Tropical Journal of Pharmaceutical Research November 2015; 14 (11): 2005-2008
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v14i11.8

Original Research Article

Acetyl-cholinesterase Enzyme Inhibitory Effect of *Calophyllum* species

Gwendoline Cheng Lian Ee[1]*, Soek Sin Teh[1], Siau Hui Mah[2], Nur Ain Jamaluddin[1], Muhamad Syarik bin Muhamad Sahimi[1] and Zuraini Ahmad[3]

[1]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, [2]School of Biosciences, Taylors University, Lakeside Campus, 47500 Subang Jaya, [3]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

For correspondence: **Email: gwen@upm.edu.my; **Tel:** +603-8946-6785*

Received: 13 October 2014 *Revised accepted: 29 August 2015*

Abstract

Purpose: *To search for new acetylcholinesterase enzyme inhibitors from Calopyllum species.*
Methods: *Six stem bark extracts of Calophyllum inophyllum, C. soulattri, C. teysmannii, C. lowii, C. benjaminum and C. javanicum were subjected to anti-cholinesterase analysis against acetylcholinesterase (AChE) enzyme using Ellman's method.*
Results: *Most of the extracts showed promising inhibitory activity against AChE at concentrations of 100 µg/mL, with the methanol extract of C. inophyllum demonstrating the strongest inhibitory effect of 81.28 % followed by the methanol extract of C. benjaminum with 74.32 % inhibition. The methanol extracts of Calophyllum inophyllum and Calophyllum bejaminum also showed significant inhibitory activity towards the acetylcholinesterase (AChE) enzyme.*
Conclusion: *Calophyllum species is capable of yielding potential lead compounds for the development for acetyl-cholinesterase enzyme inhibition drugs.*

Keywords: *Calophyllum species, Acetylcholinesterase, Inhibitors*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Alzheimer's disease (AD) which is a neurodegenerative disease commonly affecting elderly people is the result of an irreversible degeneration of cholinergic neurons. Patients lose their memory and cognitive abilities. Rapid hydrolysis by acetylcholinesterase (AChE) enzyme decreases the level of acetylcholine. Therefore, treatment of AD is based on the inhibition of AChE in order to improve cholinergic neurotransmission. Tacrine, a well-known AChE enzyme inhibitor elicits severe side effects to patients such as nausea, insomnia and salivation [1]. Hence, new inhibitors for the disease must be discovered. We chose the species of *Calophyllum*, for our pharmacognosy investigation since these plants are traditionally used as antiseptics, astringents, expectorants, diuretic, purgatives and analgesics [2]. Previous investigations on some *Calophyllum* species have shown them to possess potential pharmaceutical usage such as they indicated cytotoxicity [3], anti-HIV [4], anti-leishmanial [5], antioxidant [6], antibacterial [7], anti-fungal [8] and anti-malarial [9] properties. These promising biological effects of *Calophyllum* species have attracted attention from our research group.

The present study was conducted to evaluate the anti-cholinesterase effects of six *Calophyllum* species, namely, *Calophyllum inophyllum*, *C. soulattri*, *C. teysmannii*, *C. lowii*, *C. benjaminum* and *C. javanicum*.

EXPERIMENTAL

Plant material

Stem bark samples of *Calophyllum inophyllum* (RG 205) were collected from campus grounds in Universiti Putra Malaysia in 2010 while *Calophyllum soulattrii* (RG 202), *Calophyllum tesmannii* (RG208), *Calophyllum lowii* (RG321), *Calophyllum benjaminum* (RG 105) and *Calophyllum javanicum* (RG201) were collected from Sri Aman in Sarawak in 2011. The plant materials were identified by Dr Rusea Go of the Biology Department, Universiti Putra Malaysia where the voucher specimens were kept in the herbarium.

The stem bark of the *Calophyllum* species (except for *C. soulattri*) were extracted using conventional extraction method in which the solvent was added and removed in batches. The dried and powdered samples were extracted in n-hexane (Hex), ethyl acetate (EA) and methanol (MeOH) in a polarity-increasing order. The solvent was decanted and replaced with new solvent after 3 days. The extracts were dried under reduced pressure using a rotary evaporator to yield the hexane, ethyl acetate and methanol extracts, respectively.

The air-dried stem bark of *Calophyllum soulattri* was ground to a fine powder and extracted successively in a Soxhlet apparatus with n-hexane (60–80 °C, 3 × 2 L), dichloromethane (40 °C, 3 × 2 L), ethyl acetate (77 °C, 3 × 2 L) and methanol (65 °C, 3 × 2 L) for 24 h. The extracts were evaporated to dryness under vacuum to give n-hexane, ethyl acetate and methanol extracts. The weights of the extracts obtained from the six *Calophyllum* species are tabulated in Table 1.

AChE inhibitory activity assay

AChE inhibitory activity was measured using Ellman's method with some modifications. Acetylcholinesterase enzyme (AChE) from electric eel (Type-VI-S, C3389, Sigma) was used, while acetylthiocholine iodide (ATCI, Sigma) was employed as a substrate for the reaction. 5,5´-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma) was used for the measurement of anti-AChE activity.

Hydrolysis of ATCI was monitored by observation of the formation of the yellow 5-thio-2-nitrobenzoate anion, a result of the reaction of DTNB with thiocholines which is catalyzed by the enzyme at a wavelength of 412 nm. Every well was initially filled with 210 µL (0.15 mM/well) DTNB solution. Samples of 20 µL volume (100 µg/mL for crude extracts and serial dilutions for pure compounds - 100, 50, 25, 12.5, 6.25 µM) was added in triplicates and the control wells were filled with 20 µL (0.10 M, pH 7.4) of phosphate buffer, followed by 20 µL (0.037 U/mL per well) of AChE. The plate was then incubated for 10 minutes at 37 °C. Thereafter, 20 µL (0.25 µM/well) of ATCI were added into each well to initiate the hydrolysis reaction. The plate was immediately shaken for 2 sec and measured at 412 nm at 25 °C for 10 times consecutively. The experiments were carried out in triplicate. Tacrine (Sigma) was used as the reference drug. The percentage of inhibition was calculated as in Eq 1.

Inhibition (%) = {(A-B)/A}100 …… (1)

where A = average of (control absorbance – colour absorbance) and B is the average of (sample absorbance – colour absorbance); colour absorbance is the absorbance without the addition of AChE and ATCI.

Statistical analysis

The data are expressed as mean ± standard deviation (SD). Statistical analysis was carried out by paired t-test.

Table 1: Yield of extracts obtained from six *Calophyllum* species

***Calophyllum* species**	**Sample weight (kg)**	**Extract (g)**		
		n-Hexane	***Ethyl acetate***	***Methanol***
C. inophyllum	3.0	80.7	40.1	60.9
C. soulattri	1.0	101.2	32.4	97.3
C. teysmannii	1.5	52.4	68.1	30.0
C. lowii	1.5	50.8	47.6	40.5
C. benjaminum	3.0	22.3	11.0	221.3
C. javanicum	2.5	26.0	15.6	260.0

The level of significance used was $p < 0.05$. The statistical software used was Graph Pad Prism 5.

RESULTS

Non-aqueous extracts of six *Calophyllum* species, *Calophyllum inophyllum, C. soulattri, C. teysmannii, C. lowii, C. benjaminum* and *C. javanicum* were evaluated for their anti-AChE activity by Ellman's method. Results of the percentage inhibition of AChE are summarized in Table 2 and Figure 1. Overall, these extracts of *Calopyllum* spp. showed promising inhibitory effects against AChE at concentration of 100 µg/mL. Among the extracts, the methanolic extract of *C. inophyllum* demonstrated the strongest inhibitory activity with 81.28 % inhibition. This is followed by the methanolic extract of *C. benjaminum* and the hexane extract of *C. soulattri* which gave 74.32 % and 70.45 % inhibitions, respectively. All the percentage of inhibition extracts were statistically different at $p < 0.05$ from control.

DISCUSSION

The treatment of AD is mainly focused on the improvement of cholinergic neurotransmission by inhibiting AChE, which is the enzyme that catalyzes the hydrolysis of acetylcholine (ACh). Cholinesterase inhibitors reduce the degradation of ACh, which is a neurotransmitter in the synapses of the nervous system. In other words, AChE inhibitors diminish the formation of amyloid fibrils hence increases the neuroprotective activity. The hydrolysis reaction takes place by nucleophilic attack on the carbonyl carbon followed by acylation of the enzyme and the release of choline. It is followed by hydrolysis of the acylated enzyme to produce acetic acid and

Table 2: Acetyl-cholinesterase enzyme inhibition of extracts of six *Calophyllum* species

Plant species	Crude extract	Inhibition (%) at 100 µg/mL
C. inophyllum (CI)	Hexane	34.07 ± 2.67
	Ethyl acetate	42.94 ± 2.16
	Methanol	81.28 ± 3.20
C. soulattri (CS)	Hexane	70.45 ± 1.05
	Ethyl acetate	47.10 ± 1.27
	Methanol	54.30 ± 2.78
C. lowii (CL)	Hexane	63.05 ± 2.06
	Ethyl acetate	58.12 ± 1.57
	Methanol	46.14 ± 2.43
C. teysmannii (CT)	Hexane	50.19 ± 1.09
	Ethyl acetate	42.64 ± 2.14
	Methanol	51.87 ± 2.62
C. benjaminum (CB)	Hexane	54.68 ± 0.65
	Ethyl acetate	38.74 ± 2.97
	Methanol	74.32 ± 2.41
C. javanicum (CJ)	Hexane	42.88 ± 3.25
	Ethyl acetate	45.42 ± 2.69
	Methanol	60.62 ± 2.09

**Each data point represents mean ± SD (n = 3)*

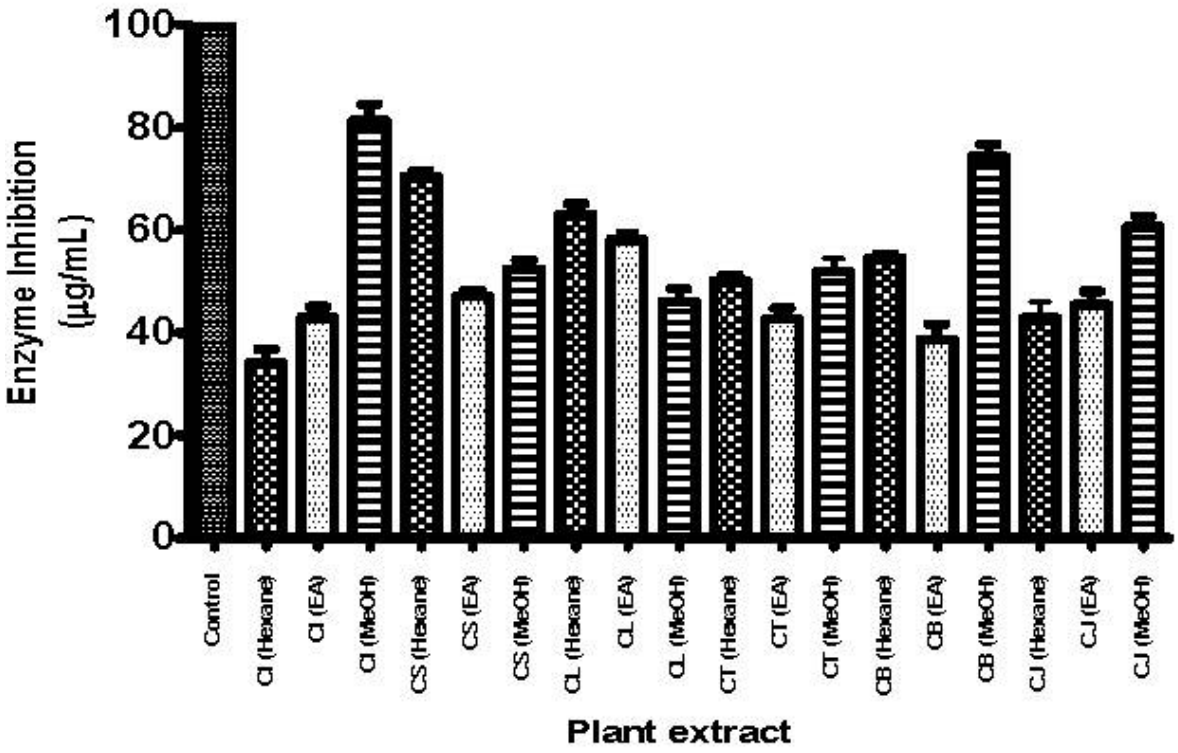

Figure 1: Inhibition of AChE by the extracts (100 µg/mL). **Each data point represents the mean ± SD (n = 3); error bars denote significant difference (p < 0.05)*

the enzyme which is in the original state. New AChE inhibitors are concerned with measuring their activity by using Ellman's method.

The AChE inhibition activities of *Calophyllum* species showed no correlation with the polarity of the solvents, which are used to extract the plant samples. For *C. inophyllum, C. benjaminum* and *C. javanicum*, the activities of the enzyme inhibition indicated a positive correlation to the polarity of the solvent used to extract the plants. The inhibition effects of these plants are directly proportional to the polarity of the non-aqueous extracts. In contrast, the activities of the non-aqueous extracts of *C. soulattri* and *C. lowii* are inversely proportional to the polarity of their extracts. Besides this, *C. teysmannii* showed no correlation with AChE inhibition.

To the best of our knowledge, the anti-AChE activity of *Calophyllum* species has not previously been investigated. This is therefore the first report and the outcome of our work has shown the first data on anti-AChE activity of this genus. *Calophyllum* species have been previously reported for the presence of diverse secondary metabolites such as xanthones [10], coumarins [11], triterpenes [12] and chromanones [13]. AChE inhibitory effects, as described, are predicted to be due to the plant metabolites mentioned. Consequently, a further study on the detailed phytoconstituent investigation of these potential plant extracts is worthwhile to obtain the plant metabolites which correspond to or are responsible for the anti-AChE activities.

CONCLUSION

The methanol extracts of *Calophyllum inophyllum* and *Calophyllum bejaminum* show significant inhibitory effects against acetylcholinesterase (AChE). *Calophyllum* species can yield potential lead compounds for the development of acetyl-cholinesterase enzyme inhibition drugs.

ACKNOWLEDGEMENT

The authors acknowledge financial support from UPM and MOSTI under the Agri Science Fund and as well as from SBC.

REFERENCES

1. *Yoshida S, Suzuki N. Antiamnesic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in rats. Eur J Pharmacol 1993; 250 (1): 117-124.*
2. *Ali MS, Mahmud S, Perveen S, Ahmad VU, Rizwani GH. Epimers from the leaves of Calophyllum inophyllum. Phytochemistry 1999; 50: 1385-1389.*
3. *Mah SH, Ee GCL, Teh SS, Rahmani M, Lim YM, Go R. Phylattrin, a New Cytotoxic Xanthone from Calophyllum soulattri. Molecules 2012; 17 (7): 8303-8311.*
4. *Spino C, Dodier M, Sotheeswaran S. Anti-HIV coumarins from Calophyllum seed oil. Bioorg Med Chem Lett 1998; 8: 3475-3478.*
5. *Brenzan MA, Nakamura CV, Prado DFB, Ueda-Nakamura T, Young MC, Aparicio GCD. Antileishmanial activity of crude extract and coumarin from Calophyllum brasiliense leaves against Leishmania amazonensis. Parasitol Res 2007; 101 (3): 715-722.*
6. *Taher M, Attoumani N, Susanti D, Ichwan SJA, Ahmad F. Antioxidant Activity of Leaves of Calophyllum rubiginosum. Am J Appl Sci 2010; 7 (10): 1305-1309.*
7. *Sakagami Y, Kajimura K, Wijesinghe WM, Dharmaratne HR. Antibacterial activity of calozeyloxanthone isolated from Calophyllum species against vancomycin-resistant Enterococci (VRE) and synergism with antibiotics. Planta Med 2002; 68 (6): 541-543.*
8. *Hay AE, Guilet D, Morel C, Larcher G, Macherel D, LeRay AM, Litaudon M, Richomme P. Antifungal chromans inhibiting the mitochondrial respiratory chain of pea seeds and new xanthones from Calophyllum caledonicum. Planta Med 2003; 69 (12): 1130-1135.*
9. *Hay AE, Helesbeux JJ, Duval O, Labaied M, Grellier P, Richomme P. Antimalarial xanthones from Calophyllum caledonicum and Garcinia vieillardii. Life Sci 2004; 75 (25): 3077-3085.*
10. *Somanathan R, Sultanbawa S, Uvais M. Chemical investigation of Ceylonese plants. Part VIII. Trapezifolixanthone, a new di-isoprenylated xanthone from the bark of Calophyllum trapezifolium Thw. (Guttiferae). J Chem Soc Perkin 1 1974: 2515-2517.*
11. *Nigam SK, Mitra CR. Constituents of Calophyllum tomentosum and Calophyllum apetalum nuts: structure of a new 4-alkyl- and of two new 4-phenyl-coumarins. Planta Med 1968; 16 (4): 450-457.*
12. *Govindachari TR, Viswanathan N, Pai BR, Rao R, Srinivasan M. Triterpenes of Calophyllum inophyllum Linn. Tetrahedron 1967; 23 (4): 1901-1910.*
13. *Wang H, Sun QY, Yang FM, Long CL,Wang YH, Tang GH, Zhao FW, Niu HM, Huang QQ, Xu JJ, Wataya Y, Ma LJ. Chromanone Derivatives from the Pericarps of Calophyllum polyanthum. Helv Chim Acta 2010; 93: 2183-2188.*