CHAPTER III
LITERATURE REVIEW

3.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia (approx. 50-60%), which can be described as an age-related neurodegenerative disorder. The causes of AD could possibly come from brain cells damaging/injury and complicated with neuron interaction, which induce brain cells to be inoperable, dysfunctional and eventually death. In the early stage, AD patients are incapable of controlling their performances and might be partially unconscious during daily activity. In along term, they may lose cognitive abilities necessary for maintaining an independent living style [14].

3.1.1 Prevalence of Alzheimer's disease

In 2010, the Alzheimer's Disease International reported that more than 35 million people around the world and 2.4 million people in South East Asia are under dementia [15]. In Thailand, the 4th National Health Examination Survey Office (NHESO) under Health Systems Research Institute (HSRI) in 2008 to 2009 suggested that 12.3% elderly people of age 60 years old and above had dementia. Among these, 8.9% were male and 15.0% were female (Table 3.1). This matter could possibly lead to longer lifespan and higher rate of AD development in women than in men [16]. Interestingly, the distribution of dementia was mostly found in the South of Thailand, followed by North, Northwest and Central regions, respectively, which might be due to particular life style, education and environment (Figure 3.1) [16].

Several types of dementia were also reported according to particular symptoms of 45 year olds patients in Chiang Mai, Thailand [17]. The most common dementia in this study was AD (approx. 75%), followed by vascular dementia, hypothyroidism, subdural hygroma and alcoholic dementia, respectively (Table 3.2). Even though no certain pathway to cure AD is available, the occurrence and

progression can be prevented and slowed down. Eventually, the cause of death in AD patients is difficult to diagnose due to possible co-symptoms with other diseases such as diabetes, renal disease and stroke.

Table 3.1 Prevalence of dementia in elderly people differentiated by age and sex [17]

Age	male subject (persons)	male (%)	female subject (persons)	female (%)	total subject (persons)	total (%)
60-69	2,498	5.6	2,559	8.3	5,057	7.1
70-79	1,566	10.6	1,652	18.1	3218	14.7
80 up	442	22.1	493	40.0	935	32.5
Total	4,506	8.9	4,704	15.0	9,210	12.3

Table 3.2 Types of dementia in Chiang Mai population of age 45 years old and above [17]

Classification	n	Percentage
Alzheimer's disease	24	75.0
Vascular dementia	4	12.5
Hypothyroidism	2	6.3
Subdural hygroma	1	3.1
Alcoholic dementia	1	3.1
Total	32	100

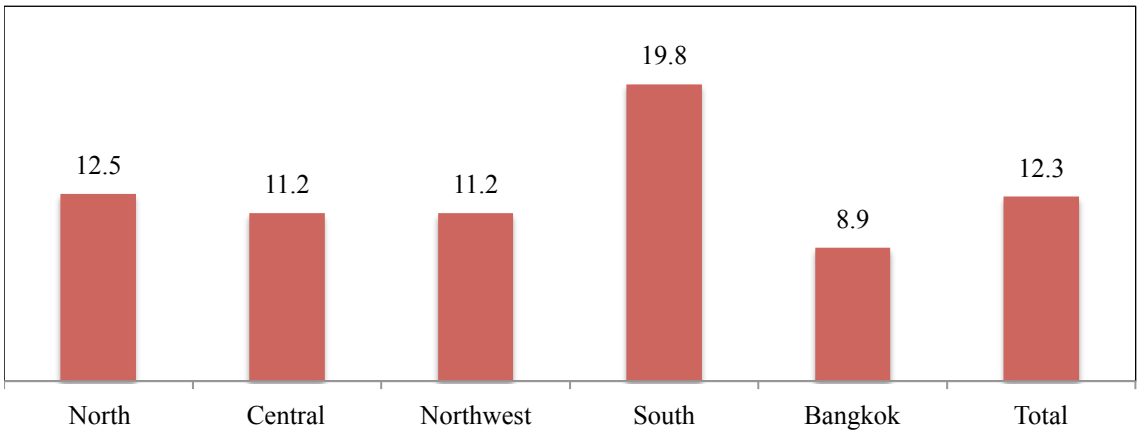


Figure 3.1 Percentage of dementia in elderly people classified by regions in Thailand, including North, Central (except Bangkok), Northwest, South and Bangkok [16].

3.1.2 Symptoms and risk

AD affects each particular individual in different ways. The most common symptom is loss of ability to recognize new memories, which might be a result of neuron cell death or dysfunction [18]. Therefore, the patients might forget some new information such as people name but are still able to recognize their daily life style. Other AD symptoms are related to declined cognition and memory alteration such as aphasia, apraxia, agnosia, constructive difficulties, disorientation, discretion problems and visual/spatial deficits [19]. The characteristics of AD symptoms can be divided in four progressions as early, mild, moderate and severe states.

In the early state, the symptoms start from memory loss or amnestic mild cognitive impairment (MCI). The patients can remember some words or sentences in recent situation and may ask same questions twice. In mild state, the patients have suffered from worse memory loss and change of cognitive function. For example, they may worry about pay bills or money, answer or have repeated questions and reiterated question. Likewise, the patients in the moderate state have suffered from severe language problems such as speech disorder from improper pronunciation, muttering and sentence repeating. In severe state, the memory and cognitive function of the patients are almost completely lost. Caretakers are required to assist the patients for basic daily performances such as eating, dressing and taking shower. After long period of time, patient's health will deteriorate along with muscle weakness, dysphagia, malnutrition and weight loss [14].

Even though nowadays states of AD symptoms are recognized, the causes of AD development are still uncertain and left to be investigated. Possible external and internal factors (such as age, lifestyle, environment, genetic and etc.) were hypothesized in attempt to explain AD progression, which, in turn, would eventually lead to AD prevention and treatment.

3.1.3 Causes of Alzheimer's disease

The causes of AD in elderly are presented progression of cognitive decline, behavior disturbances and memory loss [20, 21]. Scientific researches proposed four main hypotheses of AD development, including a loss of presynaptic markers of a cholinergic system, accumulation of beta-amyloid plaque in the brain,

neurofibrillary tangles or abnormal tau protein in brain cell and oxidative stress induction [22] (Figure 3.2).

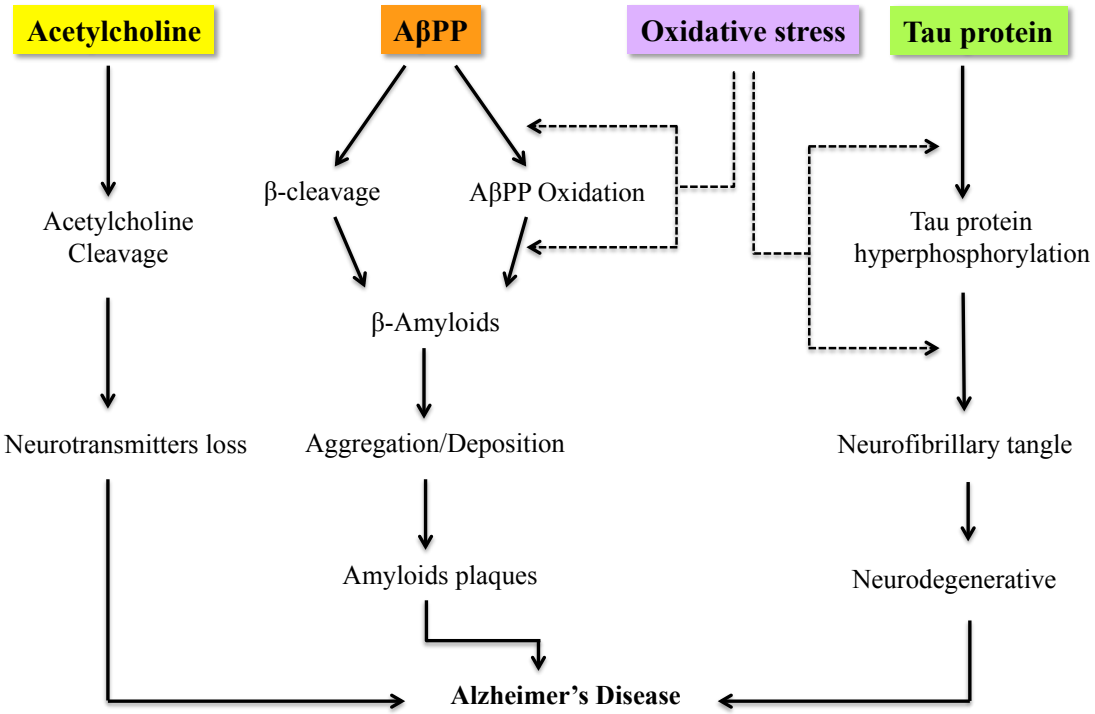


Figure 3.2 Causes of AD pathology. The four major causes are presented as loss of neurotransmitter, accumulation of beta-amyloids plaques, hyperphosphorylation of tau protein and oxidative stress induction [22].

Cholinergic systems

In healthy brain, neuron cells always communicate to each other in one-way direction *via* electrical charges through axon. Its speedy shifts in polarity are called action potential. Once the impulses reach the end of axon, depolarization causes calcium ion to enter the neuron. High concentration of intracellular calcium ion leads to release of neurotransmitters into the synaptic space. The neurotransmitters then bind to their receptor of next target cell or a postsynaptic neuron. The metabolisms are followed the neurotransmitter's transmission, which are particularly interacted with their target receptors, either ligand–gated channel receptors or messenger–linked receptors. The interaction of neurotransmitter and receptor can activate gate channels to open, which allow charges particles to flow through the membrane. Residue

neurotransmitters, however, are hydrolyzed by neurotransmitter enzymes, and converted to choline and re-use for other synapses (Figure 3.3) [23].

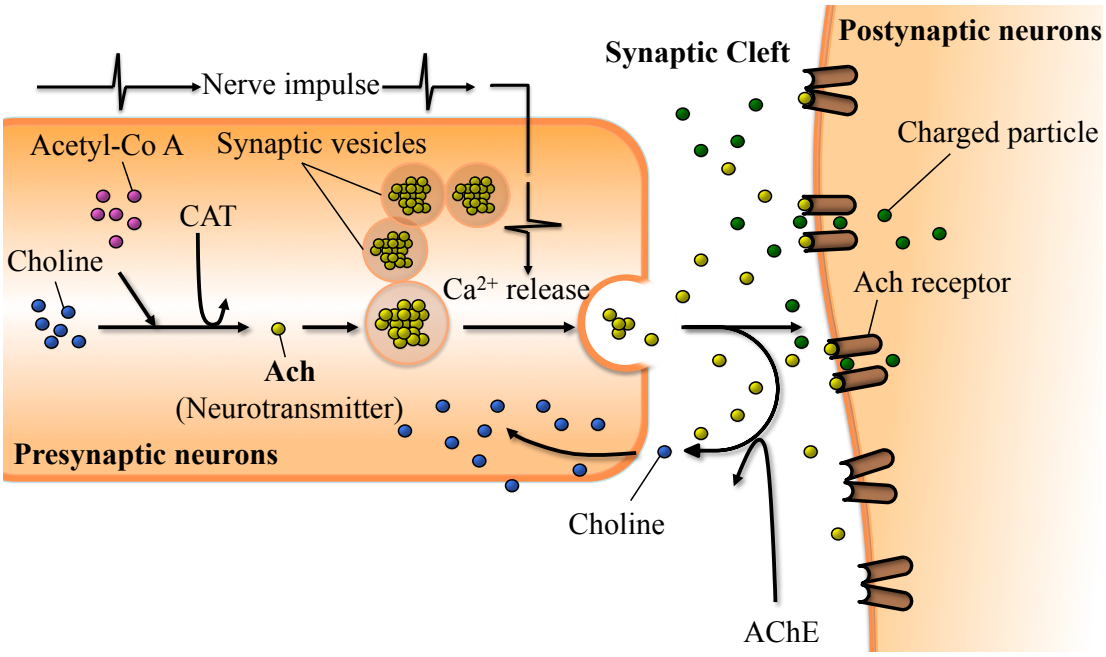


Figure 3.3 Function of acetylcholine synthesis and hydrolysis of acetylcholine in neurons. The excitatory neurotransmitter, acetylcholine (ACh), is associated with brain cognition. ACh is enzymatic synthesized from acetyl-coenzyme A and choline by acetylcholine converting enzyme (choline acetyltransferase or CAT) in presynaptic neurons. ACh interacts with targeted receptors on the post-synaptic neuron to open synaptic channel. Once the process is completed, residual or excessive ACh is enzymatically decomposed by acetylcholinesterase (AChE) into choline and re-used in presynaptic cell for other synapses [23].

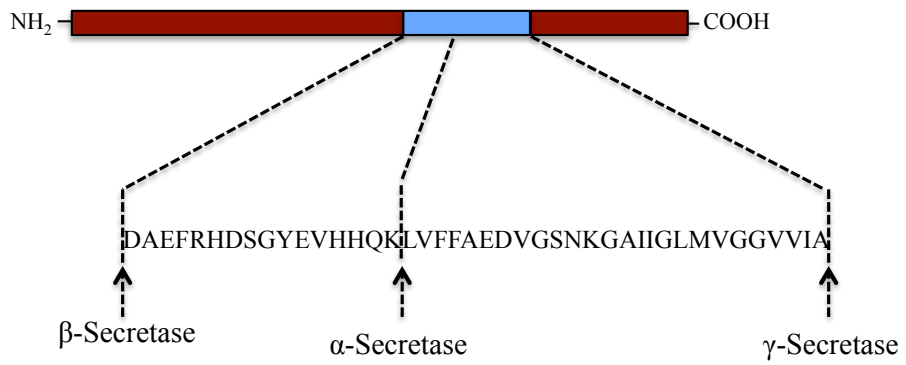
The enzymes responsible for neurotransmitter degradation are cholinesterases (ChEs), including acethylcholinesterase (AChE) and butyrylcholinesterase or pseudocholinesterase (BChE) [24]. AChE is well known for its function to terminate impulse transmission at cholinergic synapsis, while BChE role is poorly understood. Both enzymes are mostly found in vertebrate cells and presented in several tissues [25]. AChE is discovered in brain, nerve cells, muscle and erythrocyte membrane [26]. BChE is presented in liver, intestine, heart, kidney and

lung [26]. AChE and BChE share structural similarity (65%), thus BChE can hydrolyze AChE target [27]. Nevertheless, the activity of ChEs is depended on metabolic rates and affinities of neurotransmitters [28]. In cholinergic hypothesis of AD development described that concentrations of ACh and choline are rapidly declines, whereas AChE is excessively increased, causing nerve cell dysfunction [29]. Thus, cholinergic activities or neurotransmitters are decreased in the central nervous system (CNS), which is a hallmark on brain memory and cognitive function [30].

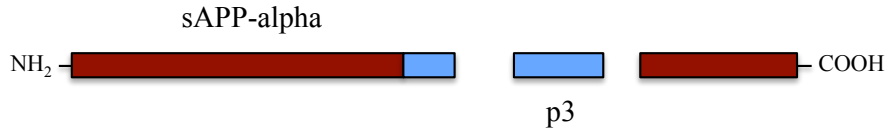
Nowadays, the treatment strategies of AD are mainly aimed to improve cholinergic neurotransmission in the brain cell [31]. This approach is focused on cholinesterase inhibitors (ChEIs) against both AChE and BChE. The ChEIs can stabilize and maintain neurotransmitter in synaptic cleft [32]. Pragmatically, the ChEIs only slow down progression of memory loss and cognitive function. However, these inhibitors cannot completely cure AD pathology, because they could not recover neurodegenerative [33].

Beta-amyloid formation

The amyloid precursor protein (APP) is a membrane glycoprotein, which is available on neuron cell membrane. Normally, it is hydrolyzed by α- and γ-secretases, producing soluble peptide APP-α (sAPP-α) and non-amyloidogenic 3kDa (p3) peptide. The sAPP-α has benefit to promote cell adhesion, neurite outgrowth and synaptogenesis, while p3 has unclear role function. Conversely, APP is also hydrolyzed by β- and γ-secretases, producing soluble peptide APP-β (sAPP-β) and amyloid peptides. The sAPP-β is associated with synaptic pruning and disturbing during development of brain cell and co-operated with amyloid peptides to interfere neuron communication (Figure 3.4) [34]. The amyloid peptides lead to amyloidogenic pathway. These amyloid peptides combine into dimmers, oligomers, and eventually amyloid plaques, which are presented in extracellular brain. The plaques can stimulate innate immune response, leading to cell inflammatory, cell abnormality, and eventually cell death (Figure 3.5) [35, 36, 37]. Besides, extracellular plaques can induce intracellular neurofibrillary tangles, the alternative feature that causes neuronal death [38].



The alpha-pathway



The beta-pathway

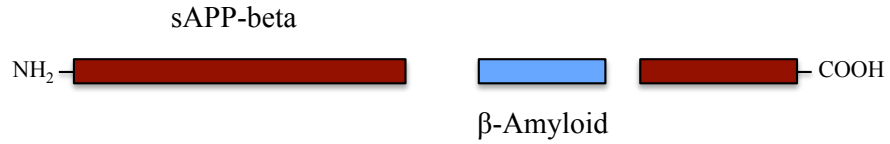


Figure 3.4 Proteolytic cleavage of amyloid precursor protein (APP). The results of α-pathway and β-pathway presented different products. In the α-pathway, the APP sequence was cleaved by α-secretase and γ-secretase and their results showed sAPP-α and p3. In another pathway, the β-secretase and γ-secretase hydrolyzed APP and release sAPP-β and β-amyloid, a cause of AD occurrence [39].

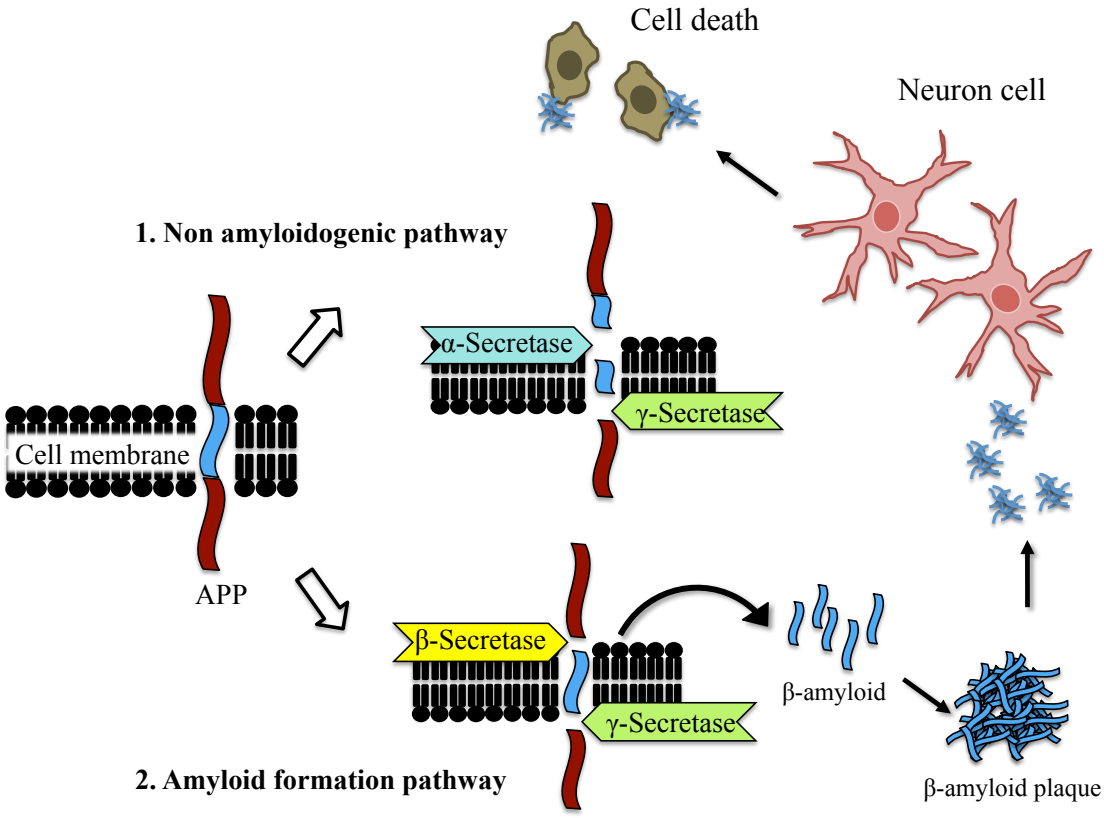


Figure 3.5 The amyloid precursor protein (APP) is a transmembrane protein cleaved by secretase enzymes. APP cleavage is occured in cell membrane, which has two pathways. The non-amyloidogenic pathway involves α- and γ-secretases, while amyloidogenic pathway involves β- and γ-secretases. The later produces β-amyloid peptides that released after cleavage of APP. These peptides can accumulate into dimers, oligomers, and eventually plaque formation. The β-amyloid plaque can interfere the communication of neuron or synapsis and ultimately cause of cell death [40].

Abnormality of tau protein

Tau proteins are axonal proteins that can stabilize and bind microtubules (MTs) inside neuron cell to support internal transport system, synaptic function and maintenance of neuron structure [41, 42, 43]. Tau proteins can be phosphorylated, depending on balance of phosphatases and multiple kinases. Normally, serine/threonine kinase and protein kinase N (PKN) were proteins functioning on tau. Their functions can control tau protein phosphorylation [44]. These proteins are over-

expressed in fetal, suggesting that fetal has highly phosphorylation in brain cell than adult [45]. The tau protein hyperphosphorylation may be regulated by age, sex, gene expression and environment such as oxidative stress induction [46].

The abnormality in phosphorylation or hyperphosphorylation of tau protein leads to MTs neurofilaments dysfunction and neuronal cytoskeleton instabilities. Post-translational modification of tau causes dimeric and oligomeric conformation, which can form self-aggregation, filament (protomers), and subsequently neurofibrillary tangles (NFTs). NFTs are insoluble filaments that can impair transport system such as nutrients and other essential molecules, leading to several neurodegenerative disorders such as AD (Figure 3.6) [47, 48, 49, 50].

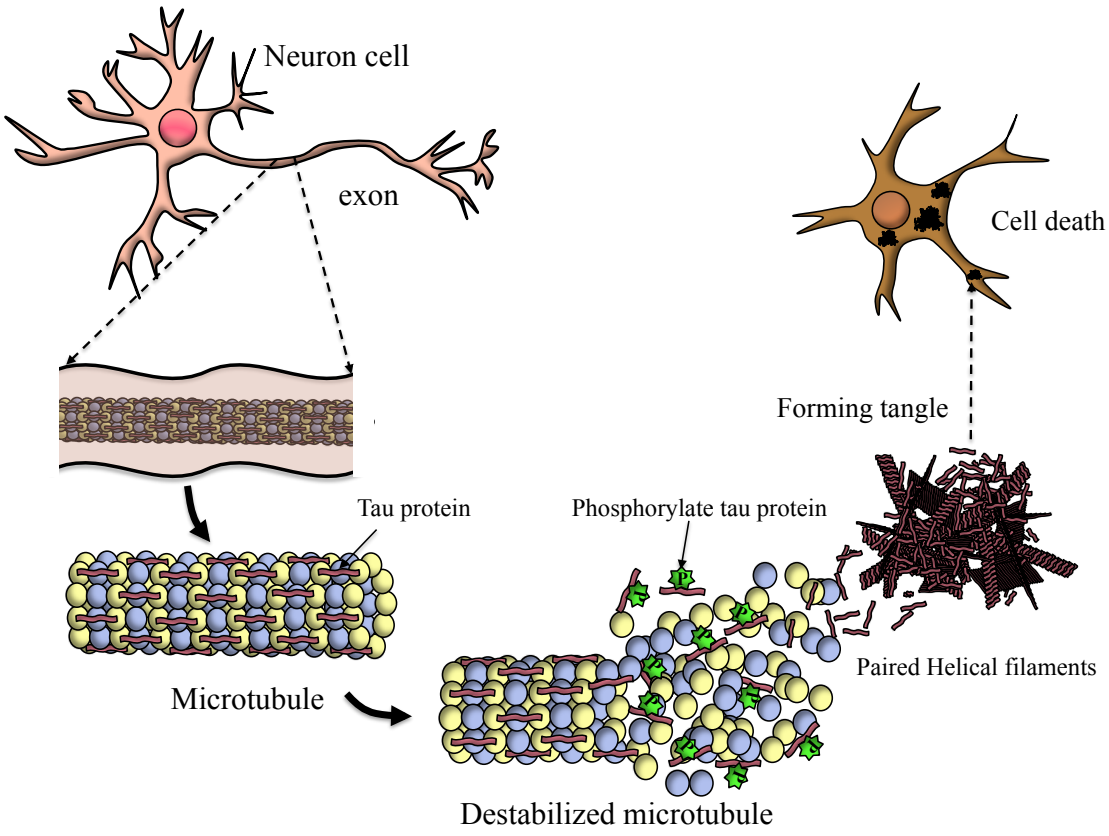


Figure 3.6 The phosphorylation and aggregation of tau protein that cause neurofibrillary tangles, a possible initiation of AD. The function of tau protein is maintained and stabilized MTs in axon. When tau protein is hyperphosphorelated, it cannot bind to MTs and aggregates into tangles. Eventually, its functionality of neuron cells is reduced, leading to development of AD [40].

Oxidative stress induction

Oxidative stress is represented as oxidant and antioxidant unbalance. It showed free radicals in system including reactive oxygen species (ROS) and reactive nitrogen species (RNS). These molecules could occur from endogenous and exogenous sources. The endogenous free radicals are generated by oxidative enzyme and respiratory chain. The exogenous ones are originated in inappropriate environments such as air pollution, ozone, smoking, X-ray and drugs. Suitable amount of free radicals plays a necessary role in gene expression, enzyme-catalytic reaction and transcription-binding protein activation. However, overloaded free radicals might lead to healthy tissues damage and cytotoxicity. Free radicals could interact with biomolecules such as DNA, RNA, protein and lipid. In scientific clinical perspective, free radicals could increase risk of some oxidative related diseases, especially chronic disease and neuronal disease such as cardiovascular disease, cancer, Parkinson's disease, diabetes and AD. The important sources of oxidative stress induced AD are also related to metal homeostasis such as copper, iron, zinc and aluminium, in which their excess quantity can induce free radicals in cell [51]. However, the biological system can maintain condition of excessive free radicals by ant-oxidative agents.

Two hypotheses of oxidative stress influencing on neurodegeneration are proposed. First, oxidative stress appears in periphery neuron, disturbs oxidant-antioxidant level in CNS and induces neurotoxicity. Second, oxidative stress initiates free radicals in periphery neuron, causing cell damage [52]. The markers of oxidative stress could be detected as DNA/RNA oxidation, protein carbonyl production and lipid peroxidation [53]. The results of oxidative stress are increased metabolism of β-amyloids aggregation and tau protein phosphorylation, which indirectly stimulated neuron cell dysfunction (Figure 3.7). In direct partway, oxidative stress would modify and adjust sequence of DNA, leading to development of AD. It was suggested that abnormality of RNA and protein synthesis could be described by amount of lymphocytes being affected by oxidative damaging [54, 55]. It has been reported that oxidative stress can lead to accumulation of β-amyloid augmentation [56, 57, 58]. Conversely, β-amyloids also produce neurotoxics, which directly increase free radicals generation in brain. Moreover, tau protein aggregation is also associated with free radicals. Tau glycation produces free radical byproducts, thus increasing oxidative

stress that promotes tau phosphorylation and accumulation [59, 60]. The oxidative stress also increases tau oxidation and nitration, leading to neurodegenerative and dysfunction [61].

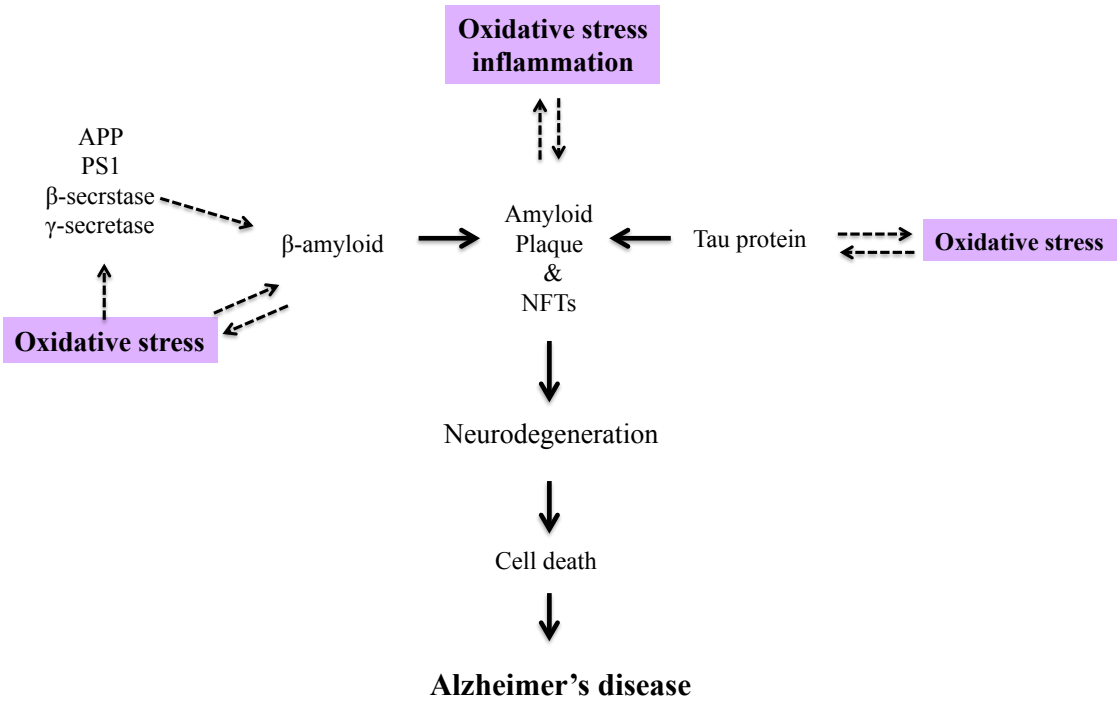


Figure 3.7 Molecular mechanisms of cause factors in AD. The oxidative stress is indirect pathway to induce AD occurrence *via* β-amyloids and tau protein pathway. [62].

Other causes

AD might not be developed through single hypothesis but several factors. It was previously reported that AD could be induced from genetic and environmental/lifestyle factors [14]. Nowadays, it is believed that gene may influence an increased risk of AD. Apolipoprotein E (APOE) gene, especially APOE ε4 allele, was found to be inherited from AD parents, leading to AD pathology [63]. However, the in-depth role of APOE needs to be investigated to expose the relationship between genetic and the disease [64]. On the contrary, environmental and lifestyle factors indirectly affect AD. It was previously found that CNS was associated with air pollution, in which neurodegeneration and neuroinflammation were discovered as increased oxidative stress in brain [65, 66]. Lifestyle is also another external factor that can lead to AD development. Certain behaviors such as high saturated fat

consumption, alcohol drinking and smoking can induce risk of AD occurrence [67]. On the other hand, nutritional diet and exercise can promote healthy lifestyle for each individual on their age, thus preventing and reducing risk of AD [68, 69].

3.1.4 Treatment of Alzheimer's disease

Treatments are important to reduce and slow down symptoms in AD patients (Figure 3.8). AD therapies can improve cognitive function and memory. Currently, therapies and drugs can slow down progression of neuron dysfunction/damage [18]. The United States Food and Drug Administration (FDA) is health service agency, which approves medicinal treatments for AD patients. Several drugs are directly involved with cholinergic hypothesis, but indirectly interacted with β-amyloids aggregation and tau protein phosphorylation. ChEIs are chemical compounds, which are capable of inhibiting hydrolytic activity of ChEs. ChEIs such as physostigmine, donepezil, galantamine, rivastigmine and tacrine can improve AD symptoms by increasing neurotransmitter in neuron cells [70]. However, these drugs have several side effects such as diarrhea, nausea, dizziness, vomiting and headaches [71]. Besides, cholinergic signaling may lead to increased β-amyloid plaque through APP processing, induced hyperphosphorylation of tau protein, which contributes worse status of AD [72].

Interestingly, drugs associated with decreased β-amyloid aggregation/ oligomers and increased β-amyloid clearance are currently under investigation as potential treatment of AD [73]. Drugs are used for prevention of tau protein abnormality and tau aggregation as well as stabilizing microtubules would be a consecutively positive step in the treatment of AD. However, these drugs (such as valproate, lithium, nicotinamide, methylene blue and davunetide) have not been wildly applied to AD patients since they might not cover all symptoms and only provided a few scientific researches in human study [73, 74].

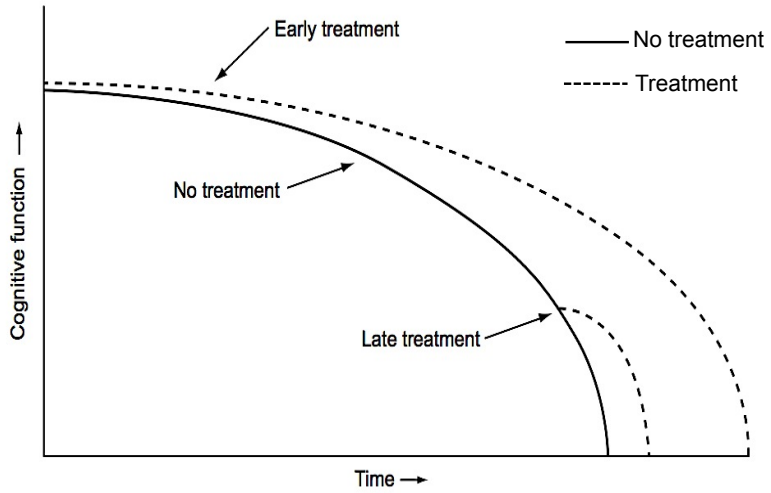


Figure 3.8 Prediction of early treatment and late treatment in AD patients. Treatment using drug plays an important role to slow down progression of AD [75].

3.2 *Pandanus amaryllifolius* Roxb.

3.2.1 Plant characteristic

Pandan (*Pandanus amaryllifolius* Roxb.) is a member of Pandanaceae family, which is subclass of monocot plant. Its actual origination is in Moluccas Islands, Indonesia. Typically, it can populate around tropical areas [76, 77] and distribute to subtropical regions such as Sri Lanka, India, Philippine, Malaysia, Singapore, Taiwan, Vietnam and Thailand (Figure 3.9) [78, 79].

Figure 3.9 Breeding of pandan in tropical and subtropical area. Pandan is tropical plant. It is widely cultivated in Asia, especially South East Asia [77, 78]

Pandan is evergreen perennial plant. It grows in moist soil and clump decumbent. The aerial roots are presented in this plant. Its stem is slender and branches, which can reach up to 1 meter tall. The characteristics of leaves are linear, thin and sharp. Its width and length of leaves are 3-5 centimeters and 40-80 centimeters, respectively. Its leaves grow spiral around the main trunk and prickles are presented on apex of leaves (Figure 3.10). Its shoot is small and covered with its leaves. Its flower is unknown or probably never produced in initial phase [80].

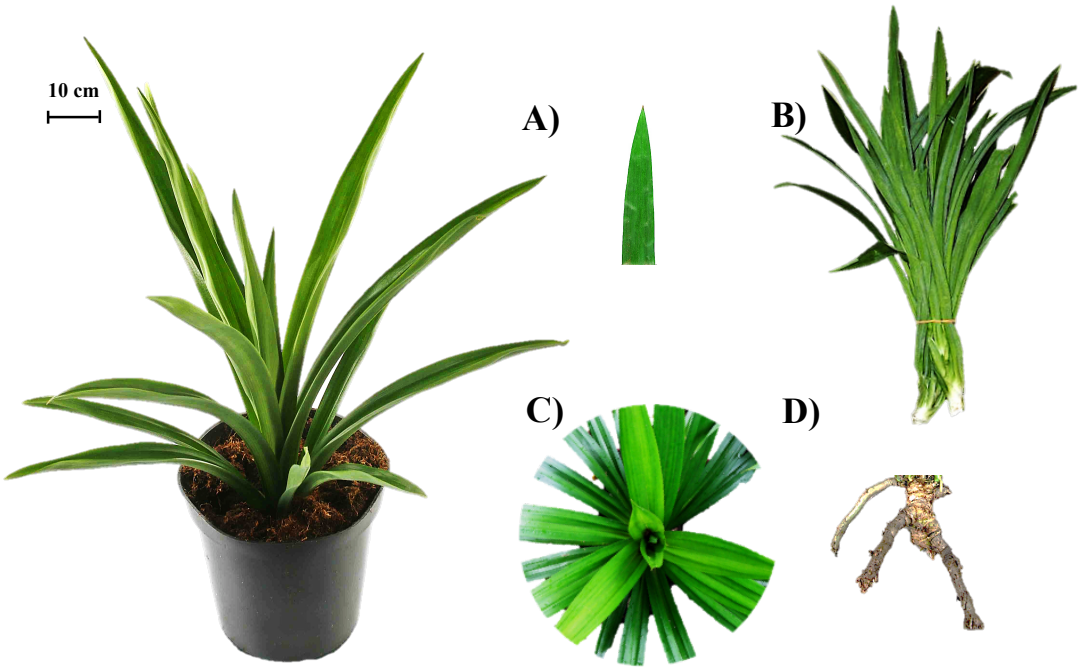


Figure 3.10 Characteristic and morphology of *Pandanus amaryllifolius* Roxb., including (A) apex of leave, (B) leaves, (C) spiral shrub and (D) aerial root [81, 82, 83]

3.2.2 Plant applications

Pandan is usually used for decorative auspicious ceremony and ritual, fragrant leaves, foodstuff, traditional medicine, handicraft and industrial materials such as rope, plaited, hat and bag [84]. Pandan leaves are used for cooking in household. Its leaves generate green color, which is popular natural food coloring. Besides, it produces fragrant, which can be used for aromatherapy. In Asia, it is widely used for flavoring food such as desserts and rice. Leaves juice is also popular, since it is delicious and its scent favor helps relaxing.

Pandan is an interested plant to use in flavor industry. A key fragrant compound, 2-acetyl-1-pyrroline (2-AP, Figure 3.11), is located in its leaves (Figure 3.12, Table 3.3). The non-oriental people identify the fragrant from pandan leaves as popcorn-like aroma. In contrast, oriental people identify this smelling as pandan-like aroma [6, 7]. Pandan fragrant, 2-AP (C_6H_9NO), is a heterocyclic volatile compound that is also a principal scent in rice varieties such as Jasmine and. It is presented in rice endosperm. Interestingly, aromatic rice contains ten times higher concentration of 2-

AP than non-aromatic rice. However, the aromatic rice contains less quantity of this volatile than pandan leaves (ten times lower) [85]. In addition, trace of 2-AP is detected in bread, cracker, sesame, French-fried, corn and beef [86].

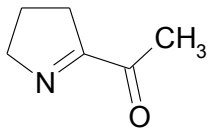


Figure 3.11 Chemical structure of 2-acetyl-1-pyrroline (2-AP), the main fragrant in pandan leaves used for aromatherapy [87].

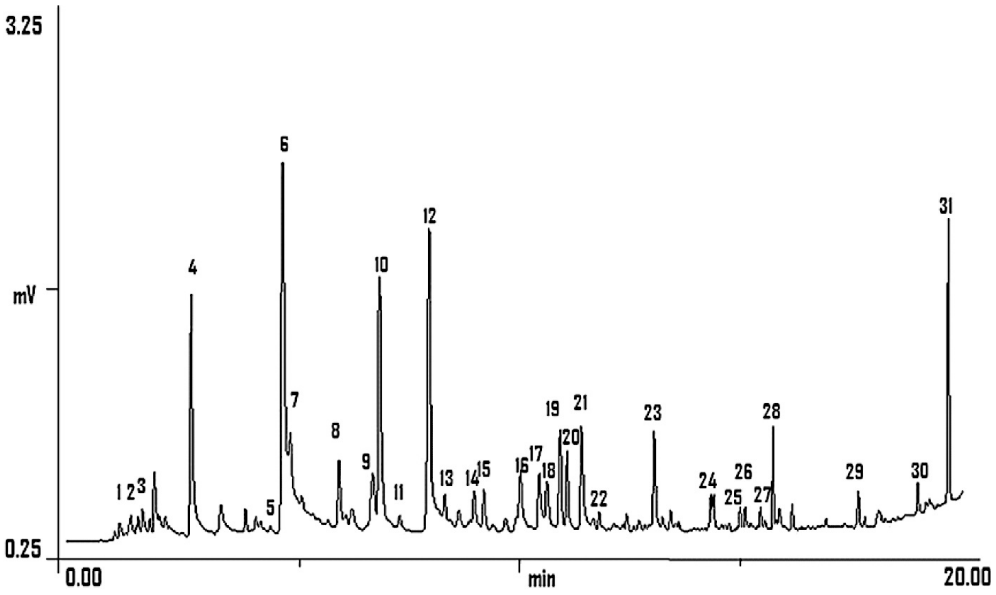


Figure 3.12 Gas chromatography of volatile compounds in pandan leaves. The chromatograph showed 31 peaks of volatile compounds as being analyzed by GC-MS [88].

Table 3.3 Volatiles compounds from pandan leaves as being detected by GC-MS [88].

Peak no.	Compounds	RT	Peak area ± SD (1000)	Percentage of total
1	Dipropyl ether*	1.11	1.41 ± 0.06	0.17
2	Ethyl acetate*	1.47	5.48 ± 1.82	0.68
3	4-Hydroxy-2-butanone*	1.73	5.34 ± 0.45	0.66
4	Hexanal*	2.81	53.44 ± 4.06	6.63
5	2-Pentylfuran*	4.58	0.70 ± 0.18	0.09
6	2-Hexenal	4.84	175.87 ± 12.46	21.87
7	3-Hexenal	5.01	5.80 ± 2.08	0.72
8	3-Methyl pyridine	6.10	21.24 ± 1.16	2.64
9	2-Penten-1-ol	6.86	18.70 ± 1.59	2.32
10	2-Acetyl-1-pyrroline	7.00	68.56 ± 1.57	8.52
11	1-Hexanol*	7.45	3.47 ± 0.34	0.43
12	Nonanal	8.10	84.62 ± 4.90	10.50
13	3-Hexen-1-ol*	8.45	4.51 ± 1.08	0.56
14	2-Octenal*	9.11	7.88 ± 0.13	0.98
15	Dodecane,2,6,11 trimethyl*	9.32	8.39 ± 0.67	1.04
16	2,4-Heptadienal	10.13	13.66 ± 4.95	1.69
17	Benzaldehyde	10.55	16.60 ± 1.08	2.06
18	2-Nonenal*	10.72	15.07 ± 1.16	1.87
19	Linalool	11.02	21.60 ± 4.33	2.70
20	1-Octanol*	11.17	16.40 ± 0.90	2.02
21	2,6-Nonadienal*	11.50	25.16 ± 5.33	3.11
22	b-Cyclocitral*	11.89	3.74 ± 0.70	0.46
23	3-Methyl-2(5H)-furanone	13.11	24.99 ± 5.53	3.12
24	a-Cyclocitrylideneacetone*	14.37	5.38 ± 0.18	0.67
25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol*	15.02	4.70 ± 0.15	0.58
26	b-Ionone*	15.13	4.09 ± 0.50	0.50
27	b-Ionon-5,6-epoxide*	15.47	4.10 ± 0.31	0.51
28	Pentadecanal*	15.75	12.39 ± 2.10	1.53
29	Decanoic acid*	17.65	5.11 ± 0.91	0.63
30	Lauric anhydride*	18.98	4.22 ± 0.29	0.52
31	Phytol*	19.65	43.29 ± 3.03	5.28
	% of identified volatiles			85.08
	% of unidentified volatiles			14.92
	Total			100

Percentage of total: the average value from three-replicated analyses
SD: standard deviation from the mean value.
* New volatile compounds in pandan leaves recently discovered in this report [89, 90].

3.2.3 Health promotion

Pandan leaves are used for health promotion in human regarding their biological properties against chronic and non-chronic diseases. Previous researches suggested the efficiency of pandan leaves extraction towards growth termination of *Staphylococcus aureus*, a pathogenic bacterium that causes food toxicity [8]. Besides, lectin and pandanin isolated from pandan leaves exhibit antiviral properties such as influenza virus (H1N1), herpes simplex virus type-1 (HSV-1) and SARS-associated

coronavirus [9, 10]. Besides, it was found that phytosterol, stigmasterols and other phytochemicals from pandan can be used for anti-cancer agents though cell apoptosis and signal transduction modulation pathway [3]. Aqueous ethanolic extracted pandan leaves could also effectively stimulate insulin secretion *in vivo* and inhibit α-glucosidase *in vitro*, suggesting that pandan leaves possessed anti-hyperglycemic effect [91]. Interestingly, pandan leaves tea could significantly decrease postprandial blood sugar in healthy individual after drinking glucose solution by oral glucose tolerance test (OGTT) method [91]. Moreover, pandan leaves could heal some diseases such as heart disease, fever, headache, sore throat, toothache, flatulence and digestion system [4, 92].

Antioxidant Activity

High level of antioxidant activity was detected in pandan leaves (86-94% with 10 g dried weight/250 mL solvent) as being measured by 2,2- diphenyl-1-picry hydrazyl radical (DPPH) radical scavenging assay [93]. Besides, it was found that pandan leaves and *Curcuma longa* leaves exhibited higher TPCs and antioxidant activities than *Etlingera elatior* flower [94]. It was suggested that pandan leave could provide high antioxidant activity more than some plants.

Several external factors can affect antioxidant activities of pandan leaves. It was previously reported that the dried sample exhibited higher TPCs and antioxidant activities than fresh sample [94]. Besides, the effect of extraction solvent suggested that propylene glycol released higher degree of antioxidants than ethanol [93]. However, propylene glycol is biohazard, thus it is not suitable for future food/drug development used for human [93]. Response surface methodology (RSM) was also applied to optimize extraction condition of antioxidants from pandan leaves [95]. The resulted showed that 78.8% (v/v) aqueous methanol as solvent extraction, 69.5°C as extraction temperature and 32.4 mL/g liquid-to-solid ratio yielded the highest total flavonoids, total phenolics and antioxidant capacity [95].

Anti-Alzheimer property

Previous researches on AD were focused on rhizome and root of ginger, ginseng and turmeric [11, 96, 97, 98, 99, 100, 101, 102]. Ginger was reported to

exhibit anti-AChE activity [96, 97], anti-BChE activity [97] and anti-β-amyloid [98, 99, 100] *in vitro*. The investigation in this area was, as well, extended into animal [101, 102] and human tests [11]. It was found that ginger supplement was more effective toward brain function than placebo group as being evaluated by memory test tools [11].

Interestingly, our preliminary data showed that tea from pandan leaves exhibited higher percentage of AChE and BChE inhibitory activities than those of ginger tea (Figure 3.13). These properties might be related to alkaloids in pandan leaves such as norpandamarilactonine, pandamarilactonine, pandanamine, pandamarilactone, pandamarilactam and pandamarine [92, 103], since it was previously reported that some alkaloids such as steroids, triterpenes and quinolizidine could inhibit ChE reactions [104]. Nevertheless, there is no scientific evidence to support this hypothesis. Therefore, this research was focused on extraction conditions, identification of bioactive compounds, and product development from pandan leaves regarding its effect on anti-AD properties.

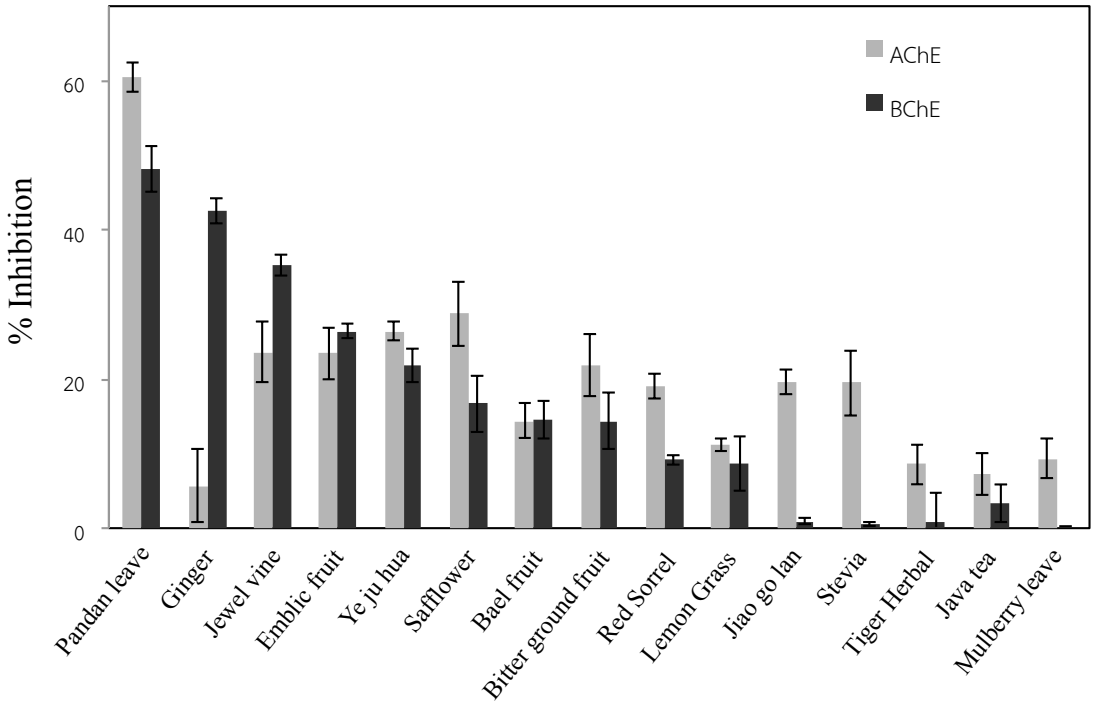


Figure 3.13 Percentage of AChE and BChE inhibitory activity in tea leaves (0.5 g of sample/20 mL of hot water).