Screening of Medicinal Plants from Taman Herba Perlis for Acetylcholinesterase Inhibitory Activity

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ABSTRACT

In a search for new potential AChE inhibitors, 31 selected medicinal plants from Perlis were collected gathered, air dried and successively extracted using hexane, dichloromethane, and alcohol. The dichloromethane and alcoholic extracts were screened for AChE inhibitory activity using Ellman's method. Out of 31 plant species, the methanol extracts of *Rhapis excelsa* leaves (97.03 ± 3.71 %), *Diospyros blancoi* leaves (95.80 ± 1.57 %) and *Phyllantus elegans* root (83.22 ± 3.08 %) showed the highest AChE inhibitory activity at the concentration of 100 µg/mL.

Keywords: *Alzheimer's disease, acetylcholinesterase, acetylcholine, acetylcholinesterase inhibitor, medicinal plants*

INTRODUCTION

Acetylcholinesterase (AChE) is an enzyme which is responsible for the termination of nerve impulse transmission by rapidly hydrolyzing neurotransmitter, acetylcholine into choline and acetic acid in the central and peripheral nervous systems [1]. One of the proposed causative factors of Alzheimer's disease is cholinergic neurotransmission impairment due to acetylcholine deficiency, either by the reduction of acetylcholine production or amplified acetylcholinesterase activity [2]. Thus, one of the strategies to enhance the cholinergic function is by increasing the acetylcholine level in the synapses between the cholinergic neurons with the aid of acetylcholinesterase inhibitor [3]. The acetylcholinesterase inhibitors prevent the breakdown of acetylcholine and thus increase the cholinergic neurotransmission in the forebrain regions.

Plants are an important source of AChE inhibitors such as galantamine and huperzine A [4]. Galantamine, an alkaloid isolated from *Galanthus woronowii* was approved by the U.S. Food and Drug Administration for mild to moderate AD treatment. Galantamine was considered to be less tolerated than the other AD drugs but the long-term tolerability could be improved with careful and gradual titration over more than three months [1]. Huperzine A is an alkaloid obtained from *Huperzia serrata* and has better penetration through the blood-brain barrier, higher oral bioavailability, and longer AChE inhibition activity when compared to the other AChE inhibitors [1]. Huperzine A was commercialized as a dietary supplement for memory support and AD symptoms treatment in China [5].

Perlis is the smallest state in Malaysia, but it is rich in biodiversity due to its unique geographical location at the Malaysia-Thailand country border. As a part of the equatorial climate, it consists of abundant tropical plants, many of which are widely used in ethnomedicine. Several plants are reserved in Taman Herba Perlis for research purposes and for future references. In search of hits for AchE inhibitors from tropical plants, 31 medicinal plants were screened for AChE inhibitory activities. The present study reports on AChE inhibitory activity of 31 medicinal plants collected from Taman Herba Perlis.

EXPERIMENTAL

General

All the solvents used for extraction and isolation were of analytical grade solvent. The chemicals and reagents for bioassay, acetylcholinesterase enzyme from electric eel (C2888-1KU), acetylthiocholine iodide (A5751), physostigmine (E8375) and 5,5'-dithiobis(2-nitrobenzoic acid) (D218200) were purchased from Sigma-Aldrich. The 96-well used in AChE assay was visualized using Spectrostar Nano BMG Labtech spectrometer.

Plant material

The plants materials were collected in January 2016 from Taman Herba Perlis, Perlis, Malaysia, and their identification was based on the plant tags at the garden. The plants were air dried and ground into powder. About 50 g of dried plant materials were macerated in 100 ml of hexane,

dichloromethane, and methanol/ethanol successively at room temperature for three days. The filtrate was evaporated *in vacuo* to obtain dry crude extracts.

Acetylcholinesterase Inhibition Activity

The acetylcholinesterase (AChE) inhibition activity was assessed using a modified method by Ellman which employed acetylthiocholine iodide (ATCI) as a synthetic substrate for AChE [6]. Sodium phosphate buffer (140 µL, 0.1 M, pH 7.4) was added into each well. The plants crude extracts diluted in methanol (20 µL, 1 mg/mL) were pipetted into the well followed by electric eel acetylcholinesterase enzyme (AChE) solution (20 µL, 0.09 U/mL). The well was incubated for 15 minutes at 25℃. The 5,5-dithiobis (2-nitrobenzoic) acid (10 µL, 10 mM) was added to the well and the reaction was initiated by the addition of ATCI (10 µL, 14 mM) solution. The formation of colored product 5-thio-2-nitrobenzoate anion from the hydrolysis of ATCI was measured at 412 nm wavelength after 10 minutes. The standard AChE inhibitor drug, physostigmine was dissolved in methanol (20 µL, 1 mg/mL) and used as a positive control. For blank, sodium phosphate buffer (160 µL, 0.1 M, pH 7.4) and methanol (20 µL) was used. The percentage of AChE inhibition was calculated using Eq. 1.

$$\text{Percentage inhibition} = (A_{blank}-A_{sample})/A_{blank} \times 100 \quad \text{(Eq. 1)}$$

Where A_{sample} is the absorbance of the sample extract and A_{blank} is the absorbance of the blank (solvent in the 0.1 M sodium phosphate buffer at pH 7.40).

The data were expressed as a mean ± standard deviation. The differences were analyzed using one-way analysis of variance (ANOVA) completed with Duncan's post hoc test and $p<0.05$ was considered as statistically significant using IBM SPSS Statistic version 23.

RESULTS AND DISCUSSION

The percentage of AChE inhibition data for all the dichloromethane and alcoholic extracts are presented in Table 1. Out of the 31 plant species, only three samples, the methanol leaves extracts of *Rhapis excelsa* (97.03 ± 3.71 %), methanol leaves extract of *Diospyros blancoi* (95.80 ± 1.57 %) and methanol root extract of *Phyllantus elegans* (83.22 ± 3.08 %) showed more than 80 % AChE inhibitory activity at the concentration of 100 µg/mL. Seven extracts, i.e. alcoholic extracts of *Acrotrema costatum* root, *Phyllanthus elegan* leaves, stem and twigs, and *Fragraea acuminatissima* leaves, dichloromethane extracts from *Morinda citrifolia* root and *Glycosmis pentaphylla* leaves exhibited 50-80 % AChE inhibition activity. The remaining samples showed weak or no activity. Physostigmine was used as a positive control with an IC_{50} value of 0.008 ± 0.004 µM.

The AChE inhibition activity of *Rhapis excelsa* (Arecaceae), *Diospyros blancoi* (Ebenaceae) and *Phyllanthus elegans* (Euphorbiaceae) crude extracts have never been reported before. *Rhapis excelsa* (Arecaceae) showed the highest AChE inhibition activity in this study. In the previous study, ethyl acetate and butanol fractions of *R. excelsa* leaves showed DPPH radical scavenging activity with the EC_{50} values of 30 ± 1.06 and 32 ± 1.26 µg/mL, respectively, probably due to its phenolic content [7]. The major phenolic compounds reported in the leaves

extract were benzoic acid and ferulic acid, in addition to four flavonoid-glycosides which may contribute to the radical scavenging activity [7]. The chemical constituents and bioactivity of *R. excelsa* were not widely reported.

Diospyros blancoi (Ebenaceae) mainly consists of triterpenes and naphthoquinones [8]. Betulin and β-sitosterol-3-*O*-glucopyranoside were previously reported to inhibit 50% of AChE at 23.98 ± 1.10 μM and 5.22 ± 0.59 μM, respectively [9]. The natural occurrences of betulin and β-sitosterol-3-*O*-glucopyranoside were widely reported in *Diospyros spp.* [8].

Limited scientific reports were found on *Phyllanthus elegans* (Euphorbiaceae). The methanolic extract of *P. acidus*, a family member of *Phyllanthus* spp, inhibited rat brain AChE and human blood BuChE with the IC_{50} values of 1009.87 μg/mL and 449.51 μg/mL, respectively [10]. Isocorilagin, a phenolic compound commonly found in *Phyllanthus* spp, showed stronger activity than galanthamine in inhibiting both acetylcholinesterase and butrylcholinesterase activities with the IC_{50} values of 0.49 μM and 4.20μM, respectively [11].

Acrotrema costatum (Dillenaceae) is used in Kerala, India for the treatment of excessive hair fall and baldness prevention by Malavedan tribe [12]. Some known triterpenes and flavonoid were reported from *A. arnottianum* [13]. There are very limited chemical constituents and biological activities reported from this genus.

Fagraeae acuminatissima (Loganiaceae/Gentianaceae) is used traditionally for the treatment of hypertension [14]. Fagraeoside, an alkaloid from *F. racemose* was reported to inhibit low to moderate AChE activity. It also inhibited the production of prostaglandin E_2 in 3T3 murine fibroblast with IC_{50} 5.1 M and was reported nontoxic to P388 murine leukemia cell [15].

Morinda citrifolia (Rubiaceae) is used traditionally for the treatment of diabetes, high blood pressure, heart disease, cancer, depression, and atherosclerosis [16]. The fruit extract and its isolated compound neostigmine were reported to inhibit AChE with the IC_{50} values of 31.84 μg/mL and 19.71 μg/mL, respectively [17]. The fruit extract was also reported to prevent STZ-induced memory impairment and significantly improved memory and cerebral blood flow [16, 18]. *M. citrifolia* is a potential plant to be developed for the treatment of Alzheimer's disease, however further studies are required to confirm its safety and efficacy.

Glycosmis pentaphylla (Rutaceae) showed strong AChE inhibition activity in this study. It has been used traditionally for the treatment of rheumatism, cancer, liver disorder and inflammation [19, 20]. The present study has successfully identified potential plants for the discovery of new hits for AChE inhibitors. Further phytochemical and biological studies need to be carried out to identify the bioactive compounds.

CONCLUSIONS

Random screening on 31 medicinal plants from Taman Herba Perlis has successfully discovered potential tropical plants with acetylcholinesterase inhibitory activity. Out of 31 plant species, the

methanol leaves extracts of *Rhapis excelsa* (97.03 ± 3.71 %), methanol leaves extract of *Diospyros blancoi* (95.80 ± 1.57 %) and methanol root extract of *Phyllantus elegans* (83.22 ± 3.08 %) showed the highest AChE inhibitory activity at the concentration of 100 µg/mL.

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Table 1 Acetylcholinesterase (AChE) inhibition activity of dichloromethane (DCM), ethanol (EtOH) and methanol (MeOH) extracts of different plant samples

Plant species	Family	Traditional uses	Extraction solvent (plant part)	AChE inhibition (%)
Strobilanthes crispa	Acanthaceae	Antidiabetic, diuretic, antilytic, laxative [21]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Cordyline terminalis	Agavaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Canangium odoratum	Annonaceae	Malaria, stomachache, asthma, hypertension [22]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Vallaris glabra	Apocyanaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Plumeria obtusa	Apocyanaceae	Bacterial and fungal infection [23]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Aglaonema nitidum	Araceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Trevesia burckii	Araliaceae	Ache, skin infection, increase woman fertility [24-26]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Rhapis excelsa	Arecaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	97.03 ± 3.71^{a}
Dracaena cantleyi	Asparagaceae	Relief body pain [27]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Vernonia amygdalina	Asteraceae	Tonsillitis [28]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Pereskia bleo	Cactaceae	Detoxification, cancer, hypertension, diabetes, stomach ache, muscle pain, inflammation [29]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Chloranthus erectus	Chloranthaceae	Localised swelling, joint pain, skin inflammation, fever, body ache [30]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Scleria lithosperma	cyperaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Acrotrema costatum	Dilleniaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	$30.41 \pm 13.70^{f,g,h}$

Table 1 Continue

Plant species	Family	Traditional uses	Extraction solvent (plant part)	AChE inhibition (%)
Acrotrema costatum	Dilleniaceae		DCM (root)	n.i.
			MeOH (root)	79.99 ± 4.43[b,c]
Diospyros blancoi	Ebenaceae	Wound healing, snakebite, spider bite, diabetes, hypertension, eczema, stomachache [31]	DCM (leaves)	n.i.
			MeOH (leaves)	95.80 ± 1.57[a,b]
Mallotus marostachyus	Euphorbiaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	45.23 ± 14.24[e,f]
Pimelodendron griffithianum	Euphorbiaceae	-	DCM (bark)	27.91 ± 11.47[g,h]
			MeOH (bark)	28.14 ± 12.48[g,h]
Phyllanthus elegan	Euphorbiaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	68.04 ± 7.68[c,d]
			MeOH (root)	83.22 ± 3.08[a,b,c]
			EtOH (twigs)	66.05 ± 5.11[c,d]
			MeOH (stem)	72.32 ± 8.86[c]
Hanguana malayana	Hanguanaceae	Fever [32]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Molineria latifolia	Hypoxidaceae	Loss of appetite [33]	DCM (leaves)	n.i.
			MeOH (leaves)	14.69 ± 4.36[h]
Fagraea acuminatisima	Gentianaceae	Hypertension [14]	DCM (leaves)	n.i.
			MeOH (leaves)	68.72 ± 5.92[c,d]
			DCM (bark)	n.i.
			MeOH (bark)	n.i.
Stachyphrynium griffithii	Marantaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Clidemia hirta	Melastomataceae	Wound healing, fever, diarrhea, irritation, bacterial infection [34]	DCM (leaves)	n.i.
			MeOH (leaves)	22.78 ± 17.40[g,h]
Piper curtisii	Piperaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.

Table 1 Continue

Plant species	Family	Traditional uses	Extraction solvent (plant part)	AChE inhibition (%)
Garcinia carinata	Rubiaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
			DCM (bark)	n.i.
			MeOH (bark)	n.i.
Morinda citrifolia	Rubiaceae	Arthritis, diabetes, high blood pressure, menstrual problems, heart disease, cancer, ulcers, depression, and atherosclerosis [16]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
			DCM (root)	79.03 ± 5.32^{c}
			MeOH (root)	n.i.
			DCM (bark)	$45.43 \pm 12.70^{e,f}$
			MeOH (bark)	n.i.
Mussaenda pubescens	Rubiaceae	Common cold, diarrhea, inflammation [35]	DCM (leaves)	n.i.
			MeOH (leaves)	$32.23 \pm 17.00^{f,g}$
Glycosmis pentaphylla	Rutaceae	Rheumatism, cancer, liver disorders, inflammation [19]	DCM (leaves)	$52.98 \pm 5.82^{d,e}$
			MeOH (leaves)	n.i.
			DCM (stem)	$18.16 \pm 5.23^{g,h}$
			MeOH (stem)	n.i.
Luvunga scandens	Rutaceae	Malaria, fatigue [36]	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.
Tectaria shahidaniana	Tetariaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	36.32 ± 5.64^{f}
Elatostema repens	Urticaceae	-	DCM (leaves)	n.i.
			MeOH (leaves)	n.i.

Data were expressed as mean ± standard deviation where n=3 while n.i.=no inhibition. Data with different superscript lower letter are significantly different ($p<0.05$)