyflavanone (**5**), 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8- γ,γ -dimethylallylflavone (**6**) and 2',4'-dihydroxy-3,4-(2'',2''-dimethylchromeno)-3'-prenyldihydrochalcone (**7**) (Abdullah et al., 2016; Lathiff et al., 2015).

Acetylcholinesterase activity

Chemicals

Buffer A: 50 mM Tris-hydrochloride (pH 8); Substrate: Acetylthiocholine iodide (ATCI); Ellman's reagent: 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB); Enzyme preparation: Acetylcholinesterase from electric eel (type VI-s, lyophilized powder, 518 U/mg solid, 844 U/mg, protein); Positive control: Galantamine hydrobromide; TLC plate: Silica gel 60F₂₅₄, Aluminium sheets, 20×20 cm (Merck).

Procedure using TLC plate

The acetylcholinesterase inhibitory assay was conducted based on Ellman's method with minor modifications (Ellman et al., 1961; Rhee et al., 2003). The crude extracts and flavonoids were dissolved in methanol at a concentration of 2 mg/mL to produce the stock sample. The stock solutions were then serially diluted to varying concentration from 2000 $\mu\text{g/mL}$ to 7.81 $\mu\text{g/mL}$. Sample solution (10 μL) was spotted on the silica gel TLC plate and developed in the solvent system of *n*-hexane:EtOAc with suitable ratio. Galantamine hydrobromide solution was also spotted as reference. Then the TLC plate was sprayed with DTNB/ATCI reagent (1 mM DTNB and 1 mM ATCI in Tris-HCl buffer) until the silica plate was saturated with the reagent. The TLC plate was left to dry in 5-7 min before it was sprayed with enzyme solution. Within five minutes, white dots caused by the inhibitory chemical will appear. The visible white spots were

observed and recorded within 15 min because they will vanish in 20-30 mins. The detection limit for each sample was established using the readily apparent white dots. The term "detection limit" refers to the lowest concentration at which a white spot is visibly present (Rhee et al., 2001).

RESULTS AND DISCUSSION

Extraction and isolation

The dried leaves (2 kg) and heartwoods (2 kg) of *A. anisophyllus* and *A. lowii* were ground and sequentially extracted using *n*-hexane, dichloromethane and ethyl acetate at room temperature for 72h. Evaporation of the solvent using rotary evaporator yielded crude extracts of *n*-hexane, dichloromethane and ethyl acetate. Fractionation and purification of each extract by chromatographic techniques had afforded seven pure flavonoids. Artocarpin (**1**) and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8- γ,γ -dimethylallylflavone (**6**) were isolated from the heartwoods of *A. anisophyllus* and *A. lowii* while isobavachalcone (**2**) was obtained from the leaves of *A. anisophyllus* and *A. lowii*. Pyranocycloartobiloxanthone A (**3**) and 2',4'-dihydroxy-3,4-(2'',2''-dimethylchromeno)-3'-prenyldihydrochalcone (**7**) were isolated from the heartwoods of *A. anisophyllus* and leaves of *A. lowii*, respectively. The purification of *A. anisophyllus* leaves extract had afforded 5,7-dihydroxy-4'-methoxy-6-prenylflavanone (**4**) and 5-hydroxy-7,8-(2,2-dimethylchromano)-4'-methoxyflavanone (**5**). The structural elucidation of these flavonoids were reported in our previous publications (Abdullah et al., 2016; Lathiff et al., 2015).

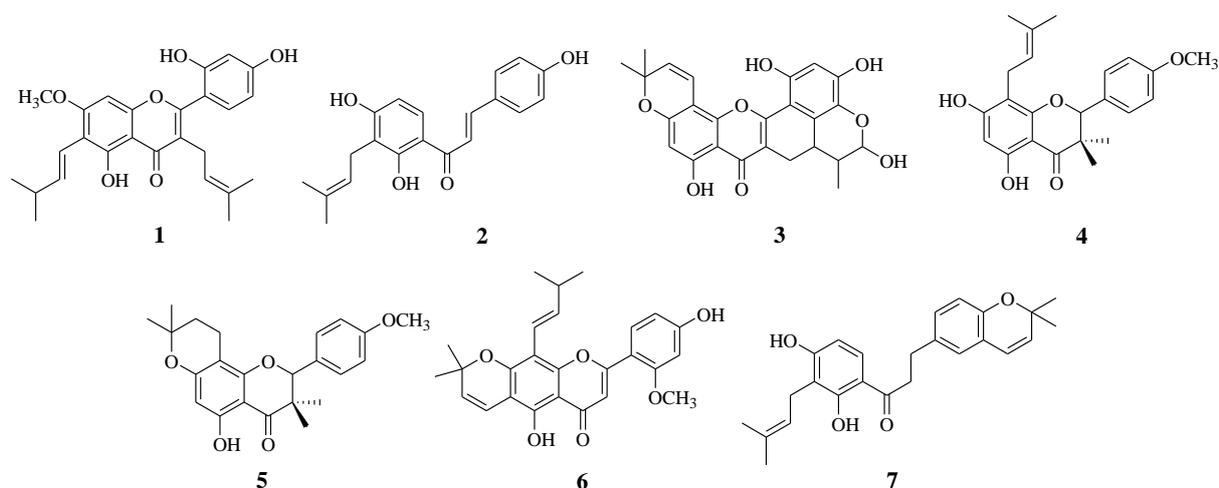


Figure 1. Isolated flavonoids from *A. anisophyllus* and *A. lowii*

Acetylcholinesterase inhibitory activity

AChE inhibitory activity was investigated in this study since *Artocarpus* species is rich in hydroxylated and prenylated flavonoids. Twelve crude extracts and seven flavonoids from the leaves and heartwoods of *A. anisophyllus* and *A. lowii* were screened on AChE inhibition activity using TLC with bioactivity staining based on the Ellman's method that developed by Rhee et al. (2003). Table 1 showed the detection limits of tested samples using TLC assay with silica gel layer. Galantamine hydrobromide was used as a positive control. All crude extracts showed no reaction and the spots were not detected on the silica gel TLC plate except dichloromethane extract of the leaves of *A. lowii* (ALLD). ALLD showed the detection limit at 250 $\mu\text{g/mL}$. Among the isolated flavonoids, pyranocycloartobiloxanthone A (**3**) showed significant acetylcholinesterase inhibitory activity towards the AChE enzyme at the lowest

detection limit same as shown by the positive control, galantamine hydrobromide (7.81 µg/mL). Artocarpin (**1**), 5,7-dihydroxy-4'-methoxy-6-prenylflavanone (**4**) and 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallylflavone (**6**) were inactive at concentration less than 125 µg/mL while 5-hydroxy-7,8-(2,2-dimethylchromano)-4'-methoxyflavanone (**5**) were inactive at concentration less than 62.5 µg/mL.

Table 1. Detection limits of extracts and flavonoids measured using TLC

Extracts	Detection Limit (µg/mL)	Flavonoids	Detection Limit (µg/mL)
AALH	ND	(1)	125
AALD	ND	(2)	500
AALE	ND	(3)	7.81
ALLH	ND	(5)	125
ALLD	250	(6)	62.5
ALLM	ND	(7)	125
AAHH	ND		
AAHD	ND	Positive control	7.81
AAHE	ND	(Galantamine	
ALHH	ND	hydrobromide)	
ALHD	ND		
ALHE	ND		

ND - not detected; AA - *A. anisophyllus*; AL - *A. lowii*; L - leaves; H - heartwoods; H - hexane; D - dichloromethane; E - ethyl acetate

Based on the results obtained, all isolated flavonoids should be further investigated for acetylcholinesterase inhibitory activity for determination of the IC₅₀ value. Even though the TLC assay is a qualitative method, the active compounds can be recognized at an early stage. Beside qualitative TLC assay, both quantitative *in vitro* assay and *in silico* analysis will provide useful information for acetylcholinesterase inhibitory activity. Previous study showed that type and substituents attached to the flavonoid's moiety influenced the acetylcholinesterase inhibition activity. Azman et al. (2020) highlighted that nonpolar substituent at C-3 have better activity towards acetylcholinesterase inhibition compared to hydroxyl group as substituents. Separate study by Vanessa et al. (2021) also supported that the hydrophobic substituents contribute to the π-π interactions while the hydroxyl substituents form hydrogen bond interactions that will improve the acetylcholinesterase inhibition activity.

CONCLUSION

Twelve crude extracts and seven flavonoids from the leaves and heartwoods of *A. anisophyllus* and *A. lowii* were screened for acetylcholinesterase inhibition activity using TLC method. The results showed that pyranocycloartobioxanthone A (**3**) had potential as acetylcholinesterase inhibitor towards AChE enzyme with the lowest detection limit comparable to the standard galantamine hydrobromide with the detection limit value of 7.81 µg/mL. Acetylcholinesterase inhibitory activity of these flavonoids depends on the number and configuration of hydroxyl groups in the molecules and also influenced by configuration of other substituents such as prenyl and methoxyl groups.

Acknowledgement

The authors would like to thank Universiti Teknologi Malaysia for the University Research Grant (RUG) for financially supported this research under vote QJ130000.2526.21H57 and the Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, for providing research facilities.

REFERENCES

- Abdullah, S.A., Jamil, S., Basar, N., Lathiff, S.M.A., & Arriffin, N.M. (2016). Flavonoids from the leaves and heartwoods of *Artocarpus lowii* King and their bioactivities. *Natural Product Research*, 31(10), 1113-1120.
- Azman, N.A.N., Alhawarri, M., Rawa, M.S.A., Dianita, R., Gazzali, A. M., Nogawa, T., & Wahab, H.A. (2020). Potential anti-acetylcholinesterase activity of *Cassia timorensis* DC. *Molecules*, 25(19), 4545-4559.
- Alzheimer's Association. (2013). Alzheimer's disease Facts and Figures. *Alzheimer's & Dementia*, 9, 208-245.
- Ellman, G.L., Courtney, K.D., Andress, V.Jr., & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88-95.
- Giovanni, S.D., Borloz, A., Urbain, A., Marston, A., Hostettmann, K., Carrupt, P.A., & Reist, M. (2008). *In vitro* screening assays to identify natural or synthetic acetylcholinesterase inhibitors: thin layer chromatography versus microplate methods. *European Journal of Pharmaceutical Sciences*, 33, 109-119.
- Jamil, S., Abdullah, S.A., Lathiff, S.M.A., & Sirat, H.M. (2014). Tyrosinase inhibitory activity of flavonoids from *Artocarpus lowii* King. *Jurnal Teknologi*, 71(1), 55-58.
- Jamil, S., Lathiff, S.M.A., Abdullah, S.A., Jemaon, N., & Sirat, H.M. (2014). Antimicrobial flavonoids from *Artocarpus anisophyllus* Miq. and *Artocarpus lowii* King. *Jurnal Teknologi*, 71(1), 95-99.
- Lathiff, S.M.A., Arriffin, N.M., & Jamil, S. (2021). Phytochemicals, pharmacological and ethnomedicinal studies of *Artocarpus*: A scoping review. *Asian Pacific Journal of Tropical Biomedicine*, 11(11), 469-480.
- Lathiff, S.M.A., Jemaon, N., Abdullah, S.A., & Jamil, S. (2015). Flavonoids from *Artocarpus anisophyllus* and their bioactivities. *Natural Products Communications*, 10(3), 393-396.
- Nomura, T., Hano, Y., & Aida, M. (1998). Isoprenoid-substituted flavanoids from *Artocarpus* plants (Moraceae). *Heterocycles*, 47, 1179-205.
- Rhee, I.K., Meent, M.V.D., Ingkaninan, K., & Verpoorte, R. (2001). Screening for acetylcholinesterase inhibitors from amaryllidaceae using thin layer chromatography in combination with bioactivity stain. *Journal of Chromatography A*, 915, 217-223.
- Rhee, I.K., Rijn, R.M.V., & Verpoorte, R. (2003). Qualitative determination of false-positive effects in the acetylcholinesterase assay using thin layer chromatography. *Phytochemical Analysis*, 14, 127-131.
- Rosdi, M.N.M., Sirat, H.M., Abdullah, S.A., Jamil, S., Muhamad, I.I., & Zulkifli, R.M. (2015). Inhibitory effect of *Artocarpus lowii* King compounds on COX-2 and 15-LO activities. *Jurnal Teknologi*, 76(1), 299-302.
- Vanessa, V.V., & Mah, S.H. (2021). Xanthone: Potential acetylcholinesterase inhibitor for Alzheimer's disease treatment. *Medicinal Chemistry*, 21(17), 2507-2529.
- Whitmore, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., & Delon, M.R. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science*, 215, 1237-1239.