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\textsubscript{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\textsuperscript{-1} when IC\textsubscript{50} value of physostigmine was 0.01±0.002 μg mL\textsuperscript{-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\textsuperscript{-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, donepezil 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 (Ch 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 AChE inhibitor has not been properly investigated. Potency of some Syzygium as antiinflammatory have already analyzed (Alkalat 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 Biopharmacy 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:

\[
\text{inhibition} (\%) = \frac{E - S}{E} \times 100
\]  
(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). IC₅₀ 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.

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

where, A is absorbance of DPPH in ethanol and B is absorbance of DPPH in ethanol plus sample solution. IC₅₀ 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 IC₅₀ 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

<table>
<thead>
<tr>
<th>Botanical names</th>
<th>Part plant used</th>
<th>Extraction solvent</th>
<th>AChE inhibitory activity IC$_{50}$ (μg mL$^{-1}$)</th>
<th>Antioxidant activity IC$_{50}$ (μg mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aromaticum</td>
<td>Leaves</td>
<td>n-hexane</td>
<td>62.05±16.62</td>
<td>24.04±1.61</td>
</tr>
<tr>
<td>S. aromaticum</td>
<td>Leaves</td>
<td>EtOAc</td>
<td>55.90±3.82</td>
<td>9.20±0.64</td>
</tr>
<tr>
<td>S. aromaticum</td>
<td>Leaves</td>
<td>MeOH</td>
<td>42.10±1.41</td>
<td>11.43±0.88</td>
</tr>
<tr>
<td>S. aromaticum</td>
<td>Bud</td>
<td>MeOH</td>
<td>45.25±6.07</td>
<td>9.26±0.25</td>
</tr>
<tr>
<td>S. polyanthum</td>
<td>Leaves</td>
<td>MeOH</td>
<td>47.30±3.54</td>
<td>21.24±1.14</td>
</tr>
<tr>
<td>S. polyanthum</td>
<td>Leaves</td>
<td>EtOAc</td>
<td>45.10±8.06</td>
<td>13.70±0.24</td>
</tr>
<tr>
<td>Physostigmine</td>
<td></td>
<td></td>
<td>0.01±0.002</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
<td>-</td>
<td>4.54±0.36</td>
</tr>
</tbody>
</table>

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

![AChE Inhibitory activity graph](image)

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±0.64-21.24±1.14 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$^{-1}$ (SatheshKumar 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 antiarrhythmic activity against scopomolamine 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 leaf 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).
ACKNOWLEDGMENTS

The authors gratefully acknowledge Analytical Division of Chemistry Department, Faculty of Mathematics and Natural Sciences and Biopharmaceutical Research Center Bogor Agricultural University for research facility and funding. Dr Yaya Rukayadi is acknowledged for critical suggestion to improve the manuscript.

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