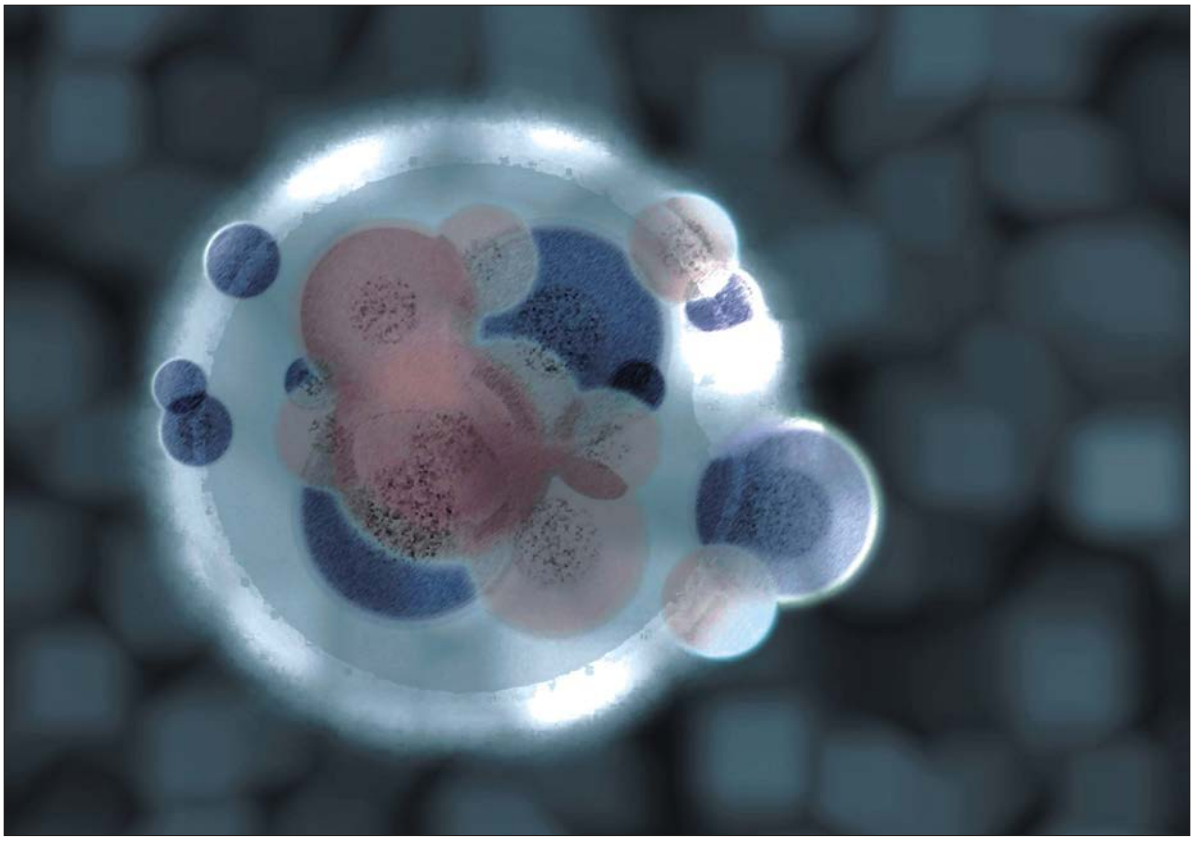

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Acetylcholinesterase Inhibition and Antioxidant Activity of *Syzygium cumini*, *S. aromaticum* and *S. polyanthum* from Indonesia

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Abstract: Acetylcholinesterase inhibition and antioxidant activity are considered to be highly correlated with Alzheimer's disease treatment. Plants from *Syzygium* genus reported as potential antioxidant, however the potency of plants as acetylcholinesterase inhibitor has not been properly investigated. The present study was design to investigate the antioxidant and acetylcholinesterase inhibitory activity of three *Syzygium* plants, *Syzygium cumini*, *S. aromaticum* and *S. polyanthum*. Leaves of *S. cumini*, *S. aromaticum*, *S. polyanthum and* bud of *S. aromaticum* extracted with gradient polarity solvent consist of *n*-hexane, ethyl acetate and methanol. Acetylcholinesterase inhibitory activity was measured with modified Ellman method at 412 nm and physostigmine was used as positive control, meanwhile antioxidant activity measured based on 1,1-diphenyl-2-picrylhydrazil free radical scavenging test. The methanol extract of *S. aromaticum* leaves, *S. aromaticum* bud, *S. polyanthum* leaves and the ethyl acetate extract of *S. polyanthum* leaves were potential as acetylcholinesterase inhibitors. The IC_{50} values of the extracts respectively were 42.10±1.41; 45.25±0.07; 47.30±3.54 and 45.10±8.06 μg mL^{-1} when IC_{50} value of physostigmine was 0.01±0.002 μg mL^{-1}. Meanwhile, antioxidant activity of potential extracts successively were 11.43±0.88, 9.26±0.25, 21.24±1.14 and 13.70±0.24 μg mL^{-1}.

Key words: Acetylcholinesterase, antioxidant, Alzheimer's disease, inhibition, *Syzygium*

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia, a progressive neurodegenerative disorder which is often observed in elderly people. AD was characterized by decrease function of thinking, orientation, calculation, learning capacity, language and decision making (Alzheimer's Association, 2012). The prominent neurochemical change in AD brain is the decrease of acetylcholine concentration, caused by degeneration of cholinergic neurons (Perry *et al*., 1999). On the other hand the increase of free radicals in brain cause a progressive accumulation of cellular damage, that oxidative stress also correlated with AD (Ho *et al*., 2010).

The cholinergic strategy through inhibiting acetylcholinesterase (AChE) to maintain or even increase acetylcholine (ACh) level in brain reported as effective therapeutic approach for the treatment of AD. Most of the prescribed drugs, such as tacrine, rivastigmine, donepenzil and galantamine are acetylcholinesterase inhibitors (AChEI) (Ballard, 2002). Beside as AChEI, tacrine and donepezil may also suppress oxidative stress (Saxena *et al*., 2008).

Although, AChEI was the most widely used medication in AD treatment, some report propound that AChEI have inclement side effect, such as tacrine cause hepatotoxicity (Wu *et al*., 2010) and galantamine induced tremor (Collins *et al*., 2011). This condition has led the research in obtaining new AD drug candidates derived from natural resources. Some works have reported the potency of natural resources as AD drug, for example formulation of Chinese herbal medicine (Seo *et al*., 2010; Jeon *et al*., 2011; Wu *et al*., 2011; Liu *et al*., 2012), Ayurvedic medicine (Liu *et al*., 2012), *Catharanthus roseus* root alkaloids (Pereira *et al*., 2010), fermented tofu (Chen *et al*., 2012), *Trigonella foenum graecum* (SatheeshKumar *et al*., 2010), *Illicium verum* (Bhadra *et al*., 2011), *Centella asiatica* (Mukherjee *et al*., 2007), *Acorus calamus* (Oh *et al*., 2004) and *Ginkgo biloba* (Das *et al*., 2002).

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Syzygium plants was reported as potential antioxidant (Shyamala *et al*., 2003; Ruan *et al*., 2008; Har and Ismail, 2012), nevertheless the potency of this plants as AChEI inhibitor has not been properly investigated. Potency of some *Syzygium* as antiamnestic have already analyzed (Alikatte *et al*., 2012), but further investigation is still needed. In present study the antioxidant and AChE inhibitory activity of *Syzygium cumini*, *S. aromaticum* and *S. polyanthum* was investigated.

MATERIALS AND METHODS

Materials: Leaves of *Syzygium cumini*, *S. aromaticum* and *S. polyanthum* were obtained from Biopharmaca research center park located at Darmaga, Bogor, Indonesia while *S. aromaticum* bud was collected from Sukabumi, Indonesia. Acetylcholinesterase (AChE), ACh, physostigmine, other chemical reagent and solvent were purchased from Sigma Aldrich.

Methods

Samples preparation and extraction: Leaves of *Syzygium cumini*, *S. aromaticum*, *S . polyanthum* and *S. aromaticum* bud were dried at 40°C. Before extracted, dried samples were grinded to size 60-80 mesh. Extraction conducted with sonication technique using gradient polarity solvent, *n*-hexane, then ethyl acetate and the last is methanol. The extracts of each sample were then dried using rotary evaporator.

Acetylcholinesterase inhibitory assay: The analysis conducted based on Ellman colorimetric assay (Ellman *et al*., 1961) with brief modification. Each samples diluted in HEPES buffer pH 8 in various concentrations. The 112 μL diluted extract was added into micro-well plate. 40 μL AChE 0.36 U mL^{-1}, 10 μL 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) 0.5 mM and 10 μL ACh 0.6 mM then added into each samples. The mixture incubated at 37°C for 20 min. The reaction terminated by adding 20 μL physostigmine 0.1 mM. Physostigmine was also used as positive control. The measurement performed at 412 nm and inhibition of AChE activity calculated as followed:

$$\text{inhibition (\%)} = \frac{E - S}{E} \times 100 \quad (1)$$

where, E is absorbance of blank (test well containing ACh and AChE), S is absorbance of test sample (test well containing ACh, AChE and sample). IC_{50} obtained by plotting the correlation of extract concentration and inhibition (%).

Antioxidant assay: Antioxidant activity of the samples measured through DPPH free radical scavenging assay. The DPPH assay carried out refers to Salazar-Aranda *et al*. (2011). The extracts diluted in ethanol, 100 μL extract solution mixed with 100 μL DPPH 125 μM in ethanol and incubated at 37°C for 30 min. The measurement conducted at 517 nm and radical scavenging activity calculated as followed.

$$\text{Radical scavenging activity (\%)} = \frac{A - B}{A} \times 100 \quad (2)$$

where, A is absorbance of DPPH in ethanol and B is absorbance of DPPH in ethanol plus sample solution. IC_{50} obtained by plotting the correlation of extract concentration and radical scavenging activity (%).

RESULTS

The extraction process using n-hexane, ethyl acetate and methanol provide varying extraction yield in range 0.88±0.16-19.03±0.77%. The highest extraction yield for *Syzygium cumini*, *S. aromaticum*, *S. polyanthum* leaves obtained when ethylacetate used as solvent. Meanwhile for *S. aromaticum* bud methanol exhibited the highest extraction yield (Fig. 1).

Result of AChE inhibitory assay signified that extracts of *S. aromaticum* and *S. polyanthum* leaves owned lower IC_{50} value compared to *S. cumini* leave and *S. aromaticum* bud extracts (Fig. 2). Further analysis exhibited that AChE inhibitory activity of n-hexane, ethyl acetate and methanol extract of *S. aromaticum* leaves; ethyl acetate and methanol extract of *S. polyanthum* leaves and methanol extract of *S. aromaticum* bud were in

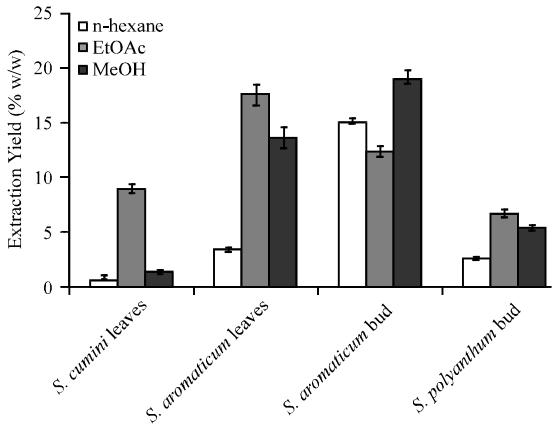

Fig. 1: Extraction yield of *Syzygium cumini*, *S. aromaticum*, *S. polyanthum* leaves and *S. aromaticum* bud

Table 1: AChE inhibitory and antioxidant activity of n-hexane, ethyl acetate and methanol extract of *Syzygium aromaticum* leaves, methanol extract of *S. aromaticum* bud, ethyl acetate and methanol extract of *S. polyanthum* leaves

Botanical names	Part plant used	Extraction solvent	AChE inhibitory activity IC_{50} (μg mL^{-1})	Antioxidant activity IC_{50} (μg mL^{-1})
S. aromaticum	Leaves	n-hexane	62.05±16.62	24.04±1.61
S. aromaticum	Leaves	EtOAc	55.90±3.82	9.20±0.64
S. aromaticum	Leaves	MeOH	42.10±1.41	11.43±0.88
S. aromaticum	Bud	MeOH	45.25±0.07	9.26±0.25
S. polyanthum	Leaves	MeOH	47.30±3.54	21.24±1.14
S. polyanthum	Leaves	EtOAc	45.10±8.06	13.70±0.24
Physostigmine			0.01±0.002	-
Quercetine			-	4.54±0.36

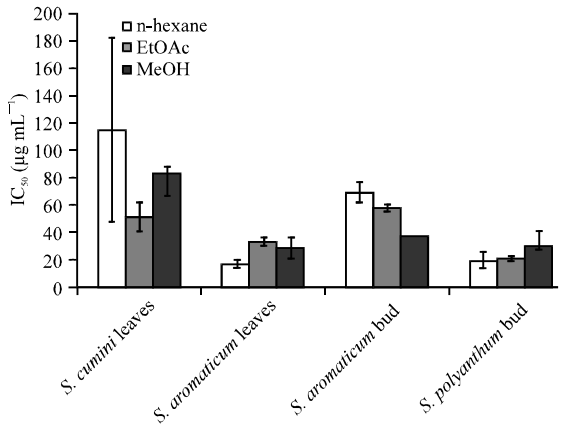

Fig. 2: AChE Inhibitory activity of *Syzygium cumini*, *S. aromaticum*, *S. polyanthum* leaves and *S. aromaticum* bud

range 42.10±1.41-62.05±16.62 μg mL^{-1} when the IC_{50} value of physostigmine was 0.01±0.002 μg mL^{-1} as shown in Table 1.

Antioxidant activity of four extracts selected based on AChE inhibitory activity were presented in Table 1. The IC_{50} value were in range 9.20±0.64-24.04±1.61 μg mL^{-1}. Ethyl acetate extract of *S. cumini* leaves signified the lowest IC_{50} value, while the IC_{50} value of quercetine as positive control was 4.54±0.36 μg mL^{-1}.

DISCUSSION

Extraction yield depend on polarity of the sample components, in accordance with the principle of like dissolve like. The components in *Syzygium cumini*, *S. aromaticum* and *S. polyanthum* leaves expected to be semipolar since optimally extracted with ethyl acetate. On the other hand, the highest yield for *S. aromaticum* bud obtained when methanol used as solvent. This result indicated that most compounds in *S. aromaticum* bud were soluble in polar solvent.

The sample extracts mostly exhibited IC_{50} value less than 100 μg mL^{-1}, it indicated that *Syzygium* used in this work were potential as AChEI. The AChE inhibitory activity of the *Syzygium* extracts was comparable to inhibitory activity of ethyl acetate fraction of *Trigonella foenum* alcohol extract with IC_{50} 53.00±17.33 μg mL^{-1} (SatheeshKumar *et al*., 2010), extract of *Illicium verum* with IC_{50} 39.89±0.32 μg mL^{-1}, oil of *Illicium verum* with IC_{50} 36.00±0.44 μg mL^{-1} (Bhadra *et al*., 2011) and Chinese sufu with IC_{50} 191 μg mL^{-1} (Chen *et al*., 2012). Alikatte *et al*. (2012) reported that *S. cumini* methanol extract possessed antiamnestic activity against scopolamine induced spatial memory impairments in rats. The results of this study were consistent with those reported by Alikatte *et al*. (2012), however this study found that the inhibitory activity of *S. aromaticum* and *S. polyanthum* leaves extracts against AChE were stronger compared to *S. cumini* leaves extracts. Further analysis were carried out to confirm the inhibitory activity of the potential extracts. The result showed that n-hexane, ethyl acetate and methanol extract of *S. aromaticum* leaves; ethyl acetate and methanol extract of *S. polyanthum* leaves and methanol extract of *S. aromaticum* bud consistently inhibit the activity of AChE.

The extracts were also potential as antioxidant since the DPPH assay showed that IC_{50} value of all extracts were less than 25 μg mL^{-1} when IC_{50} of quercetine was 4.54±0.36 μg mL^{-1}. This result was in accordance with other research reported by Shyamala *et al*. (2003) and Har and Ismail, 2012), that *S. aromaticum* and *S. polyanthum* were potential as antioxidant. Phenolic compounds contained in the plants expected to be responsible for antioxidant activity (Shyamala *et al*., 2003; Har and Ismail, 2012). The results signified that antioxidant activity of methanol and ethylacetate extracts of *S. aromaticum* leaves and bud were stronger than *S. polyanthum* leave extracts.

S. aromaticum leaves and bud extracts and *S. polyanthum* leaves extracts were potential both as AChEI and antioxidant. Some report suggest that oxidative stress are correlated with AD (Ho *et al*., 2010; Mariani *et al*., 2005; Mangialasche *et al*., 2009) and oxidative stress is suspected as a major factor implicated in the degeneration of cholinergic neurons in Alzheimer's disease (Saxena *et al*., 2008).

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