CHAPTER I

INTRODUCTION

1. Statement and significance of the problem

Alzheimer's disease (AD) is the sole cause of about half of cases of dementia in later life and a significant contributor to cognitive decline in a further quarter. It is therefore overwhelmingly the most important factor in what has been called the silent epidemic of dementia that is occurring in societies with aging populations. Since this includes the incidence of AD can be confidently expected to rise in all societies where economic progress leads to increased life expectancy, AD ranks with tuber culosis and malaria in economic and social importance. In part and for this reason, an enormous amount of research work has been performed in the last two decades and significant progress has been made in our understanding of the pathophysiology of AD. It is true that no cure is yet in sight, but progress has been substantial and there are several avenues of research that might generate significant therapeutic advances within the next few years (Margaret E. *et al*., 2004).

At present, the prevalence of AD, a neurodegenerative condition whose greatest risk factor is old age and is dramatically increased. It is also expected to continually increase during the next five decades, along with the trend to increase longevity. Currently, it is reported that AD is the main cause of senile dementia in elderly

especially in the United State of America. The number of the new cases of AD in the United State of America was expected to increase approximate 27%, 70% and 300% in 2020, 2030 and 2050 respectively. These situations were expected to increase the expenditure cost approximate 80% with in the year 2010. The prevalence of AD in Thailand was estimated to 953,904 from 64,865,523 Thai populations in 2004. Thus the expenditure cost for managing this problem is also increases dramatically. Therefore, AD is one of the important problems in Thailand and worldwide.

The protection of neurons against damage and death, with preservation of function in chronic. In the 21st century, progressive neurodegeneration in AD is claimed to be one the major challenges. Based on data from animal models, basic science, epidemiological studies and several cross sectional study, diet and products containing antioxidant activities have been reported to prevent the progression of disease in patient with moderate AD.

Since AD, one of the most common cause of death worldwide, has happened to be one of the threaten disease to public health, new treatment strategies have been focused on treatment AD. Many pharmaceutical industries put an effort to find out the natural potential sources of drug for prevention and treatment of AD. In traditional medicine, many plants have been used as nerve tonic and improve cognitive function. Therefore, the medicinal plants reputed for nerve tonic and for memory improvement in traditional folklore are very much attractive for the potential source of new drugs against and prevent AD.

Acetylcholine (ACh) is a neurotransmitter inhibited primarily by acetylcholinesterase (AChE) and secondly by butyrylcholinesterase (BuChE), considered to play a role in the pathology of AD. Both enzymes are presented in the

brain and are detected among neurofibrillary tangles and neuritic plaques. Despite the unknown etiology of AD, elevation of acetylcholine amount through AChE enzyme inhibition has been accepted as the most effective treatment strategy against AD (Arnold and Kumar, 1993). Therefore, AChE and BuChE inhibitors have become the remarkable alternatives in treatment of AD (Orhan I.B. *et al*., 2004).

Four commercial cholinesterase inhibitors (ChEIs) approved by US Food and Drug Administration (US FDA) and presently used in AD therapy are tacrine (Arrieta J.L.A.F., 1998; Qizibash N. *et al*., 1998), donepezil (Jones *et al*., 2003), rivastigmine (Polinsky R.J., 1998) and galanthamine (Jones R.W., 2003). Although all commercial ChEIs are effective in AD treatment, those ChEIs still have undesirable side effects and very expensive. Therefore, the search for new ChEIs is still ongoing from several investigators.

The prime purpose in treating AD is to preserve the memories in the brain, in addition to slowing the loss of cognition. There are herbs that serve this purpose. It makes sense to consider herbs that enhance and stimulate mental activity in the brain. Plants of the amaryllidaceae family are well-known for their ornamental value but also for the alkaloids they produce. Some of these alkaloids have been found to exhibit interesting pharmacological and/or biological properties. However, the most extensively studied effects are those of nonspecific inhibition, such as antiviral and antitumour. Galanthamine, an amaryllidaceae alkalolid, is a long acting, selective, reversible and competitive acetylcholinesterase inhibitor (AChEIs), which produces beneficial effects even after the drug treatment has been terminated. This product is marketed as a hydrobromide salt under the name Reminyl® for the treatment of AD. Plants of the genus *Narcissus* L., which belong to the amaryllidaceae family, are well-

known for their alkaloids (Susana L. *et al*., 2002). In our previous study on twelve species of amaryllidaceae plants in Thailand which found in Genus; *Hippeastrum, Zephyranthes, Crinum, Hymenocallis, Phaedranassa* and *Haemanthu.* They exhibited potential AChE inhibitory activity compared with physostigmine. The present study investigates the AChE inhibitory activity and the phytochemical characteristics of *Haemanthus multiflorus* Martyn, which is one of amaryllidaceae plants grown in Thailand.

2. Literature review

2.1. Alzheimer's disease (AD)

AD is the most common cause of dementia, afflicting 24 million people worldwide. AD is a degenerative and terminal disease for which there is currently not cure. In its most common form, it occurs in people over 65 years old although a less-prevalent early-onset form also exists. The disease can begin many years before it is eventually diagnosed. In the early stages, short-term memory loss is the most common symptom, often initially thought to be caused by aging or stress by the sufferer. Later symptoms are confusion, anger, mood swings, language breakdown, long-term memory loss, and the general withdrawal of the sufferer as his or her senses decline. Gradually the sufferer loses minor, and then major bodily functions, until death occurs. Although the symptoms are common, each individual experiences the symptoms in unique ways. The duration of the disease is estimated as being between 5 and 20 years. The symptoms of AD are generally reported to a physician when memory-loss causes concern, and on suspecting AD, the physician or healthcare specialists will confirm the diagnosis with a behavioral assessment and cognitive tests, often followed by a brain scan. The cause and progression of AD is not well understood, but is associated with plaques and tangles in the brain. Possible causes and potential cures of the disease have been conjectured, with varying evidence supporting each claim. No treatment has been found to stop or reverse the disease, and it is not known whether current treatments slow the progression, or simply manage the symptoms. Many preventative measures have been suggested for AD, but

their value is often uncertain: mental stimulation, exercise and a balanced diet are usually recommended, both as a possible prevention and as a sensible way of managing the disease. Due to the incurable and degenerative nature of the disease care-management of AD is essential. The role of the main caregiver is often taken by the spouse or a close relative. Caregivers may themselves suffer from stress, over-work, depression, and being physically hit or struck. (The GNU., 2008)

2.2 Manifestations of AD

Patients of AD generally have three kinds of symptoms. These have been grouped as the A , B and C (Chicot J.V., 2001).

'A' stands for impairment in Alzheimer of daily living. The ability to perform activities such as brushing, bathing, toilet habits and dressing is lost, usually in the more advanced stages of the disease.

'B' stands for abnormal behaviour in the patient. This can be very distressing, for example, patients may suspect their spouse of 50 years to be a thief, not recognize their own children, or make sexual advances towards servants. Some patients even become abusive, possibly in contradiction to their past behaviour. Restlessness at night is another pattern which is very troublesome to the entire family.

'C' stands for loss of cognitive functions. The intellectual capability of the patent is lost. The ability to perform activities that they were very good at, such as arithmetic calculations, planning, giving their opinion and making difficult decisions, is lost. They seem to get confused when confronted with the slightest problem.

The most striking aspect is the pattern of loss of intellectual function that follows the principle of "last learnt, first lost".

2.3. Etiology of AD

The definitive diagnosis of AD is made at autopsy. The gross neuroanatomical finding include diffuse atrophy with flattened cortical sulci and neuronal loss, synaptic loss, and granulovascular degeneration of the neurons. Senile plaques, or amyloid plaques, are most indicative of AD but may be present in down's syndrome and, to a lesser degree, in normal aging. The amount of senile plaques found in autopsy has been correlated to the severity of the dementia that affected the person with AD (Ruth A.H. and Ben A., 2000)

2.3.1. Neurotransmitter Abnormalities

The neurotransmitters most studied are ACh and norepinephrine. Both are hypothesized to be hypoactive in AD. Several studies have found specific degeneration of cholinergic neurons in the nucleus basalis of Meynert in persons in the nucleus basalis found decreases in ACh and cholinergic acetyltransferase concentrations in the brains of individuals with AD, which suggests a decrease in the number of cholinergic neurons. Because it is known that cholinergic antagonists, such as scopolamine and atropine, impair cognitive abilities, and that cholinergic agonists, such as physostigmine and arecoline, enhance cognitive abilities, the cholinergic deficit hypothesis is further supported.

Some pathological examinations of brains from individuals with AD have found a decrease in the norepinephrine-containing neurons in the locus ceruleus. Two other neurotransmitters the neuroactive peptides somatostatin and corticotropin have also been reported to be decreased in AD.

2.3.2. Amyloid Precursor Protein

There are four forms of amyloid precursor protein. One of which, the beta/A4 protein, is the major constituent of senile plaques. Current research suggest that beta-amyloid (Aβ) deposits play a central role in the development of AD.

2.3.3. Genetic Predisposition

The genetic risk factors AD have been linked to three chromosomal abnormalities. Research has found that each of these abnormalities results in an accumulation of insoluble Aβ within cortical and subcortical neurons.

Abnormal chromosome 21 has been linked to an altered amyloid precursor protein (APP). Mutations in the amyloid precursor protein gene on chromosome I are known to cause early-onset (before age 65) AD.

Abnormal chromosome 19 has been associated with late-onset AD. Varible genes on this chromosome cause production of apolipoprotein E4 (ApoE4). ApoE4 causes diffuse depositions of insoluble Aβ.

Abnormal chromosome 14, associated with onset of AD before the age of 50, leads to mutated from of the APP.

2.3.4. Other Potential Causes

Some researchers are exploring the theory that abnormality in the regulation of membrane phospholipid metabolism results in membranes that are more rigid in AD. Others are looking for a possible viral connection or a combination of aforementioned factors as causing AD.

2.4. Pathophysiology of AD

2.4.1. Neuropathology

At a macroscopic level, AD is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe , and parts of the frontal cortex and cingulate gyrus. Both amyloid plaques and neurofibrillary tangles (NFT) are clearly visible by microscopy in AD brains. Plaques are dense, mostly insoluble deposits of Aβ protein and cellular material outside and around neurons. Tangles are insoluble twisted fibers that build up inside the nerve cell. Though many older people develop some plaques and tangles, the brains of AD patients have them to a much greater extent and in different brain locations.

2.4.2. Biochemical characteristics

AD has been identified as a protein misfolding disease, or proteopathy, due to the accumulation of abnormally folded A-beta and tau proteins in the brains of AD patients. Plaques are made of a peptide called Aβ, a protein fragment snipped from a larger protein called APP. APP is a transmembrane protein; which means that it sticks through the neuron's membrane; and is believed to help neurons grow, survive and repair themselves after injury. In AD, something causes APP to be divided by enzymes through a mechanism called proteolysis. One of these fragments is Aβ. Aβ fragments (amyloid fibrils) outside the cell come together into clumps that deposit outside neurons in dense formations known as senile plaques. AD is also considered a tauopathy due to abnormal aggregation of the tau protein. Healthy neurons have an internal support structure, or cytoskeleton, partly made up of structures called microtubules. These microtubules act like tracks, guiding nutrients and molecules from the body of the cell down to the ends of the axon and back. A special kind of protein, tau, makes the microtubules stable through a process named phosphorylation and is therefore called a microtubule-associated protein. In AD, tau is changed chemically, becoming hyperphosphorylated. Hyperphosphorylated tau begins to pair with other threads of tau and they become tangled up together inside nerve cell bodies in masses known as NFT. When this happens, the microtubules disintegrate, collapsing the neuron's transport system. This may result first in malfunctions in communication between neurons and later in the death of the cells.

2.4.3. Disease mechanism

Three major competing hypotheses exist to explain the cause of the disease. The oldest, on which most currently available drug therapies are based, is known as the cholinergic hypothesis and suggests that AD is due to reduced biosynthesis of the neurotransmitter ACh. However, the medications that treat ACh deficiency only affect symptoms of the disease and neither halt nor reverse it. The cholinergic hypothesis has not maintained widespread support in the face of this evidence, although cholinergic effects have been proposed to initiate large-scale aggregation, leading to generalized neuroinflammation.

In 1991 the amyloid hypothesis was proposed, while research after 2000 is also centered on tau proteins. The two positions differ with one stating that the tau protein abnormalities initiate the disease cascade, while the other states that Aβ deposits are the causative factor in the disease. The tau hypothesis is supported by the long-standing observation that deposition of amyloid plaques does not correlate well with neuron loss, but a majority of researchers support the alternative hypothesis that Aβ is the primary causative agent. The amyloid hypothesis is compelling because the gene for the Aβ precursor is located on chromosome 21, and patients with trisomy 21 (Down Syndrome) who thus have an extra gene copy almost universally exhibit AD-like disorders by 40 years of age. The traditional formulation of the amyloid hypothesis points to the cytotoxicity of mature aggregated amyloid fibrils, which are believed to be the toxic form of the protein responsible for disrupting the cell's calcium ion homeostasis and thus inducing apoptosis. It should be noted further

that ApoE4, the major genetic risk factor for AD, leads to excess amyloid build-up in the brain before AD symptoms arise. Thus, Aβ deposition precedes clinical AD. Another strong support for the amyloid hypothesis, which looks at Aβ as the common initiating factor for AD, is that transgenic mice solely expressing a mutant human APP gene develop fibrillar amyloid plaques. (The GNU., 2008)

2.5. Treatment of AD

There is no known cure for AD, nor has any therapy shown an ability to delay or halt the progression of the disease. Available treatments offer relatively small symptomatic benefit but remain palliative care in nature. Current treatments can be divided into pharmaceutical, psychosocial and caregiving. There are also many potential treatments undergoing investigation, some of which are in clinical study. Four medications are currently approved for to treat the manifestations (dementia symptoms) of AD by regulatory agencies, including the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMEA). Three are AChEIs and the other is memantine, an *N*-methyl-D-aspartic acid (NMDA) receptor antagonist. No drug has an indication for delaying or halting the progression of the disease. Because reduction in the activity of the cholinergic neurons in the disease is well known, AChEIs are employed to reduce the rate at which ACh is broken down and so to increase the concentration of ACh in the brain, thereby combatting the loss of ACh caused by the death of the cholinergic neurons. ChEIs currently approved include donepezil (Aricept®), galantamine (Razadyne®), and rivastigmine (Exelon®, and Exelon® Patch). There is also evidence for the efficacy of these medications in

mild to moderate AD, and some evidence for their use in the advanced stage. Only donepezil is approved for treatment of AD dementia during this stage. The use of these drugs in mild cognitive impairment has not shown any effect in a delay of the onset of AD. Most common side effects include nausea and vomiting, both of which are linked to cholinergic excess. These side effects arise in approximately 10% to 20% of users and is mild to moderate in severity. Less common secondary effects include muscle cramps, decreased heart rate (bradycardia), decreased appetite and weight, and increased gastric acid.

Glutamate is a useful excitatory neurotransmitter of the nervous system, although excessive amounts in the brain can lead to cell death through a proccess called excitotoxicity. The overstimulation of glutamate receptors leads to increase glutamate levels. Excitotoxicity not only occurs in AD, but also in other neurological diseases such as Parkinson's disease or multiple sclerosis. Memantine (Akatinol®, Axura®, Ebixa®/Abixa®, Memox® and Namenda®), is a noncompetitive NMDA receptor antagonist first used as an anti-influenza agent that acts on the glutamatergic system by blocking NMDA glutamate receptors and inhibits their overstimulation by glutamate. Memantine has been shown to be moderately efficacious for the treatment of moderate to severe AD, and is the only drug currently approved for severe AD. Its effects in the initial stages are unknown. Reported adverse events with memantine are infrequent and mild, including hallucinations, confusion, dizziness, headache and fatigue.

Psychosocial interventions are used as an adjunct to pharmaceutical treatment and can be classified within behavior, emotion, cognition or stimulation oriented approaches. Research on efficacy is unavailable and rarely specific to the

disease, focusing instead on dementia. Behavioral interventions attempt to identify and reduce the antecedents and consequences of problem behaviors. This approach has not shown success in the overall functioning of patients, but can help to reduce some specific problem behaviors, such as incontinence. There is still a lack of high quality data on the effectiveness of these techniques in other behavior problems such as wandering.

Since there is no cure for AD, care giving is an essential part of the treatment. Due to the eventual inability for the sufferer to self-care, AD has to be carefully care-managed. Home care in the familiar surroundings of home may delay onset of some symptoms and delay or eliminate the need for more professional and costly levels of care. (The GNU., 2008)

2.6. Acetylcholine (ACh)

ACh was the first known neurotransmitter. It was also recognized as an important neurotransmitter in learning and memory process (Muir J.L.,1996). This neurotransmitter could be found in brain, neurotransmitter junction, spinal cord and in both the postganglionic terminal buttons of the parasympathetic division of the autonomic nervous system and the ganglia of the autonomic nervous system.

It was discovered that the nucleus basalis, particularly in the nucleus basalis of Meynert, was a source of ACh. Other central cholinergic projections were also reported including the cholinergic projection from the adjacent medial septum and diagonal band of broca to hippocampus (Squire L.R.,1987). The cholinergic system

in the neocortex particularly in the basal forebrain was postulated to be the sites for memory storage.

In AD, there are many prominent changes occurred including the degeneration of the cortex, basal forebrain and hippocampus, which led to a profound loss of memory. Although the degeneration of basal forebrain cholinergic neurons was claimed to be an important cause of cognitive impairment in AD, (Coyle J.T. *et al*.,1983) a number of other neurotransmitter systems were also severely affected, including those using the excitatory amino acids aspartate and glutamate as neurotransmitters.

2.7. ChE enzymes

ChE is a group of ubiquitous classes of serine hydrolase that role in hydrolysis of choline esters. In vertebates, two forms of ChE are identified: AChE and BuChE (Massoulie J. *et al*., 1993). The main function of AChE is the rapid hydrolysis of ACh at cholinergic synspses (Taylor P. and Radic Z., 1994). ACh has not generally been regarded only as a neurotransmitter. In the central nervous system (CNS), it has been considered to act more as an overall modulator and moderator in the cerebral cortex which amongst other things enhances the effects of glutamate so that the two systems interact very closely (Giacobini E. *et al*., 1989; Court J.A. *et al*., 1993). AChE is the principal enzyme that presents in the central and peripheral nervous system, (Brimijoin S.,1983) plasma and red blood cells (Heller M., 1972 and Szelenyi J.G. *et al*., 1982).

BuChE presents in glia cell selected CNS neurons such as the lateral geniculate bodies, the interstitial nucleus of the ventral hippocampal commissure, the islets of calleja, some cell of the lateral thalamic nucleus, the nucleus reuniens of the thalamus, the interstitial nucleus of the ventral supraoptic decussation and a few cells of the medial habenular nucleus, (Graybile A.M. and Ragadale, 1982; Shute C.C. and Lewis P.R.,1963) but mainly is synthesized in liver as acirculating plasma glycoprotein. Besides of function in ACh hydrolysis, the biological role of BuChE is still unclear. It has been proposed that it scavenges anti-ChE agent, protecting synaptic AChE from inhibition and the multitude of ACh receptor from blockade (Soreq H. *et al*.,1992). In addition, it has been suggested to function as a decoy to protect the highly essential cholinergic AChE from inhibition (Soreq H. and Zakut H., 1990; Ehrlich G. *et al*.,1994). Nevertheless, this functional view of BuChE is already changing as recent findings suggest that the BuChE may modulate cholinergic transmission in smooth muscle and substitute for AChE in the neuromuscular junction (Li B. *et al*., 2000; Norel X. *et al*., 1993). Furthermore, a reduction of BuChE activity, either due to treatment with ChEIs or due to possession of BuChE with reduced activity, may influence the cognitive function of patients with AD (Giacobini E. *et al*., 2002; Perry E. *et al*., 2003; Brienet O.J.T. *et al*., 2003).

In AD, the central BuChE has been implicated in the development of AD. BuChE may participate in the transformation of Aβ from an initially benign form to an eventually malignant form associated with neuritic tissue degeneration and clinical dementia (Gulliozet A.L. *et al*., 1997).

2.8. AChE and neuronal activity in learning and memory process

Several reports have suggested that the acute administration of AChEIs such as pyridostigmine enhanced neuronal excitability and induced long-lasting alteration in gene expression (Friedman A. *et al*., 1996; Kaufer D. *et al*., 1998). The mechanisms underlying AChE-induced changes in neuronal excitability have not been completely understood. The mechanisms may be from ACh-related mechanism (Cole A.E. and Nicoll R.A., 1984) or other mechanisms not related to ACh (Polinsky R.J., 1998; Moretto A., 1998; Gray R. *et al*., 1996). In amnesia model animal induced by scopolamine, a muscrinic receptor antagonist, several AChEIs have been reported to prevent the scopolamine-induced disruption of memory function (Honer W.G., 1987; Summers W.K. *et al*., 1986). In particular, ChEIs such as physostigmine and tacrine were reported to improve learning and memory (Davis K.L. and Mohs R.C., 1982; Summer W.K. *et al*., 1986). The mechanisms of those cognitive enhancers are still unclear. The beneficial effect in the AD patients from AChEIs could be due to its enhancement of neuronal activity.

3. Relevant research

Inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as a promising strategy for the treatment of neurological disorders such as AD, senile dementia, ataxia and myasthenia gravis. A potential source of AChEIs is certainly provided by the abundance of plants in nature.

Thirty-two plants used in Thai traditional rejuvenating and neurotonic remedies were collected and their methanolic extracts were tested for AChE inhibitory activity using Ellman colorimetric method in 96-welled microplates. The results showed that the methanolic extracts from roots of *Stephania suberosa* Forman. and *Tabernaemontana divaricata* (L.) R.Br. ex Roem.&Schult. at concentration of 0.1 mg/ml inhibited more than 90% of AChE activity. At the same concentration, four extracts, i.e. stems of *Piper interruptum* Opiz., seeds of *Piper nigrum* L., rootbarks of *Butea superba* Roxb. and roots of *Cassia fistula* L. extracts showed 50–65% inhibitory activity on AChE. The rest of the extracts showed the AChE inhibitory activity below 50% (Kornkanok I. *et al*., 2003).

Several alkaloids are produced by *Haemanthus*, a genus belonging to the amaryllidaceae (Shibnath G. *et al*., 1985). These perennial plants are used against coughs, dropsy, asthma and as topical antiseptics. Some species have shown antiviral activity on RNA viruses, e.g. Poliovirus, as well as antitumour and antineoplastic activity, but are also reported for their toxicity, probably due to the presence of alkaloids (Louw C.A.M *et al*., 2002).

Bacopa monniera and *Ginkgo biloba* are well-known cognitive enhancers in Indian and Chinese traditional medicine systems. Standardized extracts of *Bacopa monniera* and *G.biloba* both showed a dose-dependent inhibitory effect on AChE activity.

Eighty percent methanolic extract of *Myricaria elegans* Royle was found to have significant AChE inhibitory activity (Pulok K.M. *et al*., 2007).

Huperzine A, an alkaloid from *Huperzia serrata*, was found to be a reversible AChEIs and is neuroprotective. It was shown that huperzine A has a neuroprotective effect against Aβ peptide fragment 25–53, oxygen glucose deprivation and against free radical-induced cytotoxicity. It also attenuates apoptosis by inhibiting the mitochondria-caspase pathway. In cortex or synaptic plasma membranes it counteracted NMDA-induced toxicity. This may be due to the fact that huperzine A facilitates cholinergic neurotransmission by increasing the concentration of ACh in the CNS about 100 times more effectively than tacrine, a drug used for AD. In cell culture studies, huperzine A decreased neuronal cell death caused by toxic levels of glutamate.

Huperzine A (400μg) was given daily to 100 patients for 12 weeks. It was reported to be more selective for AChE than BuChE and was less toxic than the synthetic AChEIs donepezil and tacrine. Possible side effects are nausea, vomiting, diarrhea and muscle cramps have been observed. Cholinergic effects may also worsen pulmonary and peptic ulcer disease and cardiac arrhythmia (Michael A. *et al*., 2007).

The lycopodium alkaloid huperzine A related to the quinolizidines, is a potent, yet reversible, inhibitor of AChE and is used in China for treating patients with

myasthenia gravis and AD. The source of huperzine A is *Huperzia serrata*, a moss that has been used for treating contusions, strains, hematuria and swelling in Chinese folk medicine. It improved memory retention processes in cognitively impaired aged and adult rats. In a multicenter, double blind trial, huperzine A significantly improved memory and behavior in AD patients, and was reported to be more selective for AChE than BuChE and less toxic than the synthetic AChEIs donepezil and tacrine. It may also have potential in the attenuation of memory deficits and neuronal damage that occurs after ischemia, so may therefore is beneficial in the treatment of cerebrovascular- type dementia (Pulok K.M. *et al*., 2007).

The AChE inhibitory activity of huperzine A was compared to Donepezil and Rivastigmine in vivo. They found that huperzine A increased the concentration of ACh and inhibited AChE more efficiently than of Donepezil and Rivastigmine. They also found that huperzine A not only penetrated the blood–brain barrier (BBB) more efficiently but also showed long-lasting inhibitory effects on AChE (Thimmappa S. *et al*., 2005).

The Indian medicinal plants were investigated for AChEIs activity in vitro. The potent AChE inhibiting methanolic plant extracts included *Withania somnifera* (root), *Semecarpus anacardium* (stem bark), *Embelia ribes* (Root), *Tinospora cordifolia* (stem), *Ficus religiosa* (stem bark) and *Nardostachys jatamansi* (rhizome). The IC_{50} values obtained for these extracts were 33.38, 16.74, 23.04, 38.36, 73.69 and 47.21 µg/mL, respectively (Vinutha B.D. *et al*., 2007).

Seven plants in Korea's history of traditional medicine, including *Acorus calamus* rhizome, *Acorus gramineus* rhizome, *Bupleurum falcatum* root, *Dioscorea*

batatas rhizome, *Epimedium koreanum* herb, *Poria cocos* sclerotium and *Zizyphus jujuba* fruit, which are used for the general indication for improvement of cognition and memory function in old age were all tested for ChE inhibitory activity using the Ellman colorimetric method. The data revealed that extracts of *Acorus gramineus*, *Dioscorea batatas* and *Zizyphus jujuba* did not show any inhibitory effects on AChE. Significant dose-dependent inhibition of the enzyme at the high dose of 200 µg/mL was observed for methanolic extracts from *Acorus calamus* and *Epimedium koreanum* (Oh M.H. *et al*., 2004).

Acorus calamus contains essential oil with the main components β-asarone and α-asarone. Other components found in the plant are caryophyllene, α-humulene and sekishone. Methanolic extracts of the roots, which contain essential oil which the toxin β-asarone showed inhibitory effect on AChE with an IC_{50} value of 188 µg/mL (Michael A. *et al*., 2007; Oh M.H. *et al*., 2004).

The medicinal plants of Pakistan and Iran have led to the isolation of AChEIs such as buxamine B, *N,N*-dimethyl buxapapine , sarsalignone and vaganine. Indole alkaloids: coronaridine, voacangine, voacangine hydroxyindolenine and rupicoline isolated from the chloroform extract of stalk of *Tabernaemontana australis* showed AChE inhibitory activity (Pulok K.M. *et al*., 2007).

Withania somnifera extract, containing the steroidal substances sitoinodosides VII–X and withaferin. A augmented learning acquisition and memory in both young and old rats. It enhanced AChE inhibitory activity in the lateral septum and globus pallidus and decreased it in the vertical diagonal band. Receptor binding on the muscarinic M_1 receptor was enhanced in the lateral and medium septum and in the

frontal cortices. M_2 receptor binding increased in cortical regions but did neither affect γ-aminobutyric acid ($GABA_A$), benzodiazepine, nor NMDA receptor binding. The extract reversed ibotenic acid induced cognitive deficit and reversed the reduction in cholinergic markers, such as ACh (Michael A. *et al*., 2007).

Seven Lebanese plants that are used traditionally for neurological disorders as AD, epilepsy and affective disorders as depression were tested for inhibition of AChE and affinity to the $GABA_A$-benzodiazepine site and to the serotonin transporter. Ethyl acetate extracts of *Salvia triloba*, *Lavandula officinalis*, *Origanum syriacum* and *Artemisia herba-alba* exhibited weak activity in the AChE assay (Sam S.M. and Anna K.J., 2005).

The chloroform:methanol (1:1) extracts of *Rhododendron ponticu, Rhododendron luteum , Corydalis solida , Glaucium corniculatum*, and *Buxus sempervirens* showed remarkable inhibitory activity above 50% inhibition rate at 1mg/mL (Orhan I.B. *et al*., 2004).

Galanthus nivalis was used traditionally in Bulgaria and Turkey for neurological conditions. Galantamine is an amaryllidaceae alkaloid obtained from *Galanthus nivalis* L. Galantamine is reported to be more selective for AChE than BuChE, and provides complete oral bioavailability. It is licensed in Europe for AD treatment, was well tolerated and significantly improved cognitive function when administered to AD patients, in multi-center randomized controlled trials (Susana L. *et al*., 2002). Initially derived from extracts of snowdrop and daffodil bulbs, this phenanthrene alkaloid is

now synthetically produced. It is a reversible competitive AChEIs that also allosterically modulates nicotinic receptors (this effect is probably independent of its ChE inhibition). It has an elimination half-life of about 6 hours. Metabolism produces four compounds, one of which is more active as a ChEIs than galantamine itself. Over 2000 patients have been involved in double-blind placebo-controlled trials of galantamine where positive effects on cognitive symptoms have been associated with significant benefits in activities of daily living (Pulok K.M. *et al*., 2007).

The stilbene oligomer viniferin from *Caragana chamlague*, has also been identified as reversible and non-competitive inhibitor of AChE. Structure–activity relationship suggested that the nitrogen substituents at C-3 and/or C-20 of steroidal skeleton and the hydrophobic properties of the pregnane skeleton are the key structural features contributed to the inhibitory potency of pregnane-type steroidal alkaloids against AChE (Pulok K.M. *et al*., 2007).

The methanolic extract resulted in the isolation of three furanocoumarins, isoimperatorin, imperatorin and oxypeucedanin as active principles from the methanolic extract of the roots of *Angelica dahurica*, which inhibited AChE activity in a dose-dependent manner (Pulok K.M. *et al*., 2007).

AChE inhibitory activity of four isoquinoline alkaloids, corynoxidine, protopine, palmatine and berberine have been isolated from the methanolic extract of the aerial parts of *Corydalis Speciosa* (Pulok K.M. *et al*., 2007).

Plants used in Korean folk medicine as memory enhancers were screened and 70% ethanolic extract of *Corydalis turtschaninovii* Besser forma *yanhusuo* (Papaveraceae) exhibited significant AChE inhibitory activity (72.5% at 100 μg/mL) (Orhan I.B. *et al*., 2004). Previously reports showed that several species of the genera *Corydalis* have been used in the treatment of memory dysfunction in folk medicine.

Protoberberine and protopine-type alkaloids, common compounds in *Corydalis* species, are potent AChEIs. The inhibition of the enzyme was examined with methanolic and aqueous extracts of *Corydalis* species. Inhibition by methanolic extracts was generally higher than by the water extracts. Tuber- and herb-extracts were assayed at concentrations of 0.1, 0.05 and 0.025 mg/mL. With 92%, 83% and 77% for the methanolic tuber extract of *Corydalis cava* showed the best results compared to *Corydalis intermedia*, *Corydalis solida* and *Corydalis solida* ssp. *slivenensis*. Pure protopine had an IC_{50} value of 50 μM. In a passive avoidance task test mice treated with protopine exhibited diminished scopolamine-induced dementia. (Michael A. *et al*., 2007).

Screening plants used in Danish folk medicine as memory enhancers showed that a crude methanolic extract of tubers from *Corydalis cava* exhibited significant AChE inhibitory activity in a dose-dependent manner. Activity guided fractionation of the methanolic extract resulted in the isolation of three alkaloids, bulbocapnine, corydaline and corydine as active constituents. Bulbocapnine inhibited AChE as well as BuChE in a dose-dependent manner with IC_{50} values of 40.2 μM and 83.3 μM, respectively. Corydaline inhibited AChE in a dose-dependent manner with an IC_{50} value of 15.3 μM and corydine inhibited BuChE in a dose-dependent manner with an

IC_{50} value of 52.4 μM. Corydalinewas considered inactive against BuChE and corydine against AChE, due to IC_{50} > 100 μM (Anne A. *et al*., 2007).

Fifteen amaryllidaceae extracts were screened using this TLC bioautograpic method. The methanol and toluene extracts of 15 different amaryllidaceae species along with galanthamine standard were developed on a TLC plate with the solvent system chloroform:methanol 8:2. AchE inhibitory activity of extracts measured by the microplate assay *Crinum* X *powellii* Baker and *A.belladonna* L. showed strong inhibition in the micro-plate assay and also showed several inhibiting spots in the TLC assay (In K.R. *et al.*, 2001).

Anisodamine, extracted from the Chinese herb *Anisodus tanguticus*, produced cholinomimetic effects in mice when combined with the peripheral muscarinic blocker pilocarpine. When pilocarpine was administered alone, anisodamine effectively blocked cholineacetylesterase activity, but it initiated typical cholinergic side effects, such as diarrhea, hypersalivation, and bradycardia (Thimmappa S. *et al*., 2005).

Ptychopetalum olacoides, a traditional Amazonian herb, inhibited AChE activity in the frontal cortex, hippocampi, and striatal neurons of 3-month-old male Wistar rats and of 12-month-old male Swiss albino mice. In a microplate assay that measured AChE activity, 23 pure alkaloids and plant extracts from 26 species of the genus *Narcissus* from Amaryllidaceae were tested (Susana L. *et al*., 2002). In this study, seven alkaloids belonging to galantamine and lycorine skeletontype, as well as three

Narcissus species (N.*confusus*, N.*perez-chiscanoi*, and N.*Assoanus*), showed AChE inhibitory activity. Zeatin, derived from *Fiatoua villosa*, also inhibited AChE activity in PC12 cells of the rat (Thimmappa S. *et al*., 2005). In addition, several species of *Salvia* (S.*lavendulefolia*, S.*officinalis*, and S.*multiorrhiza*) showed both AChE inhibitory and antioxidant activities, thus suggesting they may be useful for dementia therapy (Perry E. *et al*., 2003).

The inhibition of the BuChE of essential oils from *Salvia fruticosa*, *Salvia officinalis* var. *purpurea*, *Salvia officinalis* and *Salvia lavendulaefolia* was examined. The IC_{50} values measured after 5 min of incubation were 0.05, 0.4, 0.03, 0.07 and 0.0.3 mg/mL, respectively. Additional pure compounds from the oil were tested, none of which could fully account for the activity of the essential oils. *Salvia lavandulaefolia* and *Salvia officinalis purpurea* oils had apparent dual cholinergic activity, as they were active on both AChE and BuChE (Michael A. *et al*., 2007).

Alkaloids from the plants were isolated and tested for their AChE inhibitory properties. Hamayne (IC_{50} 250 μM) and lycorine (IC_{50} 450 μM) showed only slight activities compared to the positive control physostigmine (IC_{50} of 0.25 μM) (Michael A. *et al*., 2007).

Extracts obtained from 10 plants used in South African traditional medicine were screened for anti-ChE activities and 21% of the plant extracts tested showed activity against the AChE enzyme in both TLC and micro-dilution assays. At the concentration of 1 mg/mL, ethyl acetate leaf extract of *Combretum kraussii* showed

the highest activity (96%). The IC_{50} value was 0.2 mg/mL. Moderate activities (<60%) were observed with extracts of *Acacia nilotica* (ethyl acetate and ethanolic, leaf), *Albizia adianthifolia* (ethanolic, bark) and *Trichilia dregeana* (ethyl acetate, bark). The lowest IC_{50} value was obtained with an ethanol bark extract of *Combretum kraussii* (0.04 mg/mL). The percentage inhibition of galanthamine (positive control) at a concentration of 20 µM was 93% and the IC_{50} value was 2 µM (Eldeen I.M.S and Van S.J., 2007).

N-(14-Methylallyl)norgalanthamine, a new natural compound, together with five known alkaloids: *N*-allylnorgalanthamine, galanthamine, epinorgalanthamine, narwedine, and lycorine were isolated from mother liquors obtained after industrial production of galanthamine hydrobromide from *Leucojum aestivum* leaves. The structures of *N*-allylnorgalanthamine and *N*-(14-methylallyl) norgalanthamine were completely determined by ^{1}H and ^{13}C NMR spectroscopy and two-dimensional experiments. *N*-allylnorgalanthamine (IC_{50} = 0.18 µM) and *N*-(14-methylallyl) norgalanthamine (IC_{50} = 0.16 µM) inhibit AChE considerably more than the approved drug galanthamine (IC_{50} = 1.82 µM) (Strahil B *et al*., 2008).

Extracts obtained from seven plants species used in Sudanese traditional medicine were screened for anti-ChE activities. 58% of the plant extracts were active at a concentration of/or below 1 mg/mL using the micro-dilution assay. The lowest IC_{50} value was 0.09 mg/mL observed with the ethanolic bark and root extracts of E.*latissima* and *Kigelia Africana* (Eldeen I.M.S and Van S.J., 2007).

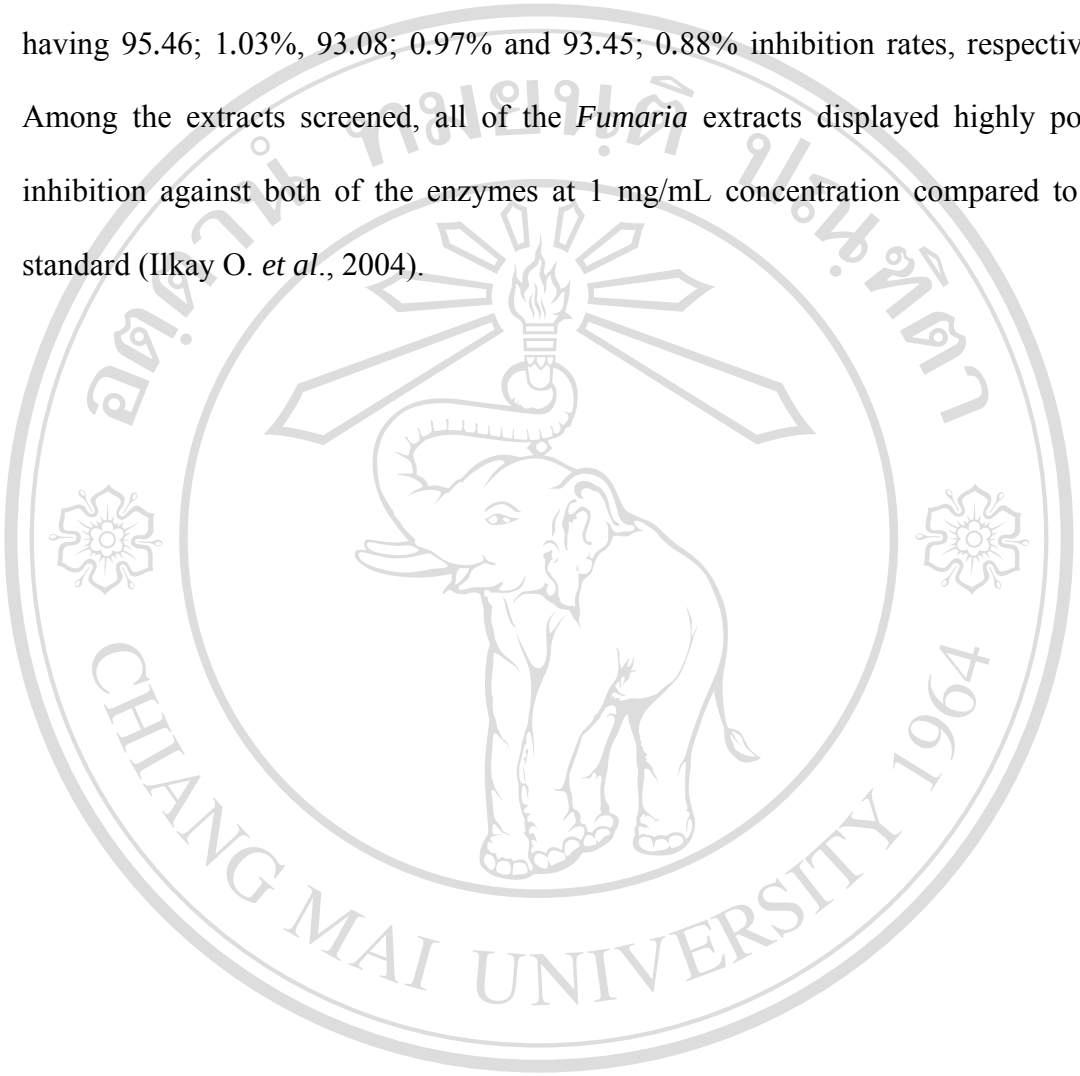
Aqueous and methanolic extracts of 11 plants, used in Danish folk medicine for improvement of memory and cognition, and 3 *Corydalis* species were tested for AChE inhibitory activity using the Ellman colorimetric method. Significant inhibitory activity in dose-dependent manner was observed for extracts of *Corydalis cava*, *Corydalis intermedia*, *Corydalis solida* ssp. *laxa* and *Corydalis solida* ssp. *slivenensis*. Extracts of *Ruta graveolens*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Petroselinum crispum* and *Mentha spicata* exhibited moderate inhibition of the enzyme, defined as more than 15% at 0.1 mg/mL (Anne A. *et al*., 2006).

The bulbs of *Crinum jagus* and *Crinum glaucum* are used in traditional medicine in southern Nigeria for memory loss and other mental symptoms associated with aging. Alkaloidal extracts of bulbs from each species showed inhibition of AChE, an activity exploited therapeutically to raise the depressed levels of acetylcholine in the brain associated with AD. Using the in situ bioautographic test method for enzyme inhibition, a number of alkaloids were isolated and their activity quantified using the Ellman spectrophotometric test. The most active alkaloids isolated were hamayne (IC_{50} 250 μM) and lycorine (IC_{50} 450μM) whilst other alkaloids were comparatively inactive with haemanthamane giving 3% inhibition and crinamine giving 4.4% inhibition at 50 mg/mL (174 μM). These contrast with the positive control physostigmine which gave IC_{50} of 0.25 μM. ChE inhibitory activity appears to be associated with the presence of two free hydroxy groups in this structural type of Amaryllidaceae alkaloid (Peter J.H. *et al.*, 2004).

AChE and BuChE inhibitory activity of the petroleum ether, ethyl acetate, chloroform, and methanol extracts, rosmarinic acid as well as the essential oil obtained from *Rosmarinus officinalis* L. growing in Turkey were tested by a spectrophotometric method of Ellman using ELISA microplate-reader at 0.2, 0.5, and 1.0 mg/mL concentrations. Rosmarinic acid was also tested for its AChE and BuChE inhibitory effect and found to exhibit 85.8% of inhibition against AChE at only 1.0 mg/mL (Ilkay O. *et al*., 2008).

The chloroform:methanol (1:1) extracts of a number of the plant species belonging to eight families, namely *Corydalis solida* (L.) Swartz sub sp. *solida* and *Glaucium corniculatum* (L.) J. H. Rudolph (Papaveraceae), *Rhododendron ponticum* L. subsp. *ponticum* and *Rhododendron luteum* Sweet. (Ericaceae), *Buxus sempervirens* L. (Buxaceae), *Vicia faba* L. (Fabaceae), *Robinia pseudoacacia* L. (Caeselpiniaceae), *Tribulus terrestris* L. and *Zygophyllum fabago* L. (Zygophyllaceae), *Lycopodium clavatum* L. (Lycopodiaceae), *Fumaria vaillantii* Lois., *Fumaria capreolata* L., *Fumaria kralikii* Jordan, *Fumaria asepala* Boiss., *Fumaria densiflora* DC., *Fumaria flabellata* L., *Fumaria petteri* Reichb. sub sp. *thuretii* (Boiss.) Pugsley, *Fumaria macrocarpa* Boiss. ex Hausskn., *Fumaria cilicica* Hauskkn., *Fumaria parviflora* Lam. and *Fumaria judaica* Boiss. (Fumariaceae) were screened for their anticholinesterase activities on AChE and BuChE enzymes by in vitro Ellman method at 10 µg/mL and 1 mg/mL concentrations. The extracts did not show any noticeable inhibitory activity against both of the enzymes at 10 µg/mL. The extracts of *Rhododendron ponticum* subsp. *Ponticum*, *Rhododendron luteum*, *Corydalis solida* subsp. *Solida*, *Glaucium corniculatum*, and *Buxus sempervirens* showed remarkable inhibitory activity above 50% inhibition rate on AChE at

1 mg/mL. Among them, *Rhododendron ponticum* sub sp. *Ponticum*, *Corydalis solida* sub sp. *solida* and *Buxus sempervirens* were the most active extracts against BuChE having 95.46; 1.03%, 93.08; 0.97% and 93.45; 0.88% inhibition rates, respectively. Among the extracts screened, all of the *Fumaria* extracts displayed highly potent inhibition against both of the enzymes at 1 mg/mL concentration compared to the standard (Ilkay O. *et al*., 2004).



4. *Haemanthus multiflorus* Martyn

4.1. Botanical aspect and distribution of *Haemanthus multiflorus* Martyn.

There are several garden plants amongst the fascinating *Haemanthus* species. About 45 species grow wild in South Africa and tropical Africa, nearly all in the summer rainfall area. Most are deciduous in winter, while one is almost evergreen in mild climates. The showiest have scarlet flowers which resemble thick shaving brushes or have heads like a growing red *Agapanthus.* Its globular inflorescence consists of as many as a hundred tiny individual flower (Eliovson S. and Reed A.W., 1968).

Bulbs or saplings can be planted during February / March in pots filled with equal parts of sand, gravel and dried cow dung powder. Its lifecycle completes in two stages. The plant grows in the first stage till November/December. The fully grown leaves die off in winter and the bulbs remain dormant. With the help of the food stored in these bulbs, the flowers bloom in the second stage. Needs more sun while blooming. Fresh leaves start appearing after the flowering stage (Alice N., 2001). (Figure 1)

Scientific name : *Haemanthus multiflorus* Martyn.

Common names : Football lily, African Blood Lily,
Powder puff Lily, Blood Flower.

Thai names : Wan saeng a thit, Wan kra thum, Wan ta kro

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Subclass : Monocots

Order : Asparagales

Family : Amaryllidaceae

Genus : *Haemanthus*

Country of origin : South Africa

Flowering period : April to May

Colour : Red, Pink

Stem : The plant grows to a height of 10 – 20 inch (Bruggeman L., 1962).

Bulb : tunicate bulb; large fleshy bulbs, borad mostly abtuse, 3 – 6 inch diameter (Bailey L.H., 1961; Eliovson S and Reed A.W. *et al*, 1968)

Leaves : 4- 7 on a large; the oblong blade, with 6 -8 veins each side of the stout midrib, narrow and erect to wavy – margined, deep green leaves than can be up to 18 inch long and 6 inch wide (Hsuan K.,

1998; Bruggeman L., 1962; Alice N., 2001; Gail K.W., 1973).

Scape : straight, 1 – 3 feet high; solid, broad and flattened, and often spotted with green or red (Dahlgren R.M.T. *et al*, 1989; Hsuan K., 1998).

Flowers : umbellate; 50 – 100 in a globose head, 4 – 6 inch across, perianth straight and erect, bright red (Hsuan K., 1998; Bruggeman L., 1962; Bailey L.H., 1961; Hsuan K, 1969).

Stamens : 6; inserted in the throat of the perianth, red filament-exserted, bearing prominent yellow anthers, style filiform and erect (Bailey L.H., 1961).

Ovary : ovary inferior; 3 loculed ovary (Bailey L.H., 1961).

Fruit : berry with globose, water rich seeds. (Dahlgren R.M.T. *et al.*, 1989)

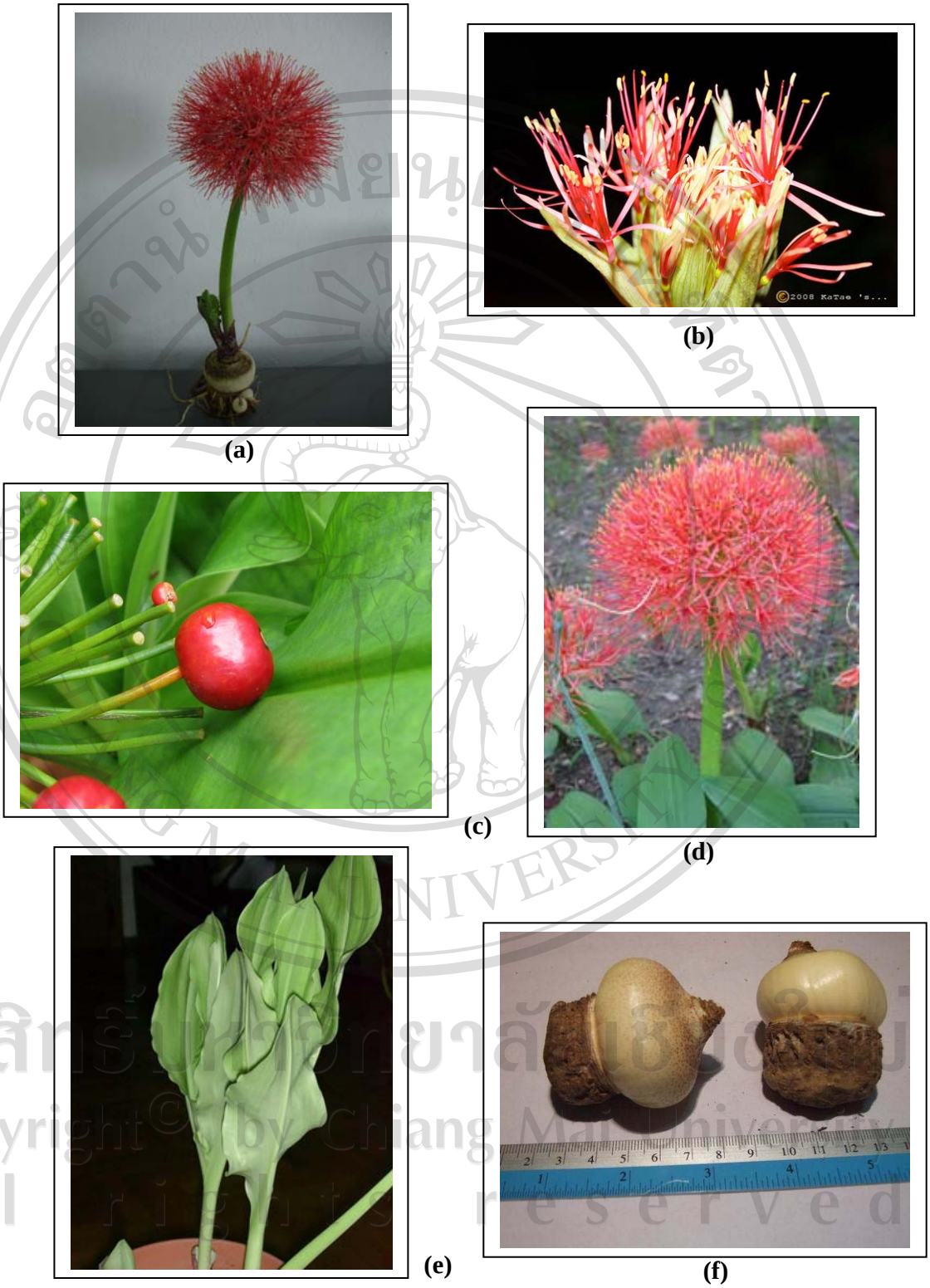


Figure 1. Whole plant (a), Stamens (b), Fruit (c), Flower (d), Leaves (e), Bulb (f) of *Haemanthus Multiflorus* Martyn.

4.2. Chemical constituents of genus *Haemanthus*

Many compounds have been found in the genus *Haemanthus*. Alkaloids were the outstanding group. The data from literature review are shown in Table 1. The number in parentheses is as shown in Figure 2.

Table 1. Chemical constituents of genus *Haemanthus*

Plant species	Part used	Chemical constituent	Category	Reference
H. albiflos	Bulb	Albiflomanthine **(3)**	Alkaloid	Baudouin G. *et al*., (1994)
	-	Albomaculine **(4)**	Alkaloid	Crouch N.R. *et al*., (2005)
	-	Coccinine **(7)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Lycorenine **(26)**	Alkaloid	Hans G.B., (1954)
	-	Homolycorine **(22)**	Alkaloid	Crouch N.R. *et al*., (2005)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. albiflos	-	Manthidine **(17)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Manthine **(18)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Montanine **(30)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Natelensine **(32)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Tazettine **(37)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955); Hans G.B., (1954)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. albomaculatus	Bulb	Albomaculine **(4)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955); Briggs C.K. *et al*., (1956)
	Bulb	Coccinine **(7)**	Alkaloid	Briggs C.K. *et al*., (1956)
	Bulb	Lycorenine **(26)**	Alkaloid	Briggs C.K. *et al*., (1956)
	-	Manthidine **(28)**	Alkaloid	Inubushi Y. *et al*., (1960)
	Bulb	Nerinine **(33)**	Alkaloid	Briggs C.K. *et al*., (1956)
	-	Tazettine **(37)**	Alkaloid	Briggs C.K. *et al*., (1956)
H. amarylloides	Bulb	Coccinine **(7)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. amarylloides	Bulb	Manthine **(29)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Montanine **(30)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
H. catherinae Baker X *H.puniceus* L.	-	Dihydro hemanthamine **(11)**	Alkaloid	Hans G.B., (1954)
	-	Dihydro hemanthidine **(12)**	Alkaloid	Hans G.B., (1954)
	-	Lycorine **(27)**	Alkaloid	Hans G.B., (1954)
	-	Hemanthamine **(16)**	Alkaloid	Hans G.B., (1954)
	-	Hemanthidine **(17)**	Alkaloid	Hans G.B., (1954)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. catherinae Baker X *H.puniceus* L.	-	*N*-methyl hemanthidine **(31)**	Alkaloid	Hans G.B., (1954)
H. coccineus L.	Bulb	Coccinine **(7)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Lycorine **(27)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Manthidine **(28)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	Bulb	Montanine **(30)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
H. deformis	-	Coccinine **(7)**	Alkaloid	Crouch N.R. *et al*., (2005)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. deformis	-	Manthidine **(28)**	Alkaloid	Crouch N.R. *et al*., (2005)
	-	Montanine **(30)**	Alkaloid	Crouch N.R. *et al*., (2005)
H. kalbreyeri	Bulb	7-deoxy narciclasine **(9)**	Alkaloid	Ghosal S. *et al*., (1989)
	Bulb	7-deoxy pancratistatine **(10)**	Alkaloid	Ghosal S. *et al*., (1989)
	-	Hemanthamine **(16)**	Alkaloid	Ghosal S. *et al*., (1985)
	-	Hemanthidine **(17)**	Alkaloid	Ghosal S. *et al*., (1985)
	-	Hippadine **(20)**	Alkaloid	Ghosal S. *et al*., (1985)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. kalbreyeri	Bulb	Kalbreclasine **(24)**	Alkaloid	Ghosal S. *et al*., (1985); Jeffrey B., (1999)
	Bulb	Kalbretorine **(25)**	Alkaloid	Ghosal S. *et al*., (1985)
	-	Lycorine **(27)**	Alkaloid	Ghosal S. *et al*., (1985)
	Bulb	Narciclasine **(32)**	Alkaloid	Ghosal S. *et al*., (1985); Ghosal S. *et al*., (1989); Jeffrey B., (1999)
	Bulb	Pancratistatine **(34)**	Alkaloid	Ghosal S. *et al*., (1989)
	-	Pratorimine **(35)**	Alkaloid	Ghosal S. *et al*., (1985)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. katherinae Bak. X H. *puniceus* L.	-	Hemanthamine **(16)**	Alkaloid	Hans G.B., (1954)
	-	Hemanthidine **(17)**	Alkaloid	Hans G.B., (1954)
	-	Lycorine **(27)**	Alkaloid	Hans G.B., (1954)
H. katherinae Bak	-	Epihemanthamine **(13)**	Alkaloid	Noqueiras C. *et al*., (1971)
	-	Galanthamine **(15)**	Alkaloid	Hans G.B. and Dopke W., (1961); Jaspersen S.R., (1970)
	Bulb	Hemanthamine **(16)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	Bulb	Hemultine **(19)**	Alkaloid	Hans G.B. and Dopke W., (1961)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. katherinae Bak	-	Hippeastrine **(21)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	-	Lycorenine **(26)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	-	Lycorine **(27)**	Alkaloid	Hans G.B. and Dopke W., (1961)
H. *montanus* Bak.	Bulb	Montanine **(30)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
H. multiflorus Martyn	Bulb	2-O-Acetyl chlidanthine **(1)**	Alkaloid	Abdallan O.M. *et al*., (1989)
	Bulb	3-O-Acetyl sanguinine **(2)**	Alkaloid	Abdallan O.M. *et al*., (1989)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. multiflorus Martyn	Bulb	Chlidanthine **(6)**	Alkaloid	Hans G.B. and Dopke W., (1958); Hans G.B. *et al*., (1958)
	-	Coccinine **(7)**	Alkaloid	Fales H.M. and Wildman W.C., (1961)
	-	Crinamine **(8)**	Alkaloid	Hans G.B. and Dopke W., (1958)
	-	Galanthamine **(15)**	Alkaloid	Ali A.A. et al., (1987); Jaspersen S.R., (1970)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. multiflorus Martyn	Bulb	Hemanthamine **(16)**	Alkaloid	Hans G.B. and Dopke W., (1958); Fales H.M. and Wildman W.C., (1961)
	Bulb	Hemanthidine **(17)**	Alkaloid	Hans G.B. and Dopke W., (1958); Hans G.B. *et al*., (1958)
	Bulb	Hemultine **(19)**	Alkaloid	Fales H.M. and Wildman W.C., (1961); Hans G.B. *et al*., (1958)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. multiflorus Martyn	Bulb	Hippesatrine **(21)**	Alkaloid	Hans G.B. and Dopke W., (1958); Hans G.B. *et al*., (1958)
	Bulb	Lycorine **(27)**	Alkaloid	Ali A.A. et al., (1987); Hans G.B. and Dopke W., (1958); Hans G.B. *et al*., (1958)
	-	Manthine **(29)**	Alkaloid	Fales H.M. and Wildman W.C., (1961)
	-	Monthanine **(30)**	Alkaloid	Fales H.M. and Wildman W.C., (1961)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. multiflorus Martyn	-	Sanguinine **(36)**	Alkaloid	Ali A.A. et al., (1987)
H. natalensis Pappe ex Hook.	-	Apohemanthidine **(5)**	Alkaloid	Goosen J. *et al*., (1960)
	-	Epihemanthidine **(14)**	Alkaloid	Goosen J. *et al*., (1960)
	Bulb	Hemanthamine **(16)**	Alkaloid	Warren F.L. and Wright W.G., (1958); Jeffs P.W., (1962)
	-	Hemanthidine **(17)**	Alkaloid	Jeffs P.W., (1962)
	-	6-hydroxy crinamine **(23)**	Alkaloid	Jeffs P.W., (1962)
H. nelsonii Bak	Bulb	Lycorenine **(26)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. otavinsis Dinter.	bulb	Galanthamine **(15)**	Alkaloid	Jaspersen S.R., 1970
H. pauculifolius	-	Homolycorine **(22)**	Alkaloid	Crouch N.R. *et al*., (2005)
	-	Manthidine **(28)**	Alkaloid	Crouch N.R. *et al*., (2005)
	-	Montanine **(30)**	Alkaloid	Crouch N.R. *et al*., (2005)
H. puniceus L.	bulb	Hemanthamine **(16)**	Alkaloid	Warren F.L and Winifred G.W., 1958; Wildman W.C. and Kaufman C.J., (1955)
	-	Hemanthidine **(17)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
H. sacculus E. Philips.	bulb	Galanthamine **(15)**	Alkaloid	Jaspersen S.R., 1970

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. spp	-	Coccinine **(5)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Galanthamine **(15)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	Bulb	Hemanthamine **(16)**	Alkaloid	Hans G.B. and Dopke W., (1961); Jeffrey B., (1999)
	-	Hemanthine **(18)**	Alkaloid	Goosen A. *et al*., (1961)
	-	Hemultine **(19)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	-	Hippeastrine **(21)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	-	Lycorenine **(26)**	Alkaloid	Hans G.B. and Dopke W., (1961)
	-	Lycorine **(27)**	Alkaloid	Hans G.B. and Dopke W., (1961)

Table 1. Chemical constituents of genus *Haemanthus* (continued)

Plant species	Part used	Chemical constituent	Category	Reference
H. spp	-	Manthidine **(28)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Manthine **(29)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
	-	Montanine **(30)**	Alkaloid	Wildman W.C. and Kaufman C.J., (1955)
H. *tigrinus* Jacq.	-	Coccinine **(7)**	Alkaloid	Inubushi Y *et al.*, 1960
	-	Manthine **(29)**	Alkaloid	Inubushi Y *et al.*, 1960
	-	Montanine **(30)**	Alkaloid	Inubushi Y *et al.*, 1960

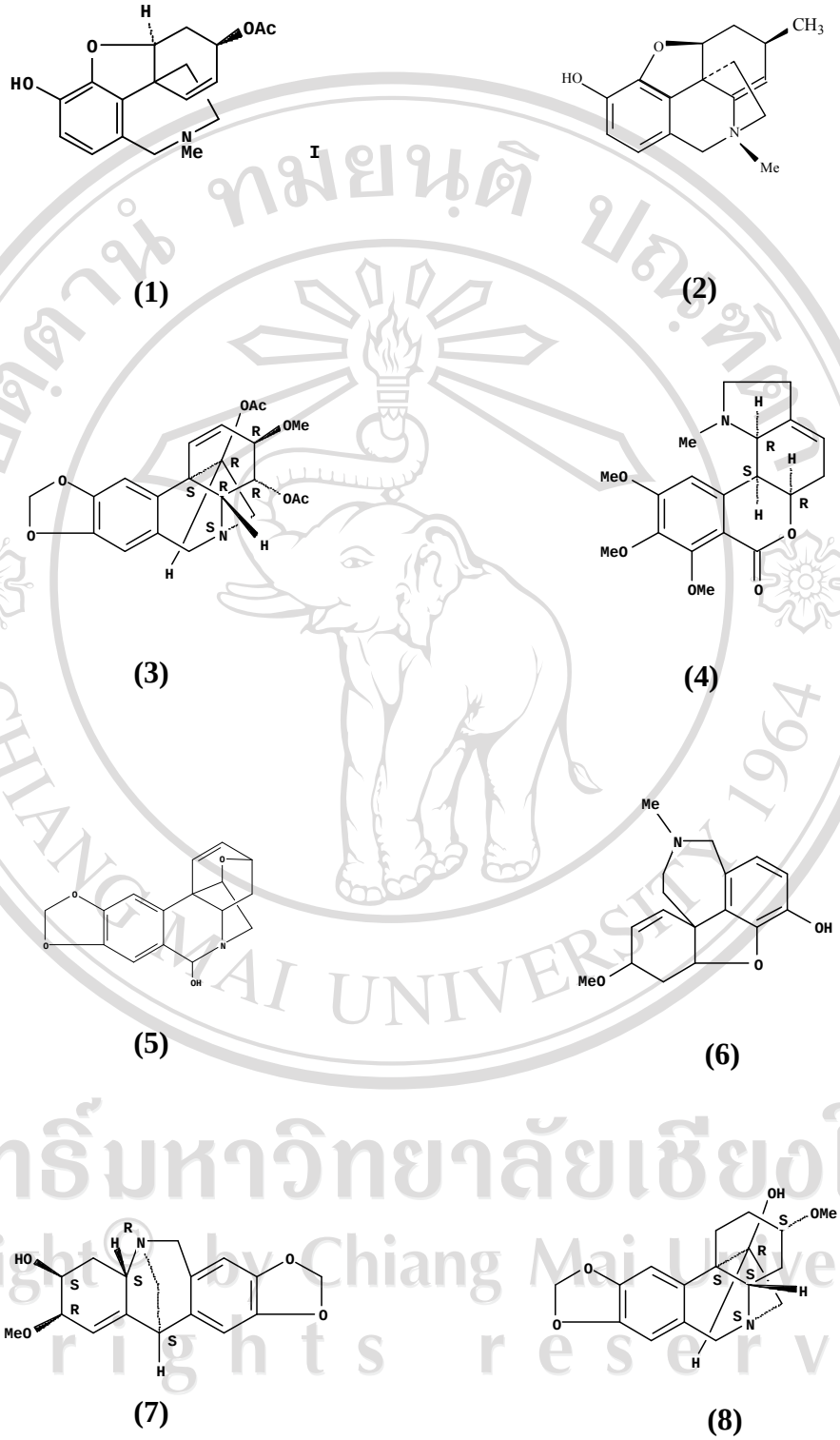
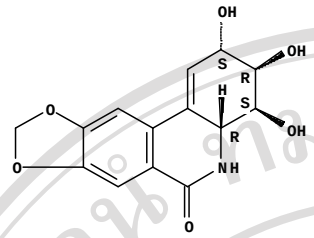


Figure 2 Structures of compounds previously isolated from *Haemanthus spp.*



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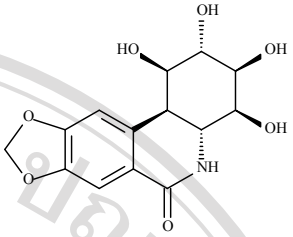

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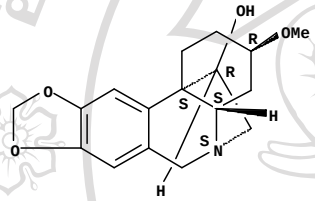

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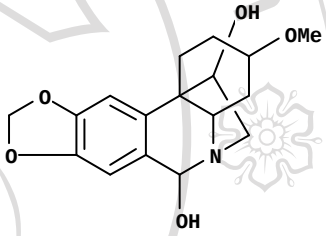

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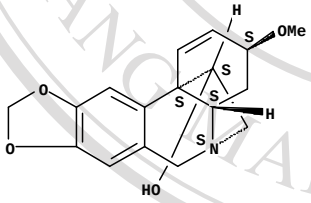

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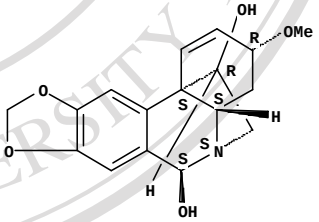

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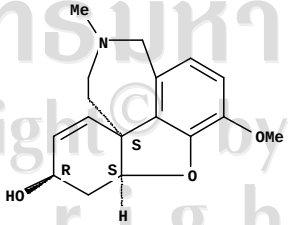

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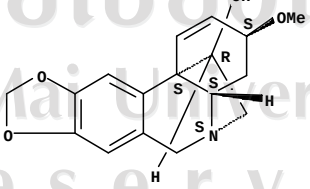
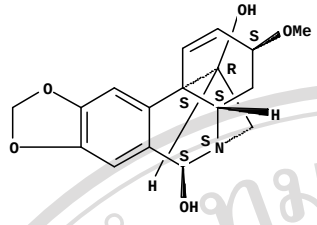


(16)

Figure 2 Structures of compounds previously isolated from *Haemanthus spp.*

(continued)



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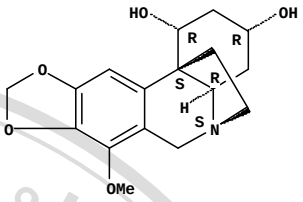

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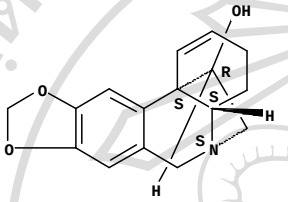

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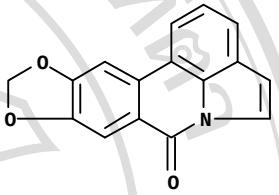

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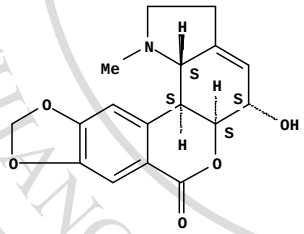

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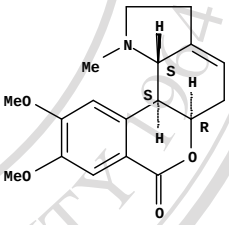

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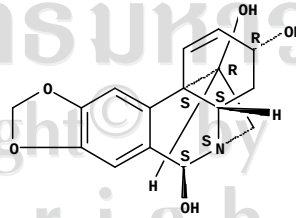

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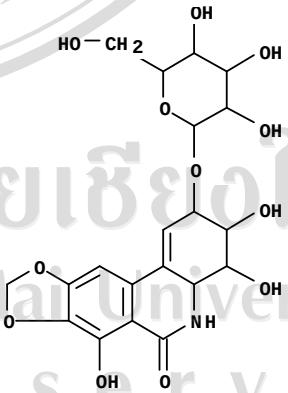
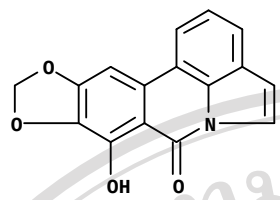


(24)

Figure 2 Structures of compounds previously isolated from *Haemanthus spp.*

(continued)



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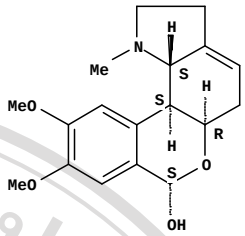

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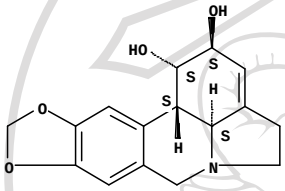

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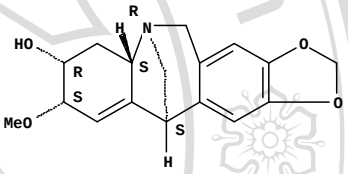

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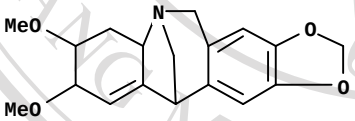

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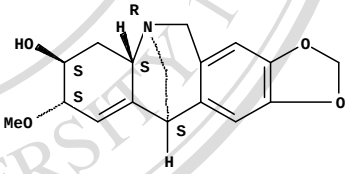

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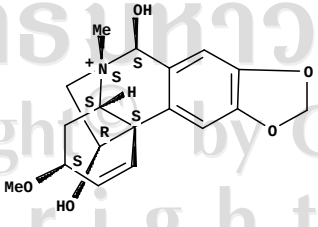

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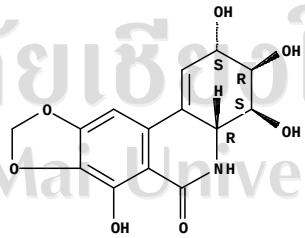
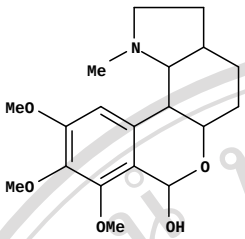


(32)

Figure 2 Structures of compounds previously isolated from *Haemanthus spp.*

(continued)



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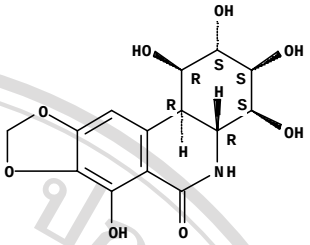

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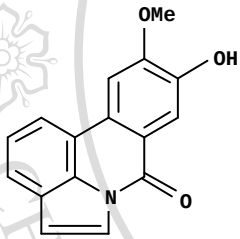

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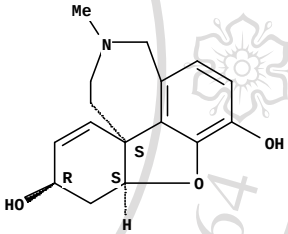

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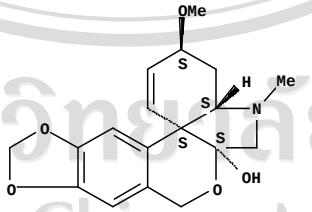

(37)

Figure 2 Structures of compounds previously isolated from *Haemanthus spp.*

(continued)

5. Objectives of the Study

1. To investigate for acetylcholinesterase inhibitory activity of *Haemanthus multiflorus* Martyn's Bulbs.

2. To separate and study the phytochemistry of acetylcholinesterase inhibitors from *Haemanthus multiflorus* Martyn's Bulbs.