Original Article

Thai Journal of Pharmaceutical Sciences

Screening of acetylcholinesterase inhibitory activity in essential oil from Myrtaceae

Tipsara Petrachaianan, Saran Chaiyasirisuwan, Sirivan Athikomkulchai, Vipaporn Sareedenchai

Department of Pharmacognosy, Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhonnayok 26120, Thailand

Corresponding Author:
Vipaporn Sareedenchai, Department of Pharmacognosy, Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhonnayok 26120, Thailand. Tel: (037)395094-5. Fax: (037)395096.
E-mail: vsareedenchai@yahoo.com

Received: May 25, 2018
Accepted: Oct 11, 2018
Published: Dec 30, 2018

ABSTRACT

Objective: This research was to investigate acetylcholinesterase inhibitory (AChEI) activity of the essential oil from Myrtaceae and its components. **Materials and Methods:** The essential oils were extracted from a fresh leave of Myrtaceae plants: *Eucalyptus globulus* Labill (Eucalyptus), *Melaleuca cajuputi* Powell (Samed-Khao), *Melaleuca citrina* (Curtis) Dum. Cours (Bottlebrush tree), *Psidium guajava* Linn. (Gauva), *Syzygium cumini* (L.) Skeel (Wha), and *Syzygium samarangense* (Blume) Merr. and L. M. Perry (Chompoo Nam Dok Mai) by hydrodistillation and analyzed chemical constituent by gas chromatography. The AChE inhibition was determined based on Ellman's method. **Results:** The essential oil from *M. citrina* presented the greatest inhibitory activity (71.77 ± 2.11%) and followed by *E. globulus* (47.65 ± 2.26%), *P. guajava* (24.96 ± 2.38%), *M. cajuputi* (21.18 ± 0.54%), *S. cumini* (19.97 ± 1.10%), and *S. samarangense* (13.78 ± 1.52%), respectively. Alpha-pinene was found in the essential oil of 6 species. 1,8-Cineole was the main compound of the essential oil from *M. citrina* and acts as an active constituent on AChEI. The essential oil from *M. cajuputi*, *S. cumini,* and *S. samarangense* contained a small amount of α-pinene, and without 1,8-cineole, then they were less potency on AChEI. **Conclusion:** The essential oil containing higher amount of 1,8-cineole presented strong activity to inhibit the AChE. Moreover, there are also other constituents in the essential oils that may affect the AChEI activity and also present synergistic effect for AChEI.

Keywords: 1,8-Cineole, acetylcholinesterase inhibitor, essential oil, myrtaceae

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease of cholinergic neurons, accompanied by loss of the neurotransmitter acetylcholine (ACh). Acetylcholine is involved in the regulation of cognitive function. The causes of AD are still unknown, but it was found in clinical studies that by giving acetylcholinesterase inhibitors (AChEI) to patients resulted in the increase of ACh in the central nervous system. The used of AChEI is one of the methods to prevent ACh from breaking down the process, consequently delaying disease progression.[1,2]

Several herbs has been evaluated for their biological activity on AChEI. There have been a lot of research, searching a new compounds for management the AD.[3-5] Some of Amaryllidaceae alkaloids were reported for AChEI activity. Galantamine, alkaloids, from the bulb of *Galanthus caucasicus,* was licensed as a medicine for treating AD. Furthermore, numerous essential oils and their monoterpene constituents were tested for AChEI. The essential oil of *Salvia lavandulaefolia* and its terpenoinds, composing of 1,8-cineole, camphor, α-pinene, α-pinene, borneol, caryophyllene oxide, linalool, and bornyl acetate, was investigated an activity of AChE inhibition. The studies indicated that the major compounds of essential oils, 1,8-cineole, α-pinene, and α-pinene, presented a potency on AChEI.[6-8] Thymoquinone constituent from the essential oil of *Thymus vulgaris* L. exhibited the strongest AChEI.[9] The commercial essential oils, *Artemisia dracunculus* L., *Inula graveolens* L., *Lavandula officinalis* Chaix, and *Ocimum sanctum* L. inhibited AChE activity. The terpenoids from the essential oil were investigated. 1,8-cineole, α-pinene, eugenol, α-terpineol, and terpinene-4-ol were showed AChEI.[10] The extracted and essential oil from *Satureja thymbra* L. showed an activity on AChEI. Carvacrol was a major component of essential oil but less potency on AChEI than thymol.[11] The 5 essential oils from the commercial, Eucalyptus, Cajuput, Sweet Marjoram, Camphor, and Rosemary, composing 1,8-cineole, showed a potency on AChEI.[12] Moreover, the essential oil from *Salvia*

lavandulaefolia inhibits the acetylcholinesterase in vivo. The essential oil of *S. lavandulaefolia* was administered orally in rat for 5 days. The cortex, hippocampus and striatum were dissected and evaluated AChE activity. Acetylcholinesterase activity was decrease in the striatum and hippocampus but no change in the cortex of the rat brain. That was supported, the constituents of essential oil reach the brain and inhibit AChE in specific areas.[13] Many studies on essential oil indicated that 1,8-cineole and α-pinene are strong inhibitors of AChE. 1,8-cineole and α-pinene can be found in the various genus of Myrtaceae; Eucalypts, Melaleucas, or Leptospermums.[14]

Myrtaceae originate from the central and south of America. They distribute in tropical and subtropical regions. Myrtaceae plant, shrub or tree, is the accumulation of essential oils in schizogenic glands. Myrtaceae is an important economic in the product from timber, fruits, spices, and essential oil. The essential oil from the member of Myrtaceae was enriched in terpene; 1, 8-cineole, α-pinene, limonene, linalool, and terpinen-4-ol, etc. For this reasons, the leaves, flowers, and fruits, provided essential oils, used for several purposes as a food, medicine, spice, or fragrance.[15,16] The objective of this study was to investigate AChEI activity form the essential oil of Myrtaceae species which was grown in Thailand and its components using gas chromatography (GC).

MATERIALS AND METHODS

Plant Materials and Distillation of Essential Oils

Fresh plant of 5 species, *Eucalyptus globulus* Labill (Eucalyptus), *Melaleuca cajuputi* Powell (Samed-Khao), *Melaleuca citrina* (Curtis) Dum. Cours (Bottle brush tree), *Psidium guajava* Linn. (Gauva), *Syzygium cumini* (L.) Skeel (Wha), and *Syzygium samarangense* (Blume) Merr and L.M. Perry, were collected from Nakhonnayok. Only *M. citrina* (Curtis) Dum. Cours (Bottle brush tree) was collected from Nakhon Si Thammarat. The voucher specimen of *M. cajuputi* was kept at Faculty of Pharmacy, Srinakharinwirot University. Fresh leaves of 6 species were cleaned and cut into small piece. The essential oils were extracted from the fresh leave by hydrodistillation with Clevenger apparatus for 4 h. Anhydrous sodium sulfate was added in the essential oils for eliminate water. They were kept in an amber bottle and stored at 4°C for analyzed by gas chromatography–mass spectrometry (GC-MS).

GC-MS

The essential oil was analyzed the component using GC on a Finnigan Trace GC-MS ultra (Thermo Electron Corporation, USA). The detector was Finnigan DSQ Quadrupole MS. The column was BPX5 fused silica column (30 m × 0.25 mm, 0.25 uM film thickness). The carrier gas was Helium, flown in 1 ml/min. The injector temperature was 180°C. 1 μl of sample was injected by splitter (1:100). The oven temperature programming was 60°C for 1 min. then ramp to 240°C with the rate of 3°C/min. MS was performed by EI positive mode at 70 eV ionization voltages. The compounds were identified by comparing by matching their mass spectra and retention indices with Adams EO Mass Spectral library and NIST05 Mass Spectral library.

AChE Inhibition Assay

The AChEI was determined in a 96-well plate assay based on the colorimetric Ellman's method (1961)[17] and modified method from Salah and Jäger.[18] In briefly, 25 μl of 1.5 mM acetylcholine iodide, 25 μl of sample, and 125 μl of 3mM of 5,5'-dinitrobis-2-nitrobezoic acid in 50 μl of 50 mMTris/HCl buffer pH8 were added in 96 well plates. Each sample was dissolve with methanol to 10 mg/ml for stock solution, diluted with buffer to 1 mg/ml. 1,8-Cineol and essential oil from *M. citrina* were prepared in various concentration (1.0–0.25 mg/ml) for investigated IC_{50}. The absorbance was measured in kinetics mode (OD/min), every 13 s for five cycles, by Anthos Zenyth, model 200 RT at 405 nm. Then, 25 μl of 0.20 U/ml AChE was added in the reaction. The hydrolysis of acetylcholine iodide was monitor by the formation of the 5-thio-2-nitrobenzoate which was measured again every 13 s for eight cycles. The reactions were performed in triplicate. 1,8-Cineole was used as positive control. The inhibitory activity was calculated from this formula.

$$\% \text{ Inhibition} = [1 - \frac{\text{OD Sample}}{\text{OD Control}}] \times 100$$

RESULTS AND DISCUSSION

The essential oil was obtained by hydrodistillation of the fresh leave of plant. The yield of essential oils was shown in Table 1.

Table 1: The inhibition activity on AChE of the essential oils *E. globulus*, *M. cacajuputi, M. citrina, P. guajava, S. cumini, S. samarangense*, and 1,8-cineol, final concentration at 0.10 mg/ml (n=3)

Sample	% yield v/w	% Inhibition±SD at 0.10 mg/ml	IC_{50} (mg/ml)
Eucalyptus globulus	0.40	47.65±2.26	NE
Melaleuca cajuputi	0.31	21.18±0.54	NE
Melaleuca citrina	0.18	71.77±2.11	0.037
Psidium guajava	0.19	24.96±2.38	NE
Syzygium cumini	0.24	19.97±1.10	NE
Syzygium samarangense	0.08	13.78±1.52	NE
1,8-cineol	-	66.18±0.53	0.052

NE: Non evaluated. *E. globulus: Eucalyptus globulus, M. cajuputi: Melaleuca cajuputi, M. citrine: Melaleuca citrine, P. guajava: Psidium guajava, S. cumini: Syzygium cumini, S. samarangense: Syzygium samarangense.* AChE: Acetylcholinesterase

E. globulus provided the highest yield of essential oil at 0.40% v/w, followed by essential oil from *M. cajuputi* (0.31%), *S. cumini* (0.24%), *P. guajava* (0.19%), and *M. citrina* (0.18%). The lowest yield was found in *S. samarangense* (0.08%). The chemical composition of essential oil was analyzed by GC-MS. The essential oil composed various constituents [Table 2] and the GC chromatograms of essential oil were shown in Figure 1. The essential oil was consisted of a mixture of a monoterpene, sesquiterpene, and phenolic compounds. They were in a various functional groups such as acid, alcohol, aldehydes, ester, ketones, or lactones. The major compounds (>10%) of each essential oil were found as the following: *E. globulus* were γ-terpinene and *p*-cymene; *M. cajuputi* were terpinolene, γ-terpinene and *E*-caryophyllene; *M. citrina* were 1,8-cineole, and α-pinene; *P. guajava* was limonene and α-pinene; *S. cumini* were terpinolene, γ-terpinene and *E*-caryophyllene; and *S. samarangense* was *o*-cymene. All major components were monoterpene; only *E*-caryophyllene was bicyclic sesquiterpene. Alpha-pinene was found all 6 species and had been report AChEI, but it was less potency than

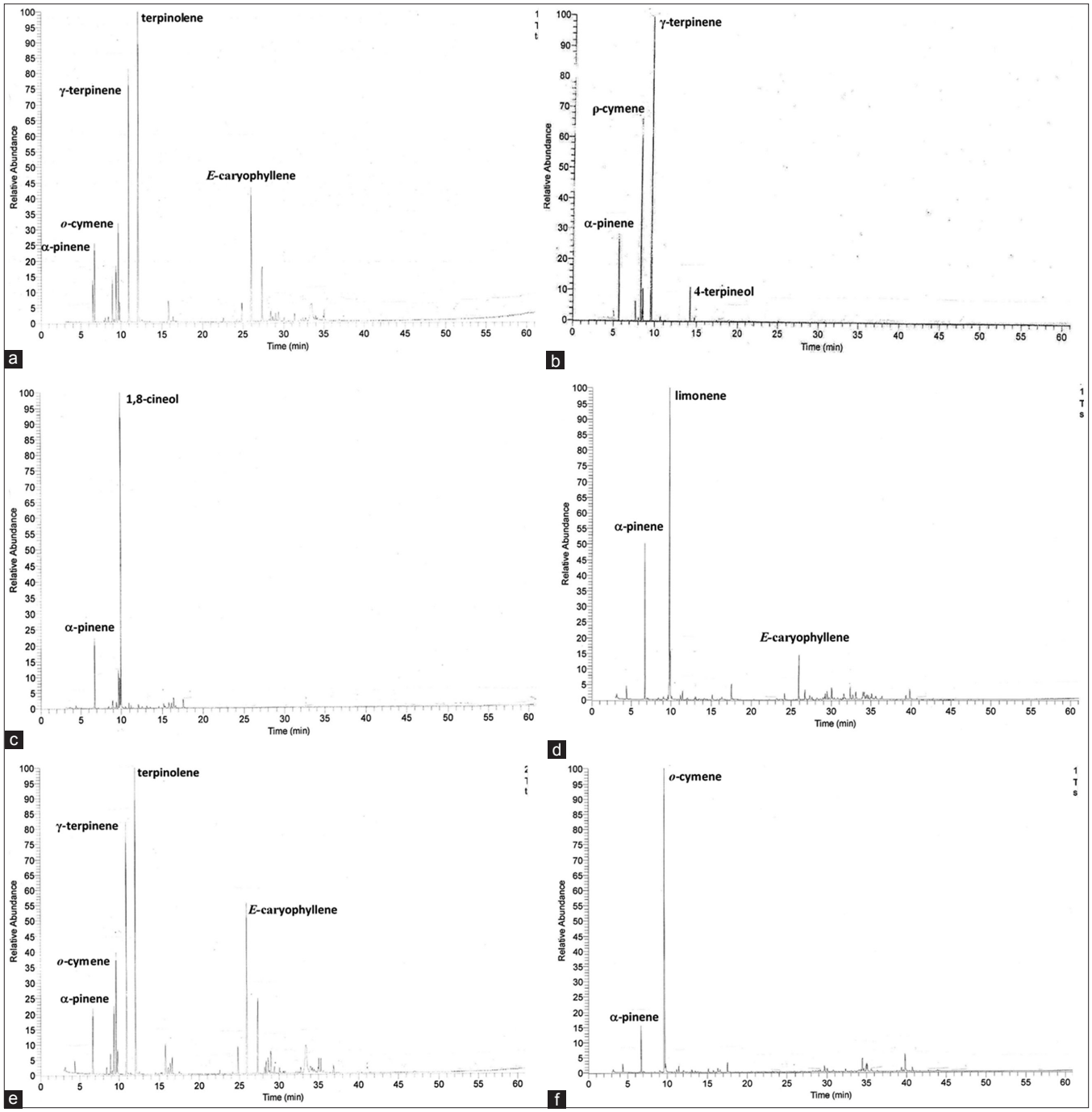

Figure 1: Gas chromatography of essential oil from (a) *Eucalyptus globulus*, (b) *Melaleuca cajuputi*, (c) *Melaleuca citrina*, (d) *Psidium guajava*, (e) *Syzygium cumini,* and (f) *Syzygium samarangense*

Table 2: The chemical constituent of the essential oils from Myrtaceae plant by GC-MS.

Compound	Area percentage (%)					
	E. globulus	***M. cajuputi***	***M. citrina***	***P. guajava***	***S. cumini***	***S. samarangense***
α-Amorphene		1.16			0.85	
Aromadendrene				1.88		
β-Bisabolene				1.46		
α-Bisabolene				1.02		
Bornyl Chloride			1.23	0.77		1.11
γ-Cadinene						1.64
epi-α-Cadinol						4.30
Camphene			0.11			
1,8-Cineole	4.48		58.06	7.37		
E-Caryophyllene		12.22		7.22	12.25	
Trans-Calamenene				2.70		1.03
o-Cymene		7.34	7.56	0.60	7.34	54.33
p-Cymene	28.75					
β-Elemene		1.65			2.02	
Globulol				1.46		
α-Humulene		4.92		0.77	5.58	
Limonene		1.85	6.23	36.55	1.69	2.79
epi-α-Muurolol						2.07
E-Nerolidol				1.48		
α-Phellandrene	2.78	2.66	1.68		1.14	
α- Pinene	8.57	4.15	10.98	14.70	3.41	7.51
β- Pinene	0.09	8.03	0.66			
Piperitone	0.31					
Platyphyllos		7.50			9.69	
D-sylvestrene	2.00					
α-Terpinene	0.41	3.48	1.14		3.82	
γ-Terpinene	44.60	17.81			16.63	
4-Terpineol	5.42	2.02	1.45		2.47	0.57
α-Terpineol	0.55	0.75	2.69	0.65	1.19	
α-Terpineol-acetate			2.03		0.34	
Terpinolene	0.82	21.61			19.08	0.49
α-Thujene		2.46				
Thymol methyl ether						1.19

E. globulus: Eucalyptus globulus, M. cajuputi: Melaleuca cajuputi, M. citrine: Melaleuca citrine, P. guajava: Psidium guajava, S. cumini: Syzygium cumini, S. samarangense: Syzygium samarangense, GC-MS: Gas chromatography–mass spectrometry

1,8-cineole.[8,10] *Eucalyptus* spp. and *Melaleuca* spp. are enrich in 1,8-cineole[10]. This resulted, 1,8-cineole was found only from *M. citrina*, *E. globulus,* and *P. guajava*. *E*-caryophyllene had been report as a main component of Thai *P. guajava*.[20] This contrasting pattern of the result, *E*-caryophyllene was found a small amount in the essential oil of *P. guajava*.

The essential oils from six species were tested on various concentration in AChEI assay which was limited the final concentration at 0.1 mg/ml. The essential oil was not possible to test in the higher concentrations than 0.1 mg/ml cause the appearance of turbidity in the test solution. All essential oils from this studying inhibited AChE [Table 1]. At 0.1 mg/ml, the essential oil of *M. citrina* showed a high potency on AChEI activity (71.77 ± 2.11%), followed with *E. globulus* (47.65 ± 2.26%), *P. guajava* (24.96 ± 2.38%), *M. cajuputi* (21.18 ± 0.54%), *S. cumini* (19.97 ± 1.10%), and *S. samarangense* (13.78 ± 1.52%). They compared with 1,8-cineole [Figure 2]. The essential oil from *Syzygium* spp. was less activity to inhibit AChE. Furthermore, the essential oil of *M. citrina* and 1,8-cineole was investigated IC_{50} values and showed 0.037 mg/ml and 0.052 mg/ml, respectively [Table 1]. Phrompittiyarat *et al*. (2014) investigated AChEI

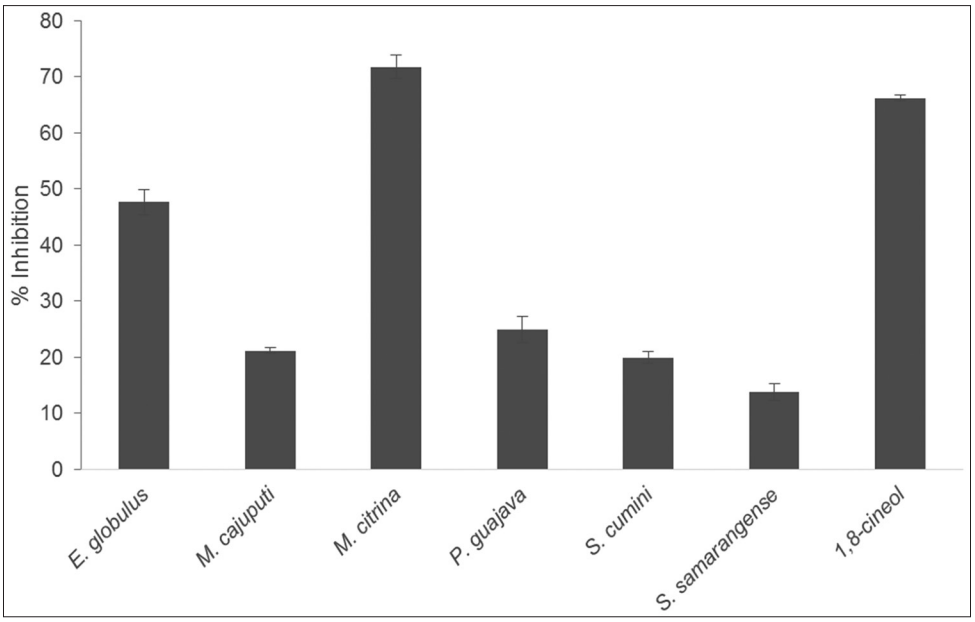

Figure 2: The inhibition acetylcholinesterase activity of essential oils from *Eucalyptus globulus*, *Melaleuca cacajuputi*, *Melaleuca citrina, Psidium guajava*, *Syzygium cumini*, *Syzygium samarangense,* and 1, 8 cineole at 0.10 mg/ml (n=3)

activity from the commercial essential oil. Eucalyptus oil, cajuput oil, and sweet majoram oil showed the potency on AChEI at 68.49%, 68.68%, and 63.51%, respectively, and they performed a high content of 1,8-cineole at 83.30%, 70.16%, and 63.51%, respectively, while the current study, eucalyptus oil and cajuput oil from distillation presented an AChEI activity at 47.65±2.26% and 21.18%. The essential oil from *E. globulus* contained 1,8-cineole only 4.48% and *M. cajuputi* do not show any 1,8-cineole.[12] This has been confirm that the essential oil containing higher amount of 1,8-cineole showed potency on AChEI. The essential oil from *M. citrina* had 1,8-cineole and α-pinene as a main constituent and presented higher inhibition activity on AChE. The essential oils of *M. cajuputi*, *S. samarangense,* and *S. cumini* showed weak activity in AChEI, due to absent of 1,8-cineole. Then they presented less potency to inhibit on AChE. The essential oil from *E. globulus* had γ-terpinene and *p*-cymene as the main constituent but composed small amounts of 1,8-cineole. Even though the essential oil from *E. globulus* contained 1,8-cineole (4.48%), small amount than the essential oil from *P. guajava* (7.37%) but it presented moderate AChEI activity (47.65%). Gamma-terpinene had been report AChEI activity[11] and showed a competitive inhibitor on AChE,[16] but *p*-cymene did not present any inhibition on AChE[11]. Gamma-terpinene, α-pinene, and 1,8-cineole responded for AChEI in the essential oil of *E. globulus*. The essential oil from *P. guajava* had limonene and α-pinene and contained a small amount of 1,8-cineole (7.37%). Miyazawa *et al*. (1997) studied AChEI activity on monoterpene. At 1.2 mM, (−)-limonene and (+)-limonene inhibited AChE at 25% and 22%, respectively, they showed less potency on AChEI[19]. The AChEI activity of the essential oil from *P. guajava* should be from α-pinene, 1,8-cineole, and limonene. *E*-caryophyllene, *o*-cymene, and terpinolene had not been report any AChEI activity; then they should be further study on AChEI activity. The essential oil contained 1,8-cineole which present a potency on the inhibition of AChE. Savelev *et al*. had been investigate the AChEI activity by combinations of terpenoids. The synergy was apparent in 1,8-cineole/α-pinene and 1,8-cineole/caryophyllene oxide. Antagonism was found in 1,8-cineole/camphor. The AChEI activity of essential oil resulted from the complex interaction between its constituents cause synergistic and antagonistic activity.[8] The components on essential oil are important on the biological activity of the plant. Not only major compound but also minor chemical constituent in essential oil influence on their activity. They could presented a synergist or an antagonist to AChEI activity. In addition, the synergism or antagonism of the substance from essential oil should be further investigated.

CONCLUSION

The essential oil from Myrtaceae contained higher constituent of 1,8-cineole that presents potency on AChEI. Moreover, there are also other constituents in the essential oils that may affect the AChEI activity by a synergistic and antagonistic activity.

ACKNOWLEDGMENT

The work was granted by Srinakharinwirot University (No.079/2557).

REFERENCES

1. Munoz-Torrero D. Acetylcholinesterase inhibitors as a disease-modifying therapies for Alzheimer's disease. Curr Med Chem 2008;15:2433-55.
2. Perry E, Howes MJ. Medicinal plants and dementia therapy: Herbal hopes for brain aging? CNS Neurosci Ther 2011;17:683-98.
3. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J Ethnopharmacol 2003;89:261-4.
4. Ferreira A, Proença C, Serralheiro ML, Araújo ME. The *in vitro*

screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J Ethnopharmacol 2006;108:31-7.
5. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine 2007;14:289-300.
6. Perry NS, Houghton PJ, Theobald A, Jenner P, Perry EK. *In-vitro* inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. J Pharm Pharm 2000;52:895-902.
7. Perry NS, Bollen C, Perry EK, Ballard C. Salvia for dementia therapy: Review of pharmacological activity and pilot tolerability clinical trial. Pharm Biochem Behav 2003;75:651-9.
8. Savelev S, Okello E, Perry NS, Wilkins RM, Perry EK. Synergistic and antagonistic interactions of anticholinesterase terpenoids in salvia lavandulaefolia essential oil. Pharmacol Biochem Behav 2003;75:661-8.
9. Jukic M, Politeo O, Maksimovic M, Milos M, Milos M. *In vitro* acetylcholinesterase inhibitory properties of thymol, carvacrol and their derivatives thymoquinone and thymohydroquinone. Phytother Res 2007;21:259-61.
10. Dohi S, Terasaki M, Makino M. Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. J Agric Food Chem 2009;57:4313-8.
11. Özturk M. Anticholinesterase and antioxidant activities of Savoury (*Sature jathymbra* L.) with identified major terpenes of the essential oil. Food Chem 2012;134:48-54.
12. Phompittayarat W, Hongratanaworakit T, Tadtong S, Sareedenchai V, Ingkaninan K. Survey of acetylcholinerease inhibitory activity in essential oil derived from aromatic plants. Int J Med Arom Plants 2014;4:1-5.
13. Perry NS, Houghton PJ, Jenner P, Keith A, Perry EK. Salvia lavandulaefolia essential oil inhibits cholinesterase *in vivo*. Phytomedicine 2002;9:48-51.
14. Cheryll W. Medicinal Plant in Australia Gums, Resins, Tannin and Essential Oils. 1st ed. Vol. 2. New South Wales: Rosenberg Publishing; 2011.
15. Cock IE. Medicinal and Aromatic Plants-Australia in Ethnopharmacology section, Biological, Physiological and Health Science, Encyclopedia of Life Support Systems (EOLSS), Developed Under the Auspices of the UNESCO, Oxford, UK: EOLSS Publishers; 2011.
16. Brophy JJ, Craven LA, Doran JC. *Melaleucas*. Their Botany, essential Oils and Uses. Australia: Can Print Communication; 2013.
17. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharm 1961;7:88-95.
18. Salah SM, Jäger AK. Screening of traditionally used Lebanese herbs for neurological activities. J Ethnopharmacol 2005;97:145-9.
19. Miyazawa M, Watanabe H, Kameoka H. Inhibition of acetylcholinesterase activity by monoterpenenoids with a –Menthane skeleton. J. Agric Food Chem 1997;45:677-9.
20. Prapasanobon D, Intuprapa P. Thai Essential Oil. Vol. 1. Pathum Thani: Thailand Institute of Scientific and Technological Research (TISTR); 2005. p. 56-9.