RESEARCH PAPER

Anticholinesterase activity of essential oils and their constituents from Thai medicinal plants in human neuroblastoma SK-N-SH cells

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Abstract

Essential oils had strong potency to inhibit acetylcholinesterase in the brain because of their lipophilicity and small molecular size of the constituents. The anticholinesterase activity of thirteen essential oils from Thai medicinal plants was investigated. The inhibitory activity of the oils and their constituents was determined by modified Ellman's coloric method. Additionally, anticholinesterase activity of these oils was examined in SK-N-SH cells. Only six out of thirteen essential oils; galanga, turmeric, fingerroot, holy basil, plai and betel vine, showed the strong inhibition of acetylcholinesterase activity by using TLC-assay method. IC50 values of galanga, turmeric and fingerroot oils demonstrated the stronger inhibition than the rest. These oils also exhibited strong inhibitory effect in SK-N-SH cells. However, the oils itself exhibited higher anticholinesterase activity than their active constituents. Turmeric, galanga and fingerroot oils showed strong inhibitory effect on both pure and cellular acetylcholinesterase enzyme. The anticholinesterase activity of the oils may be the consequence of the interactions between active constituents as well as other constituents in the oils.

INTRODUCTION

Dementia is a significant and severe disease in elderly population and the most common form of dementia is Alzheimer's disease (AD). AD patients suffer from the impairment of mental health functions, for example, memory loss. The most important pathology is the plaques and neurofibrillary tangles in the brain (Mölsä et al, 1995). Although the cause of AD is not clearly understood, two main strategies, cholinergic and non-cholinergic, are existed. Acetylcholine is recognized as the most important neurotransmitter involved in the regulation of cognitive function. The cholinergic hypothesis is based upon marked reduction of acetylcholine level in hippocampus (Cummings, 2004). On the other hand, non-cholinergic agents focus on the other pathways such as anti-amyloid strategies, transition metal chelators, growth factors, hormones, antioxidants and herbs (Doraiswamy, 2002). Currently, drugs with cholinergic hypothesis are the mainstream (Park, 2010). Acetylcholinesterase (AChE) is the enzyme that breakdowns acetylcholine to choline and acetate in the synaptic cleft. Acetylcholinesterase inhibitors have been studied for their inhibitory action on the progression of AD as they improve the cognitive function in these patients. Physostigmine, huperzine-A and galanthamine which are the proto-types of acetylcholinesterase inhibitor are originally obtained from the plants (Hostettmann et al, 2006, Liu et al, 1986, Nesterenko, 1965). Thus, natural products are of interest as a potential candidate for the treatment of AD especially as an inhibitor of acetylcholinesterase enzyme.

Plants and phytoconstituents with anticholinesterase activity have been reviewed. Many alkaloids have been reported to have acetylcholinesterase activity (Mukherjee et al, 2007). The methanol extracts from many parts of Thai medicinal plants; stem bark, roots, seeds, fruits, flowers, leaves and whole plant, have been reported for their anticholinesterase activity (Ingkaninan et al, 2003). Among all parts of the plant, essential oils exhibit strong potency to inhibit acetylcholineesterase in the brain because of the lipophilicity and small molecular size of their constituents. *Salvia* spp. is the plants with extensive studies for anticholinesterase activity both *in vitro* and *in vivo* (Perry et al, 1996, Perry et al, 2000, Perry et al, 2002, Senol et al, 2010, Tel et al, 2010). The essential oils of *Salvia* species have volatile constituents which are likely to cross the blood brain barrier and exert their effect. In addition, the constituents of many essential oils, such as essential oils from *Salvia* species, *Mentha* species, *Citrus paradise*, *Artemisia dracunculus* L., *Inula graveolens* L., *Lavandula officinalis* Chaix, and *Ocimum sanctum* L., have been investigated for their anticholinesterase activity (Miyazawa et al, 1998, Miyazawa et al, 2001, Savelev et al, 2003, Savelev et al, 2004, Dohi et al, 2009). However, the anticholinesterase activity of essential oils from Thai plants and their constituents have not been reported. The aim of this study is to investigate the anticholinesterase activity of the essential oils from Thai medicinal plants as well as their active constituents. Furthermore, anticholinesterase activity of the active oils was determined in neuroblastoma cells. Thus, this paper is the first report to confirm anticholinesterase activity of essential oils from Thai medicinal plants in human neuroblastoma SK-N-SH cells.

Materials and methods

Thirteen essential oils from Thai medicinal plants (see below) were investigated for anticholinesterase activity using TLC bioassay and microplate assay. The oils which showed strong inhibitory activity were selected for further investigation. The active constituents of these active oils were identified by TLC and gas chromatography-mass spectrometry (GC-MS) and then determined for their anticholinesterase activity using microplate assay. The anticholinesterase activity of the active oils was also investigated in SK-N-SH cells.

Essential oils

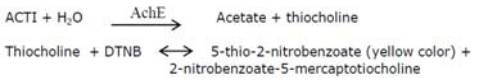
Thirteen essential oils were obtained from Thai-China Flavours and Fragrances Industry Co., Ltd. (Bangkok, Thailand) including essential oils from betel vine leaves (*Piper betle* L.), black pepper fruits (*Piper nigrum* L.), galanga rhizomes (*Alpinia galanga* (L.) Willd), guava leaves (*Psidium guajava* L.), holy basil leaves (*Ocimum tenuiflorum* L.), kaffir lime peel (*Citrus hystrix* DC.), kaffir lime leaves (*Citrus hystrix* DC.), lemongrass stem (*Cymbopogon citratus* Stapf), fingerroot rhizomes (*Boesenbergia pandurata* (Roxb.) Schltr.), plai rhizomes (*Zingiber cassumunar* Roxb), pomelo peel (*Citrus maxima (Burm.f.) Merr.*), sweet basil leaves (*Ocimum basilicum* L.), and turmeric rhizome (*Curcuma longa* L.).

Chemicals

Acetylthiocholine iodide (ATCI), acetylcholinesterase type VI-s, lyophilized powder, 425.94 units/mg solid, 687 U/mg protein (AChE, purified enzyme from electric eels; *Electrophorus electricus*), 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), galanthamine and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). Fifty millimolar Tris–HCl (pH 8.0) was used as a buffer throughout the experiment. The lyophilized enzyme was prepared in buffer to obtain 100 U/ml stock solution and further dilute with buffer containing 0.1% BSA to get 10 U/ml for TLC assay and 0.22 U/ml for microplate assay. Camphor, camphene, 1,8-cineole, geraniol, linalool, methyl eugenol, methyl cinnamate, terpinen-4-ol and *trans*-caryophyllene were purchased from Sigma (St. Louis, MO, USA). All organic solvents (analytical-reagent grade) were obtained from Merck (Darmstadt, Germany).

Bioassay detection for AchE inhibition by thin layer chromatography (TLC)

A qualitative method for AChE activity measurement was based on Ellman's coloric method (Ellman et al, 1961). The detection for AChE inhibition using TLC was modified from the study of Rhee (Rhee et al, 2001). Two silica gel GF_{254} plates (20x20 cm) (Merck, Darmstadt, Germany) were used as a stationary phase and toluene-ethyl acetate (93:7 v/v) was used as the mobile phase. Samples were dissolved in dichloromethane to a concentration of 50 mg/ml. Then, 3-20 µl of each sample was spotted on two siliga gel TLC plates and developed in the solvent system. Galanthamine (1 mM) was used as a positive control. The inhibitory activity of the developed spots was observed after successive spraying with the substrate (30 mM ACTI in buffer), dye (20 mM DTNB in buffer) and enzyme (10 U/ml AChE).



After the two plates have been developed, the first plate (plate A)

was sprayed with 30 mM ATCI followed by 20 mM DTNB. It was allowed to dry at room temperature for 5 min, then sprayed with 10 U/ml AChE. Acetylcholinestrase inhibiting spots were observed within 15 min after spraying the enzyme. The enzyme hydrolysed the substrate (ACTI) resulting in the production of thiocholine which reacts with the dye or Ellman's reagent (DTNB) to produce 5-thio-2-nitrobenzoate and 2-nitrobenzoate-5-mercaptotiocholine which can be detected at 405 nm. After spraying the enzyme the spot with anticholinesterase activity was a white spot on the yellow background. The second plate (plate B) was sprayed with anisaldehyde-sulfuric acid and used as a reference plate.

Microplate assay for AChE activity

AChE inhibitory activity was assayed by modified Ellman's coloric method using 96-well microplates (Rhee et al, 2001). Briefly, 125 µl of 3 mM DTNB in buffer containing 0.1 M NaCl and 0.02 M $MgCl_2.2H_2O$, 25 µl of 15 mM ATCI in deionized water, 50 µl of buffer containing 0.1% BSA and 25 µl of sample (in buffer containing 50% methanol) were added to the wells followed by 25 µl of 0.22 U/ml AChE. The absorbance was measured at 405 nm before adding the enzyme and was measured again every 45 seconds for 5 cycles after adding the enzyme by a microplate reader (M200, Tecan, Switzerland). Inhibitory activity was calculated from differences between absorbance values of control and sample, and resulted in percent of acetylcholinesterase inhibition. The assay was performed in triplicate. The percentage of enzyme inhibition was calculated as follows:

$$\% \text{ Acetylcholinesterase inhibition} = \frac{[(A-B) - (C-D)]}{(A-B)} \times 100$$

Where:
A: Control (reagent + methanol + enzyme)
B: Blank of control (reagent + methanol)
C: Sample (reagent + sample + enzyme)
D: Blank of sample (reagent + sample + methanol)

The concentration that inhibited 50% of AChE activity (IC_{50}) was obtained from the graph that plot between the percentage of acetylcholinesterase inhibition and concentration of the oil.

Determination of active constituents of essential oils by bioautography and gas chromatography-mass spectrometry (GC-MS)

The active constituents of active essential oils were identified by bioautography using thin layer chromatography (TLC). Three silica gel GF_{254} plates (A, B, C) were prepared. Plate A was used for bioautography method, plate B was used as a reference chromatogram and plate C was prepared for the isolation of active constituents of the oil. Three plates were subjected to separation by using toluene – ethyl acetate (93:7 v/v) as developing solvent. After development, plate A and B were performed as described in section 2.3, while plate C was used as preparative TLC.

To isolate the active components, the inhibition zones were scraped off the plate, then the active component was eluted from the silica gel plate with hexane and the eluate was evaporated to dryness under *vacuo*. The components were further identified by GC-MS with GCMS-QP 2010 gas chromatograph mass spectrometer (Shimadzu) using a DB-5ms bonded phase fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA). Helium was used as carrier gas at a constant flow rate of 0.68 mL/min. The oven temperature programs for galanga, turmeric and fingerroot oils were modified from the method of

Jirovetz et al. (2003), Richmond et al. (1997) and Bin Jantan et al. (2001), respectively. Injection temperature: 250 °C, interface temperature: 250 °C and MS detection was performed with electron impact (EI) mode at 70 eV by operating in the full-scan acquisition mode in the 40-400 *m/z* range. The identification of the active constituents was performed by comparing the obtained mass spectra with those from the Wiley and NIST spectral library and their retention times with those of the reference standards. The identified active constituents of essential oils were further evaluated for their AChE inhibitory activity using microplate assay.

Cell lines

SK-N-SH human neuroblastoma cells (HTB-11) were obtained from American Type Culture Collection (ATCC). Cells were grown in minimum essential medium from Gibco (California, USA) supplemented with 10% heat inactivated fetal bovine serum, 2% supplementary amino acid solution, 1% Glutamax (Gibco, California, USA), 1% penicillin/streptomycin in a 5% CO_2–95% O_2 atmosphere at 37 °C. Cells were seeded into 96-well microplates (Nunc, Roskilde, Denmark) at a density of 1×10^5 cells/ml (100 µl in each well). Experiments were carried out after 24 hour of seeding. All chemicals were obtained from Sigma (St. Louis, MO, USA).

Inhibition of acetylcholinesterase activity was assayed based on Ellman's coloric method (Ellman et al, 1961). Cellular AChE was used as an enzyme source instead of purified AChE of electric eels. The condition was slightly modified to enable the cellular enzyme to work properly. Briefly, 100 µl of 3 mM DTNB in buffer containing 0.1 M NaCl and 0.02 M $MgCl_2.2H_2O$, 25 µl of 15 mM ATCI in deionized water and 25 µl of sample dissolved in buffer containing 50% methanol were added to the well containing 100 µl of the cell. The absorbance at 405 nm was measured every 45 seconds for 5 cycles by a microplate reader (M200, Tecan, Switzerland). Inhibitory activity and IC_{50} value were determined by the same method as using enzyme from electric eels.

Statistics

Data were expressed as mean ± standard deviation (SD) of the value obtained from three parallel measurements. The anticholin-esterase activity was demonstrated as IC_{50} values and % inhibition values at 10 and 50 µg/ml using purified and cellular enzymes, respectively. Since cellular enzyme was not as pure as purified enzyme from electric eels, the concentration of sample used to inhibit AChE activity of the former is lower than the latter.

Results and Discussion

Table 1 Anticholinesterase activity of essential oils

Inhibitor	% inhibition	IC_{50} (µg/ml)
Galanthamine (reference)	89.18±2.84[1]	1.73±0.12
Turmeric oil	54.81±0.97[3]	34.70±3.10[3]
Galanga oil	52.13±0.47[3]	44.29±0.97[3]
Fingerroot oil	49.85±2.62[2]	59.03±4.28[3]
Plai oil	27.14±3.91[2]	ND
Betal vine oil	12.97±2.77[2]	ND
Holy basil oil	8.84±2.40[2]	ND

Note : mean ± SD of 3 parallel measurements [1] measured at 20 µg/ml, [2] measured at 50 µg/ml, [3] Calculated from the dose-response equations, ND - Not determined (the maximum level of inhibition below 50%)

After investigation of AChE inhibitory activity by using TLC-assay method, only six out of thirteen essential oils;

galanga, turmeric, fingerroot, holy basil, plai and betel vine, showed the strong inhibition. IC_{50} values of these essential oils were further examined by microplate assay.

Table 2 Active component(s) of essential oils identified by GC-MS

Essential oils	Identified active constituents (% in oil)
Turmeric oil	ar-tumerone (44.68%) β-sesquiphellandrene (6.36%) 1,8-cineole (1.85%) β-bisabolene (1.69%) zingiberene (1.46%) trans-caryophyllene (1.43%) camphene (0.37%) *and traces β-tumerone, α-tumerone, Ar-curcumene*
Galanga oil	1,8-cineole (34.5%) methyl eugenol (1.78%) chavicol acetate (1.67%) farnesol (1.16%) terpinene-4-ol (0.99%) α-cadinol (0.85%) *and traces; p-mentha-2,8-dien-1-ol, eugenol acetate, (-)-caryophyllene oxide, β-bisabolene*
Fingerroot oil	camphor (23.71%) 1,8-cineole (16.92%) geraniol (10.91%) methyl cinnamate (3.11%) linalool (1.77%) α-terpineol (0.75%) α-bergamotene (0.33%) *and traces; linalyl propionate, trans-geraniol, borneol, terpinene-4-ol, Z-citral, E-citral, geraniol formate, geranyl acetate, nerol acetate, α-farnesene*

The anticholinesterase activity of 6 oils reported as %inhibition values at their maximum solubilities, as follows: 16.18±3.65 at 100 µg/ml for holy basil oil, 37.88±.13 at 90 µg/ml for betal vine oil, 27.14±3.91 at 50 µg/ml for plai oil, 49.85±2.62 at 50 µg/ml for fingerroot oil, 49.09 ±1.04 at 40 µg/ml for galanga oil and 53.11±0.15 at 40 µg/ml for turmeric oil. %inhibition of betal vine and holy basil oils at 50 µg/ml were 12.97±2.77 and 8.84±2.40, respectively. IC_{50} of turmeric, galanga and fingerroot oils calculated from dose-response equations were 34.70±3.10, 44.29±0.97 and 59.03±4.28 µg/ml, respectively whereas that of the reference compound, galanthamine, being 1.73±0.12 µg/ml (Table 1).

Table 3 Anticholinesterase activity of active constituents of essential oils and their combinations

Inhibitor	IC_{50} (µg/ml)	% inhibition
Galanthamine (reference)	1.73 ± 0.12	
Turmeric oil	34.70 ± 3.10	54.81 ± 0.97
1. *trans*-Caryophyllene		33.01 ± 2.92
2. 1,8-Cineole		17.83 ± 0.63
3. Camphene		12.76 ± 0.68
4. *trans*-Caryophyllene +1,8- Cineole		40.70 ± 0.82
5. *trans*-Caryophyllene +Camphene		13.28 ± 0.57
6. 1,8-Cineole + Camphene		57.53 ±3.22
7. *trans*-Caryophyllene + 1,8-Cineole + Camphene		71.87 ± 0.59
Galanga oil	44.29 ± 0.97	52.13 ± 0.47
1. 1,8-Cineole		17.83 ± 0.63
2. Terpinene-4-ol		14.96 ± 0.48
3. Methyl eugenol		13.6 ± 0.78
4. 1,8-Cineole +Terpinene-4-ol		48.71 ± 0.79
5. 1,8-Cineole + Methyl eugenol		45.06 ± 1.28
6. Terpinene-4-ol + Methyl eugenol		5.82 ± 1.15
7. 1,8-Cineole + Terpinene 4-ol + Methyl eugenol		18.35 ± 2.21
Fingerroot oil	59.03 ± 4.28	49.85 ± 2.62
1. 1,8-Cineole		17.83 ± 0.63
2. Methyl cinnamate		13.35 ± 3.34
3. Linalool		11.27 ± 0.93
4. Camphor		9.58 ± 0.32
5. Geraniol		7.99 ± 0.60
6. 1,8-Cineole + Linalool		47.80 ± 1.41
7. 1,8-Cineole + Methyl cinnamate		42.30 ± 0.52
8. 1,8-Cineole + Geraniol		37.16 ± 0.25
9. 1,8-Cineole + (+)-Camphor		33.13 ± 0.69
10. 1,8-Cineole + (-)-Camphor		31.86 ± 1.00
11. 1,8-Cineole + Methyl cinnamate + Linalool + Camphor + Geraniol		20.02 ± 2.20

Note : mean **±** SD of 3 parallel measurements

The anticholinesterase activity of the six oils was reported as %inhibition values (at maximum solubility) because of the limit of their solubilities in the test system. The %inhibition values (at maximum solubility) were 16.18 ± 3.65 (at 100 µg/ml) for holy basil oil, 37.88 ± 3.13 (at 90 µg/ml) for betal vine oil, 27.14 ± 3.91 (at 50 µg/ml) for plai oil, 49.85 ± 2.62 (at 50 µg/ml) for fingerroot oil, 49.09 ± 1.04 (at 40 µg/ml) for galanga oil and 53.11 ± 0.15 (at 40 µg/ml) for turmeric oil. However, at the same concentration of 50 µg/ml, fingerroot, plai, betal vine and holy basil oils showed %inhibition values of 49.85 ± 2.62, 27.14 ± 3.91, 12.97 ± 2.77 and 8.84 ± 2.40, respectively. IC_{50} values of turmeric, galanga and fingerroot oils calculated from the dose-response equations were 34.70 ± 3.10 µg/ml, 44.29 ± 0.97 µg/ml and 59.03 ± 4.28 µg/ml, respectively whereas IC_{50} values of galanthamine used as positive control was 1.73 ± 0.12 µg/ml (Table 1). The result suggested that turmeric oil showed stronger inhibition of cholinesterase than galanga, fingerroot, plai, betal vine and holy basil oils. Based on the results of TLC bioautography assay, turmeric, galanga and fingerroot oils were selected for the identification of active constituents responsible for their AChE inhibitory activity. The identification of the fractions obtained from active essential oils by preparative TLC was further confirmed by GC-MS analysis. The mass spectroscopic data of the active fraction when compared with those from the Wiley and NIST spectral library and their retention times with those of the reference standards revealed the active constituents responsible for their AChE inhibitory activity as shown in Table 2. Most of the active constituents were found to be major constituents of the essential oil. ar-Tumerone and 1,8-cineole are the major constituents of turmeric and galanga oil, respectively. The major constituents of fingerroot oil were camphor, 1,8-cineole and geraniol.

After identification of active constituents of the essential oils, the commercial available reference compounds were subjected to the anticholinesterase assay including; (a) *trans*-caryophyllene, 1,8-cineole and camphene of turmeric oil (b) 1,8-cineole, terpinene-4-ol and methyl eugenol of galanga oil and (c) 1,8-cineole, methyl cinnamate, camphor, linalool and geraniol of fingerroot oil. Because of the solubility limit, the anticholinesterase activity of the constituents was demonstrated in term of %inhibition values compared to the oil at the same concentration of 50 µg/ml (Table 3). Among active constituents of the oils, *trans*-caryophyllene was the strongest constituents in turmeric oil whereas 1,8-cineole was the strongest constituents in galanga and fingerroot oils. However, the result showed that all of the oils had stronger activity than each active constituent. *Trans*-caryophyllene exhibited highest inhibitory activity of 33.01 ± 2.92% whereas turmeric oil exhibited 54.81 ± 0.97%. 1,8-cineole was the strongest constituents in galanga and fingerroot oil. It exhibited 17.83 ± 0.63% while galanga and fingerroot oil exhibited 52.13 ± 0.47% and 49.85 ± 2.62%, respectively. In addition, the anticholinesterase activity of the combination of the active constituents of each oil was tested. The ratio between the active constituents used in this study was according to the result from GC-MS. The additive, synergistic as well as inhibitory effects between the active constituents were found.

The above data showed that none of the active constituents exhibited stronger inhibitory activity than the

essential oils. Chemical interactions of constituents in these oils were suggested. Therefore, the active oils were further determined for their anticholinesterase activity in the cell lines.

Table 4 Anticholinesterase activity of the essential oils in SK-N-SH cells[a]

Inhibitor	%inhibition	IC_{50} (µg/ml)
Galanthamine (reference)	76.16±0.34[1]	0.23±0.02
Turmeric oil	74.16±2.35[2]	1.34±0.14
Galanga oil	64.67±0.58[2]	2.26±0.30
Fingerroot oil	65.02±1.04[2]	2.93±0.20

Note : mean ± SD of 3 parallel measurements, [1] at 1 µg/ml, [2] at 1 µg/ml

SK-N-SH cells were human neuroblastoma cell lines widely used to study gene expression and activity of neuronal cells. They also were used for activity testing of a novel cholinesterase inhibitor (Lahiri et al, 2000, Ezoulin et al, 2005). In this study, cellular AChE from human neuroblastoma SK-N-SH cells was used as enzyme source instead of the purified enzyme from electric eels. The anticholinesterase activity of the oils was demonstrated in term of %inhibition values at 10 µg/ml and IC_{50} values. Table 4 showed that turmeric oil exhibited the strongest inhibitory activity with IC_{50} values of 1.34 ± 0.14 µg/ml. Galanga and fingerroot oil showed IC_{50} values of 2.26 ± 0.30 and 2.93 ± 0.20 µg/ml, respectively whereas IC_{50} value of galanthamine was 0.23 ± 0.02 µg/ml.

Turmeric, galanga and fingerroot oils, the active oils in this study, inhibited AChE enzyme at the similar range of concentration as other essential oils from previous reports. Essential oil from *A. dracunculus* L. exhibited IC_{50} values of 58 µg/ml (Dohi et al, 2009), whereas those from *Salvia lavandulaefolia* showed IC_{50} values of 50 µg/ml (Savelev et al, 2003). Interestingly, 1,8-cineole was found in all of the active oils. There are many reports that supported the anticholinesterase activity of 1,8-cineole (Miyazawa et al, 1998, Perry et al, 2000, Savelev et al, 2003, Savelev et al, 2004, Dohi et al, 2009).

Most of the combination of active constituents in this study resulted in additive or synergistic effect except for the combination of *trans*-caryophyllene and camphene and the combination of terpinene-4-ol and methyl eugenol which exhibited the antagonistic response. It was noted that combination of 1,8-cineole and other constituents tended to give synergistic response. The acetylcholinesterase inhibitory activity of the combination of all available active constituents was higher than turmeric oil whereas galanga and fingerroot oils had higher inhibitory activity than that of the combinations. The finding revealed that the anticholinesterase activity of the oil may be the result of the interaction between its active constituents. However, the constituents other than those were tested in this experiment may play a role in both synergistic and antagonistic effect. There was a report that anticholinesterase activity of Spanish sage (*S. lavandulaefolia*) oil came from the synergistic effect among the constituents; 1,8-cineole and α-pinene (Perry et al, 2000). The active constituents of essential oils from *Mentha* species; viridiflorol, elemol and 1,8-cineole, and *Citrus paradise*; nootkatone and auraptene, showed less potent inhibition than the essential oils (Miyazawa et al, 1998, Miyazawa et al, 2001). Similar result was reported in *S. lavandulaefolia*'s essential oil, of which synergistic effect was observed in the combination of 1,8-cineole and α-pinene, whereas the antagonistic effect was shown in the combination

of 1,8-cineole and camphor (Savelev et al, 2003). Synergistic effects of active constituents of essential oil from *Salvia* species including 3-carene, β-caryophyllene and 1,8-cineole was also suggested (Savelev et al, 2004). Although eugenol was the major component accounted for the inhibitory activity of *O. sanctum* oil, the anticholinesterase activity of this oil was proposed to be due to the combined effect of eugenol, β-caryophyllene and α-humulene (Dohi et al, 2009). In addition, all of the active oils demonstrated the inhibition of cholinesterase enzyme from SK-N-SH cells. Thus, inhibitory effect of the oils did not depend on the sources of the enzyme.

Therefore, the result of this present study suggested that turmeric, galanga and fingerroot oils had shown potential as functional food in AD patients. However, the data of toxicity and anticholinesterase activity in animal model should be further examined.

Conclusion

Among 13 essential oils, turmeric, galanga and fingerroot oils showed strong inhibitory effect on both pure and cellular acetylcholinesterase enzyme. However, the oil itself exhibits the stronger anti-cholinesterase activity than its active constituents. The anticholinesterase activity of the combination of the active constituents of each oil demonstrated additive, synergistic as well as the inhibitory effect. The result suggested that anti-cholinesterase activity of the oil may be the consequence of the interactions between its active constituents as well as other constituents in the oil.

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