Potential Malaysian Medicinal Plants for The Treatment of Alzheimer’s Disease

Kesevan Rajah Kumaran, Mohamad Anuar Ahad, Mira Syahfriena Amir Rawa, Habibah Wahab, Zurina Hassan

Abstract
Alzheimer’s disease (AD) is a chronic neurodegenerative disease that leads to dementia. AD is characterized by progressive loss of structure and function of neurons that results in neuronal death over time. In the late stage, patients have difficulties to carry out their daily activity as they lose bodily function and needs assistance of a caretaker. The cause of AD is poorly understood and there is no treatment to reverse the progressive loss of neurons in patients until now. Progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine (ACh) in the brain are the cause of cognitive deterioration in patients suffering AD. Inhibition of cholinesterase (ChE) which are involved in breakdown of ACh into acetic acid and choline may prevent ACh level from decreasing. Currently available drugs that act against ChE are donepezil, rivastigmine and galantamine. These drugs provide symptomatic relief and delays the progress of AD. However, this drugs often produce side effects in consumers. Recently, plants have been given attention as a source of drug to treat neurodegenerative diseases due to its efficiency and less side effect properties. Hence, this review highlights previously and newly discovered potential plants with anti-cholinesterase activity to treat AD.

Key words: Alzheimer’s disease, Malaysian plants, Cholinesterase

Introduction
Alzheimer’s disease (AD) is a leading cause of dementia in elderly people. It is estimated about 15 million people worldwide to be affected with the disease. AD is a progressive neurodegenerative brain disorder and contributes to significant disruption of normal brain structure and physiology. AD is characterized by a progressive loss of neurons, especially pyramidal cells that mediate higher cognitive functions (Mann, 1996; Norfray et al., 2004). In addition, this disease may disrupt the neural circuits communication related to memory and learning process (Selkoe, 2002). The progression of AD starts with degeneration of medial temporal lobe, specifically in the entorhinal cortex and hippocampus (Jack et al., 1997).

The pathogenesis of AD is associated with the cholinergic neurons. Bartus (2000) stated that dysfunction of cholinergic neurons in the central nervous system (CNS) contributes significantly to the cognitive functions; it impairs learning and movement associated with ageing and AD. The ChE is an enzyme involved in cholinergic nerve transmission processes and bound to the cellular membrane of excitable tissues. There are two major enzymes namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The biological function of these enzymes is to catalyse the hydrolysis of the neurotransmitter known as ACh into acetate and choline, which is a reaction necessary for a cholinergic neuron to return to its resting phase after activation (Holmsted, 1971; Lawson et al., 1987; Barr et al., 1988). Excessive breakdown of ACh may lead to cognitive impairment. ACh is a neurotransmitter produced from cholinergic neuron and bind to the specific receptors at post-synaptic neuron (Colovic et al., 2013).
The ChE inhibitor drugs currently used for the treatment of AD are donepezil, rivastigmine and galantamine (Kaduszkiewicz et al., 2005; Graham et al., 2017). Despite of their effectiveness as inhibitors, they may produce adverse effects such as diarrhoea, vomiting and weight loss (Kaduszkiewicz et al., 2005; Hansen et al., 2008). Hence, current research for the treatment of AD is focusing on improvement of cognitive impairment through prevention of the ACh breakdown and development of the AChE inhibitors from medicinal plants (Terry et al., 2003).

2. Detection assay. Cholinesterase assay is a method for the determination of both AChE and BuChE activities. Several reported techniques for ChE assay are colorimetric, spectrophotometric, fluorometric, radiometric, or electrochemical techniques. The assay is used to screen the potential drug for AD (Sramek et al., 2000). The most common method for AChE assay is Ellman’s method. The principal of this assay is using an alternative substrate known as acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). The assay reaction results in production of 5-thio-2-nitrobenzoate that exhibits yellow colour due to the shift of electrons to the sulphur atom. The method was established by Ellman and co-workers in the early 1960s (Ellman et al., 1961) and it is utilized until today with several modifications (Phonaka, et al., 2008).

In this review, we have highlighted some of the previously reported and newly discovered plants with anti-cholinesterase activity by performing the method described above with some modifications. Briefly, for AChE inhibitory assay, 140µL of 0.1 M sodium phosphate buffer (pH 8) was added to each well of 96-well microplate followed by 20µL of the test sample (in DMSO) and 20µL of 0.09 unit/mL AChE. After 15 minutes of pre-incubation at room temperature, 10µL of 10 mM DTNB was added into each well followed by 10µL of 14mM acetylthiocholine iodide as substrate. Absorbance of the end product was measured using Multiskan Go at 412 nm for 30 minutes after initiation of enzymatic reaction. For BuChE inhibitory assay, the same procedure as described above was followed except for the use of enzyme and substrate, which were BuChE and butyrylthiocholine iodide respectively. Absorbance of the test samples were corrected by subtracting the absorbance of their respective blank (test samples in DMSO with substrate and DTNB, but without enzyme). A set of five concentrations was used to estimate the IC50.

Percentage inhibition was calculated using the following formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{absorbance of control} - \text{absorbance of extract}}{\text{absorbance of control}} \right) \times 100
\]

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>Parts</th>
<th>Concentration (µg/mL)</th>
<th>AChE inhibition activity (%)</th>
<th>IC50 (µg/mL)</th>
<th>Concentration (µg/mL)</th>
<th>BuChE inhibition activity (%)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syzygium polyanthum</td>
<td>Salam</td>
<td>Leaves</td>
<td>25</td>
<td>94.65</td>
<td>8.275</td>
<td>20</td>
<td>85.14</td>
<td>6.539</td>
</tr>
<tr>
<td>Coccoloba uvifera</td>
<td>Sea grape</td>
<td>Stems</td>
<td>12.5</td>
<td>97.86</td>
<td>3.782</td>
<td>20</td>
<td>84.33</td>
<td>5.936</td>
</tr>
<tr>
<td>Mimusops elengi</td>
<td>Tanjung</td>
<td>Leaves</td>
<td>25</td>
<td>89.52</td>
<td>9.7</td>
<td>50</td>
<td>90.6</td>
<td>11.879</td>
</tr>
<tr>
<td>Syzygium jambos</td>
<td>Jamba mawar</td>
<td>Leaves</td>
<td>50</td>
<td>85.09</td>
<td>16.049</td>
<td>50</td>
<td>93.39</td>
<td>15.251</td>
</tr>
<tr>
<td>Garcinia mangostana</td>
<td>Mangosteen</td>
<td>Pericarp</td>
<td>5</td>
<td>82.19</td>
<td>1.28</td>
<td>5</td>
<td>44.96</td>
<td>7.17</td>
</tr>
</tbody>
</table>

Table 1 represents the list of plants with their percentage of AChE and BuChE inhibition activities and IC50 values.

3. Cholinesterase inhibitory activities of potential Malaysian medicinal plants

AChE inhibitors are used as a diagnostic modality to prolong the duration of action of the remaining ACh leading to enhancement of cholinergic transmission in AD (Ballard, 2002). Cholinesterase inhibitory activities of some potential Malaysian medicinal plants were listed in Table 1. Garcinia mangostana shows the best AChE inhibitory activity (82% for AChE) with the lowest concentration 5 µg/mL. The definition of IC50 value based on this assay is the concentration of plant extract at which 50% of enzyme is inhibited. Therefore, the lower the IC50 value of plant extract, the more effective the inhibition activity of plant extract with less off-target effect. Among the plant extract against AChE activity, Garcinia mangostana and Coccoloba uvifera...
extract shows lower IC50 value which are 1.28 and 3.78 µg/mL respectively, followed by Syzygium polyanthum, Mimusops elengi, and Syzygium jambos with IC50 value of 8.27, 9.7 and 16.1 µg/mL respectively. For the BuChE inhibition activity, Coccoloba uvifera, Syzygium polyanthum and Garcinia mangostana shows lower IC50 value of 5.94, 6.54 and 7.17 µg/mL respectively followed by Syzygium jambos with IC50 value of and 15.25 µg/mL respectively. The results obtained indicate all plant extracts possess good AChE and BuChE inhibition activities and have potential to be used as medicine to treat AD.

4. Botanical origin and pharmacological properties of potential Malaysian medicinal plants

4.1 Syzygium polyanthum. Syzygium polyanthum or Salam belongs to family member of Myrtaceae that grows in temperate, tropical and subtropical regions of the world. The leaves of this plant are commonly used in cooking to enhance pleasing aroma of local dishes (Shanmugapriya et al., 2012). Besides using as a spice in cooking, Salam leaves have been used traditionally as medicine or therapeutic agents against ulcer, hypertension, hyperuricemia, diarrheal, gastritis, skin diseases and inflammation. In Indonesia, decoction of Salam leaves is used as one of the ingredients in “Jamu” that are prepared specifically to treat diabetes. An in vitro study conducted by Widyawati et al. (2015) revealed that the methanol extract of Salam leaves inhibited the absorption of glucose from the intestine and augment uptake of glucose in muscle tissues. Methanol extract of Salam leaves is able to reduce microbial population in chicken and shrimp with good sensory acceptability, hence may have the potential to be developed as a natural food sanitizer in future (Ram Li et al., 2017). So far there is only one study reported that Salam leaves extract has lower IC50 value for AChE inhibition when compared to other plant in Syzygium genus like S. cumini leave and S. aromaticum bud extracts (Darusman et al., 2013). Our finding further supports the AChE inhibition activity of these plant leaves. In spite of that, there are no studies performed on the BuChE inhibition activity previously. Further studies are needed to reveal the neuroprotective properties of this plant.

4.2 Coccoloba uvifera. Coccoloba uvifera or sea grape belongs to the buckwheat family, Polygonaceae. Sea grape is a sprawling evergreen shrub or small tree that can grow up to 8 meters. This tree is found in coastal beaches throughout tropical America and the Caribbean, including southern Florida, the Bahamas, Barbados and Bermuda. This plant produces green fruit in large grape-like clusters. The fruits gradually change colour to purplish when ripens. Each fruit contains a large pit that constitutes most of the volume of the fruit (Campos, Ruiz, Chel-Guerrero & Ancona, 2015). Sea grape leaves were found to reduce blood glucose level and acts as an antioxidant (Povi et al., 2015). The fruit is a good source of nutrients and shows in vitro antioxidant properties comparable to those of conventional fruits. The antioxidant effects appeared to be due to the phytochemical contents like polyphenols, flavonoids, anthocyanins and ascorbic acid (Campos, Ruiz, Chel-Guerrero & Ancona, 2015). So far, there are no studies performed on neuroprotective effect of this plant. Hence, this is the first report to reveal the AChE and BuChE inhibition activities of this plant. This underutilized plant may provide tremendous health benefits to human. Therefore, more studies are needed to reveal the therapeutic properties along with toxicity of this plant.

4.3 Mimusops elengi. Mimusops elengi or locally known as Tanjung is a 30-feet-tall tree, with a wavy and dull green leaf, greyish brown fissured bark, oblong berry fruit and flowers with creamy fragrant found in tropical and subtropical regions (Amir et al., 2013). The bark extracts have been proven to have anti-anxiety in mice, anti-urolithiatic in rats, anti-ulcer, anti-hyperlipidemic, anti-convulsant, wound healing effect, analgesic, anti-inflammatory and anti-inflammatory activity. β-amyrin caprylate and betulinic acids found in Tanjung bark contribute to the anti-inflammatory action and anti-HIV activity respectively (Prasad et al., 2012). The leaves extract was found to have anti-helminthics activity against Pheretima posthuma, anti-atherosclerotic, anti-diabetic and diuretic activities. Spinasterol in leaves extract has anti-bacterial and anti-inflammatory activity (Amir et al., 2013). Although previously, there are no studies conducted on anti-BuChE activity of this plant, there are quite several studies conducted to investigate the anti-AChE potential and other neuroprotective effect of this plant. An in vitro study conducted by Sainiar et al. (2015) revealed that methanolic extract from lower and leaves of Tanjung can inhibit AChE. Moreover, the inhibition activity of the flower was found to be higher compared to the leaves. Based on in vivo studies, treatment with hydroalcoholic Tanjung flower extract in rats with colchicine-induced AD shows improvement in memory and cognitive function and reduced AChE...
activity were found in the brain of treated rats (Chitra et al., 2016). Ethanolic extract of Tanjung flower up to 2g/kg of body weight does not show any toxic effect (Hadaginhal et al., 2010). Besides flower extract, methanol extract of Tanjung leaves were found to improve memory in mice with amnesia due to normal ageing and administration of scopolamine and diazepam, which suggest that Tanjung leaves may have the potential to be used as a memory invigorating agent. Treatment of 100 and 200mg/kg of Tanjung bark extract is able to decrease whole brain AChE activity significantly and improve amnesia induced by diazepam and scopolamine in both young and old mice (Josji & Parle, 2012). This study revealed that the leaves, flowers and bark of Tanjung plant could have a memory improving potential which can be used as supplements for AD patients to reverse their memory decline.

4.4 Syzygium jambos. Syzygium jambos or also known as “rose apple” is a large bush or small to medium sized woody fruit tree, originated from Southeast Asia, but now widely grown in the tropics (Nawwar et al., 2016). All parts of the plant are reported to have medicinal values. In China, the leaves of rose apple are consumed as herbal tea. The leaves decoction is used as diuretic and to treat rheumatism, sore eyes (Li et al., 2015) and diabetes. The leaves extract was also proven to have anti-bacterial activity against Salmonella typhi (Murugan et al., 2011) and anti-viral activity against vesicular somatitis virus, herpes simplex type 1 and type 2 (Athikomkulchai et al., 2008). The fruits were used as diuretic and tonic for brain and liver. The flowers were believed to reduce fever and the seeds were used to treat diarrhoea, dysentery and catarrh. The bark decoction is administered to relieve asthma and bronchitis. Rose apple extract also had an analgesic effect on muscle hyperalgesia and inflammatory cutaneous pain with an efficacy higher than that of diclofenac (known as an anti-inflammatory drug). It also possesses long-lasting analgesia effect for thermal cutaneous pain with onset time and efficacy comparable to that of morphine (potent opioid analgesic) without involving opioid receptor (Avila-Pena et al., 2007).

To date, there is no in vitro or in vivo studies that reveals the potential of this plant as neuroprotective agent to treat neurodegenerative diseases. This is the first report on the AChE and BuChE inhibition activity of this plant. This plant has the potential to be used in improving cognitive and memory as myricetin present in this plant leaves has been reported to significantly reverse cognitive deficits in scopolamine-induced mice by inhibiting AChE activity (Wang et al., 2017).

4.5 Garcinia mangostana. The tropical plant, Garcinia mangostana or locally known as mangosteen belongs to Guttiferae family. This plant can be found in the Asian region for instance Malaysia, Myanmar, Thailand, Philippines, Sri Lanka and India. Several in vitro and in vivo studies have shown a wide range of pharmacologic actions of mangosteen, including anti-malarial, anti-carcinogenic, anti-bacterial, anti-fungal and anti-atherogenic activities along with neuroprotective properties in AD (Obolskiy et al., 2009). A randomized, double blinded, placebo-controlled study using 59 healthy human subjects with age ranging between 40 to 60 years old, pointed out that the intake of mangosteen product significantly enhances immunity and improves self-appraisal on subject’s overall health condition (Tang et al., 2009). Mangosteen pericarp extract with concentration of 100µg/mL showed 51.93% inhibition activity against AChE meanwhile the positive control, eserine hemisulphate showed 93.87% inhibitory activity at 0.25 µg/mL. The IC₅₀ values of mangosteen and eserine hemisulphate were 42.05 and 0.020 µg/mL respectively. This inhibitory effect of mangosteen pericarp extract proposed that mangosteen has potential application in the treatment of AD (Raghavendra et al., 2011). Huang et al. (2014) found that treatment with pericarp extract significantly decreases the cell mortality and increases the Brain-derived neurotrophic factor (BDNF) level in vitro. After 8 months of dietary supplementation of mangosteen pericarp diet (5000 ppm), a significant improvement on cognitive impairment associated with anti-inflammation, increasing BDNF level and decreasing p-tau were observed in older B6 mice. Besides that, administration of this diet supplement to triple Transgenic Alzheimer’s disease (3×Tg-AD) mice, exerted neuroprotective, anti-oxidative, anti-inflammatory effects and reduces the amyloid-β (Aβ) deposition along with p-tau (S202/S262) levels in the hippocampus, which might further attenuate the deficit in spatial memory retrieval. Sattayasai et al., (2013) found that mangosteen extract reduces reactive oxygen species level and caspase-3 activity as well as improves memory in scopolamine induced mice.

The pericarps of mangosteen are rich in xanthones. Among the xanthones, α-mangostin has received much attention due to its high antioxidant properties (Tjahjani et al., 2014). α-mangostin was identified as an
an AChE selective inhibitor as this compound significantly inhibits more AChE as compared to BuChE. On the other hand, γ-mangostin is classified as a dual inhibitor as it shows almost equal selectivity towards both enzymes. Furthermore, Wang et al. (2012) discovered the potential of α-mangostin to reduce Aβ oligomers induced neurotoxicity in vitro. Molecular docking and dynamics simulations were utilized to demonstrate binding of α-mangostin to Aβ and stabilize a-helical conformation. It was found that α-mangostin could detach Aβ (1-40) and Aβ (1-42) oligomers directly via blotting with oligomer specific antibodies. The disruption of pre-formed fibrils as well as hindering of fibril formation were observed in ThioflavinT fluorescence assay and electron microscopy imaging.

Besides possessing good health benefits, α-mangostin shows low cytotoxic effects against normal liver cells (WRL-68) in vitro. Acute treatment of α-mangostin via oral gavage at single doses of 100, 500, and 1,000 mg/kg does not produce any toxicity in Institute of Cancer research (ICR) mice, a commonly used outbred mice. There were no adverse effects observed on body weight, organ weight, serum biochemistry, histopathology and oxidative stress biomarkers in α-mangostin treated mice (Ibrahim et al., 2015). Additionally, oral administration of α-mangostin did not produce any toxic effect in rats till the dose 1250 mg/kg body weight (Kumar et al., 2016). Due to their high safety and results from the above studies revealed that mango-steen pericarp extract might offer a promising supplementary diet to attenuate cognitive dysfunction in AD.

5. Conclusion. In conclusion, there is still no cure for AD until today. The medicines available in the market will only provide symptomatic reliefs for a short period of time but the degeneration of neurons is continuous gradually. In recent years, medicinal plant-derived natural compounds have received extensive attention as major sources of new therapeutic agents for treating neurodegenerative diseases or neurological disorders. The drugs prepared from plants are considered moderate in efficacy, less toxic and relatively low cost as compared to commonly used pharmaceutical drugs. Hence, utilization of this abundantly available natural resources will provide beneficial health effect on ageing population that are prone to AD.

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References


About the author

Our research focuses on discovering potential bioactive compounds from plants found in Malaysia against cholinesterase enzymes for the treatment of Alzheimer’s disease. Initially, we have screened 300 plant extracts using in vitro enzymatic assay and discovered a number of plant extracts with high AChE and BuChE inhibition activities. Currently, the selected plant extracts are being tested in vivo to determine the effectiveness of this plant extracts in improving memory and inhibiting AChE and BuChE activities in rat’s brain. We also working on isolation and purification of bioactive compounds against AChE and BuChE activities based on bioassay guidance. We hope our research findings could contribute to the development of more effective anti-cholinesterase drugs with less side effects to improve memory in ageing population.