EFFECTS OF THE EXTRACT FROM THE FRUIT HULL OF MANGOSTEEN (*Garcinia mangostana* L.) ON LEARNING MEMORY AND THE CENTRAL CHOLINERGIC SYSTEM OF MALE WISTAR RATS

Naiyana Nontamart

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in Environmental Biology

Suranaree University of Technology

Academic Year 2013

ผลของสารสกัดจากเปลือกมังคุด (*Garcinia mangostana* L.) ต่อการเรียนรู้และระบบสารสื่อประสาทอะซิทิลโคลีนในสมองของหนูขาวเพศผู้พันธุ์วิสตาร์

นางสาวนัยนา นนทะมาตย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีววิทยาสิ่งแวดล้อม
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2556

EFFECTS OF THE EXTRACT FROM THE FRUIT HULL OF MANGOSTEEN (*Garcinia mangostana* L.) ON LEARNING MEMORY AND THE CENTRAL CHOLINERGIC SYSTEM OF MALE WISTAR RATS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

_____
(Asst. Prof. Dr. Nooduan Muangsan)
Chairperson

_____
(Asst. Prof. Dr. Rungrudee Srisawat)
Member (Thesis Advisor)

_____
(Dr. Walaiporn Tongjaroenbuangam)
Member

_____
(Asst. Prof. Dr. Benjamart Chitsomboon)
Member

_____
(Prof. Dr. Sukit Limpijumnong)
Vice Rector for Academic Affairs and Innovation

_____
(Assoc. Prof. Dr. Prapun Manyum)
Dean of Institute of Science

นัยนา นนทะมาตย์ : ผลของสารสกัดจากเปลือกมังคุด (*Garcinia mangostana* L.) ต่อการเรียนรู้และระบบสารสื่อประสาทอะซิทิลโคลีนในสมองของหนูขาวเพศผู้พันธุ์วิสตาร์ (EFFECTS OF THE EXTRACT FROM THE FRUIT HULL OF MANGOSTEEN (*Garcinia mangostana* L.) ON LEARNING MEMORY AND THE CENTRAL CHOLINERGIC SYSTEM OF MALE WISTAR RATS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.รุ่งฤดี ศรีสวัสดิ์, 115 หน้า.

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดจากเปลือกมังคุดต่อการเรียนรู้และความจำที่ทดสอบโดยวิธี Morris water maze และการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนซีรีบรัลคอร์เท็กซ์ ฮิปโปแคมปัส และเบซอลฟอร์เบรนของหนูขาวเพศผู้พันธุ์วิสตาร์ (หนูที่ได้รับน้ำเกลือ หนูที่ถูกเหนี่ยวนำให้ความจำเสื่อมด้วยยาซาโคโปลามีน หนูหนุ่มปกติ และหนูแก่) การศึกษาพบว่าหนูที่ได้รับน้ำเกลือร่วมกับสารสกัดจากเปลือกมังคุด (250 500 และ 1000 มก./มล./ กก.) เป็นเวลา 7 วัน ใช้เวลาในการว่ายวนในพื้นที่ที่เคยมีแท่นซ่อนอยู่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม (P<0.05) แต่ไม่พบในหนูที่ถูกเหนี่ยวนำให้ความจำเสื่อมด้วยยาซาโคโปลามีน (1 มก./ มล./ กก. ทางช่องท้อง เป็นเวลา 7 วัน) ในหนูที่ได้รับน้ำเกลือที่ได้รับสารสกัดจากเปลือกมังคุด มีผลลดการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนซีรีบรัลคอร์เท็กซ์ (ที่ 500 และ 1000 มก./ มล./ กก.) และสมองส่วนฮิปโปแคมปัส (ที่ 250 และ 500 มก./ มล./ กก.) อย่างมีนัยสำคัญทางสถิติ แต่มีผลเพิ่มการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนเบซอลฟอร์เบรนเมื่อเทียบกับกลุ่มควบคุม ในหนูที่ถูกเหนี่ยวนำให้ความจำเสื่อมด้วยยาซาโคโปลามีนที่ได้รับสารสกัดจากเปลือกมังคุดมีผลเพิ่มการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนซีรีบรัลคอร์เท็กซ์ (ที่ 500 มก./ มล./ กก.) และ ฮิปโปแคมปัส (ที่ 250 และ 500 มก./ มล./ กก.) อย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม (P<0.05) การศึกษาในหนูหนุ่มปกติและหนูแก่ที่ได้รับสารสกัดจากเปลือกมังคุดทางปาก (500 มก./ มล./ กก.) เป็นเวลา 30 วัน พบเวลาในการว่ายวนในพื้นที่ที่เคยมีแท่นซ่อนอยู่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม (P<0.05) หนูหนุ่มปกติที่ได้รับสารสกัดจากเปลือกมังคุด (1000 และ 2000 มก./ มล./ กก.) พบการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสลดลงอย่างมีนัยสำคัญทางสถิติในสมองส่วนซีรีบรัลคอร์เท็กซ์และสมองส่วนเบซอลฟอร์เบรน และสารสกัดจากเปลือกมังคุด (500 มก./ มล./ กก.) มีผลลดการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนเบซอลฟอร์เบรน อย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับกลุ่มควบคุม (P<0.05) หนูแก่ที่ได้รับสารสกัดจากเปลือกมังคุดทางปาก (1000 มก./ มล./ กก.) มีผลเพิ่มการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสในสมองส่วนฮิปโปแคมปัสอย่างมีนัยสำคัญทางสถิติ แต่ไม่มีผลในสมองส่วนซีรีบรัลคอร์เท็กซ์ และเบซอล

ฟอร์เบรน โดยสรุป การศึกษาครั้งนี้แสดงให้เห็นถึงผลในการเพิ่มความทรงจำและยับยั้งการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสของสารสกัดจากเปลือกมังคุดในหนูหนุ่มปกติและหนูแก่ แต่ไม่พบในหนูที่ถูกเหนี่ยวนำให้ความจำเสื่อมด้วยยาซาโคโปลามีน ผลของการเพิ่มความจำของสารสกัดจากเปลือกมังคุดอาจเป็นผลของการยับยั้งการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรส การได้รับสารสกัดจากเปลือกมังคุดเสริมอาจมีประโยชน์สำหรับการป้องกันการพัฒนาการหรือการดำเนินไปของความจำเสื่อมที่มีสาเหตุมาจากความชราตามธรรมชาติ

สาขาวิชาชีววิทยา ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2556 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

NAIYANA NONTAMART : EFFECTS OF THE EXTRACT FROM THE FRUIT HULL OF MANGOSTEEN (*Garcinia mangostana* L.) ON LEARNING MEMORY AND THE CENTRAL CHOLINERGIC SYSTEM OF MALE WISTAR RATS. THESIS ADVISOR : ASST. PROF. RUNGRUDEE SRISAWAT, Ph.D. 115 PP.

MANGOSTEEN/ MORRIS WATER MAZE TEST/ MEMORY/ DEMENTIA/ CENTRAL CHOLINERGIC SYSTEM

The present study aimed to investigate the effects of the crude extract from the fruit hull of mangosteen (GME) on learning and memory using Morris water maze test and acetylcholinesterase (AChE) activity in cerebral cortex, hippocampus, and basal forebrain of male Wistar rats (normal saline treated rats, scopolamine (SCOP)–induced amnesic rats, healthy adult rats, and aged rats). Results showed that oral administration of GME (250, 500, or 1000 mg/ml/kg) for 7 days significantly increased time spent in target quadrant of the normal saline treated rats when compared to the vehicle control (P<0.05), but had no effect in SCOP (1 mg/ml/kg, i.p., 7 days)-induced amnesic rats. In normal saline treated rats, GME exposure also significantly decreased AChE activity in cerebral cortex (at 500 and 1000 mg/ml/kg) and hippocampus (at 250 and 500 mg/ml/kg), while GME at all doses significantly increased AChE activity in basal forebrain when compared to the vehicle control (P<0.05). In SCOP–induced amnesic rats, GME exposure significantly increased AChE activity in cerebral cortex (at 500 mg/ml/kg) and hippocampus (at 250 and 500 mg/ml/kg) when compared to the vehicle control (P<0.05). Subchronic exposure to

GME (500 mg/ml/kg, p.o.) for 30 days significantly increased time spent in target quadrant in both healthy adult rats and aged rats, compared to the vehicle control (P<0.05). In healthy adult rats, GME (1000 and 2000 mg/ml/kg) significantly decreased AChE activity in both cerebral cortex and basal forebrain, and GME (500 mg/ml/kg) significantly decreased AChE activity in basal forebrain, compared to the vehicle control (P<0.05). GME (1000 mg/ml/kg) significantly increased AChE activity in hippocampus, but had no effect on cerebral cortex and basal forebrain of aged rats. In conclusion, the present study demonstrated memory enhancing and anti-acetylcholinesterase actions of GME in normal adult and normal aging models, but not in scopolamine-induced amnesia model. Memory enhancing effects of GME may be caused by its anti-acetylcholinesterase activity. Supplementation of GME may be beneficial for the prevention of the development or progression of cognitive impairment caused by natural aging.

School of Biology

Academic Year 2013

Student's Signature_____

Advisor's Signature_____

Co-advisor's Signature _____

ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor, Asst. Prof. Dr. Rungrudee Srisawat for her consistent supervision, advice encouragement, valuable suggestion, and support throughout my thesis. Special thanks are also extended to Dr. Walaiporn Tongjaroenbuangam for her valuable suggestion and guidance given as thesis co-advisor.

I would like to thank to all my thesis committees, Asst. Prof. Dr. Nooduan Muangsan and Asst. Prof. Dr. Benjamart Chitsomboon for giving the comments and suggestions.

I am also grateful to all the faculty and staff members of the Center for Scientific and Technological Equipment, Suranaree University of Technology for their help and assistance throughout the period of this research work and I would like to thank all members in Dr. Rungrudee Srisawat's lab.

I am extremely grateful for the National Research Council of Thailand (NRCT) and Suranaree University of Technology thesis support grant for providing the financial support and giving me the opportunity to conduct and complete my thesis.

Finally, I would also like to thank my family. They are always supporting me and encouraging me with their best wishes.

Naiyana Nontamart

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

CHAPTER I

INTRODUCTION

1.1 Background/Problem

Dementia is characterized by loss of memory and cognitive abilities. Dementia is not a disease, but rather a group of symptoms caused by disorders that affect the brain (Alzheimer's Association, 2011; 2012). Dementia is a symptom complex of continuous global weak of intellectual function. It becomes a major medical, economic, and social problem that is deterioration as the number of elderly people in the general population increases (Maxine and Papadakis, 2009). Different types of dementia, such as Alzheimer's disease (AD), vascular disorders, dementia with Lewy bodies and Parkinson's disease have been associated with distinct patterns of symptom and brain abnormalities manner (Alzheimer's Association, 2010).

AD is the most common type of dementia. It is also characterized by loss of cognitive function, behavior and activities of daily living. It is also characterized by an age-dependent loss of memory and an impairment of multiple cognitive functions that primarily affects the elderly population (Howes and Houghton, 2003; Francis et al., 1997; Ingkaninan et al., 2003; Jalbert et al., 2008; Pakaski and Kalman, 2008; Thimmappa et al., 2005; Uabundit et al., 2010).

Dysfunction of cholinergic neurotransmission in the brain that causes cognitive decline has been reported in AD. The gradual death of cholinergic cells in AD is accompanied by loss of the acetylcholine (Vinutha et al., 2007). A cholinergic deficit has been shown to be associated with memory loss and the severity of AD. Loss of the cholinergic markers, acetylcholinesterase (AChE) is neurological changes consistently found in the brains of AD patients (Court and Perry, 1991). Hence, it has been suggested that elevation of acetylcholine level might be helpful in attempt to improve the symptoms of cognitive deficit in AD. One of the most promising approaches for treatment of this disease is to enhance the acetylcholine level in the brain using acetylcholinesterase inhibitors (Chattipakorn et al., 2007; Ingkaninan et al., 2003; Vinutha et al., 2007).

The use of herbal remedies is an alternative treatment for AD. Many medicinal plants such as pomegranate, grape seed, curcumin, soy, *Ginkgo biloba,* and *Paeonia suffruticosa* have been reported as potential treatments for AD (Jung and Park, 2007; Lenta et al., 2007). These plants are good sources of polyphenols and other antioxidant components. Polyphenols found in plants and fruits (*Allanblackia monticola, Symphonia globulifera, Agrimonia pilosa, Salvia albimaculata, Salvia cyanescens, Pistacia atlantica, Pistacia lentiscus,* strawberry, walnut, rosemary, and soybean) could inhibit the activity of AChE (Benamar et al., 2010; Jung and Park, 2007; Lee et al., 2004; Lenta et al., 2007; Orhan et al., 2008; Orhan et al., 2007). Extracts from plants and fruits containing polyphenols (*Thunbergia laurifolia* L, *Centella asiatica*, and *Mangifera indica*) showed significant improvement in learning and memory impairment (Kumar et al., 2009; Veerendra et al., 2002). Moreover,

polyphenol extracts from green tea and wild blueberry could attenuate memory impairment and AChE activity (Kim et al., 2004; Papandreou et al., 2009).

The fruit hull of mangosteen was reported to contain polyphenols (xanthone, tannin, flavonoid, and anthocyanins) (Asai et al., 1995; Chairungsrilerd et al., 1996; Pedraza-Chaverri et al., 2009; Chen et al., 2008; Chomnawang et al., 2007; Cui et al., 2010; Ji et al., 2007; Jung et al., 2006; Maisuthisakul et al., 2007; Yu, et al., 2007; Zadernowski et al., 2009). These polyphenolic compounds have been found to be efficient scavenger of reactive oxygen species (ROS), and to possess neuroprotective properties (Pedraza-Chaverri et al., 2009; Weinreb et al., 2004). These findings suggest the potential role of polyphenols found in the fruit hull of mangosteen in the treatment of neurodegenerative disease.

It is hope that natural antioxidant found in the fruit hull of mangosteen could improve memory and prevent the onset of dementia. Therefore, the effects of the crude extract from the fruit hull of mangosteen on memory and the central cholinergic system were investigated.

1.2 Research objectives

The experiments were designed to clarify the followings:

1. To study the effects of the crude extract from the fruit hull of mangosteen on cognitive impairment and acetylcholinesterase activity in the hippocampus, basal forebrain and cerebral cortex of amnesic rats induced by repeated doses of scopolamine.

2. To study the subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the hippocampus, basal forebrain, and cerebral cortex of adult rats.

3. To study the subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the hippocampus, basal forebrain, and cerebral cortex of aged rats.

1.3 Research hypothesis

The crude extract from the fruit hull of mangosteen has beneficial role in prevention and treatment of dementia.

1.4 Expected results

The expected results from this study were:

1. The crude extract from the fruit hull of mangosteen could enhance memory performance and attenuate acetylcholinerase activity in amnesic rats induced by scopolamine.

2. The crude extract from the fruit hull of mangosteen could enhance memory performance and attenuate acetylcholinerase activity in both adult and aged rats.

CHAPTER II

LITERATURE REVIEW

2.1 Mangosteen

Mangosteen (*Garcinia mangostana* L.) is a tropical evergreen tree with leathery and glabrous leaves. The tree can attain 6-25 m in height and is mainly found in Thailand, India, Sri Lanka, Vietnam, Singapore, Philipines and Myanmar. It can be found in Indonesia, Southern India and Brazil. It is known as “the queen of fruits”. It has dark purple to red purple fruits. The edible fruit aril is white, soft with slightly sour taste (Ji et al., 2007). The fruit hull (pericarp) of mangosteen has been used in Southeast Asia traditional medicine for the treatment of skin infections wound, and diarrhea (Jung et al., 2006; Suksamrarn et al., 2003). The fruit hull of mangosteen is the rich source of polyphenols (xanthone, tannin, flavonoid and anthocyanins) (Asai et al., 1995; Chen et al., 2008; Chomnawang et al., 2007; Cui et al., 2010; Ji et al., 2007; Maisuthisakul et al., 2007; Yu et al., 2007; Zadernowski et al., 2009). These polyphenols are of plant secondary metabolites. The most common roles of secondary compounds in plants are ecological roles that govern interactions between plants and other organisms. Many secondary compounds are brightly colored pigments like anthocyanin that color flowers. Plant secondary metabolites have also been reported to possess a wide range of pharmacological activities, including antimicrobial, anti inflammatory, anti-diabetic and acetylcholinesterase inhibitory activities (Eldeen et al., 2005). The fruit hull of mangosteen contained four types of the xanthones:

α-mangostin, gartanin, γ-mangostin and β-mangostin (Mahabusarkam and Wiriyachitra, 1986). These compounds are the major secondary metabolites of mangosteen which are classified into prenylated xanthone derivatives (Pedraza-Chaverri et al., 2009).

Xanthone is a class of plant polyphenols that can be found in the whole mangosteen fruit (the fruit hull, edible arils and seed) (Moongkarndi et al., 2004). Xanthones of the fruit hull of mangosteen exhibits a variety of biological activities, including antibacterial, anti-inflammatory and anticancer effects (Akao et al., 2008). The α-mangostin of mangosteen could prevent lipid peroxidation in rats treated with isoproterenol by reducing the activity of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) and increased antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Isoproterenol causes severe oxidative stress in the myocardium (Devi Sampath and Vijayaraghavan, 2007). Furthermore, Chomnawang et al. (2007) found that the ethanol extract of *Garcinia mangostana* showed antioxidant activity with the IC_{50} of 6.13 μg/ml as measured by inhibition of the formation of DPPH radicals. The extract of *G. mangostana* reduced the ROS production of polymorphonuclear leucocytes (PML) with 77.8% of superoxide anion inhibition ratio. Besides, the α-mangostins could decrease the human low density lipoproteins (LDL) oxidation induced by copper or peroxyl radical. α-Mangostins prolonged lag time of conjugated dienes at 234 nm in a dose-dependent manner, decreased thiobarbituric reactive substances (TBARS) production, and decreased the α-tocopherol consumption induced by LDL oxidation (Williams et al., 1994). α-Mangostins and their synthetic derivatives prevent the

decrease of the α-tocopherol consumption induced by LDL oxidation (Mahabusarakam et al., 2000). The *G. mangostana* extract significantly diminished intracellular ROS production on SKBR3 cell in the dose dependent manner as well as exposure time dependent manner, as measured by 2, 7-dichlorofluorescein diacetate (DCFH-DA) (Moongkarndi et al., 2004). The four extracts (water, 50% ethanol, 95% ethanol and ethyl acetate) of mangosteen fruit hull showed antioxidant by the diphenylpicrylhydrazyl (DPPH) method and neuroprotective activities. Water and ethanolic (50%) extracts showed high antioxidant capacity (34.98 and 30.76 μg/ml, respectively). The antioxidant capacity of these extracts was tested on a neuroblastoma cell line (NG108-15) exposed to hydrogen peroxide (H_2O_2); both extracts at concentration of 50 μg/ml exhibited neuroprotective activity. These results suggested that the water and 50% ethanol extracts from the fruit hull of *G. mangostana* may be potent neuroprotectants (Weecharangsan et al., 2006).

Structural modifications of α-mangostin could modify the antioxidant activity. For example, substitution of C-3 and C-6 with aminoethyl derivatives enhanced the antioxidant activity. The xanthone induced apoptosis in HL60 cells. α-Mangostin induced caspases 9 and 3 activation, loss of mitochondrial membrane potential, and release of ROS and cytochrome C. These results indicated that mitochondria play a pivotal role in induction of apoptosis by α-mangostin (Matsumoto et al., 2004). The prenylated xanthones from the fruit hull of mangosteen exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the minimum inhibitory concentration value of 6.25 μg/ml (Suksamrarn et al., 2003). Xanthones isolated from the fruit powder of *G. mangostana* showed HO·-scavenging activity (IC_{50}=0.2 μg/ml). All xanthones, except α-mangostin, were found to induce activity of quinone reductase

(QR, phase II drug-metabolizing enzyme), using murine hepatoma cells (Hepa 1c1c7) *in vitro* (Chin et al., 1996). Mangiferin, a xanthone found in *Mangifara indica*, showed enhances recognition memory through a mechanism that might involve an increase in neurotropin and cytokine levels and an increase of supernatant level of nerve growth factor *in vitro* in human U138-MG glioblastoma cell (Pardo Andreu et al., 2010). Young stage of *G. mangostana* fruit generally contained larger amounts of α-mangostin, a xanthone isolated from mangosteen fruit, than that of the old stage (Pothitirat et al., 2009). By high performance liquid chromatography (HPLC) method, the mature fruit rind extract contained α-mangostin (13.63±0.06% w/w) about 2 times higher than that of the young fruit rind extract (8.07±0.11% w/w) (Pothitirat et al., 2009). The yellowish excretion of the fruit hull of *G. mangostana*, a crystalline mixture consisting mainly α- and γ-mangostin showed an inhibitory effect on cAMP phosphodiesterase (Chairungsrilerd et al., 1996). α-Mangostin, a xanthone isolated from mangosteen fruit, could reduce oxidative damage in rat brain tissue exposed to the toxic actions of a free radical generator (ferrous sulfate), an excitotoxic agent (quinolinate), and mitochondrial toxin (3-nitropropionate) (Márquez-Valadez et al., 2009). Tannins are polyphenolic secondary compounds of plants. They can be found in *Garcinia mangostana* fruit hull (Pothitirat et al., 2009). Young stage of *G. mangostana* fruit generally contained larger amounts of tannin than that of the old stage. The contents of total tannins in young and mature fruit rind extracts of *G. mangostana* were 51.25 and 36.66 TAE/100g, respectively (Pothitirat et al., 2009). Tannins constitute a complex group of naturally occurring polymers, and a rigorous chemical definition is difficult. The term “tannin” was originally used to describe vegetable components that are responsible for converting animal hides into leather in

the process of tanning by forming stable complexes with skin collagen. Thus, tannins are considered to be polyphenolic metabolites of plants with a molecular weight larger than 500 and with the ability to precipitate gelatin and other proteins from solution (Bennick, 2002). Various chemical structures of tannins occurring in medicinal and food plants that are utilized world-wide showed several remarkable biological and pharmacological activities that are often very specific to certain tannin structures, and significant for human health. Tannin has been evaluated as antibacterial, antiviral, radical scavenging and complement modulating agent (Okuda, 2005). Tannin-containing plant extracts were used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors (Khanbabaee and Ree, 2001). Curcumin, a polyphenol extracted from the rhizome of *Curcuma longa*, is well known to have antioxidative, anti-cancerous and anti-inflammatory activities, and anti-ageing and neuroprotective potential effects. Curcumin could play a crucial role in preventing against aluminium (Al) induced neurotoxicity in young and old rats, which is known to be involved in the etiology of several neurodegenerative disorders like Alzheimer's and Parkinson's diseases. Curcumin treatment could attenuate the Al-induced alterations at biochemical, behavioral and ultra-structural levels which was well reflected in the electrophysiological recordings, indicating the ability of curcumin to bind redox active metals and cross the blood–brain barrier (Sethi et al., 2009).

Flavonoids are a subclass of the polyphenols, which are consisted of an aromatic ring. There are six groups of flavonoids: anthocyanins, flavones, flavanones, catechins, isoflavonoids, and flavonols. Flavonoids found in fruits and vegetables contribute to the blue, orange or purple colors. Flavonoids found in certain beverages have diverse beneficial biochemical and antioxidant effects (Beecher, 2003; Hooper et

al., 2008). The importance of flavonoids in enhancing cell resistance to oxidative stress goes beyond simple scavenging activity and be of most interest in pathologies in which oxidative stress plays an important role (Weinreb et al., 2004). Flavonoids were shown to activate key enzymes in mitochondrial respiration and to protect neuronal cells by acting as antioxidants, thus breaking the vicious cycle of oxidative stress and tissue damage. Furthermore, recent data showed favorable effect of flavonoids on neuro-inflammatory events. Whereas, most of these effects have been shown *in vitro*, limited data *in vivo* are available, suggesting a rather low penetration of flavonoids into the brain (Schmitt-Schillig et al., 2005). The neuroprotective actions of dietary flavonoids involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to contain neuro-inflammation, and the potential to promote memory, learning and cognitive function (Spencer, 2009). The *in vivo* study of neuro-protective showed that green tea extract and (-)-epigallocatechin-3-gallate (EGCG) possessed highly potent activities in preventing striatal dopamine depletion as well as substantia nigra dopaminergic neuron loss in mice induced by the parkinsonism-inducing neurotoxin, N-methyl-4-pheny l-1,2,3,6-tetrahydropyridine (MPTP). One possible mechanism underlying the effectiveness of green tea and EGCG against MPTP neurotoxicity may involve its catechol-like structure, since it is known that catechol-containing compounds are potent radical anti-oxidants and chelators of ferric ion (Weinreb et al., 2004). Krikorian et al. (2010) showed that wild blueberry juice supplementation for 12 weeks improved memory function in older adults with early memory decline. This is the first human trial evaluate the potential benefit of blueberry supplementation on neuro-cognitive function in older adults with increased risk for dementia. The FLDK-P70, a

standardized flavonoid extract, significantly increased the survival of hippocampal CA1 pyramidal neurons after transient global brain ischemia, reduced the lesion of the insulted brain hemisphere and improved the neurological behavior using *in vivo* rat model of focal ischemia/reperfusion (I/R) injury induced by middle cerebral artery occlusion (MCAO) (Bei et al., 2009). *Ginkgo biloba* extracts such as EGb-761 containing flavonoids, proanthocyanidins and terpenoids have been suggested to have a multitude of beneficial effects on CNS function, from enhancing cognitive function in dementia to facilitating recovery from acute forms of neural damage such as hypoxia/ischemia. *Ginkgolide* B, one of the major components of EGb-761 is also regarded as having neuro-protective effects in the CNS (Maclennan et al., 2002).

Anthocyanins are naturally occurring compounds that impart color to fruits, vegetables and plants. It is probably the most important group of visible plant pigments besides chlorophyll a part from imparting color to plants, anthocyanins have an array of health-promoting benefits, as it can protect against a variety of oxidants through a various number of mechanisms (Kong et al., 2003). Many claims have been made reference to the benefits to our health from eating a variety of fruits and vegetables, especially those with strong colors, such as carrots, apricots, blackcurrants, broccoli, purple grapes, and red wine (Anthocyanins.net, 2010). Anthocyanins have been shown to inhibit the growth of cultured human malignant cells and have demonstrated excellent anti-inflammatory and antioxidant properties (Pasqua et al., 2009). Anthocyanins could inhibit carcinogenic activity against multiple cancer cell types *in vitro* and tumor types *in vivo* (Wang and Stoner, 2008). Anthocyanins have been shown to inhibit the development of tumors induced in mice following the subcutaneous injection of lung tumor cells (Ding et al., 2006).

Anthocyanins from purple sweet potato improved cognitive performance as measured by passive avoidance tests in ethanol-treated mice, and also effectively inhibited lipid per-oxidation as measured by DPPH radicals in rat brain tissues (Cho et al., 2003). Incorporation of anthocyanin from elderberry extract by endothelial cells improved resistance to the damaging effects of ROS and protected against hydrogen peroxide induced loss in cell viability (Youdin et al., 2000). The dietary supplementation with a blueberry (*Vaccinium* spp.) extract originally composed of anthocyanins retarded age-related declines in aspects of neurological function, motor behavioral performance on the rod walking and accelerated tasks, and Morris water maze performance (Joseph et al., 1999).

2.2 Dementia

Dementia is characterized by loss of memory and cognitive abilities. Dementia is not a disease, but rather a group of symptom caused by disorders that affect the brain manner (Alzheimer's Association, 2011; 2012; 2013). Dementia is caused by various diseases and conditions that result in damaged brain cells or connections between brain cells. People with dementia may not be able to think well enough to do normal activities, such as getting dressed or eating. They may lose their ability to solve problems or control their emotions. Their personalities may change. They may become agitated or see things that are not there. Dementia is caused by a variety of diseases and injuries that primarily or secondarily affect the brain, such as AD. Common types of dementia and their typical characteristics are shown in Table 2.1. Dementia is one of the major causes of disability and dependency among older people worldwide. It is overwhelming not only for the people who have it, but also for their

caregivers and families. There is often a lack of awareness and understanding of dementia, resulting in stigmatization and barriers to diagnosis and care. The impact of dementia on caregivers, family and societies can be physical and psychological (WHO, 2012). Dementia is a symptom complex of continuous global weak of intellectual function. It becomes a major medical, economic, and social problem that is deterioration as the number of elderly people in the general population increases (Maxine and Papadakis, 2009). Worldwide, 35.6 million people have dementia, with just over half (58%) living in low- and middle-income countries. Every year, there are 7.7 million new cases. The estimated proportion of the general population aged 60 and over with dementia at a given time is between 2 to 8 per 100 people. The total number of people with dementia is projected to almost double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050. Much of this increase is attributable to the rising numbers of people with dementia living in low- and middle-income countries (WHO, 2012).

Table 2.1 Different types of dementia**.**

Type of dementia	Characteristics
Alzheimer's disease (AD)	Most common type of dementia; accounts for an estimated 60% to 80% of cases. Difficulty remembering names and recent events is often an early clinical symptom; apathy and depression are also often early symptoms. Later symptoms include impaired judgment, disorientation, confusion, behavior changes, and difficulty speaking, swallowing, and walking. Hallmark abnormalities are deposits of the protein fragment beta-amyloid (plaques) and twisted strands of the protein tau (tangles).
Vascular dementia (also known as multi-infarct or post-stroke dementia or vascular cognitive impairment)	Considered the second most common type of dementia. Impairment is caused by decreased blood flow to parts of the brain, often because of a series of small strokes that block arteries. Symptoms often overlap with those of AD, although memory may not be as seriously affected.
Mixed dementia	Characterized by the hallmark abnormalities of AD and another type of dementia, most commonly vascular dementia, but also other types, such as dementia with Lewy bodies.
Dementia with Lewy bodies (DLB)	Pattern of decline may be similar to AD, including problems with memory and judgment as well as behavior changes. Alertness and severity of cognitive symptoms may fluctuate daily. Visual hallucinations, muscle rigidity, and tremors are common. Hallmarks include Lewy bodies (abnormal deposits of the protein alpha-synuclein) that form inside nerve cells in the brain.

(Alzheimer's Association, 2011; 2012; 2013).

Table 2.1 Different types of dementia. (Continued)

Type of dementia	Characteristics
Parkinson's disease (PD)	Many people who have Parkinson's disease (a disorder that usually involves movement problems) also develop dementia in the later stages of the disease. The hallmark abnormality is Lewy bodies (abnormal deposits of the protein alpha-synuclein) that form inside nerve cells in the brain.
Frontotemporal dementia	Nerve cells in the front and side regions of the brain are especially affected. Typical symptoms include changes in personality and behavior and difficulty with language. No distinguishing microscopic abnormality is linked to all cases. Pick's disease, characterized by Pick's bodies (nerve cells containing an abnormal accumulation of fibers made of the protein tau), is one type of frontotemporal dementia.
Creutzfeldt–Jakob disease	Rapidly fatal disorder that impairs memory and coordination and causes behavior changes caused by the misfolding of prion protein throughout the brain. Variant Creutzfeldt–Jakob disease is believed to be caused by consumption of products from cattle affected by mad cow disease.
Normal pressure hydrocephalus	Caused by the buildup of fluid in the brain. Symptoms include difficulty walking, memory loss, and inability to control urination. Can sometimes be corrected with surgical installation of a shunt in the brain to drain excess fluid.

(Alzheimer's Association, 2011; 2012; 2013).

AD is the most common type of dementia. It is also characterized by loss of cognitive function, behavior and activities of daily living. It is also characterized by an age-dependent loss of memory and an impairment of multiple cognitive functions that primarily affects the elderly population (Howes and Houghton, 2003). Dysfunction of cholinergic neurotransmission in the brain that cause cognitive decline has been reported in AD. The gradual death of cholinergic cells in AD is accompanied by loss of the acetylcholine (Vinutha et al., 2007). A cholinergic deficit has been shown to be associated with memory loss and the severity of AD. Loss of the cholinergic markers, choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) are neurological changes consistently found in the brains of AD patients (Court and Perry, 1991). Hence, it has been suggested that elevation of acetylcholine level might be helpful in attempt to improve the symptoms of cognitive deficit in AD. One of the most promising approaches for treatment of this disease is to enhance the acetylcholine level in the brain using acetylcholinesterase inhibitors (Chattipakorn et al., 2007; Ingkaninan et al., 2003; Vinutha et al., 2007).

2.3 Morris Water Maze Test: procedures for assessing spatial learning and memory

The Morris Water Maze (MWM) is one of the most widely used tests to measure hippocampal-dependent spatial-based learning and memory. This test is affected by aging, hormonal changes, and amnesia producing as well as cognition enhancing drugs as well as genetic manipulations of genes associated with cognition. Various protocols exist for these tests, which are used depending on a plethora of variables. Some of the protocols may be too diffuse/easy and others too

intense/difficult for the animal to learn. Variables include age, strain, experimental manipulation, or predisposition to stress, therefore a careful study of these variables is necessary before carrying out the test. Test Specifics: The rodent is placed in a pool of water where it must use and remember visual cues located in the room to find a platform hidden underneath the surface of the water. The task is carried out across days to determine learning. Distance swam, latency to reach the platform, and swim speed are common measures of this test. The capacity of the animal to retrieve and retain information learned or flexibility to purge and re-learn new strategies can be determined using a probe trial & reversal trial. In the probe trial the platform is taken out and the animals are allowed to swim in the pool. Time spent in the region that previously contained the platform, crossings over the platform area, and time to reach platform location is measured. The reversal trial is identical to the training trials but in this case the platform is switch to the opposite region of the pool, thus the animal has to have the cognitive flexibility necessary to re-learn the new location. A cued version of this task can also be used to measure none spatial strategies as well as visual acuity by rendering the platform visible (Morris, 1981).

2.4 Central cholinergic system

Acetylcholine (ACh) is the transmitter at the neuromuscular junction, in autonomic ganglia, and in postganglionic parasympathetic nerve-target organ junctions and some postganglionic sympathetic nerve-target junctions. It is also found within the brain, including the basal forebrain complex. ACh is the acetyl ester of choline, is largely enclosed in small, clear synaptic vesicles in high concentration in the terminal boutons of neurons that release ACh (cholinergic neurons). Synthesis of

ACh involves the reaction of choline with acetate. ACh is synthesized in the presynaptic terminal by ChAT, and is broken down after release into the synaptic cleft by AChE. These enzymes are synthesized in the neuronal cell body and are carried by axonal transport to the presynaptic terminal. ChAT joins acetyl-CoA to choline to from ACh. Neurons that synthesize and release ACh are termed cholinergic neuron. When nerve impulse arrive nerve terminal, a voltage-gated calcium channel will open. The influx of calcium ions (Ca^{2+}) will stimulate the exocytosis of presynaptic vesicles containing ACh, which is thereby released into the synaptic cleft. Once released, ACh must be removed rapidly by AChE in order to allow repolarization to take place, this step, hydrolysis, is carried out by the enzyme. AChE metabolizes ACh into acetate and choline (Adamson, 1997, Figure 2.1).

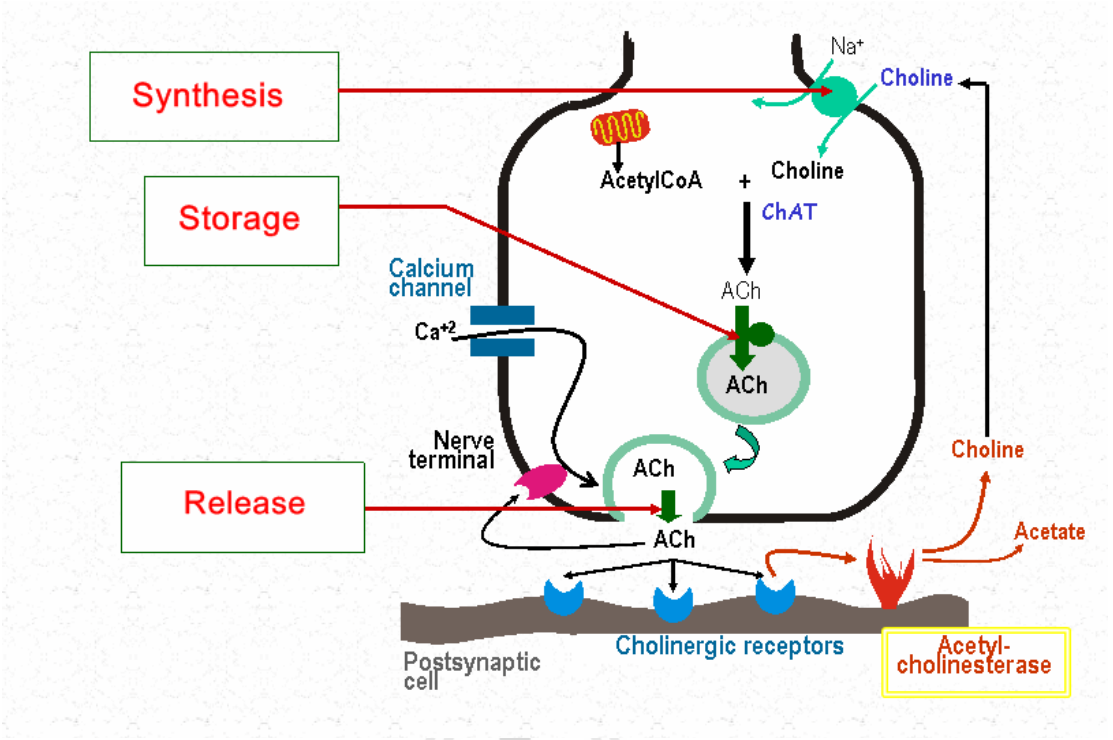


Figure 2.1 Schematic diagram showing the role of acetylcholinesterase (AChE) and acetylcholine (ACh) in cholinergic transmission (CMU medicine pharmacology, 2010).

Central cholinergic pathways are ideally suited to regulate global functions of global cerebral cortex; such functions include attention, arousal, motivation, memory and consciousness (Woolf, 1991; Woolf, 1996). There are two groups of neurons, forebrain base cholinergic: (1) a group in the middle wall (Nucleus, medial wall and vertical diagonal band : ms and VDB) cholinergic axons that project hippocampus and parahippocampal gyrus and (2) the nucleus basalis group group (nucleus basalis, substantia innominata and horizontal diagonal band: bas, si, hdb) that project cholinergic axons to all parts of the neocortex, some of the cortical limbic and amygdala cholinergic. The cholinergic pontomesencephalon neurons (laterodorsal tegmental and pedunculopontine tegmental nuclei: ldt and ppt) project onto hindbrain,

thalamus, hypothalamus and basal forebrain. the nucleus basalis group (nucleus basalis, substantia innominata and horizontal diagonal band: bas, si, hdb) that project cholinergic axons to all parts of the neo-cortex, parts of limbic cortex and to the amygdale are shown in Figure 2.2.

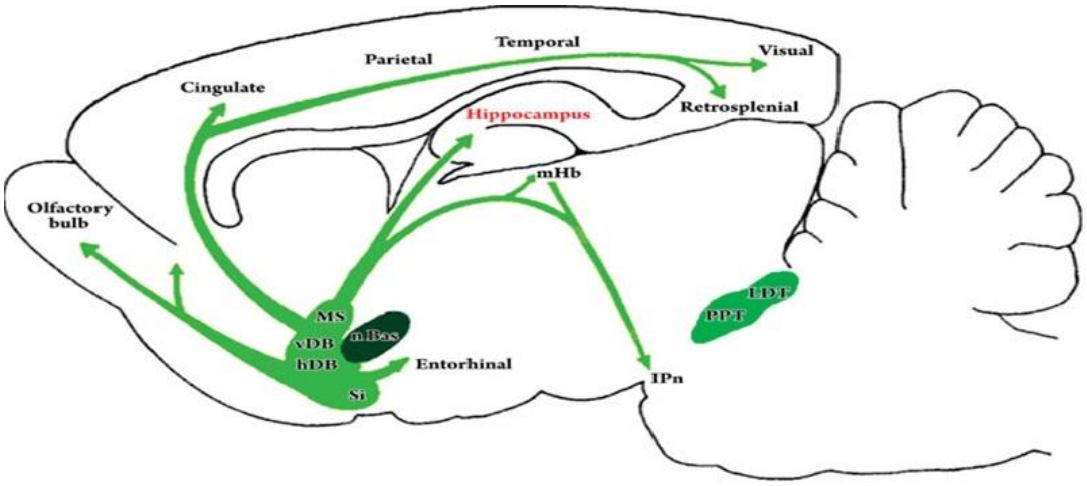


Figure 2.2 Schematic diagram showing the central cholinergic pathway and acetylcholine and memory. (MS: medial septum, n Bas: nucleus Basalis, vDB: vertical Diagonal Band, hDB: horizontal Diagonal Band, SI: sublenticular substantia innominata, mHb: medial Habenula, LDT: Laterodorsal Tegmentum, PPT: Pedunculopontine Tegmentum, IPn: Interpeduncular nucleus) (Placzek et al., 2009).

The major cholinergic afferent projections to the hippocampus are shown in Figure 2.2. The hippocampus receives cholinergic innervations mainly from the medial septum-diagonal band complex *via* the fimbria-fornix. A fine network of cholinergic fibers projects to the hippocampus and dentate gyrus, and synaptic contacts are made onto pyramidal cells, granule cells, inter-neurons, and neurons of the hilus. In addition to direct synaptic connections, ACh may spill out of synaptic

contacts and also produce volume transmission within the hippocampus *via* non-synaptic signaling (Placzek et al., 2009). In humans and other mammals, the hippocampus is located inside the medial temporal lobe, beneath the cortical surface. It belongs to the limbic system and plays important roles in long-term memory and spatial navigation.

The basal forebrain is a collection of structures located ventrally to the striatum**:** the nucleus basalis, diagonal band of Broca, and medial septal nuclei. It is considered to be the major cholinergic output of the CNS. ACh produced from these areas affects the ability of brain cells to transmit information to one another, and also encourages plasticity, or learning. Thus, damage to the basal forebrain can reduce the amount of acetylcholine in the brain and impair learning and memory.

The cerebral cortex is a sheet of neural tissue that is outer most to the cerebrum of the mammalian brain. It plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness (Wikipedia, 2010). It is divided into 4 main lope: the frontal lope involved in conscious thought and plays an important part in processing short-term memories and longer-term memories; the parietal lope was involved in integrating sensory information from the various senses, and in the manipulations of objects in determining spatial sense and navigation; the temporal lobe was involved with the sense of smell and sound, the processing of semantics in both speech and vision and plays a key role in the formation of long-term memory; the occipital lope was mainly involved with the sense of sight.

AD is a successive age-related disorder that is characterized by the regression of neurological function showed in the loss of cognitive ability, loss of reasoning ability and other disturbances of effect. Progressive lost of ACh occurs during the

progression of AD. According to the cholinergic hypothesis, the replacement of ACh can delay the loss of cognitive ability. AChE inhibitors have been shown to function by increasing ACh within the synaptic region, by which restoring deficient cholinergic neurotransmission (Felder et al., 2000). In AD, the hippocampus is one of the first regions of the brain to suffer damage; memory problems and disorientation appear among the first symptoms. Damage to the hippocampus can also result from oxygen starvation (hypoxia), encephalitis, or medial temporal lobe epilepsy. People with extensive hippocampal damage may experience amnesia, the inability to form or retain new memories (Wikipedia, 2010).

From *in vitro* studies, polyphenols from aqueous tea infusions of strawberry and walnut showed 45% and 42.5% inhibition of AChE activity, respectively (Kulisic-Bilusic et al., 2007). The aqueous tea infusions from walnut (*Juglans regia* L.), strawberry (*Fragaria´ananassa* L.), peppermint (*Mentha´piperita* L.), lemon balm (*Melissa officinalis* L.), immortelle (*Helichrysum arenarium* L.) and sage (*Salvia officinalis* L.) showed high rate of antioxidant activity determined by LDL oxidation method, DPPH radical scavenging assay, β-carotene bleaching method and Rancimat method. The highest inhibition of LDL oxidation was found in aqueous infusions of strawberry and lemon balm. Screening of the AChE inhibitory activity by Ellman´s method showed high inhibitory activity in walnut and strawberry aqueous infusions, suggesting their possible role on the treatment of Alzheimer´s disease (Kuli-Bilu et al., 2008). Polyphenols from petroleum ether extract of *Salvia albimaculata* and chloroform extract of *Salvia cyanescens* were potently inhibited AChE activity at 0.2 mg/ml (Orhan et al., 2007). Phenolic acid found in rosemary (rosmarinic acid) showed 85.8% inhibition against AChE activity at 1.0 mg/ml (Orhan et al., 2008). Xanthones

and benzophenones were isolated from the fruit extract of *Allanblackia monticola* and the leaf extract of *Symphonia globulifera.* Isolated xanthones showed potent anticholinesterase activity (Lenta et al., 2007). The aqueous extract of *Pistacia atlantica* and *Pistacia lentiscus* containing flavonoid showed high AChE inhibitory activity (% inhibition of 81.90%) (Benamar et al., 2010). Mukhergee et al. (2006) and Yang et al. (2008) reported that *N. nucifera* rhizome extract inhibited AChE activity and improved learning and memory by enhancing neurogenesis in the dentate gyrus of hippocampus.

From *in vivo* studies, isoflavones from the extract of soybean improved learning and memory performance, and reduced age-related neuronal loss and cognition decline in elderly male rats that was tested by water maze test. In addition, soybean extract also increased the ChAT activity in basal forebrain but decreased the AChE activity in cerebral cortex and hippocampus of aged rats (Lee et al., 2004). Procyanidins extracted from the lotus seed pod significantly reversed scopolamine induced learning and memory impairment in mice. In addition, the extract was found to inhibit acetylcholiesterase activity (Xu et al., 2009). Green tea extract could inhibit AChE activity in scopolamine-induced amnesia mice (Kim et al., 2004). Polyphenol-rich wild blueberry extracts attenuated brain oxidative stress, increased brain ascorbate and glutathione (GSH) levels, and decreased AChE activity in mice whole brain homogenates. This extract also exhibited a significant improvement in learning and memory tested by the passive avoidance behavioral test (Papandreou et al., 2009). *Centella asiatica* extract were found to contain triterpenes namely asiaticoside, asiatic acid and madecassic acid. The extract also contained high phenolic contents, which exhibit strong association with its anti-oxidative activities. The extract could alter the

levels of markers of brain oxidative stress: a significant decrease in the rat brain levels of malondialdehyde (MDA) with simultaneous significant increase in levels of glutathione. In addition, aqueous extract of whole plants of *Centella asiatica* showed an improvement in learning and memory of the rats in both shuttle box and step through paradigms (Veerendra et al., 2002). *Mangifera indica* fruit extract which contained polyphenol, flavonoids, tannins, saponins, and flavonols could prevent cognitive deficits and amnesic effect in aging mice and scopolamine-treated young mice in passive avoidance task and elevated plus maze task (Kumar et al., 2009). Grape seed extract containing polyphenol such as proanthocyanidin (condensed tannin) could promote memory in aging. Decreased latency time, decreased path length but increased swimming speed as measured by Morris Water Maze test in male aged rat were shown (Sarkaki et al., 2007). Nine weeks administration of grape seed proanthocyanidin extract could increase ChAT activity and decrease AChE activity in adult rat's hippocampus (Devi et al., 2006). The co-effect of procyanidins extracted from the lotus seed pod (LSPC) and bilobalide (BIL) on ameliorating scopolamine-induced learning and memory impairment in young mice showed significantly shorter escape latency and swimming distance in the Morris water maze test (Zhang et al., 2009).

2.5 Vitamin E

Vitamin E is a fat-soluble compound that includes both tocopherols and tocotrienols. Of the many different forms of vitamin E, γ-Tocopherol can be found in corn oil, soybean oil, margarine, and dressings. α-tocopherol, the most biologically active form of vitamin E, is the second-most common form of vitamin E. This variant

can be found most abundantly in wheat germ oil, sunflower, and safflower oils. (Wikipedia, 2013) The various tocopherol forms rather than α- tocopherol alone may be important in the vitamin E protective association with Alzheimer disease by the administration of 4 cognitive tests and clinical evaluations for Alzheimer disease. Dietary assessment was done by food frequency questionnaire. (Morris et al., 2005). Vitamin E is function as natural antioxidant scavenging free radicals in cell membranes and protecting unsaturated fatty acids from lipid peroxidation (Meydani, 1995). In addition, the combination of vitamine E (10, 25 and 50 μg/rat) with nicotine (0.1 μg/rat) or pilocarpine (0.5 μg/rat) increased memory retention using a step-through passive avoidance paradigm (Eidi et al., 2006). Aged 24-month-old male Sprague-Dawley rats treated for 4–5 months with daily i.p. injections of α-tocopherol (200 mg/kg) can improve cognitive function during aging (Socci et al., 1995). Vitamin E supplementation affects learning behavior and protect against the deterioration in passive avoidance response in aging rats (Ichitani et al., 1992). Vitamin E improves cognitive performance in aged animals and prevents the oxidative damage induced by ß-amyloid in cell. And vitamin E (2000 IU 1342 α-tocopherol equivalents) vitamin E can delay or prevent a clinical diagnosis of Alzheimer disease in elderly persons with mild cognitive impairment (Grundman, 2000).

2.6 Scopolamine

Scopolamine, known by the name levo-duboisine and hyoscine (rINN), sold as Scopoderm, is a tropane alkaloid drug that has anticholinergic properties and muscarinic antagonist effects. It is among the secondary metabolites of plants from Solanaceae (nightshade) family of plants, such as belladonna, brugmansia, henbane,

mandrake, and datura. Scopolamine exerts its effects by acting as a competitive antagonist at muscarinic acetylcholine receptors, specifically M1 receptors; it is thus classified as an anti-cholinergic, anti-muscarinic drug. (Wikipedia, 2013). Scopolamine has legitimate medical applications in very minute doses. As an example, in the treatment of motion sickness, the dose, gradually released from a transdermal patch, is only 330 microgrammes (µg) per day (Neurosoup, 2013). Scopolamine can be administered orally, subcutaneously, ophthalmically and intravenously, as well as via a transdermal patch (White, 2007). The transdermal patch (e.g., Transderm Scōp) for prevention of nausea and motion sickness employs scopolamine base, and is effective for up to three days (Transdermscop, 2013). Scopolamine provides a suitable pharmacological model of the memory defects associated with cortical or sub-cortical dementias. Scopolamine clearly produces deficits on some measures of anterograde memory, the present findings question whether anti-cholinergic drugs adequately mimic the full range of memory impairments observed in cortical or sub-cortical dementia (Beatty et al., 1986). Oh et al. (2009) showed that *Nelumbo nucifera* semen extract (1g/kg) can improve memory on rats with scopolamine (1mg/kg)-induced amnesia by inhibiting AChE activity. The relatively low doses of certain muscarinic acetylcholine-receptor antagonists were found to induce transient cognitive deficits in young human volunteers that resembled those observed in elderly subjects (Drachman et al., 1974). Ebert and Kirch (1988) found that scopolamine could alter certain features of the human electroencephalogram (e.g., delta, theta, alpha, and beta activities) in a fashion that mimics some of the changes observed in patients with AD. Scopolamine (0.2 mg) impaired new learning in the elderly as measured by Selective Reminding Test (SRT)

(P<0.04) and SRT delayed recall (Zemishlany and Thorn, 1991). Interestingly, scopolamine appears to negatively affect cognitive performance to a greater extent in elderly subjects than in younger subjects (Flicker et al., 1992), and it impairs subjects with AD more dramatically than nondemented elderly subjects (Sunderland et al., 1974) Similarly, aged rodents display cognitive impairments in many learning and memory tasks (Ingram et al., 1994) and are more sensitive to the disruptive effects of scopolamine than young rats (Gallagher et al., 1974).

CHAPTER III

MATERIALS AND METHODS

3.1 Plant material and preparation of extract

3.1.1 Plant material

Mangosteen (*Garcinia mangostana* L.) fruits were purchased from local market in Nakhon Ratchasima province during May-October 2007.

3.1.2 Preparation of plant extract

The fruit hull of mangosteen was washed with copious amounts of water. The fruit hull was then cut into small thin pieces and allowed to air dry at room temperature. The dried hull was powdered using an electric mill with a 1.0 mm mesh. The dried powder was extracted in 85% aqueous ethanol (1 g of dried powder : 5 ml of 85% ethanol) using maceration method for 7 days in the dark at room temperature. The extract was filtered through Whatman filter paper No. 1 (Whatman Internation Ltd., Maidstone, England). After filtration, the extract was evaporated (Rotavapor® model R-205, Buchi, Switzerland) under vacuum to absolute dryness. The aqueous extract was lyophilized (Labconco Corporation Ltd., Missouri, USA) and kept at -20 °C until further used.

3.1.3 Determination of plant extract yield

The yield of evaporated dried extracts based on dry weight basis was calculated from the following equation (Stanojevic et al., 2009).

$$\% \text{ Yield (g/100 g of dry plant material)} = (W_1 \times 100) / W_2$$

where W_1 is the weight of the extract after the solvent evaporation and W_2 is the weight of the dry plant material.

3.1.4 Determination of total phenolic contents in the crude extract from the fruit hull of mangosteen

Chemicals:

Ethanol (C_2H_5OH, Analytical Grade ≥ 99.8%, Carlo Erba Reagents, France), sodium carbonate (Na_2CO_3, BDH Ltd., UK)**,** Folin-Ciocalteu reagent (Merck Millipore, Germany), gallic acid $C_7H_6O_5$, grade ≥ 98.0 %, Carlo Erba, France)

Preparation:

2% sodium carbonate**:** dissolved 2 g of sodium carbonate in double deionized distilled (DDD) water and then adjusted the final volume to 100 ml.

10% ethanol: added 10 ml of ethanol to 90 ml of DDD water.

Folin-Ciocalteu reagent: dissolved Folin-Ciocalteu reagent with 10%Ethanol (1:1).

Gallic acid stock solution (1 mg/ml): dissolved 10 mg of gallic acid in 10% ethanol and then adjusted the final volume to 10 ml.

Table 3.1 Working standard solutions of gallic acid.

Conc. (mg/ml)	Stock (µl)	10% EtOH (µl)
0	0	100
0.05	5	95
0.10	10	90
0.20	20	80
0.30	30	70

The total phenolic compounds of the crude extract from the fruit hull of mangosteen were measured according to the Folin-Ciocalteu reagent method that was adapted from the method of Minussi et al. (2003). Briefly, the crude extract from the fruit hull of mangosteen was dissolved in 10% ethanol as shown in Table 3.1. The reaction mixtures consisted of the crude extract from the fruit hull of mangosteen solution (100 µl) and 2 ml of 2% sodium carbonate and were mixed. Two minutes later, 100 µl of Folin-Ciocalteu reagent (Merck Millipore, Germany) was added and incubated at room temperature for 30 min. The absorbance of mixtures was measured by using a spectrophotometer (CECIL 1011, England) at 750 nm. The total phenolic compounds were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry extract. All determinations were performed in triplicate.

3.1.5 Determination of radical scavenging activity of the crude extract from the fruit hull of mangosteen

Antioxidant activity of the crude extract from the fruit hull of mangosteen was determined by the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) method and measured by spectrophotometer at 517 nm (Singleton and Rossi, 1965).

All determinations were performed in triplicate. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation:

$$\%\text{DPPH scavenging} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the mixture containing extracts. The DPPH radical scavenging (%) was plotted against the plant extract concentrations (mg/ml) to determine the concentration of extract necessary to decrease DPPH radical scavenging by 50% (IC_{50}). This part of work was conducted by Science and Technology Service Center, Faculty of Science, Chiang Mai University, Thailand.

3.1.6 Determination of total anthocyanin contents of the crude extract from the fruit hull of mangosteen

The contents of total anthocyanin of the crude extract from the fruit hull of mangosteen were determined by modified method of Lohachoompol et al. (2004). The crude extract from the fruit hull of mangosteen was dissolved in 25 ml of methanol:HCl (0.1 M) at a ratio of 85:15. The absorbance reaction mixture was measured by spectrophotometer at 538 nm. The results were expressed as anthocyanindin equivalents in micrograms per gram of dry extract. This part of work was conducted by Science and Technology Service Center, Faculty of Science, Chiang Mai University, Thailand.

3.2 Drug solutions

10% Tween 80 (200 ml):

Preparation: mixed 20 ml of Tween 80 (BDH Ltd., UK) with DDD water and then adjusted the final volume to 200 ml.

100 mg/ml vitamin E (2 ml):

Stock solution: 0.95 g/ml vitamin E (Sigma-Aldrich; St. Louis, USA)

Preparation for 100 mg/ml vitamin E: mixed 0.21 ml of 0.95 g/ml vitamin E with 10% Tween 80 and then adjusted the final volume to 2 ml.

1 mg/ml scopolamine (8 ml):

Stock solution: 50 mg/ml scopolamine (1 g of scopolamine Sigma-Aldrich, New Delhi) was dissolved in 20 ml DDD water.

Preparation for 1 mg/ml scopolamine: mixed 0.16 ml of 50 mg/ml scopolamine with normal saline solution (0.9% NaCl, Otsuka, Ltd, Thailand) and then adjusted the final volume to 8 ml.

2000 mg/ml *Garcinia mangostana* extract (GME2000) (40 ml):

Preparation: dissolved 80 g of *Garcinia mangostana* extract (GME) in 10% Tween 80 and then adjusted the final volume to 40 ml.

1000 mg/ml *Garcinia mangostana* extract (GME1000) (30 ml):

Preparation: mixed 15 ml of GME2000 with 15 ml of 10% Tween 80.

500 mg/ml *Garcinia mangostana* extract (GME500) (20 ml):

Preparation: mixed 10 ml of GME1000 with 10 ml of 10% Tween 80.

250 mg/ml *Garcinia mangostana* extract (GME1000) (20 ml):

Preparation: mixed 10 ml of GME500 with 10% Tween 80.

Ice cold normal saline solution (0.9% NaCl) (4000 ml):

Preparation: dissolved 32.4 g of sodium chloride in deionized distilled (DD) water and then adjusted the final volume to 4000 ml.

3.3 Animals

Male Wistar rats (8 weeks and 10 months old) were obtained from Institutional Animal Care, Suranaree University of Technology (SUT). They were maintained under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 20±1°C) with free access to food and water. This study was conducted under permit of SUT Animal Care and Use Committee.

3.4 Morris Water Maze Test

The Morris water maze test developed by Richard Morris at the University of St Andrews in Scotland (Morris, 1981) is widely used to study spatial memory and learning in rodent. Briefly, animals are placed into open pool of water which contains an escape platform hidden below the water that is colored opaque with milk powder or non-toxic paint. Visual cues, such as colored shapes, are placed around the pool in plain sight of the animal. The animal swims around the pool in search of an exit and on subsequent trials the rat is able to locate the platform more rapidly.

Apparatus: The apparatus for Morris water maze test used in the present study consisted of large circular pool (2.3 m in diameter and 63 cm in height) which contains no internal cues, stimuli, markings on objects, but is surrounded by stable, salient extra-maze cues. The pool was filled to a depth of 21.5 cm with water at temperature of 25 °C and made opaque with white non-toxic water paint (TOA, Co., Ltd, Thailand). The pool was divided into four quadrants of equal areas (Q1, Q2, Q3 and Q4 (with platform)) and surrounded by 4 extra maze distal visual cues of different shape on the wall of each quadrant. A white platform (19.5 cm in diameter and 28.5

cm in height) was submerged 2 cm below the water surface and placed in the center of Q4 and it was located in the same position on every trials. A video camera (Sony Handycam, Japan) was placed above the centre of the pool to capture images of the swimming animal.

Training Trial: On the day before training trials, the animal was placed in the pool and allowed to swim for 60 s with the absent of the escape platform. During the seven training days, the animal was given three trials sessions each day with an inter-trial interval of 60 s. The trial began when the animal was randomly placed in the water at one of the locations (Q1, Q2, and Q3) with its head facing the wall of the pool and allowed 60 s to swim, search and climb up for the platform. Once the animal found the platform, rat was allowed to remain on the platform for 60 s. The escape latency to the platform (time to find platform) was recorded. If the animal did not locate the platform within 60 s, the animal was guided to the platform by experimenter and let the animal sit on platform for 15s. If animal jumped off, guided it back. The repeated three trial training processes for all animals were performed consecutively. Once the animal had completed all three trials, dried it off with a towel.

Probe trial: After the completion of training trial on day 7, the experimenter conducted a probe trial in which the escape platform was removed from the pool. The animals were released from the quadrant opposite to quadrant where the platform was located and allowed to swim for 60 s, after which the rat was taken out of the pool. Generally, a well-trained rat swam to the target quadrant of the pool and repeatedly acrossed the former location of the platform until starting to search elsewhere. The time spent in target quadrant and the numbers of entries into the target quadrant were recorded (Morris et al., 1982; Morris, 1981; 1984; 2008).

3.5 Determinaton of acetylcholinesterase (AChE) activity

3.5.1 Preparation of tissue homogenates

Three brain regions (cerebral cortex, hippocampus and basal forebrain) obtained from all rats in experiment 1, 2, and 3 were used to determine AChE activity. The tissue homogenates were prepared by modified methods of Chattipakorn et al. (2007) and Papandreou et al. (2009) as described in appendix A. Briefly, the brain tissues were homogenized (Ultrasonic, Sonic & Material, Inc, USA) with ice cold 10% of 0.1 M phosphate buffer (pH 7.4) containing 1% Triton-X 100 at 15,000 rpm for 15 min at 4 °C. The rat brain homogenates were used to determine acetylcholinesterase activity.

3.5.2 Determination of acetylcholinesterase activity

Determination of AChE activity was based on colorimetric method adapted from the method of Ellman et al. (1961), Chattipakorn et al. (2007), and Nakdook et al. (2010). The assay method is shown in appendix B. Briefly, the assay mixture consisted of 50 µl of brain homogenates, 25 µl of 0.1 M phosphate buffer (pH 7.4), 125 µl of 0.1 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Sigma) and 25 µl of 1 mM acetylthiocholine iodide (ATCI, Sigma) was mixed well. The absorbance of the assay mixture was measured by Benchmark Plus microplate Spectrophotometer (Benchmark Plus, BIO-RAD, Japan) at wavelength of 405 nm and monitored over period of 6 min. All determinations were performed in triplicate. AChE activity was assessed by following formula:

$$R = [\Delta A / (1.36 \times 10^4)] \times [1 / (Co \times \text{mg of protein})]$$

$$R = \Delta A / [1.36 \times 10^4 \times (50/225) \times \text{mg of protein}]$$

R = rate of enzyme activity (expressed as mole of acetylcholine iodide hydrolyzed/ minute/ mg of protein),

ΔA = the change in absorbance per minute,

Co = original concentration of tissue (mg/ml) 50/225 is volume correction 1.36×10^4 is the extinction coefficient of the yellow product.

3.5.3 Determination of protein content

The method for measurement of protein content in brain homogenates was adapted from the method of Lowry et al. (1951). The total protein concentration is exhibited by a color change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques. The protocol for method use in the present study is shown in appendix C. Bovin serum albumin (BSA) was used as a standard protein. The blank samples contained the same mixture solution without the sample. Protein was determined by comparison of the values obtained from the standard curve.

3.6 Experimental design

3.6.1 Experiment 1: Effects of the crude extract from the fruit hull of mangosteen on cognitive impairment and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of scopolamine-induced amnesic rats

Eight weeks old male Wistar rats (n=60) were randomly assigned to ten groups of six animals each as follows: group 1; vehicle + normal saline solution (NSS), group 2; vitamin E+ NSS, group 3; GME250 + NSS, group 4; GME500 + NSS, group 5; GME1000 + NSS, group 6; vehicle + scopolamine (SCOP), group 7; vitamin E+

SCOP, group 8; GME250 + SCOP, group 9; GME500 + SCOP, and group 10; GME1000 + SCOP.

Note: - GME is the *Garcinia mangostana* extract.

- Vitamin E (100 mg/kg, p.o., Alzoubi et al., 2011).

- Scopolamine (1 mg/ml, i.p.) is a muscarinic receptor (M1) antagonist that can induce amnesia (Oh et al., 2009).

All rats were tested for their spatial memory using the Morris Water Maze test. The protocol consisted of 21 training trials (3 times per day for 7 days) and probe trial on the day 7. Rats in group 1 and group 6 received daily oral dose of vehicle (10% Tween 80, 1 ml/kg), rats in group 2 and group 7 received daily oral dose of vitamin E (100 mg/ml/kg), rats in group 3 and group 8 received daily oral dose of GME250 (GME, 250 mg/ml/kg), rats in group 4 and group 9 received daily oral dose of GME500 (GME, 500 mg/ml/kg), and rats in group 5 and group 10 received daily oral dose of GME1000 (GME, 1000 mg/ml/kg), for 7 days. One hour after drug administration on each day, rats in group 1, 2, 3, 4 and 5 were treated a single intraperitoneal injection of normal saline solution (0.9% NaCl, 1ml/kg) and rats in group 6, 7, 8, 9 and 10 were treated with a single intraperitoneal injection of scopolamine (1 mg/ml/kg). Thirty minutes later, all rats were tested for 21 training trials per day. One hour after last training trials one day 7, all rats were tested probe trials (Figure 3.1). At the end of the behavioral observation, rats were anesthetized with pentobarbital sodium (Nembutal, CEVA SANTE ANIMMALE, France, 60 mg/kg, i.p.) and perfused with ice-cold normal saline solution (0.9% NaCl). Rats were decapitated and brains were rapidly removed and placed on petri dish filled with ice. The cerebral cortex, hippocampus and basal forebrain were dissected out after

localization according to the atlas of Paxinos and Watson (2009). The dissected brain structures were weighed, immediately frozen on dry ice and stored at -80 °C until assay for AChE activity. Determination of AChE activity was based on colorimetric method adapted from the method of Ellman et al. (1961).

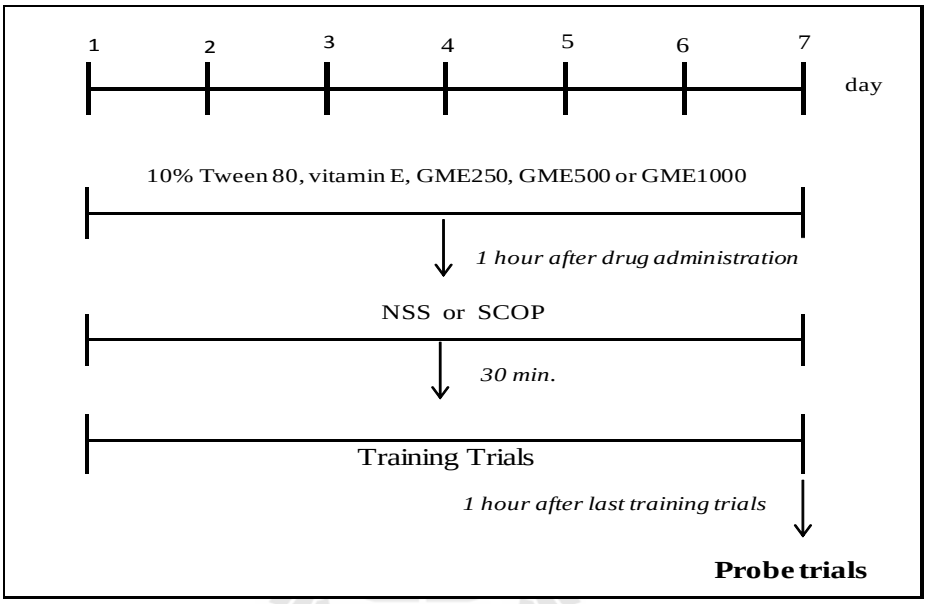


Figure 3.1 Schematic diagram showing protocol for experiment 1.

3.6.2 Experiment 2: Subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of healthy adult rats

Eight weeks old male Wistar rats (n=30) were randomly assigned to five groups of six animals each as follows: group 1 (vehicle), group 2 (vitamin E), group 3 (GME500), group 4 (GME1000), and group 5 (GME2000).

All rats were tested for their spatial memories using the Morris Water Maze test. The protocol consisted of 21 training trials (3 times per day for 7 days, on day 24-day 30) and probe trial on the day 30. Rats in group 1 received daily oral dose of

vehicle (10% Tween80, 1 ml/kg), rats in group 2 received daily oral dose of vitamin E (40 mg/ml/kg), rats in group 3 received daily oral dose of GME500 (GME, 500 mg/ml/kg), rats in group 4 received daily oral dose of GME1000 (GME, 1000 mg/ml/kg), and rats in group 5 received daily oral dose of GME2000 (GME, 2000 mg/ml/kg), for 30 days. The dosing volume was 1 ml/kg/day for all groups. One hour after administration on day 24 to day 30, all rats were tested for 21 training trials (3 times per day). On day 30, 1 hour after last training trials, all rats were tested for probe trial (Figure 3.2). At the end of the behavioral observation, rats were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg, i.p.). Blood samples (3-4 ml from each rat) were collected *via* cardiac puncture into heparinized tubes. Heparinized blood was centrifuged at 2000 rcf for 5 minutes, obtained plasma was stored at -20 °C until further biochemical analysis. The plasma biochemical parameters [glucose, triglyceride, cholesterol, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were determined by A15 Automatic Clinical Chemistry (BioSysyems S.A., Spain). This part of work was conducted by Suranaree Medical Center, Suranaree University of Technology, Thailand. After blood collection, all rats were perfused with ice-cold normal saline solution (0.9% NaCl). Rats were decapitated and brains were rapidly removed and placed on petri dish filled with ice. The cerebral cortex, hippocampus and basal forebrain were dissected out after localization according to the atlas of Paxinos and Watson (2009). The dissected brain structures were weighed, immediately frozen on dry ice and stored at -80 °C until assay for AChE activity. Determination of AChE activity was based on colorimetric method adapted from the method of Ellman et al. (1961).

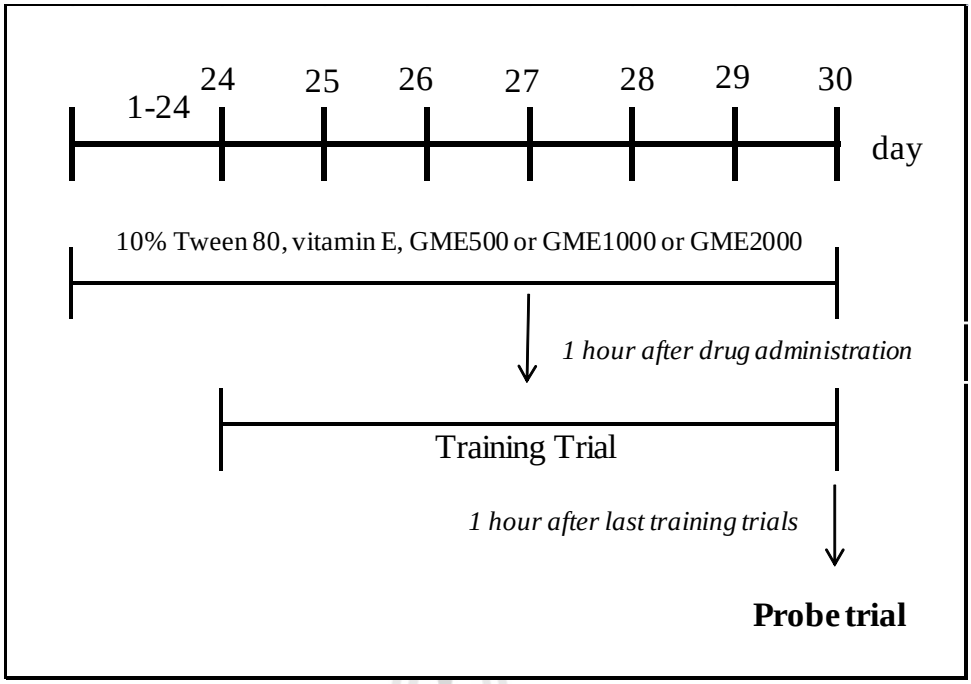


Figure 3.2 Schematic diagram showing protocol for experiment 2.

3.6.3 Experiment 3: Subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of aged rats.

Ten months old male Wistar rats (n=24) were randomly assigned to four groups of six animals each as follows: group 1 (vehicle), group 2 (vitamin E), group 3 (GME500), and group 4 (GME1000).

All rats were tested for their spatial memories using the Morris Water Maze test. The protocol consisted of 21 training trials (3 times per day for 7 days, on day 24-day 30) and probe trial on the day 30. Rats in group 1 received daily oral dose of vehicle (10% Tween80, 1 ml/kg), rats in group 2 received daily oral dose of vitamin E (40 mg/ml/kg), rats in group 3 received daily oral dose of GME500 (GME, 500 mg/ml/kg), and rats in group 4 received daily oral dose of GME1000 (GME, 1000 mg/ml/kg), for 30 days. The dosing volume was 1 ml/kg/day for all groups. One hour

after day administration on day 24 to day 30, all rats were tested for 21 training trials (3 times per day). On day 30, 1 hour after last training trials, all rats were tested for probe trial (Figure 3.3). At the end of the behavioral observation, rats were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg, i.p.). Blood samples (3-4 ml from each rat) were collected *via* cardiac puncture into heparinized tubes. Heparinized blood was centrifuged at 2000 rcf for 5 minutes, obtained plasma was stored at -20 °C until further biochemical analysis. The plasma biochemical parameters [glucose, triglyceride, cholesterol, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] were determined by A15 Automatic Clinical Chemistry (BioSysyems S.A., Spain). This part of work was conducted by Suranaree Medical Center, Suranaree University of Technology, Thailand. After blood collection, all rats were perfused with ice-cold normal saline solution (0.9% NaCl). Rats were decapitated and brains were rapidly removed and placed on petri dish filled with ice. The cerebral cortex, hippocampus and basal forebrain were dissected out after localization according to the atlas of Paxinos and Watson (2009). The dissected brain structures were weighed, immediately frozen on dry ice and stored at -80 °C until assay for AChE activity. Determination of AChE activity was based on colorimetric method adapted from the method of Ellman et al. (1961).

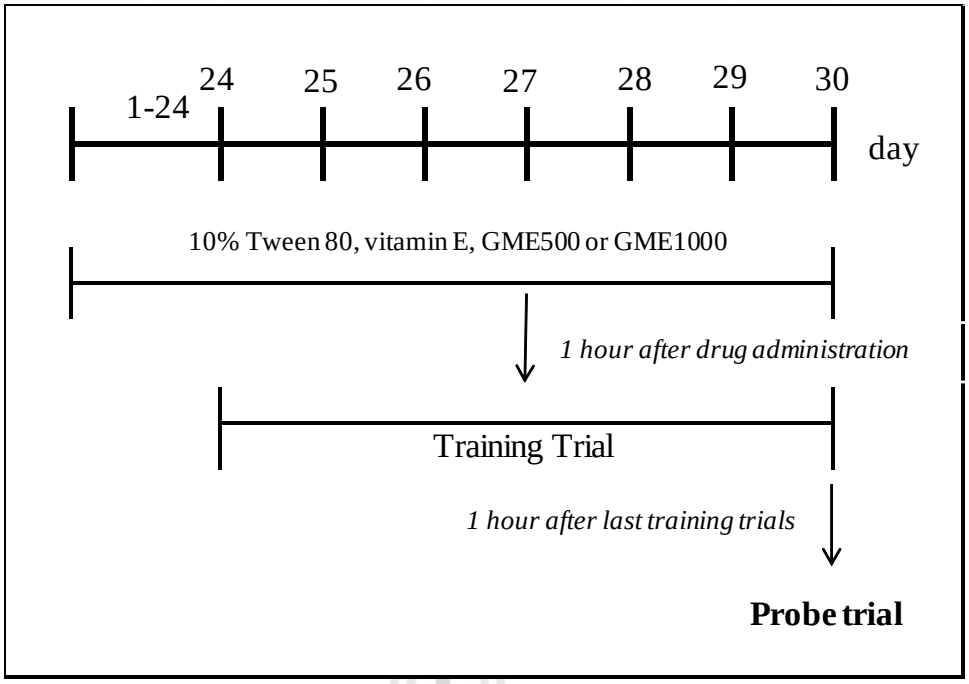


Figure 3.3 Schematic diagram showing protocol for experiment 3.

3.7 Statistics

Results were expressed as mean ± S.M.E. Time to find platform in Morris Water Maze test on training day 1 to day 7 was analyzed using two way repeated measures ANOVA for differences between groups and days followed by Student-Newman-Keuls test (Sigma Stat version 3.5, Systat Software, Inc., USA). The time spent in target quadrant, the numbers of entries into the target quadrant and AChE activity were analyzed using one way ANOVA followed by Student-Newman-Keuls test. *P*-value less than 0.05 ($P<0.05$) was considered statistically significant.

CHAPTER IV

RESULTS

In the present study, the percentage yield, total phenolic content, antioxidant activity measured by DPPH scavenging assay (50% inhibition concentration or IC50), total anthocyanin content of the fruit hull of mangosteen extract were determined. The present findings indicated that the percentage yield of 85% ethanolic extract from the fruit hull of mangosteen was 27.7%. Total phenolic content of the fruit hull of mangosteen extract was 170.94 ± 0.009 mg gallic acid/g dry extract, while total anthocyanin content of the fruit hull of mangosteen was 168.2 µg/g of dry sample. Antioxidant activity of the fruit hull of mangosteen extract was 3.55 mg/ml sample extract.

4.1 Effects of the crude extract from the fruit hull of mangosteen on cognitive impairment and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of scopolamine-induced amnesic rats

The effects of the crude extract from the fruit hull of mangosteen and vitamin E on memory were examined in normal saline treated rats and scopolamine-induced amnesic rats using Morris water maze test. Time to find platform in training trials,

time spent in target quadrant and number of entries into the target quadrant in probe trial were recorded. Rats received daily oral dose of vehicle (10% Tween 80, 1 ml/kg), vitamin E (100 mg/kg), or the crude extract from the fruit hull of mangosteen (GME) at doses of 250, 500 and 1000 mg/ml/kg followed by either normal saline solution (NSS, 1 ml/kg) or scopolamine (SCOP, 1 mg/ml/kg) for 7 days. In both normal saline treated rats and scopolamine-induced amnesic rats, all doses of GME and vitamin E administration significantly decreased time to find platform on training day 4, 5, 6 and 7 when compared to day 1, (P<0.05, two way repeated measures ANOVA); however no significant difference was found in time to find platform between groups on any training days (Figure 4.1). There was no significant difference in time to find platform between groups on the last day of training trials (day 7) (Figure 4.2). In probe trial on day 7, GME250+NSS, GME500+NSS, GME1000+NSS, and vitamin E+NSS significantly increased time spent in target quadrant in normal saline treated rats compared to vehicle+NSS (P<0.05, one way ANOVA, Figure 4.3). In scopolamine-induced amnesia rats, time spent in target quadrant of all treatment groups was not different from vehicle+SCOP group (Figure 4.3). Moreover, when compared to their corresponding control groups (normal saline treated rats), time spent in target quadrant of scopolamine-induced amnesic rats which also received GME250, GME500, GME1000, and vitamin E was significantly decreased (Figure 4.3). In both normal saline treated rats and scopolamine-induced amnesic rats, all doses of GME and vitamin E did not cause any significant change in number of entries into the target quadrant in probe trial on day 7 (Figure 4.4). Seven days administration of GME500+NSS tended to increase, but did not significantly different, number of entries into the target quadrant. In scopolamine treated group, all doses of GME

tended to increase, but not significantly different, number of entries into the target quadrant when compared to normal saline treated group (Figure 4.4).

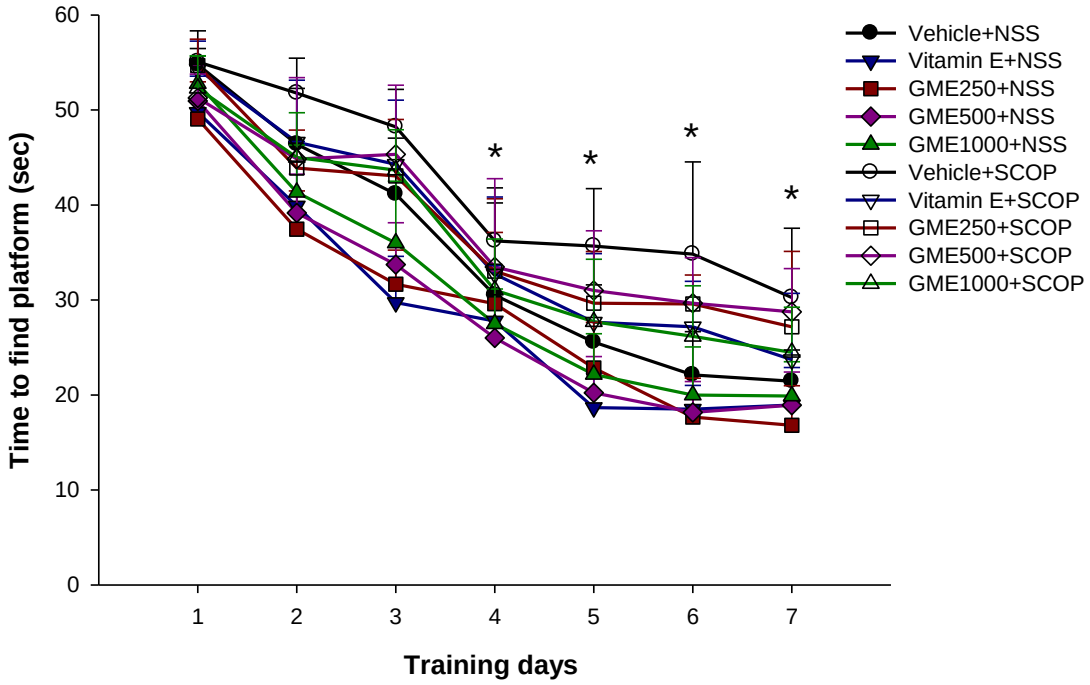


Figure 4.1 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 1 to day 7 of normal saline treated rats and scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicate significant differences of all treatment groups compared with day 1 within group (P<0.05).

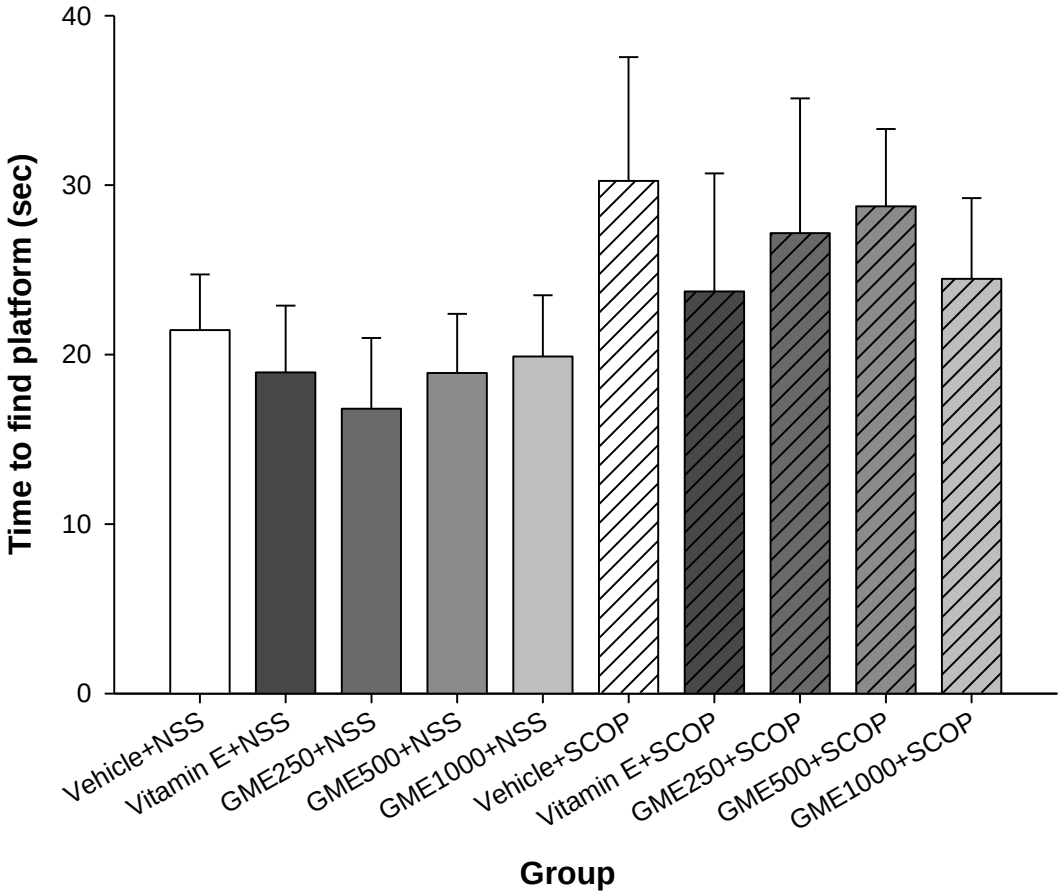


Figure 4.2 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 7 of normal saline treated rats and scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

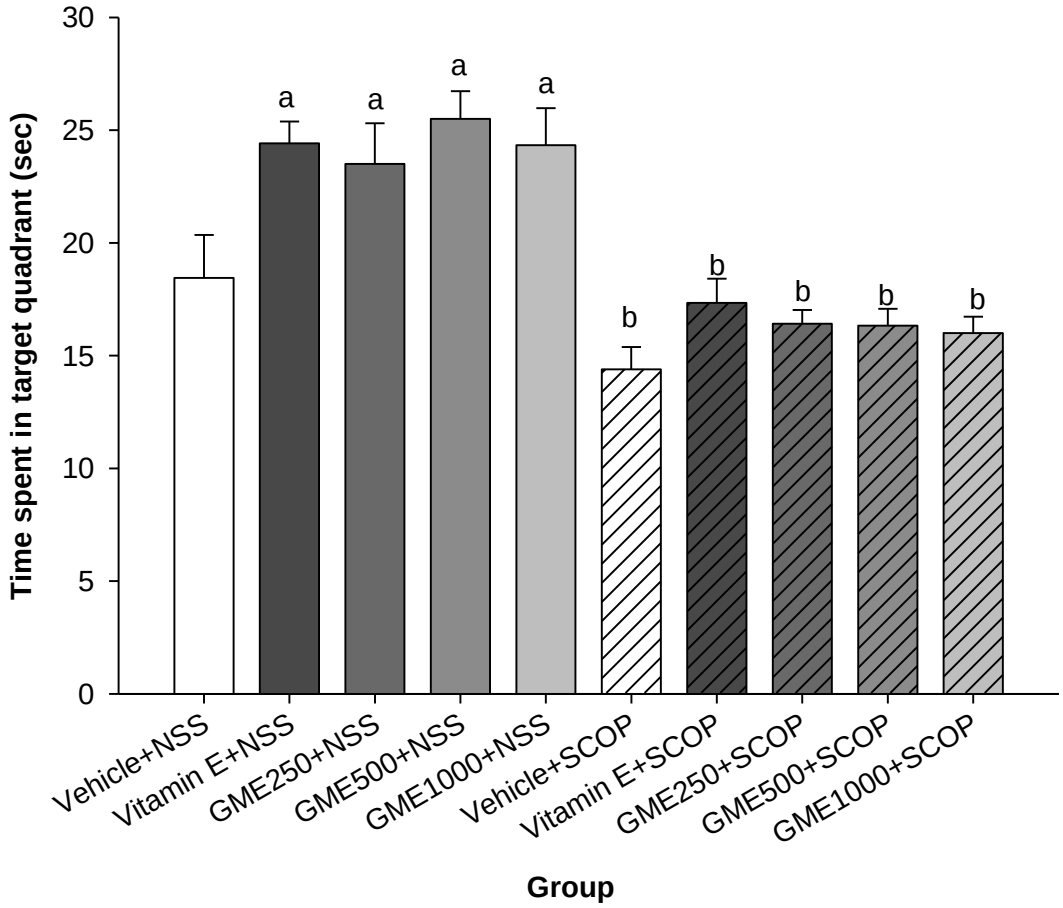


Figure 4.3 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on time spent in target quadrant in Morris water maze test on probe trial of normal saline treated rats and scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. [a] indicates a significant difference compared to vehicle+NSS group (P<0.05). [b] indicates a significant difference compared to their corresponding control groups (P<0.05). There was no significant difference among SCOP groups.

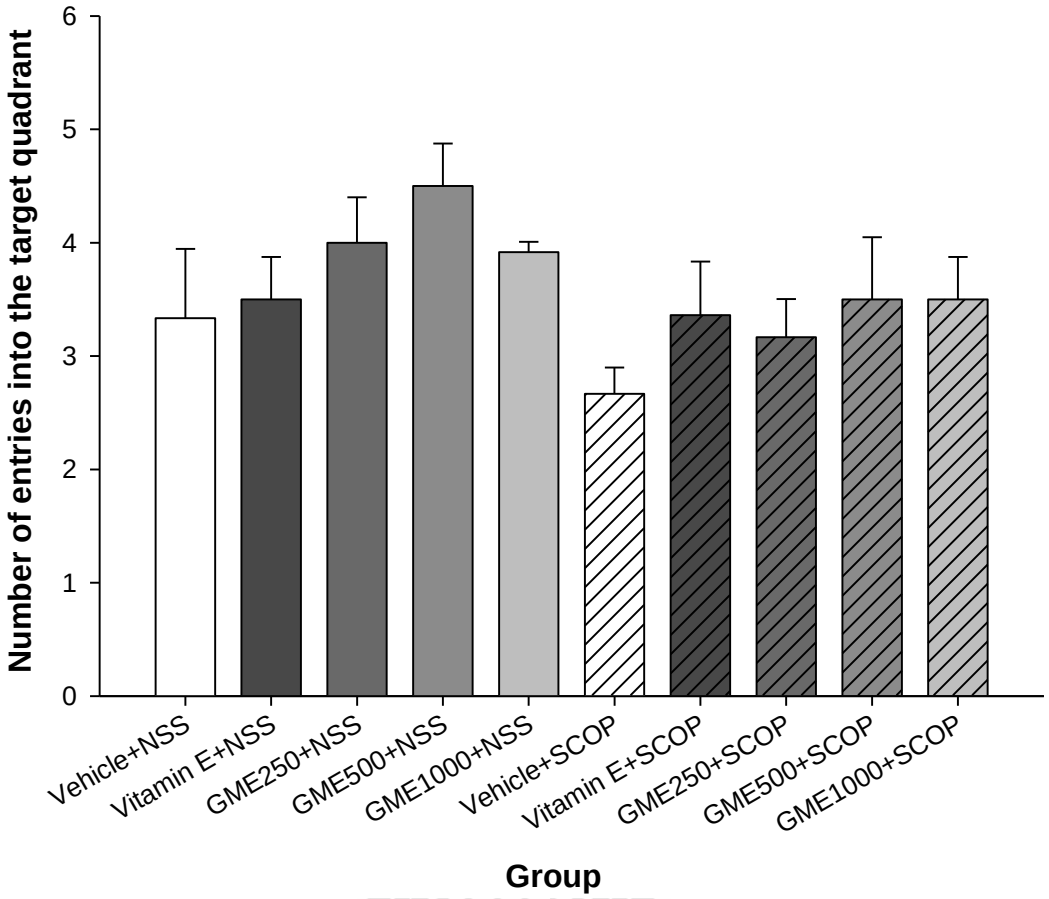


Figure 4.4 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on number of entries into the target quadrant in Morris water maze test on probe trial of normal saline treated rats and scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among group.

The effects of daily oral administration of the crude extract from the fruit hull of mangosteen (250, 500 and 1000 mg/ml/kg) and vitamin E for 7 days on AChE activity in the cerebral cortex, hippocampus, and basal forebrain of normal saline treated rats and scopolamine-induced amnesic rats were determined. The results of normal saline treated rats showed that administration of GME500+NSS, GME1000+NSS, but not GME250+NSS, and vitamin E significantly decreased AChE activity in cerebral cortex when compared to vehicle+NSS (P<0.05, one way ANOVA, Figure 4.5). The results of scopolamine-induced amnesic rats showed that administration of GME500+SCOP, but not GME250+SCOP, GME1000+SCOP, and vitamin E, significantly decreased AChE activity in cerebral cortex when compared to vehicle+SCOP (P<0.05, one way ANOVA, Figure 4.6). AChE activity in cerebral cortex of GME500+SCOP group was significantly higher than that of GME250+SCOP group and GME100+SCOP group (P<0.05, one way ANOVA). In normal saline treated rats, administration of GME250+NSS, GME500+NSS, and vitamin E+NSS, but not GME1000+NSS, significantly decreased AChE activity in hippocampus when compared to vehicle+NSS (P<0.05, one way ANOVA, Figure 4.7). In scopolamine-induced amnesic rats, administration of GME250+SCOP, GME500+SCOP and vitamin E, but not GME1000+SCOP, significantly increased AChE activity in hipocampus when compared to vehicle+SCOP (P<0.05, one way ANOVA, Figure 4.8). In normal saline treated rats, all doses of GME significantly increased AChE activity in basal forbrain when compared to vehicle+NSS (P<0.05, one way ANOVA, Figure 4.9). Vitamin E+NSS significantly decreased AChE activity in basal forebrain in normal saline treated rats (P<0.05, one way ANOVA, Figure 4.9). The results of scopolamine-induced amnesic rats showed that administration of

GME250+SCOP and GME500+SCOP, but not GME1000+SCOP and vitamin E, significantly decreased AChE activity in basal forebrain when compared to vehicle+SCOP (P<0.05, one way ANOVA, Figure 4.10).

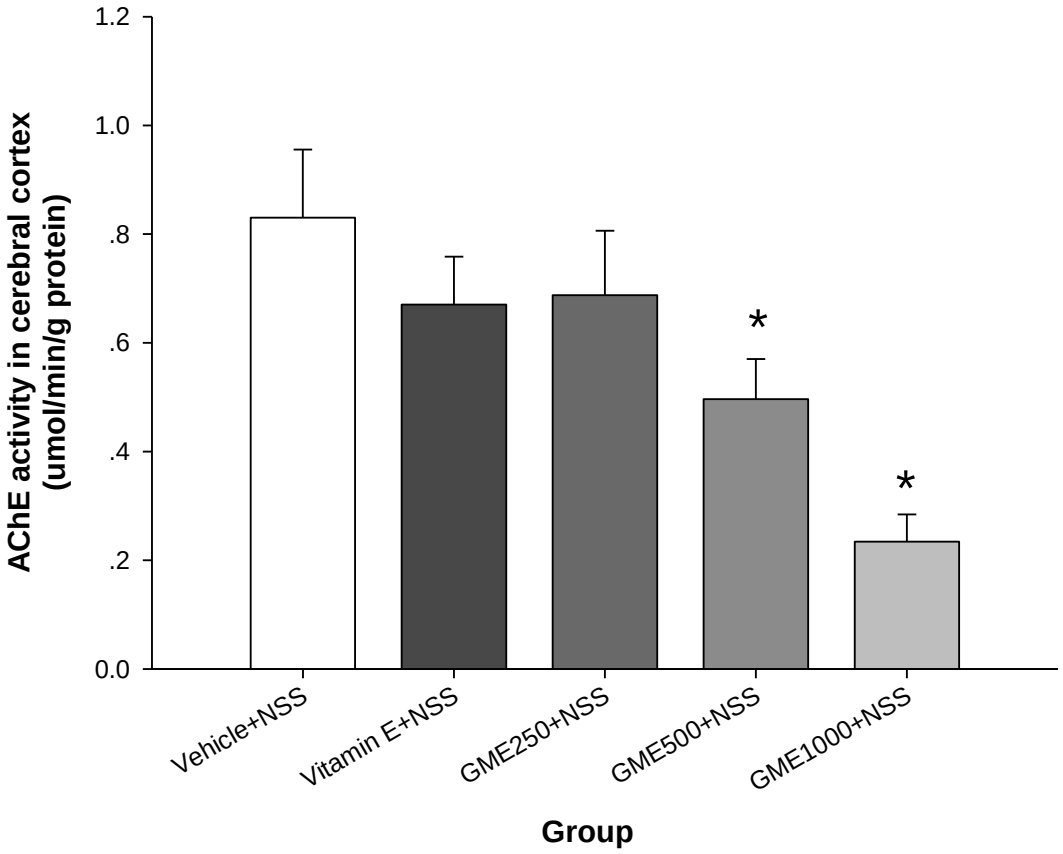


Figure 4.5 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in cerebral cortex of normal saline treated rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+NSS group (P<0.05).

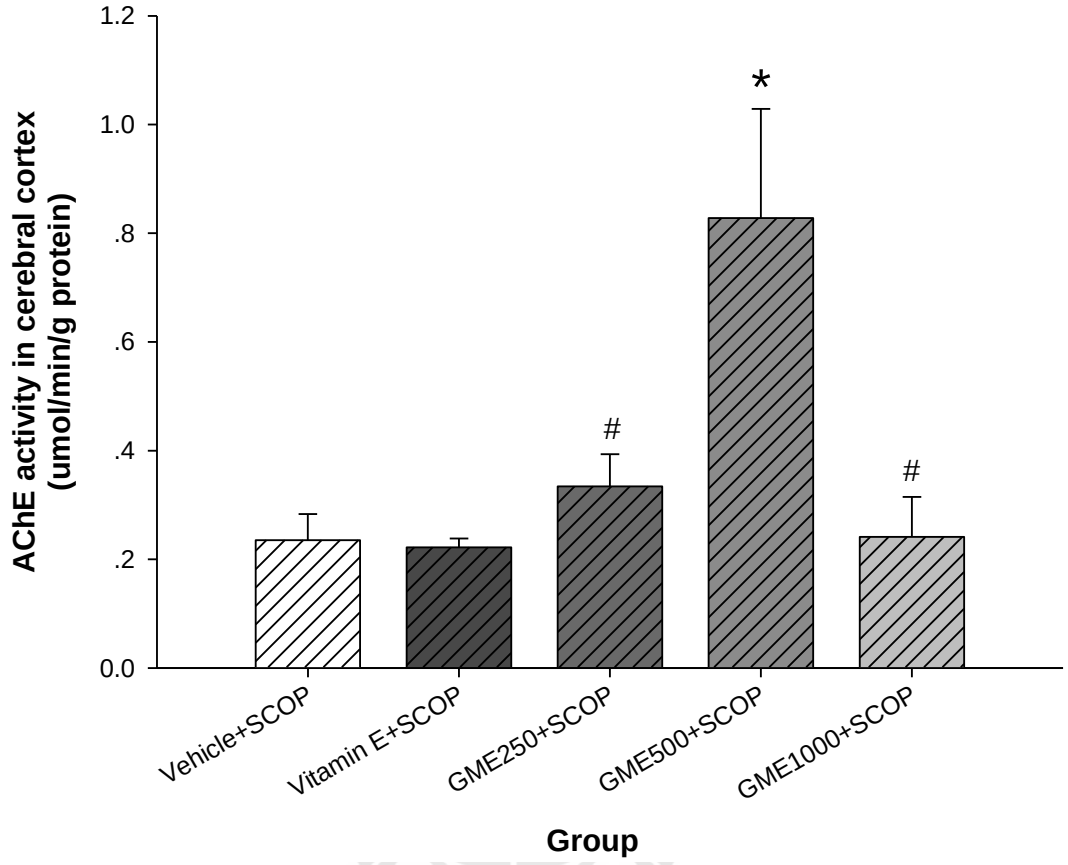


Figure 4.6 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in cerebral cortex of scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+SCOP group (P<0.05). # indicates a significant difference compared to GME500+SCOP group (P<0.05).

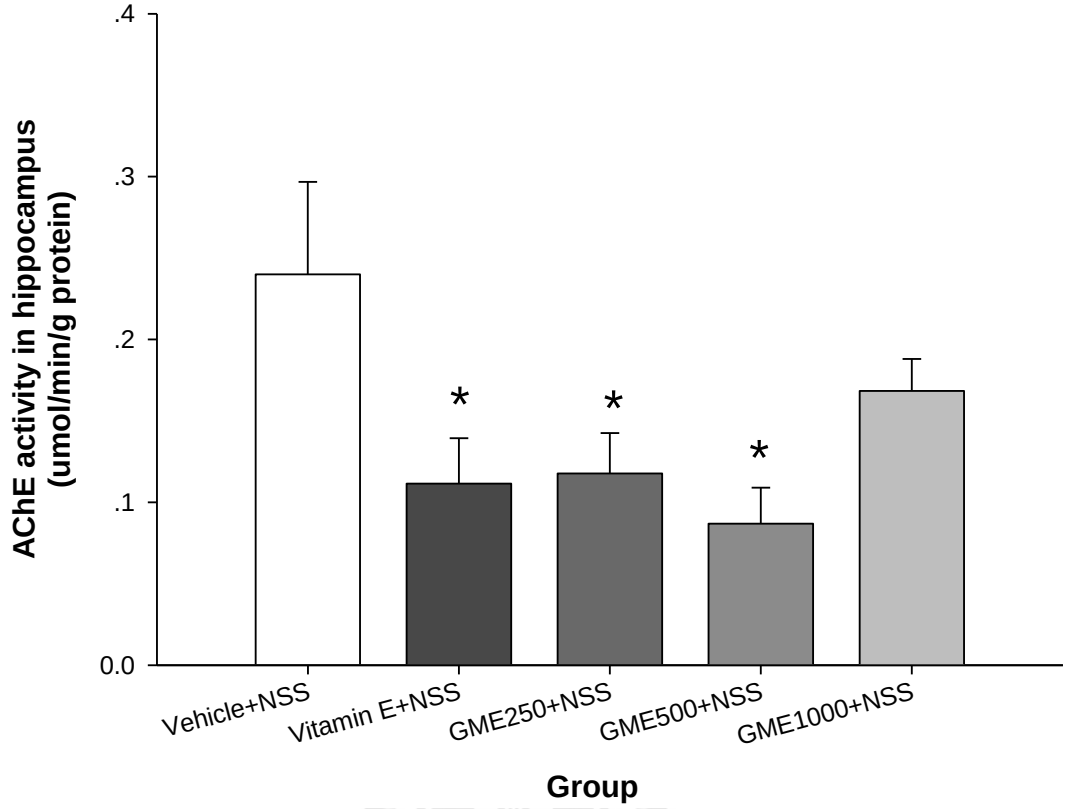


Figure 4.7 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in hippocampus of normal saline treated rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+NSS group (P<0.05).

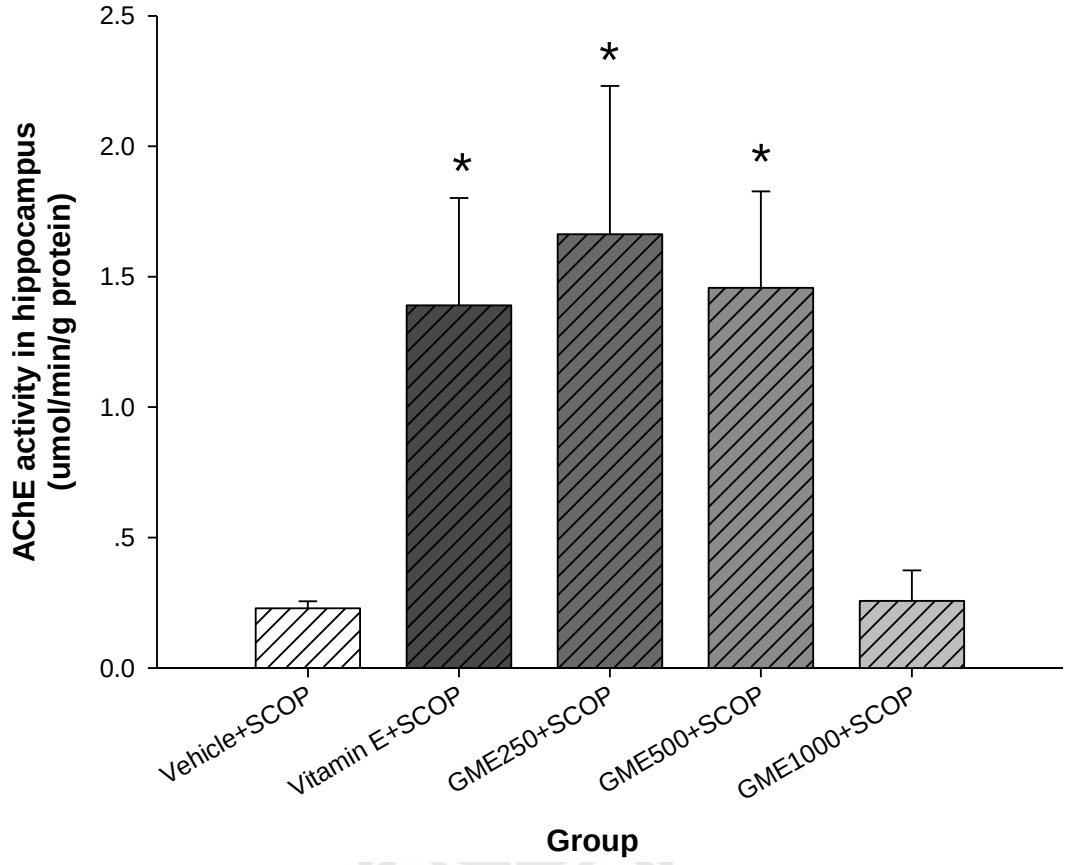


Figure 4.8 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in hippocampus of scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+SCOP group (P<0.05).

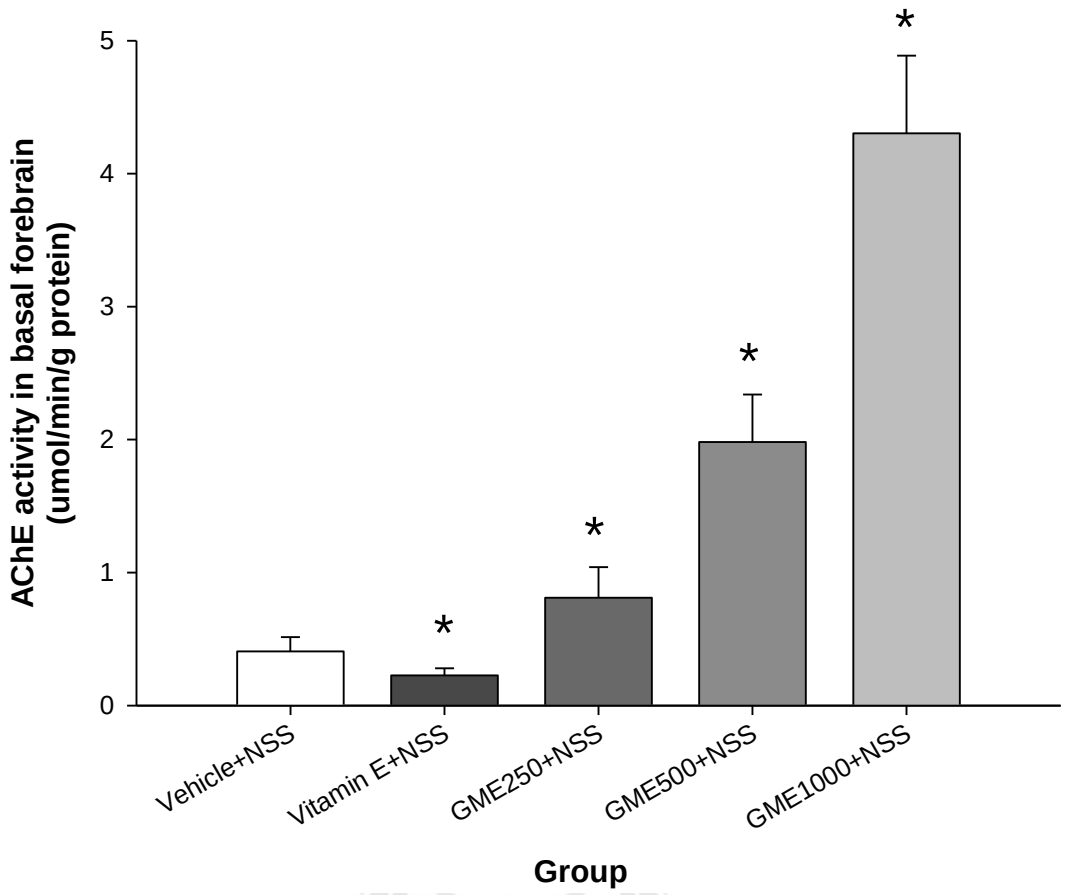


Figure 4.9 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in basal forebrain of normal saline treated rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+NSS group (P<0.05).

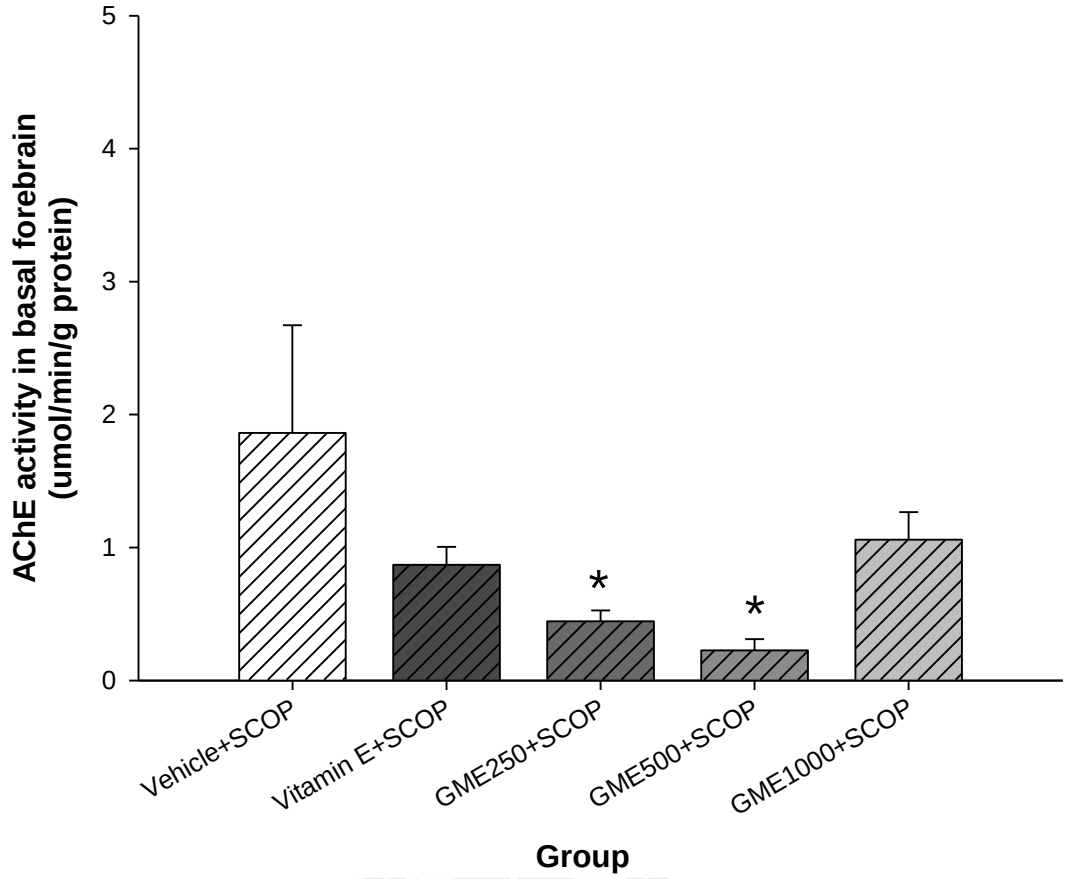


Figure 4.10 Effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in basal forebrain of scopolamine-induced amnesic rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to vehicle+SCOP group (P<0.05).

4.2 Subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of healthy adult rats

The effects of the crude extract from the fruit hull of mangosteen and vitamin E on memory were examined in healthy adult rats using Morris Water Maze test. Time to find platform in training trials, time spent in target quardrant and number of entries into the target guardrant in probe trial were recorded. Rats received daily oral dose of vehicle, vitamin E (40 mg/ml/kg), or the crude extract from the fruit hull of mangosteen (GME) at various doses ranging from 500, 1000, and 2000 mg/ml/kg for 30 days. In healthy adult rats, all doses of GME and vitamin E administration significantly decreased time to find platform on training day 5, 6 and 7 when compared to day 1 ($P<0.05$, two way repeated measures, ANOVA) however, no significant difference was found in time to find platform between groups on any training days (Figure 4.11). There was no significant difference in time to find platform between groups on the last day of training trials (day 7) (Figure 4.12). In probe trial on day 7, GME500 and vitamin E, but not GME1000 and GME2000, significantly increased time spent in target quadrant compared to control group ($P<0.05$, two way repeated measures, ANOVA, Figure 4.13). Thirty days administration of all doses of GME and vitamin E did not cause significant difference in number of entries into the target quadrant when compared to control group (Figure 4.14).

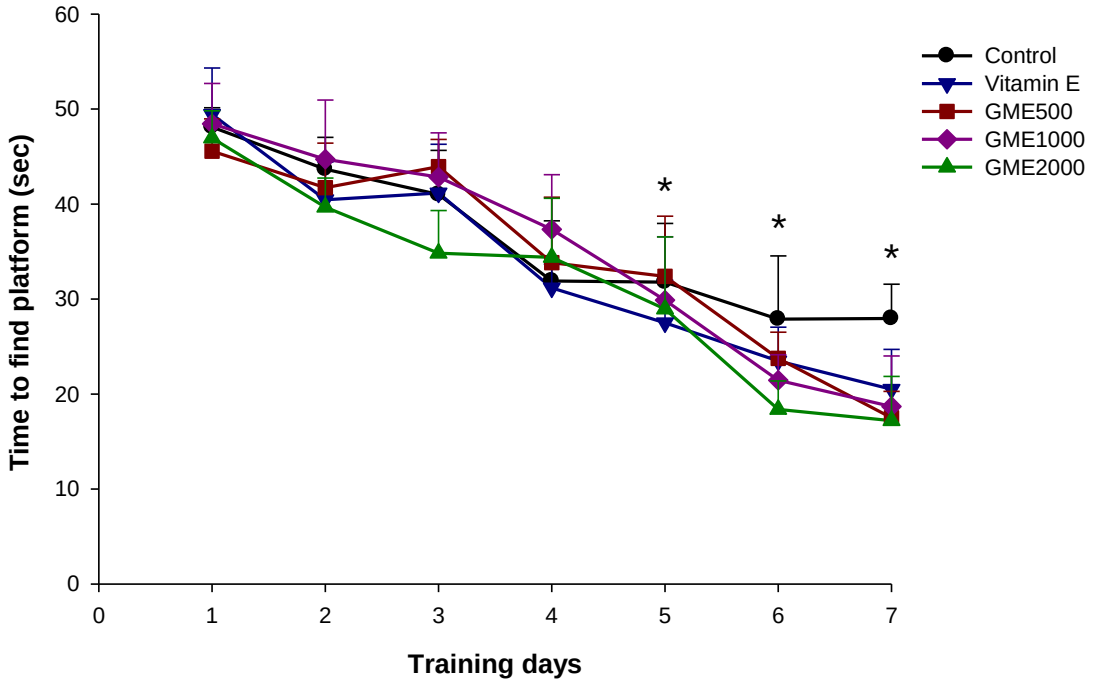


Figure 4.11 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 1 to day 7 of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference of all treatment groups compared with day 1 within group (P<0.05).

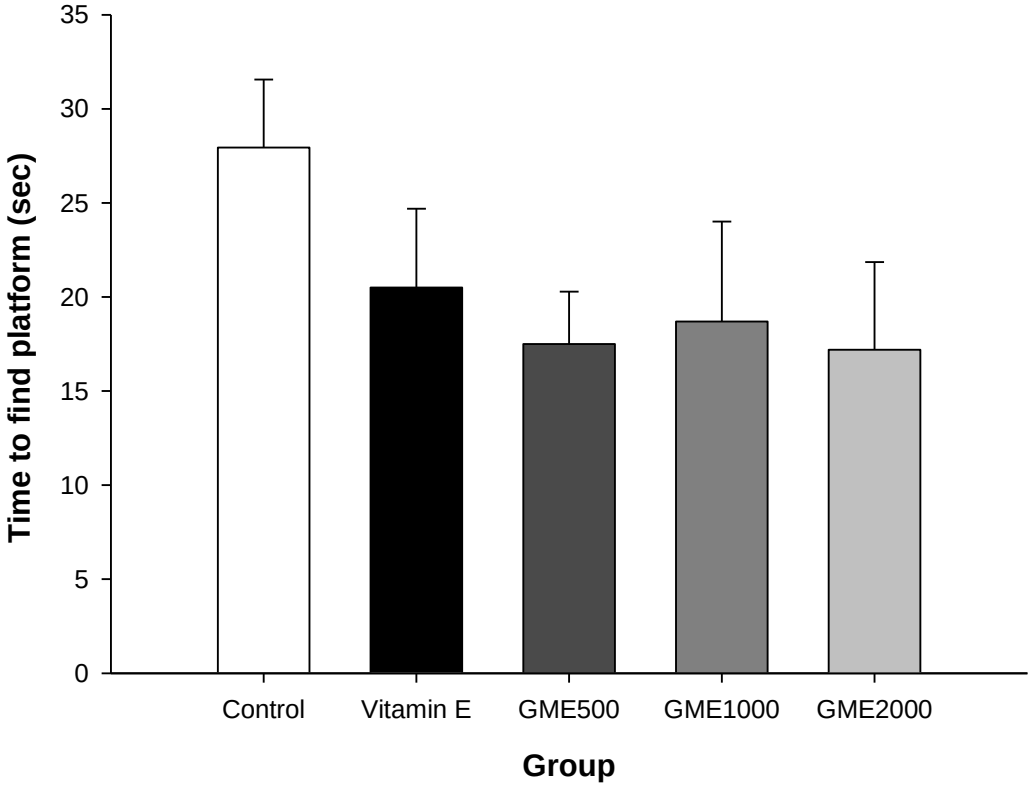


Figure 4.12 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 7 of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

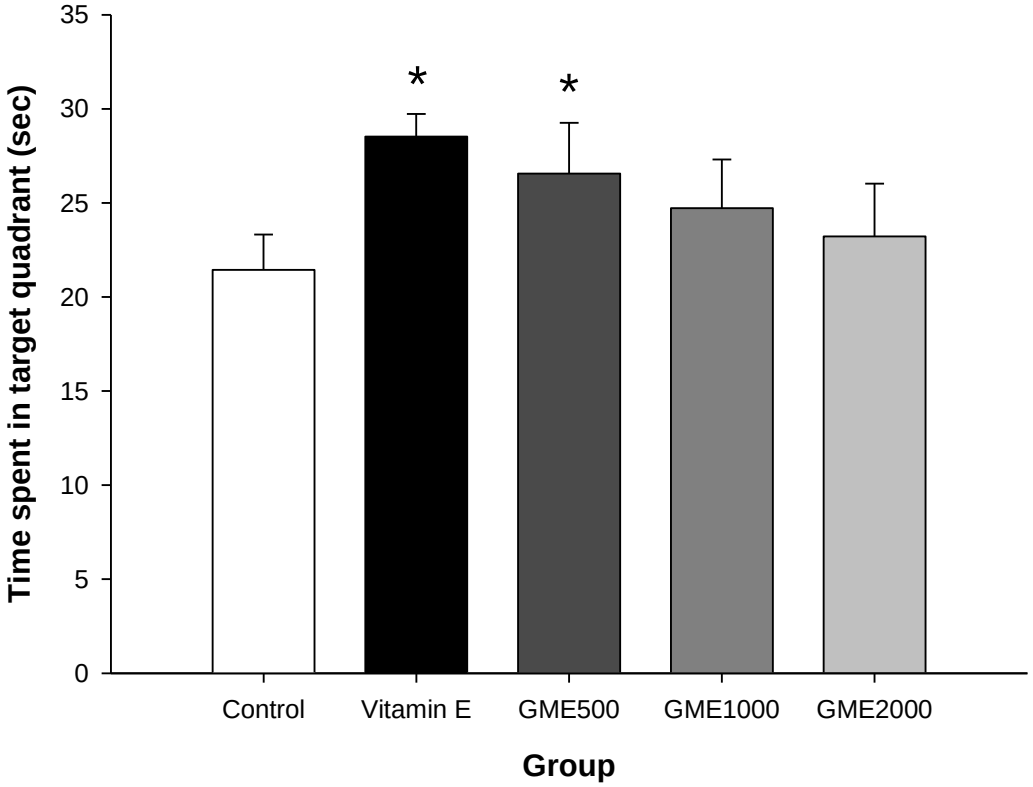


Figure 4.13 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time spent in target quadrant in Morris water maze test on probe trial of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to control group (P<0.05).

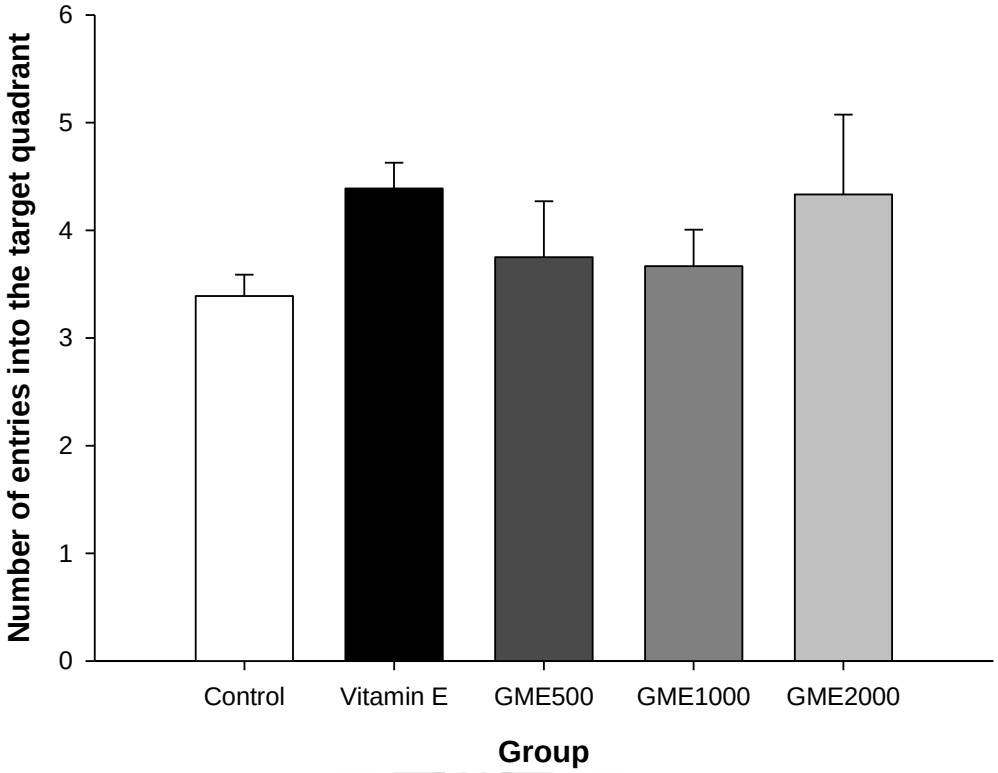


Figure 4.14 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on number of entries into the target quadrant in Morris water maze test on probe trial of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

The effects of daily oral administration of the crude extract from the fruit hull of mangosteen (500, 1000 and 2000 mg/ml/kg) and vitamin E for 30 days on AChE activity in the cerebral cortex, hippocampus, and basal forebrain of adult rats were determined. The results of healthy adult rats showed that administration of GME1000 and GME2000 significantly decreased AChE activity in cerebral cortex when compared to control group (P<0.05, one way ANOVA, Figure 4.15). The effects of GME on AChE activity in cerebral cortex were in dose-dependent manner.

AChE activity in cerebral cortex of GME500 and GME1000 was significantly higher than that of GME2000 (P<0.05, one way ANOVA). All doses of GME and vitamin E did not show significant difference of AChE activity in hippocampus when compared to control (P<0.05, one way ANOVA, Figure 4.16). Administration of GME500, GME1000, GME2000, and vitamin E significantly decreased AChE activity in basal forebrain when compared to control (P<0.05, one way ANOVA, Figure 4.17).

Table 4.1 showed plasma biochemical parameters [glucose, triglyceride, cholesterol, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] of healthy adult male Wistar rats orally administration with the crude extract from the fruit hull of mangosteen and vitamin E for 30 day. No significant difference was found in all plasma biochemical parameter between all treatment groups. Subchronic administration of the crude extract from the fruit hull of mangosteen and vitamin E did not affect behavior of healthy adult rats.

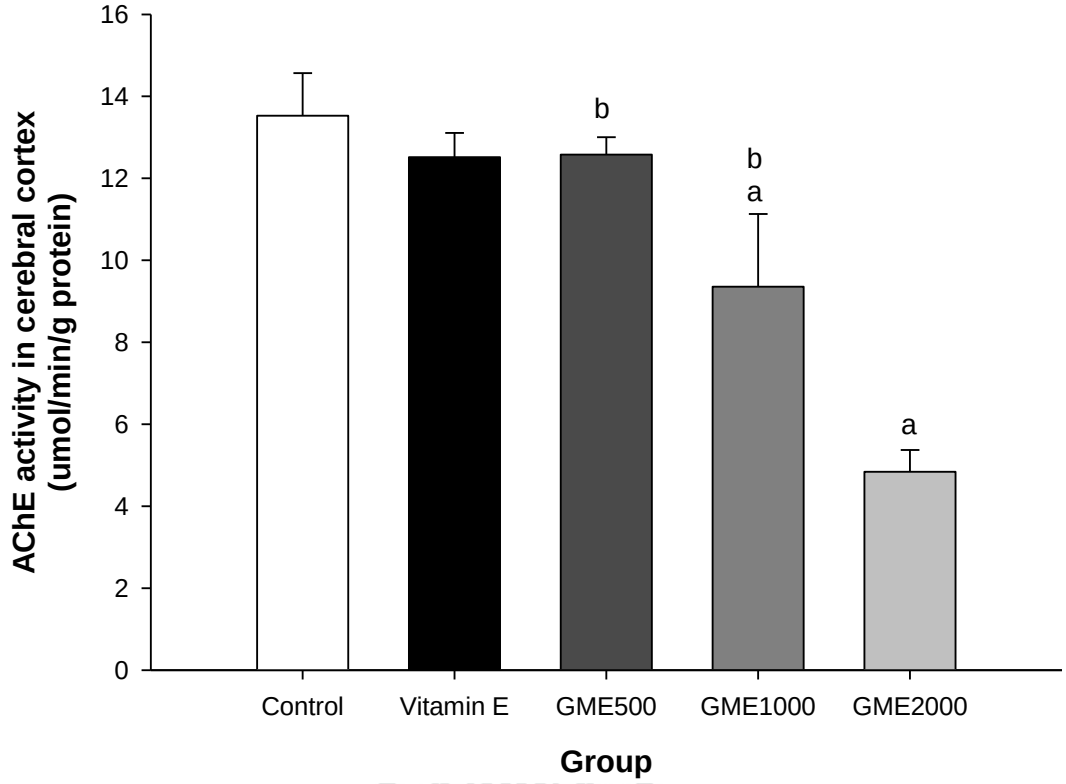


Figure 4.15 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in cerebral cortex of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. [a] indicates a significant difference compared to control group (P<0.05). [b] indicates a significant difference compared to GME2000 group (P<0.05).

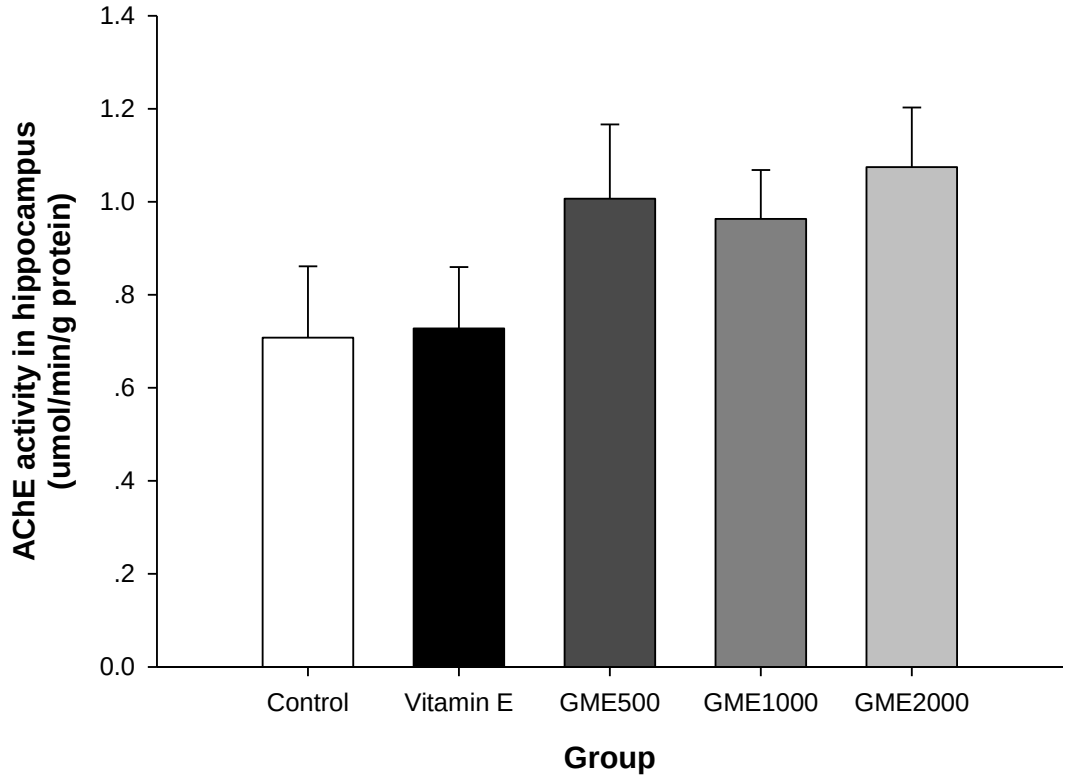


Figure 4.16 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in hippocampus of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

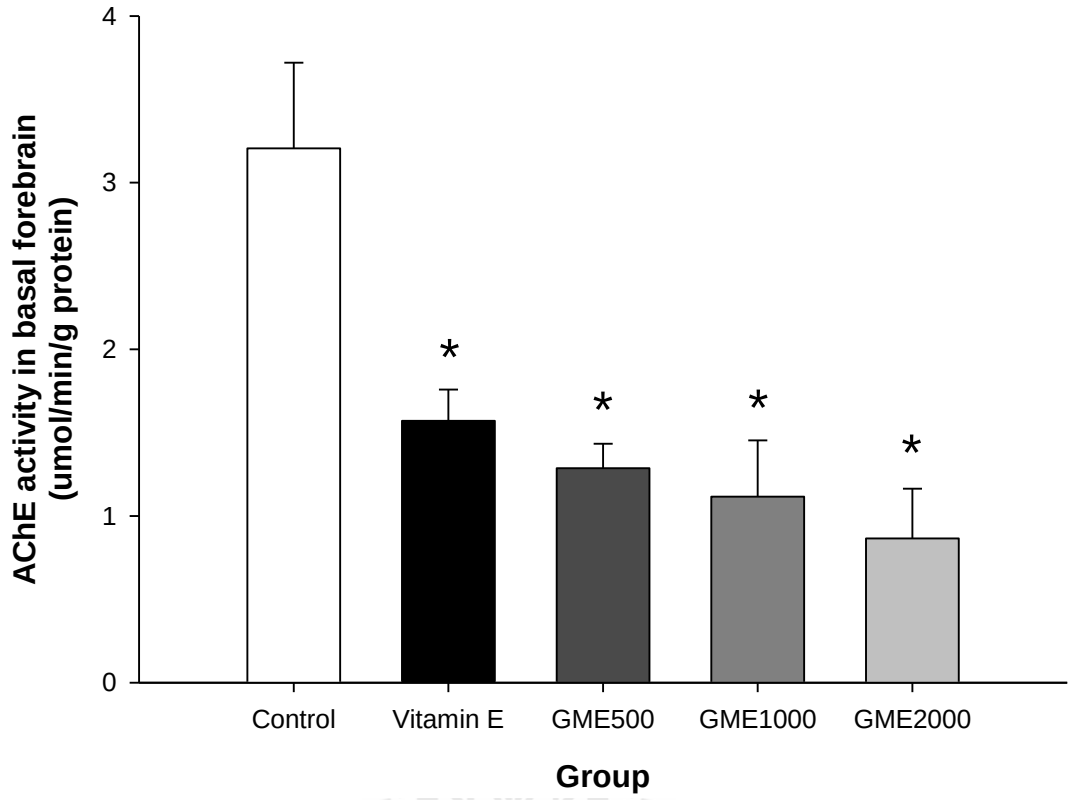


Figure 4.17 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in basal forebrain of adult rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to control group (P<0.05).

Table 4.1 Subchronic effects of the crude extract from the fruit hull of mangosteen on plasma biochemical parameters of healthy adult rats.

Parameters	control (n=6)	Vitamin E (40 mg/ml/kg) (n=6)	The crude extract from the fruit hull of mangosteen (mg/ml/kg)		
			500 (n=6)	1000 (n=6)	2000 (n=6)
Glucose (mg%)	225.50 ± 13.47	214.50 ± 22.16	218.17 ± 5.75	229.17 ± 12.61	227.33 ± 16.66
Triglyceride (mg%)	89.83 ± 6.54	82.50 ± 6.78	86.17 ± 8.47	86.17± 5.75	91.17 ± 7.41
Cholesterol (mg%)	121.00 ± 9.84	137.50 ± 24.45	135.67 ± 13.08	141.17 ± 8.47	137.50 ± 19.52
BUN (mg/dl)	22.18 ± 2.79	22.55 ± 4.93	23.47 ± 2.03	21.45 ± 2.28	21.45 ± 1.94
Creatinine (mg%)	1.45 ± 0.12	1.49 ± 0.18	1.47 ± 0.09	1.39 ± 0.07	1.49 ± 0.26
AST (U/L)	137.50 ± 6.02	135.67 ± 9.67	139 .33 ± 8.03	137 .50 ± 10.66	133.83 ± 9.55
ALT (U/L)	66.00 ± 6.22	66.00 ± 7.62	62.33 ± 7.41	64.17 ± 7.24	66.00 ± 7.62

Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.
BUN; Blood urea nitrogen, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase.

4.3 Subchronic effects of the crude extract from the fruit hull of mangosteen on memory and acetylcholinesterase activity in the cerebral cortex, hippocampus, and basal forebrain of aged rats

The effects of the crude extract from the fruit hull of mangosteen and vitamin E on memory were examined in aged rats using Morris water maze test. Time to find platform in training trials, time spent in target guardant and number of entries into the target quadrant in probe trial were recorded. Rats received daily oral dose of vehicle (10% Tween 80), vitamin E (40 mg/ml), or the crude extract from the fruit hull of mangosteen (GME) at doses of 500 and 1000 mg/ml/kg for 30 days. The results of aged rats showed that administration of GME1000 significantly decreased time to find platform on training day 5, 6 and 7 when compared to day 1 ($P<0.05$, two way repeated measures ANOVA, Figure 4.18). Administration of GME500 and vitamin E significantly decreased time to find platform on training day 7 when compared to day 1 ($P<0.05$, two way repeated measures ANOVA, Figure 4.18). No significant difference was found in time to find platform between groups on training day 1 to day 6 (Figure 4.18). On training day 7, administration of GME500, GME1000 and vitamin E significantly decreased difference in time to find platform among groups on last day of training trials (day 7) ($P<0.05$, one way repeated measures ANOVA, Figure 4.19). In probe trial on day 7, GME500 and vitamin E, but not GME1000, significantly increased time spent in target quadrant in aged rats compared to control group ($P<0.05$, one way repeated measures ANOVA, Figure 4.20). Thirty days administration of all doses of GME and vitamin E tended to increase, but not

significantly different, in number of entries into the target quadrant when compared to control group (Figure 4.21).

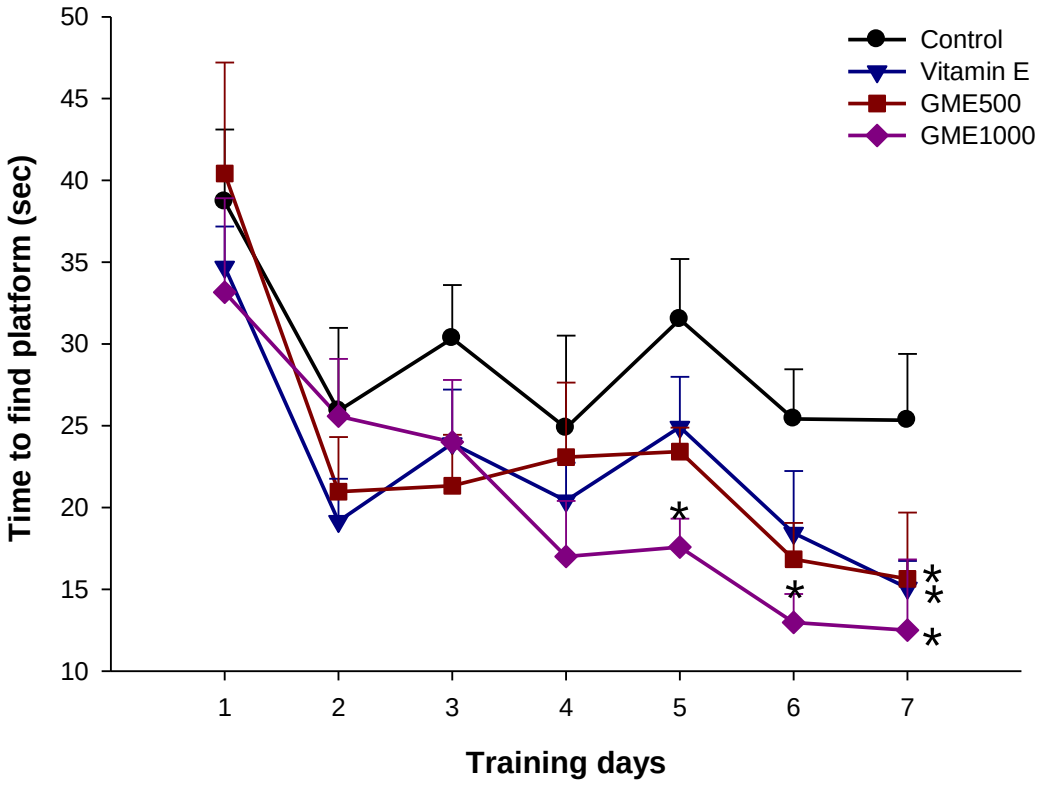


Figure 4.18 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 1 to day 7 of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference of all treatment groups compared with day 1 within group (P<0.05).

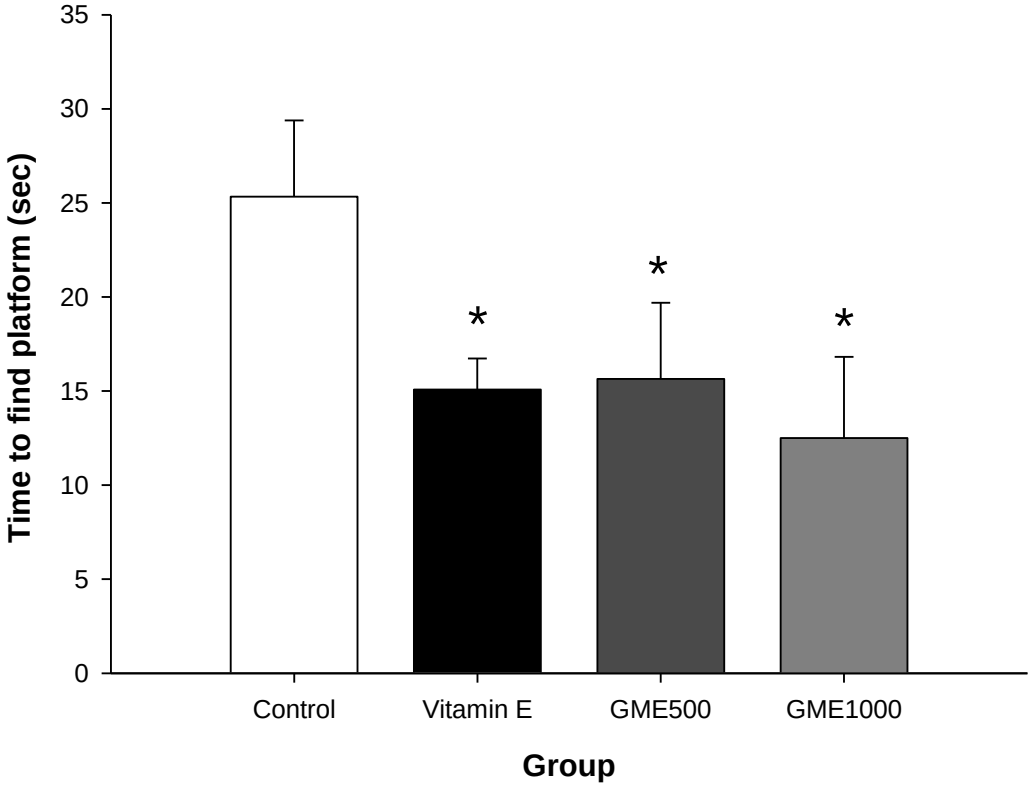


Figure 4.19 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time to find platform in Morris water maze test on training day 7 of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to control group (P<0.05).

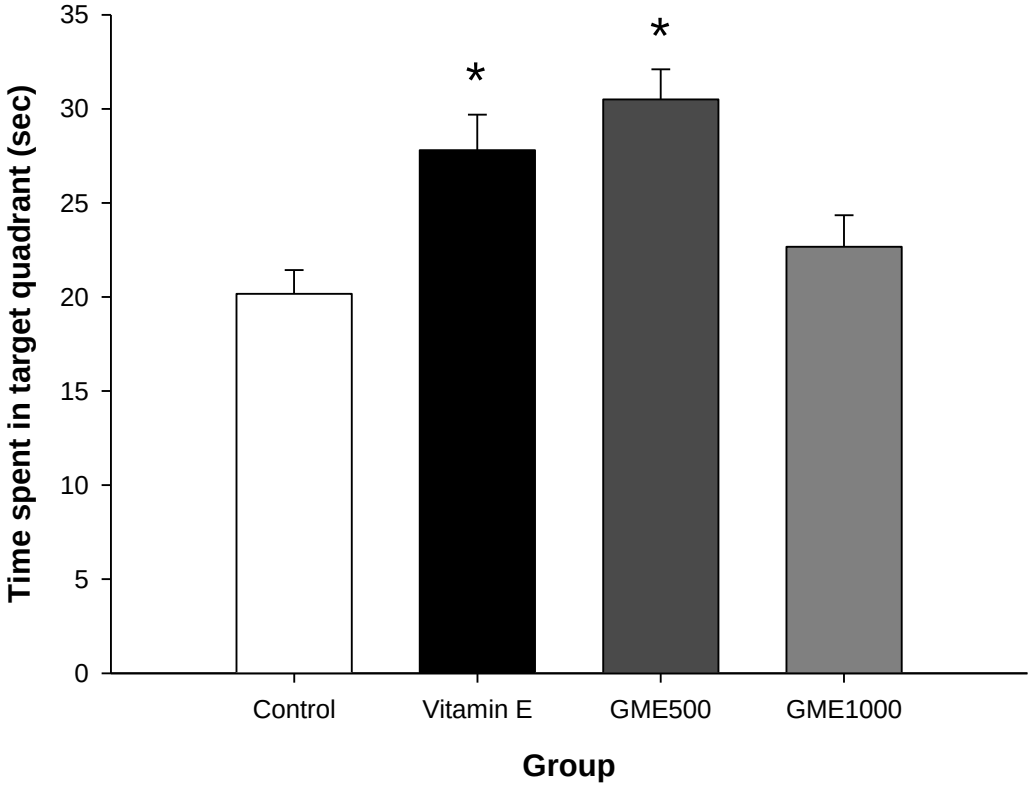


Figure 4.20 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on time spent in target quadrant in Morris water maze test on probe trial of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to control group (P<0.05).

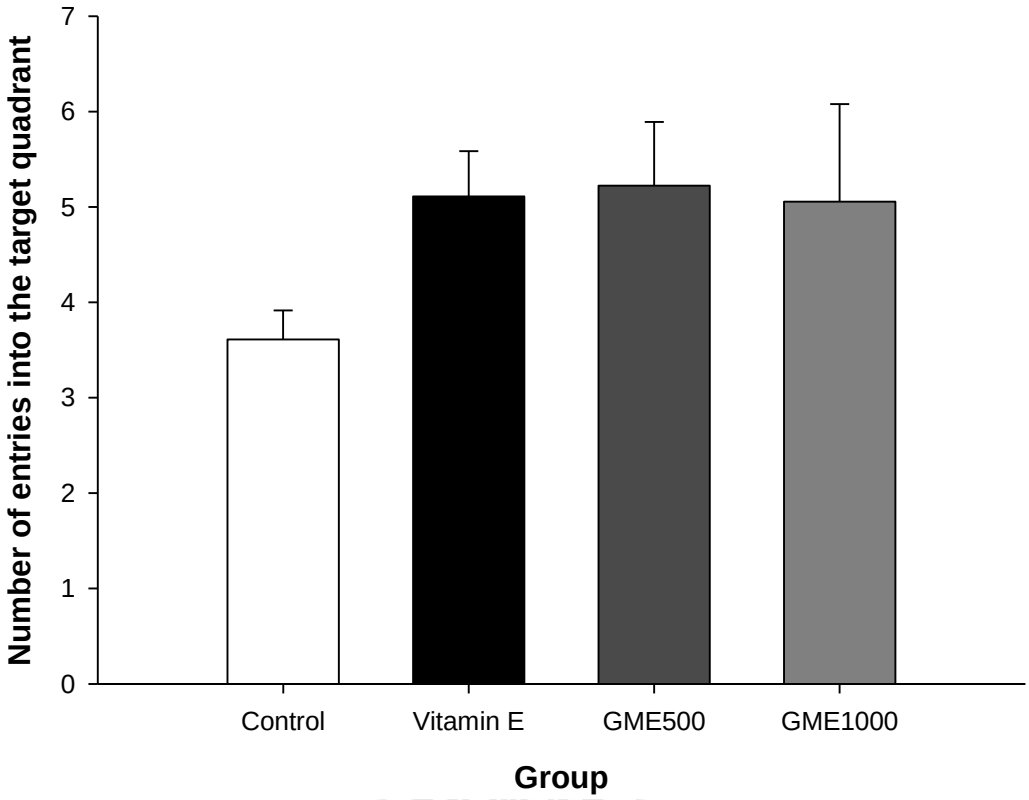


Figure 4.21 Subchornic effects of the crude extract from the fruit hull of mangosteen and vitamin E on number of entries into the target quadrant in Morris water maze test on probe trial of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

The effects of daily oral administration the crude extract from the fruit hull of mangosteen (500 and 1000 mg/ml/kg) and vitamin E for 30 days on AChE activity in the cerebral cortex, hippocampus, and basal forebrain of aged rats were determined. The results of aged rats showed that administration of GME500 and GME1000 tended to decrease, but did not cause significant difference, in AChE activity in cerebral cortex when compared to control group (Figure 4.22). Administration of GME1000, but not GME500 and vitamin E, significantly increased AChE activity in hippocampus when compared to control group ($P<0.05$, one way ANOVA, Figure 4.23). All doses of GME and vitamin E did not show significant difference in AChE activity in basal forebrain when compared to control group ($P<0.05$, one way ANOVA) as shown in (Figure 4.24).

Table 4.2 showed plasma biochemical parameters [glucose, triglyceride, cholesterol, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] of aged male Wistar rats orally administration with the crude extract from the fruit hull of mangosteen and vitamin E for 30 day. No significant difference was found in all plasma biochemical parameter between all treatment groups. Subchronic administration of the crude extract from the fruit hull of mangosteen and vitamin E did not affect behavior of aged rats.

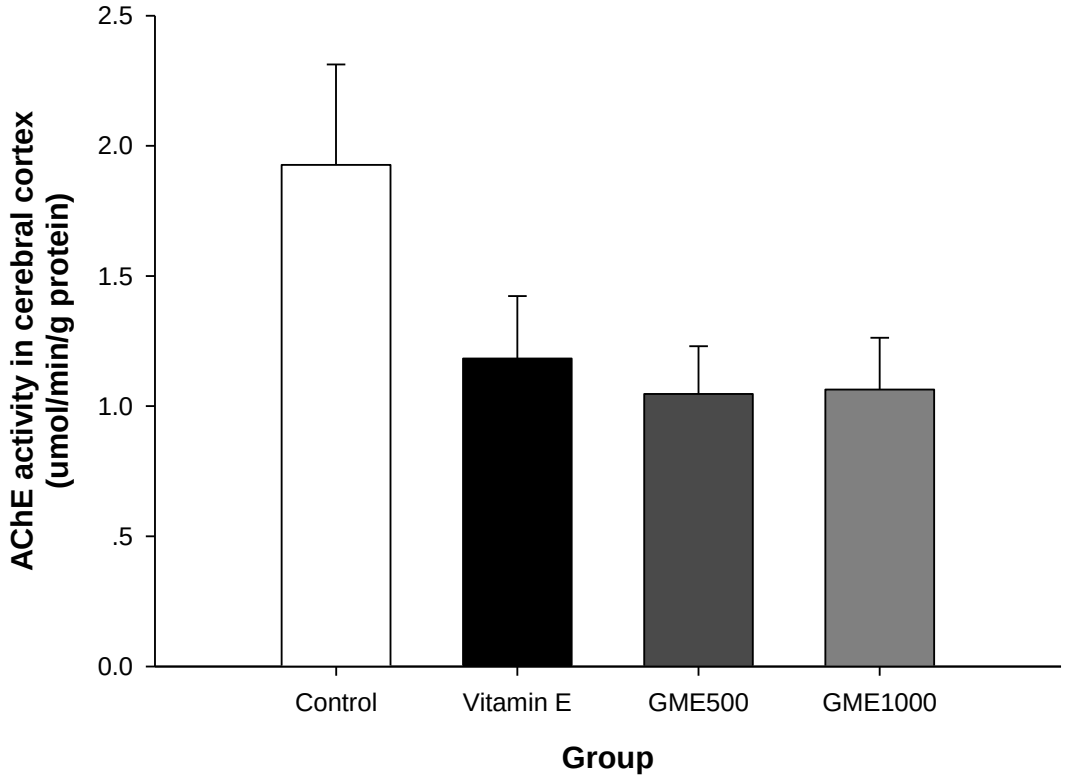


Figure 4.22 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in cerebral cortex of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among groups.

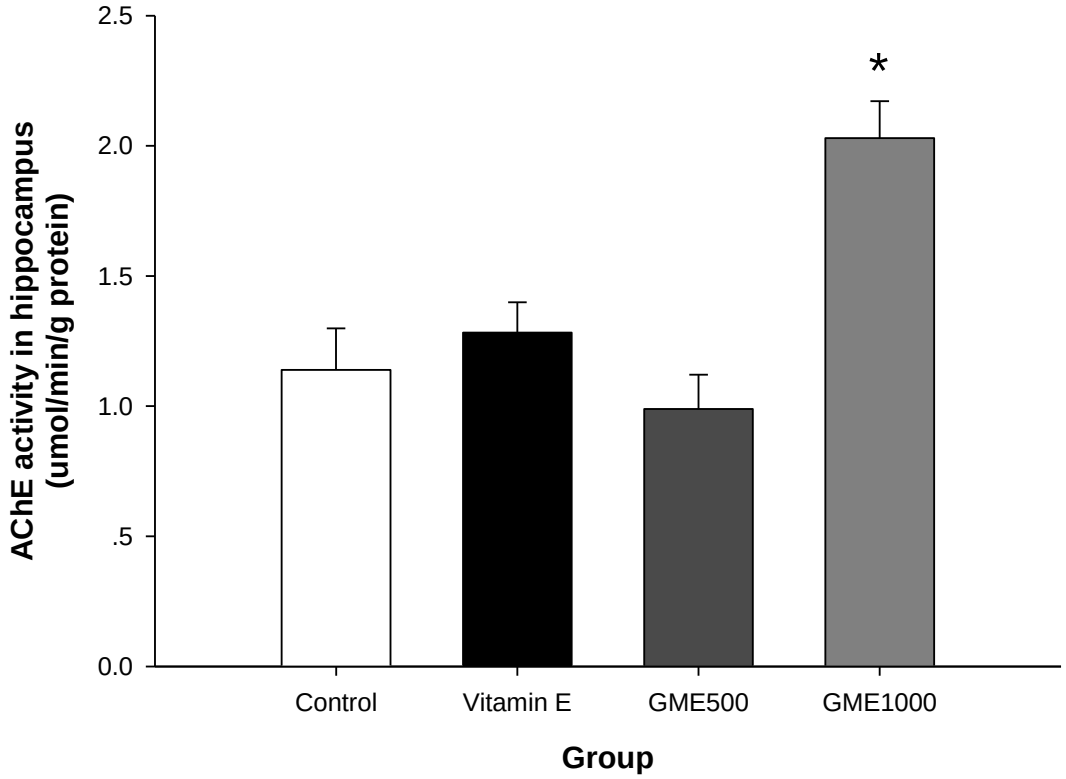


Figure 4.23 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in hippocampus of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. * indicates a significant difference compared to control group (P<0.05).

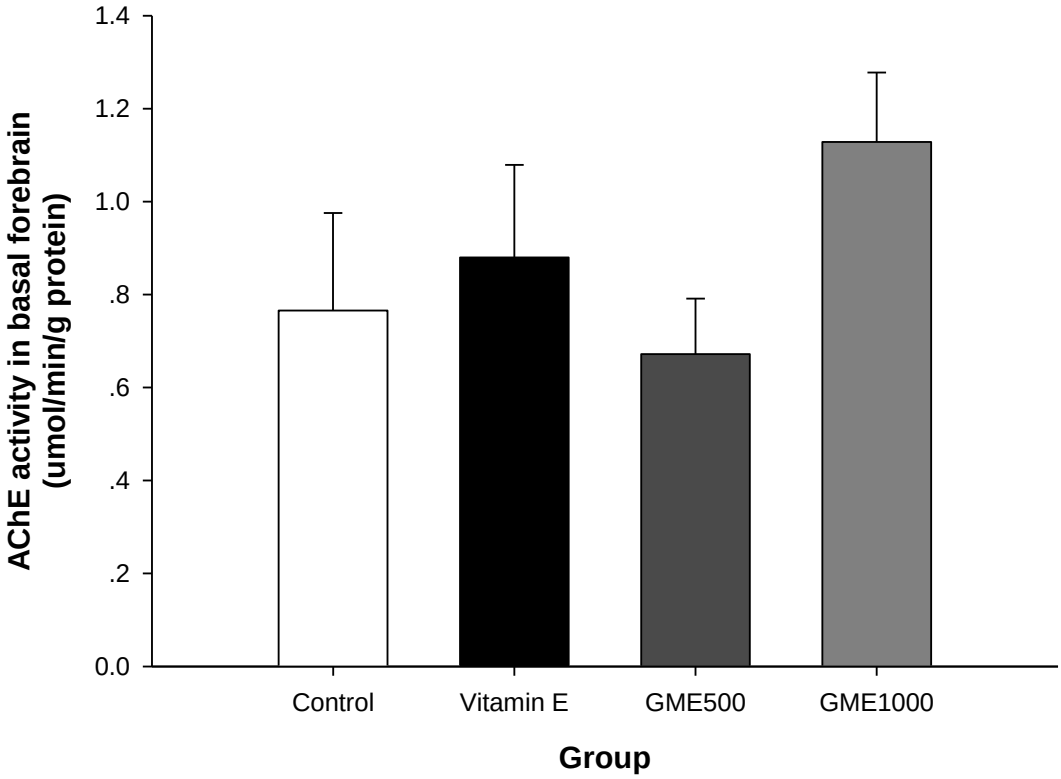


Figure 4.24 Subchronic effects of the crude extract from the fruit hull of mangosteen and vitamin E on acetylcholinesterase activity in basal forebrain of aged rats. Values are expressed as means ± S.E.M; n = 6 per group. There was no significant difference among group.

Table 4.2. Subchronic effects of the crude extract from the fruit hull of mangosteen on plasma biochemical parameters of aged rats.

Parameters	Control (n=6)	Vitamin E (40 mg/ml/kg) (n=6)	The crude extract from the fruit hull of mangosteen (mg/ml/kg) 500 (n=6)	1000 (n=6)
Glucose (mg%)	179.00 ± 7.08	165.00 ± 6.73	155.00 ± 13.83	169.00 ± 9.20
Triglyceride (mg%)	51.00 ± 7.35	55.00 ± 5.73	56.00 ± 9.24	51.00 ± 10.97
Cholesterol (mg%)	70.00 ± 2.19	74.00 ± 2.77	71.00 ± 3.95	70.00 ± 6.50
BUN (mg/dl)	34.10 ± 1.19	30.30 ± 0.86	30.10 ± 1.49	31.00 ± 1.58
Creatinine (mg%)	0.44 ± 0.04	0.47 ± 0.08	0.53 ± 0.09	0.56 ± 0.10
AST (U/L)	105.00 ± 10.29	107.00 ± 8.21	115.00 ± 8.56	118.00 ± 4.38
ALT (U/L)	50.00 ± 4.38	55.00 ± 5.21	51.00 ± 7.73	58.00 ± 6.04

Values are expressed as means ± S.E.M; n = 6 per group.

There was no significant difference among groups. BUN; Blood urea nitrogen, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase.

CHAPTER V

DISCUSSION AND CONCLUSION

Dementia is a symptom complex of continuous global weak of intellectual function. It becomes a major medical, economic, and social problem that is deterioration as the increasing number of elderly people. Recently, Youdin and Joseph (2001), Perez-Perez and Rodriguez-Malaver (2005) and Mancuso et al. (2007) reported that antioxidants are effective in the amelioration of dementia process, the involvement of oxidative stress component in dementia or Alzheimer's disease. The dementia associated with increased brain oxidative stress during brain aging could be reversed by antioxidants (Socci et al., 1995). Moreover, degradation of the central cholinergic system resulting in low levels of acetylcholine is correlated well with severity of dementia. Currently, available drug therapies for Alzheimer’s disease and other diseases that cause dementia consist primarily of acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine, Huperzine A), an N-methyl-D-aspartate receptor antagonist (memantine) and some neuroprotective agents (Olivares et al., 2012). These drugs cause side effects, thus an alternative bioactive compounds from plants and fruits with little or no side effects should be used to replace these drugs. Polyphenolic compounds may be one of candidates because of their antioxidative and anti-aging properties. Polyphenols from many fruits and plants are known to possess cognitive enhancing effects and acetylcholinesterase inhibitory activity (Papandreou

et al., 2009). High amount of polyphenolic compound can be found in the crude extract from the fruit hull of mangosteen. Mangosteen is a source of bioactive compounds with potential health-promoting activity. The crude extract from the fruit hull of mangosteen has potent antioxidant profile (Chomnawang et al., 2007). Several studies revealed that the fruit hull of mangosteen is the rich source of polyphenols (xanthone, tannin, flavonoid and anthocyanins) (Asai et al., 1995; Chen et al., 2008; Chomnawang et al., 2007; Cui et al., 2010; Ji et al., 2007; Maisuthisakul et al., 2007; Yu et al., 2007; Zadernowski et al., 2009). Thus, the crude extract from the fruit hull of mangosteen may possess biological activities related to polyphenolic compounds. The present study demonstrated for the first time that the crude extract from the fruit hull of mangosteen could enhance memory probably by inhibiting brain acetylcholinesterase activity in healthy adult and normal aging male Wistar rats.

The percentage yield of the crude extract from the fruit hull of mangosteen from the present study was 27.7% that was higher than previously found (9.21±0.30 %) in the study of Arazo et al. (2011). The total phenolic content found in crude extract from the fruit hull of mangosteen was 170.94 ± 0.009 mg gallic acid/g dry extract that was higher than previously found (158.0 mg gallic acid/g dry extract) in the study of Chaovanalikit et al. (2012). Total anthocyanin content of the crude extract from the fruit hull of mangosteen was 168.2 µg/g of dry sample which was similar to that previously found in the study of Chaovanalikit et al. (2012). Antioxidant activity (IC_{50}) of the crude extract from the fruit hull of mangosteen was 3.55 mg/ml sample extract which was similar to that previously found in the study of Arazo et al. (2011).

The present study provided the first evidence that the crude extract from the fruit hull of mangosteen and vitamin E possessed memory enhancing effects in normal

saline treated rats by increasing time spent in the target quadrant using Morris water maze test (Figure 5.1). In scopolamine-induced amnesic rats, the crude extract from the fruit hull of mangosteen and vitamin E did not cause any change in time spent in the target quadrant and number of entries into target quadrant. These results were consistent with the results of AChE activity in cerebral cortex and hippocampus, but not basal forebrain. AChE activity in cerebral cortex and hippocampus was increased by the crude extract from the fruit hull of mangosteen in scopolamine-induced amnesic rats, but was decreased in normal saline treated rats. In contrast to those findings, AChE activity in basal forebrain was decreased by the crude extract from the fruit hull of mangosteen in scopolamine-induced amnesic rats (Figure 5.2). The crude extract from the fruit hull of mangosteen increased AChE activity in basal forebrain in normal saline treated rats (Figure 5.2). These findings suggested that the antiamnesic effect of the crude extract from the fruit hull of mangosteen could be due to its anti-acetylcholinesterase action in cerebral cortex and hippocampus of normal saline treatment, but not is scopolamine-induced amnesia.

Neuroprotective effects of the crude extract from the fruit hull of mangosteen and vitamin E were demonstrated in the present study. In both healthy adult and normal aging rats, subchronic administration of the crude extract from the fruit hull of mangosteen could enhance spatial memory by increasing time spent in the target quadrant without changing in number of entries into the target quadrant using Morris water maze test (Figure 5.1). The present findings were consistent with the results of AChE activity in cerebral cortex and basal forebrain, but not hippocampus, of healthy adult rats. AchE activity in cerebral cortex and basal forebrain were decreased by the crude extract from the fruit hull of mangosteen and vitamin E in healthy adult rats

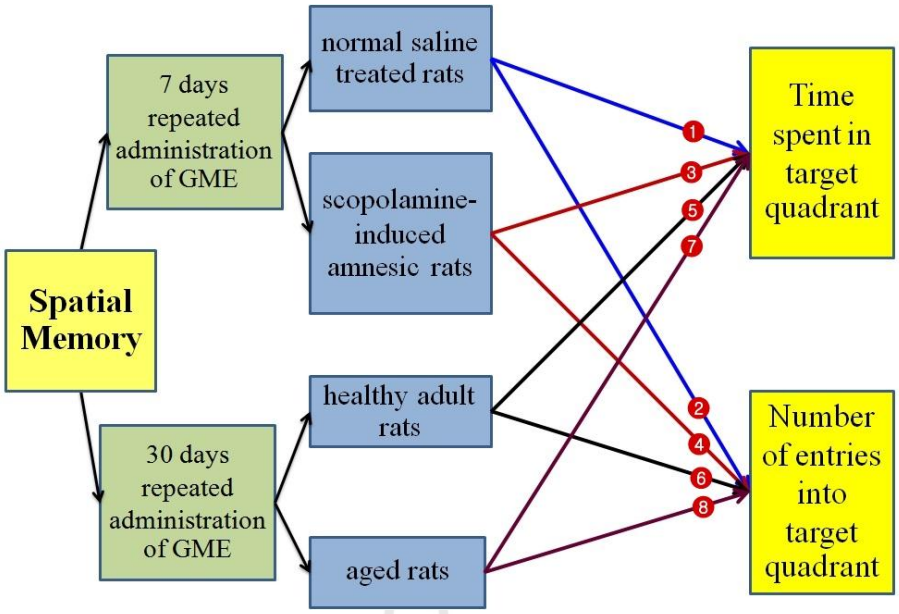
(Figure 5.2). In aging rats, AChE activity in hippocampus was increased, while in cerebral cortex and hippocampus was not changed after subchronic administration of the crude extract from the fruit hull of mangosteen (Figure 5.2). Cognitive dysfunction such as learning impairment and delayed amnesia are the most striking age-related changes observed in human being and animals (Foster et al., 1994; Gray et al., 2008).

The cholinergic system is responsible for the storage and retrieval of item in memory and it degradation correlates well with the severity of cognitive and memory impairment. Therefore, it has been suggested that elevation of the acetylcholine level might be helpful in to improve the symptoms of cognitive deficit (Gasparin et al., 1998). Loss of cholinergic innervations, demonstrated by reduced choline acetyltransferase (ChAT) and elevated acetylcholinesterase (AChE) activity, is correlated with the degree of dementia (Zubenko et al., 1989). Many different theories have been offered regarding the cause of AD; a well-established theory suggests that acetylcholine levels are too low in the brain of AD patients (Davies and Maloney 1976). Therefore, one approach for treating AD is via the inhibition of AChE. Based on the cholinergic hypothesis, a defect in the cholinergic system is involved in AD (Francis et al., 1999). Polyphenols from plants and fruits can enhance memory by inhibiting AChE activity. The polyphenol-rich extract from lotus seed pods was found to inhibit AChE activity (Xu et al., 2009). Green tea extract containing polyphenols could inhibit AChE activity in scopolamine-induced amnesia mice (Kim et al., 2004). Polyphenol-rich wild blueberry extracts attenuating brain oxidative stress, increasing brain ascorbate and glutathione (GSH) levels, and decreasing AChE activity in mice whole brain homogenates, exhibited a significant improvement in learning and memory tested by the passive avoidance behavioral test (Papandreou et al., 2009).

Mukhergee et al. (2006) and Yang et al. (2008) reported that N. *nucifera* rhizome extract inhibited AChE activity and improved learning and memory by enhancing neurogenesis in the dentate gyrus of hippocampus.

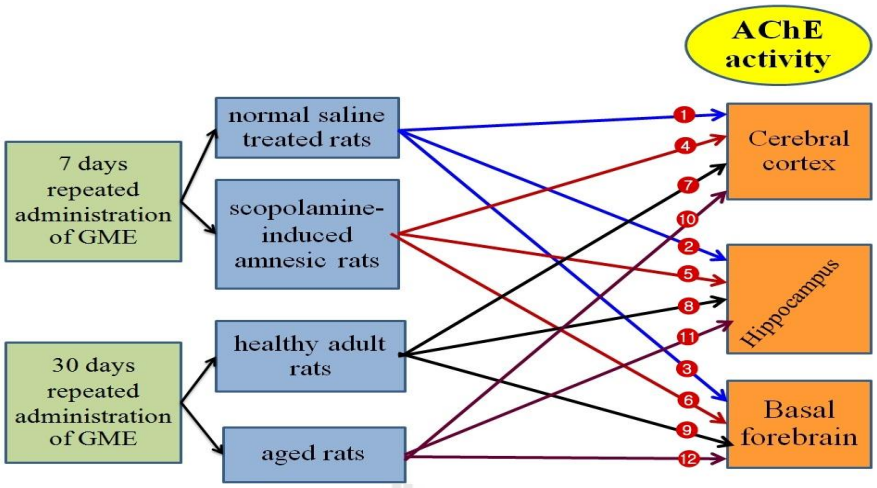
The present study also demonstrated that the crude extract from the fruit hull of mangosteen at any tested doses did not affect the animals' behavior and health status and did not produce any plasma biochemical parameters abnormality of healthy adult and aged male Wistar rats during the period of treatment for 30 days, suggesting that the crude extract from the fruit hull of mangosteen is medically safe for liver and kidneys of rats.

In conclusion, we demonstrated remarkable memory enhancing and acetylcholinesterase inhibitory effects of the crude extract from the fruit hull of mangosteen in healthy adult and normal aging models, but not in scopolamine-induced cognitive impairment model. Memory enhancing effects of the crude extract from the fruit hull of mangosteen may be a result of its anti-acetylcholinesterase activity (Figure 5.3). Thus, the crude extract from the fruit hull of mangosteen may play a useful role in the protection and treatment of cognitive impairment caused by normal aging.



	Spatial memory	
	No Effect	**Increased**
1		↑ GME250, GME500, GME1000, GME2000,Vitamin E (100 mg/ml/kg)
2	↔ GME250, GME500, GME1000, GME2000,Vitamin E (100 mg/ml/kg)	
3	↔ GME250, GME500, GME1000, GME2000,Vitamin E (100 mg/ml/kg)	
4	↔ GME250, GME500, GME1000, GME2000,Vitamin E (100 mg/ml/kg)	
5	↔ GME1000, GME2000	↑ GME500, Vitamin E (40 mg/ml/kg)
6	↔ GME500, GME1000, GME2000, Vitamin E (40 mg/ml/kg)	
7	↔ GME1000	↑ GME500, Vitamin E (40 mg/ml/kg)
8	↔ GME500, GME1000, Vitamin E (40 mg/ml/kg)	

Figure 5.1 Summary for the effects of the crude extract from the fruit hull of mangosteen (GME) on spatial memory in male Wistar rats using Morris Water Maze test. Abbreviations: GME250; 250 mg/ml/kg GME, GME500; 500 mg/ml/kg GME, GME1000; 1000 mg/ml/kg GME, GME2000; 2000 mg/ml/kg GME.



	AChE activity		
	No Effect	**Decreased**	**Increased**
1	↔ GME250, Vitamin E (100 mg/ml/kg)	↓ GME500, GME1000	
2	↔ GME1000	↓ GME250, GME500, Vitamin E (100 mg/ml/kg)	
3		↓Vitamin E (100 mg/ml/kg)	↑ GME250, GME500, GME1000
4	↔ GME1000, GME2000		↑ GME500
5	↔ GME1000		↑ GME250, GME500, Vitamin E (100 mg/ml/kg)
6	↔ GME1000, Vitamin E (100 mg/ml/kg)	↓ GME250, GME500	
7	↔ GME500, Vitamin E (40 mg/ml/kg)	↓ GME1000, GME2000	
8	↔ GME500, GME1000, GME2000, Vitamin E (40 mg/ml/kg)		
9		↓ GME500, GME1000, GME2000, Vitamin E (40 mg/ml/kg)	
10	↔ GME500, GME1000, Vitamin E (40 mg/ml/kg)		
11	↔ GME500, Vitamin E (40 mg/ml/kg)		↑ GME1000
12	↔ GME500, GME1000, Vitamin E (40 mg/ml/kg)		

Figure 5.2 Summary for the effects of the crude extract from the fruit hull of mangosteen (GME) on acetylcholinesterase (AChE) activity in cerebral cortex, hippocampus, and basal forebrain of male Wistar rats. Abbreviations: GME250; 250 mg/ml/kg GME, GME500; 500 mg/ml/kg GME, GME1000; 1000 mg/ml/kg GME, GME2000; 2000 mg/ml/kg GME.

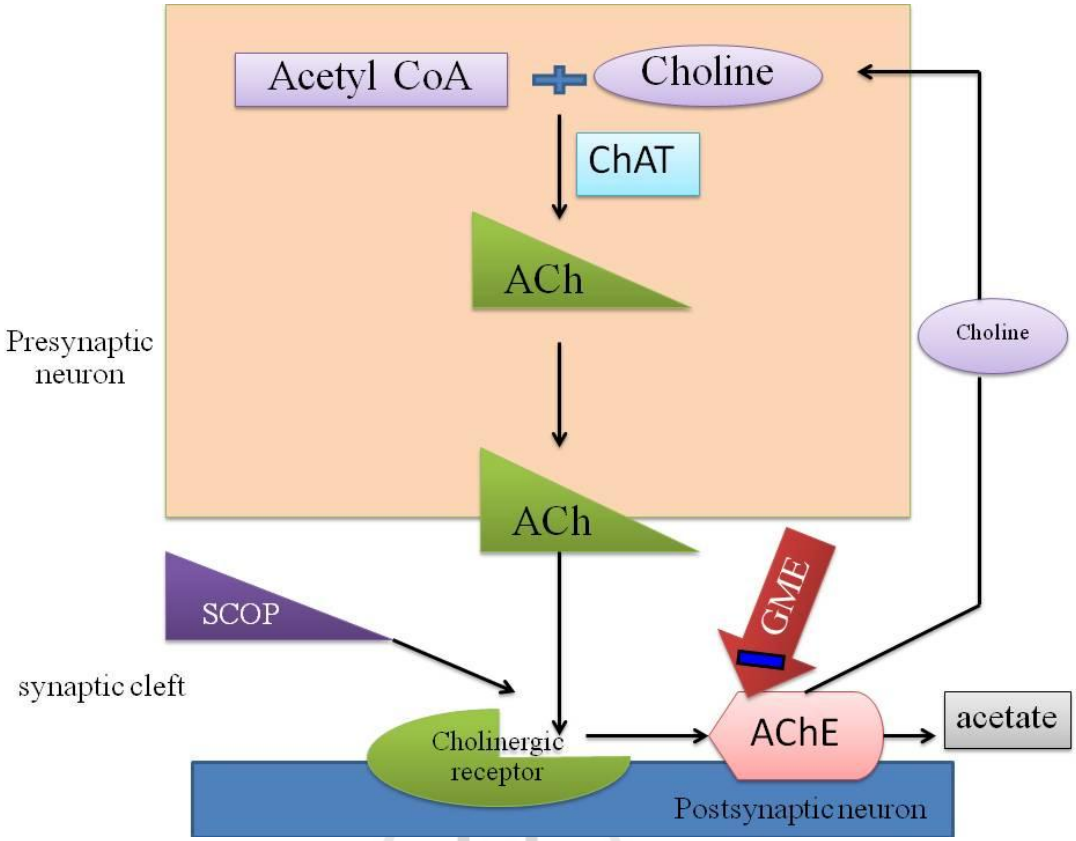


Figure 5.3 The possible mechanisms underlying the memory enhancing effects of the crude extract from the fruit hull of mangosteen (GME). Abbreviations: SCOP; scopolamine, ACh; acetylcholine, AChE; acetylcholinesterase enzyme, ChAT; choline acetyltransferase enzyme.

REFERENCES

REFERENCES

Adamson, E. D. (1997). Acetylcholinesterase in mouse brain erythrocytes and muscle. **Journal of Neurobiochemistry.** 8(2): 605-654.

Agrawal, R., Tyagi, E., Shukla, R. and Nath, C. (2008). Effect of insulin and melatonin on acetylcholinesterase activity in the brain of amnesic mice. **Behavioural Brain Research.** 189(2): 381-386.

Akao, Y., Nakagawa, Y., Iinuma, M. and Nozawa, Y. (2008). Anti-cancer effects of xanthones from pericarps of mangosteen. **International Journal of Molecular Sciences.** 9(3): 355-370.

Alzheimer's Association. (2011). Alzheimer's & Dementia: 2011 Alzheimer's disease facts and figures. **The Journal of the Alzheimer's Association.** 7(2): 208-244.

Alzheimer's Association. (2012). Alzheimer's & Dementia: 2012 Alzheimer's disease facts and figures. **The Journal of the Alzheimer's Association.** 8(2): 131-168.

Alzheimer's Association. (2013). Alzheimer's & Dementia: 2013 Alzheimer's disease facts and figures. **The Journal of the Alzheimer's Association.** 9(2): 208-245.

Alzoubi, K. H., Khabour, O. F., Rashid, B. A., Damaj, I. M. and Salah, H. A. (2011). The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. **Behavioural Brain Research.** 226(1): 205-210.

Anagnostaras, S. G., Murphy, G. G., Hamilton, S. E., Nathanson, N. M. and Silva, A.J. (2000). Learning and memory in cholinergic muscarinic M1 receptor knock out mice. **Abstract Society Neuroscience.** 26: 1809.

Anthocyanins.net. (2010). **Anthocyanins and resveratrol** [on-line]. Available: http:// www.anthocyanins.net.

Arazo, M., Bello, A., Rastrelli, L., Montelier, M., Delgado, L. and Panfet, C. (2011). Antioxidant properties of pulp and peel of yellow mangosteen fruits. **Emirates Journal of Food and Agriculture.** 23(6): 517-524.

Asai, F., Tosa, H., Tanaka, T. and Iinuma, M. (1995). A xanthone from pericarps of *Garcinia mangostana*. **Phytochemistry.** 39(4): 943-944.

Beatty, W. W., Butters, N. and Janowsky, D. (1986). Memory failure after scopolamine treatment: implications for cholinergic hypothesis of dementia. **Behavioral and Neural Biology.** 45(2): 196–211.

Beecher, G. R. (2003). Proceeding of the third international scientific symposium on tea and human health: role of flavonoid in the diet. **American Society for Nutritional Sciences.** 133: 3248-3254.

Bei, W., Zang, L., Guo, J., Peng, W., Xu, A., Good, D. A., Hu, Y., Wu, W., Hu, D., Zhu, X., Wei, M. and Li, C. (2009). Neuroprotective effects of a standardized flavonoid extract from Diospyros kaki leaves. **Journal of Ethnopharmacology.** 126 (1): 134-142.

Benamar, H., Rached, W., Derdour, A. and Marouf, A. (2010). Screening of algerian medicinal plants for acetylcholinesterase inhibitory activity. **Journal of Biological Sciences.** 10(1): 1-9.

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. **Critical Reviews in Oral Biology & Medicine.** 13(2): 184-196.

Chairungsrilerd, N., Takeuchi, Y., Ohizumi, Y., Nozoe, S. and Ohta, T. (1996). Mangostanol, A prenyl xanthone from *Garcinia mangostana*. **Phytochemistry.** 43(5): 1009-1102.

Chaovanalikit, A., Mingmuang, A., Kitbunluewit, T., Choldumrongkool, N., Sondee, J. and Chupratum, S. (2012). Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products. **International Food Research Journal.** 19(3): 1047-1053.

Chattipakorn, S., Pongpanparadorn, A., Pratchayasakul, W., Pongchaidacha, A., Ingkaninan, K. and Chattipakorn, N. (2007). *Tabernaemontana divaricata* extract inhibits neuronal acetylcholinesterase activity in rats. **Journal of Ethnopharmacology.** 110(1): 61-68.

Chen, L. G., Yang, L. L. and Wang, C. C. (2008). Anti-inflammatory activity of mangostins from *Garcinia mangostana*. **Food and Chemical Toxicology.** 46(1): 688-693.

Chin, Y. W., Jung, H. A., Chai, H., Keller, W. J. and Kinghorn, A. D. (1996). Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). **Phytochemistry.** 69(3): 754-758.

Cho, J., Kang, J. S., Long, P. H., Jing, J., Back, Y. and Chung, K. S. (2003). Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and cordyceps mushroom extract. **Archives of Pharmacal Research.** 26(10): 821-825.

Chomnawang, M. T., Surassmo, S., Nukoolkarn, V. S. and Gritsanapan, G. (2007). Effect of *Garcinia mangostana* on inflammation caused by Propionibacterium acnes. **Fitoterapia.** 78(6): 401-408.

CMU medicine pharmacology. (2010). **Cholinergic transmission** [on-line]. Available: http://www.med.cmu.ac.th/dept/pharmaco/pharmacology.

Court, J. A. and Perry, E. K. (1991). Dementia: the neurochemical basis of putative transmitter orientated therapy. **Pharmacology & Therapeutics.** 52(3): 423-443.

Cui, J., Hu, W., Cai, Z., Liu, Y., Li, S., Tao, W. and Xiang, H. (2010). New medicinal properties of mangostins: Analgesic activity and pharmacological characterization of active ingredients from the fruit hull of *Garcinia mangostana* L. **Pharmacology, Biochemistry and Behavior.** 95: 166-172.

Davies, P. and Maloney, A.J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. **Lancet.** 2: 1457-1459.

Devi, A., Jolitha, A. B. and Ishii, N. (2007). Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. **Medical Science Monitor.** 12(4): 124-129.

Devi Sampath, P. and Vijayaraghavan, K. (2007). Cardioprotective effect of α-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. **Journal of Biochemical and Molecular Toxicology.** 21(3): 336-339.

Ding, M., Feng, R., Wang, L., Bowman, L., Lu, Y., Qian, Y., Castranova, V., Jiang, B. H. and Shi, X. (2006). Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. **The Journal of Biological Chemistry.** 281(25): 17359-17368.

Drachman, D. A. and Leavitt, J. (1974). Human memory and the cholinergic system: a relationship to aging. **Archives of Neurology.** 1974(30): 113-121.

Ebert, U. and Kirch, W. (1998). Scopolamine model of dementia: electroencephalogram findings and cognitive performance. **European Journal of Clinical Investigation.** 28(11): 944-949.

Eidi, A., Eidi, M., Mahmoodi, G. and Oryan, S. (2006). Effect of vitamin E on memory retention in rats: possible involvement of cholinergic system. **European Neuropharmacology.** 16(2): 101-106.

Eldeen, I. M. S., Elgorashi, E. E. and van Staden, J. (2005). Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. **Journal of Ethnopharmacology.** 102(2): 457-464.

Ellman, G. L, Courtney, K. D., Andres, V. Jr. and Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. **Biochemical Pharmacology.** 7(2): 88-95.

Felder, C. C., Bymaster, F. P., Ward, J. and DeLapp, N. (2000). Therapeutic opportunities for muscarinic receptors in the central nervous system. **Journal of Medical Chemistry.** 43(23): 4333-4353.

Flicker, C., Ferris, S. H. and Serby, M. (1992). Hypersensitivity to scopolamine in the elderly. **Psychopharmacology Berlin.** 107(2-3): 437-441.

Foster, M. J., Dubey, A., Dawson, K. M., Stutts, W. A., Lal, H. and Sohal, R. S. (1994). Age- related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. **Proceeding of the National Acad emy of Sciences of the United States.** 93(10): 4765-4769.

Francis, P. T., Palmer, A. M., Snape, M. and Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. **Journal of Neurology, Neurosurgery & Psychiatry.** 66(2): 137-147.

Gallagher, M. and Colombo, P. J. (1995). Ageing: the cholinergic hypothesis of cogn itive decline. **Current Opinion Neurobiology.** 5(2): 161–168.

Gasparin, L., Rasshi, M., Binetti, G., Tra bucchi, M., Solerte, S. B., Alkon, D., Etche-berrigary, R., Gibson, G., Blass, J., Paletti, R. and Govoin, S. Peripheral markers in testing pathophysiological hypothesis and diagnosing Alzheimer's disease. (1998). **The Journal of the Federation of American Societies of Experimental Biology.** 12: 17-34.

Gray, S. L., Anderson, M. L., Crane, P. K., Breitner, J. C., McCormick, W., Bowen, J. D., Teri, L. and Larson, E. (2008). Antioxidant vitamin supplement use and risk of dementia or Alzheimer's disease in older adults. **Journal of American Geriatrics Society.** 56(2): 291-295.

Grundman, M. (2000). Vitamin E and Alzheimer's disease: the basis for additional clinical trials. **The American Journal of Clinical Nutrition.** 71(2): 630-636.

Hooper, L., Kroon, P. A., Rimm, E. B., Chon, J. S., Harvey, I., Cornu, K. A. L. and Ryder, J. J. (2008). Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. **American Journal of Clinical Nutrition.** 88(1): 38-50.

Howes, M. J. R. and Houghton, P. J. (2003). Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. **Pharmacology, Biochemistry and Behavior.** 75(3): 513-527.

Ichitani, Y., Okaichi, H., Yoshikawa, T. and Ibata, Y. (1992). Learning behaviour in chronic vitamin E-deficient and -supplemented rats: radial arm maze learning and passive avoidance response. **Behavioural Brain Research.** 51(2): 157-164.

Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T. and Thongnoi, W. (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. **Journal of Ethnopharmacology.** 89(2-3): 261-264.

Ingram, D. K., Joseph, J. A., Spangler, E. L., Roberts, D., Hengemihle, J. and Fanelli, R. J. (1994). Chronic nimodipine treatment in aged rats: analysis of motor and cognitive effects and muscarinic-induced striatal dopamine release. **Neurobiology Aging.** 15(1): 55-61.

Jalbert, J. J., Daiello, L. L. and Lapane, K. L. (2008). Dementia of the Alzheimer type. **Epidemiologic Reviews.** 30(1): 15-34.

Ji, X., Avula, B. and Khan, I. A. (2007). Quantitative and qualitative determination of six xanthones in *Garcinia mangostana* L. by LC–PDA and LC–ESI-MS. **Journal of Pharmaceutical and Biomedical Analysis.** 43(4): 1270-1276.

Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Bielinski, D., Martin, A., McEwen, J. J. and Bickford, P. C. (1999). Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. **The Journal of Neuroscience.** 19(18): 8114-8121.

Jung, H. A., Su, B. N., Keller, W. J., Mehta, R. G. and Kinghorn, A. D. (2006). Anti-oxidant xanthones from the pericarp of *Garcinia mangostana* (mangosteen). **Journal of Agricultural and Food Chemistry.** 54(6): 2077-2082.

Jung, M. and Park, M. (2007). Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. **Molecules.** 12(9): 2130-2139**.**

Khanbabaee, K. and van Ree, T. (2001). Tannins: classification and definition. **Natural Product Reports.** 18(6): 641-649.

Kim, H. K., Kim, M., Kim, S., Kim, M. and Chung, J. H. (2004). Effects of green tea polyphynol on cognitive and acetylcholinesterase activities. **Bioscience, Biotechnology, and Biochemistry.** 68(9): 1977-1979.

Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F. and Brouillard, R. (2003). Analysis and biological activities of anthocyanins. **Phytochemistry.** 64(5): 923-933.

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt Hale, B. and Joseph, J. A. (2010). Blueberry supplementation improves memory in older adults. **Journal of Agricultural and Food Chemistry.** 58(7): 3996-4000.

Kuli-Bilu, T., Katalinic, V., Dragovi-Uzelac, V., Ljubenkov, I., Kri, A., Dejanovi, B., Juki, M., Politeo, O., Pifat, G. and Milo, M. (2008). Antioxidant and acetylcholinesterase inhibiting activity of several aqueous tea infusions *in vitro*. **Food Technology and Biotechnology.** 46(7): 368-375.

Kumar, S., Kishore, K. and Vijender, S. (2009). Effects of mangifera indica fruit extract on cognitive deficits in mice. **Journal of Environmental Biology.** 30(4): 563-566.

Lee, Y. U., Lee, H. J., Won, M. H., Hwang, I. K., Kang, T. C., Lee, J. Y., Nam, S. Y., Kim, K. S., Kim, E., Cheon, S. H. and Sohn, H. S. (2004). Soy isoflavones improve spatial delayed matching-to-place performance and reduce cholinergic neuron loss in elderly male rats. **Journal of Nutrition.** 134(7): 1827-1831.

Lenta, B. N., Vonthron-Senecheau, C., Weniger, B., Devkota, K. P., Ngoupayo, J., Kaiser, M., Naz, Q., Choudhary, M. J., Tsamo, E. and Sewald, N. (2007). Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds from *Allanblackia monticola* and *Symphonia globulifera*. **Molecules.** 12(8): 1548-1557.

Lohachoompol, V., Srzednicki, G. and Craske, J (2004). The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. **Journal of Biomedicine and Biotechnology.** 5(5): 248-252.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. **The Journal of Biological Chemistry.** 193(1): 265-75.

Maclennan, K. M., Darlington, C. L. and Smith, P. F. (2002). The CNS effects of *Ginkgo biloba* extracts and *ginkgolide* B. **Progress in Neurobiology.** 67(3): 235-257.

Mahabusarakam, W., Proudfoot, J., Taylor, W. and Croft, K. (2000). Inhibition of lipoprotein oxidation by prenylated xanthones derived from mangostin. **Free Radical Research.** 33(5): 643-659.

Mahabusarkam, W. and Wiriyachitra, P. (1986). Chemical constituents of *Garcinia mangostana*. **Journal of Natural Products.** 50(3): 474-478.

Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. **Food Chemistry.** 100(4): 1409-1418.

Mancuso, C., Bates, T. E., Butterfield, D. A., Calafato, S., Cornelius, A., De Lorenzo, C., Kostova, A. T. and Calabrese, V. (2007). Natural antioxidants in Alzheimer's disease. **Expert Opinion on Investigational Drugs.** 16(12): 1921-1931.

Márquez-Valadez, B., Lugo-Huitrón, R., Valdivia-Cerda, V., Miranda-Ramírez, L. R., Pérez-De La Cruz, V., González-Cuahutencos, O., Rivero-Cruz, I., Mata, R., Santamaría, A. and Pedraza-Chaverrí, J. (2009). The natural xanthone α-mangostin reduces oxidative damage in rat brain tissue. **Nutritional Neuroscience.** 12(1): 35-42.

Matsumoto, K., Akao, Y., Yi, H., Ohguchi, K., Ito, T., Tanaka, T., Kobayashi, E., Iinuma, M. and Nozawa, Y. (2004). Preferential target is mitochondria in α-mangostin-induced apoptosis in human leukemia HL60 cells. **Bioorganic and Medicinal Chemistry.** 12(22): 5799-5806.

Maxine, M. J. and Papadakis, M. A. (2010). **Current medical diagnosis and treatment 2010.** (49th. ed.). LANGE CURRENT Series. United States: McGraw-Hill Medical.

Meydani, M. (1995). Vitamin E. **The Lancet.** 345(8943): 170-175.

Minussi, R. C., Rossi, M., Bologna, L., Cordi, L., Rotilio, D., Pastorea, G. M. and Durán, N. (2003). Phenolic compounds and total antioxidant potential of commercial wines. **Food Chemistry.** 82(3): 409-416.

Moongkarndi, P., Kosem, N., Kaslungka, S., Luanratana, O., Pongpan, N. and Neungton, N. (2004). Antiproliferation, antioxidation and induction of apoptosis by *Garcinia*

mangostana (mangosteen) on SKBR3 human breast cancer cell line. **Journal of Ethnopharmacology.** 90(3): 161-166.

Morris, M. C., Evans, D. A., Tangney, C. C., Bienias, J. L., Wilson, R. S., Aggarwal, N. T. and Scherr, P. A. (2005). Relation of the tocoperol forms to incident Alzheimer disease and to cognitive change. **The American Journal of Clinical Nutrition.** 81(2): 508-514.

Morris, R. G. M. (1981). Spatial localization does not depend on the presence of local cues. **Learning and Motivation.** 12(2): 239-260.

Morris, R. G. M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. **Journal of Neuroscience Methods.** 11(1): 47-60.

Morris, R. G. M. (2008). Morris water maze. **Scholarpedia.** 3(8): 6315.

Morris, R. G. M., Garrud, P., Rawlins, J. N. and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. **Nature.** 297(5868): 681-683.

Mukherjee, P. K., Kumar, V. and Houghton, P. J. (2007). Screening of Indian medical plants for acetylcholinesterase inhibitory activity. **Phytotherapy Research.** 21: 1142-1145.

Nakdook, W., Khongsombat, O., Taepavarapruk, P., Taepavarapruk, N. and Ingkaninan, K. (2010). The effects of *Tabernaemontana divaricata* root extract on amyloid beta-peptide 25-35 peptides induced cognitive deficits in mice. **Journal of Ethnopharmacology.** 130(1): 122-126.

Neurosoup. (2013). **Scopolamine** [on-line]. Available: http://neurosoup.com/scopolamine.

Oh, J. H., Choi, B. J., Chang, M. S. and Park, S. K. (2009). *Nelumbo nucifera* semen extract improves memory in rats with scopolamine-induced amnesia through the induction of choline acetyltransferase expression. **Neuroscience Letters.** 461(1): 41-44.

Okuda, T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. **Phytochemistry.** 66(7): 2012-2031.

Olivares, D., Deshpande, V. K., Shi, Y., Lahiri, D. K., Greig, N. H., Rogers, J. T. and Huang, X. (2012). N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. **Current Alzheimer Research.** 9(6): 746-758**.**

Omar Dev, R., Mohamed, S., Hambali, Z. and Samah, B. A. (2009). Comparison on Cognitive Effects of *Centella Asiatica* in Healthy Middle Age Female and Male Volunteers. **European Journal of Scientific Research.** 4: 553-565.

Orhan, I., Aslan, S., Kartal, M., Sener, B. and Baser, K. H. C. (2008). Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. **Food Chemistry.** 108(2): 663-668.

Orhan, I., Kartal, M., Naz, Q., Ejaz, A., Yilmaz, G., Kan, Y., Konuklugil, B., Sener, B. and Choudhary, M. L. (2007). Antioxidant and anticholinesterase evaluation of selected Turkish *Salvia* species. **Food Chemistry.** 103(4): 1247-1254.

Pakaski, M. and Kalman, J. (2008). Interactions between the amyloid and cholinergic mechanisms in Alzheimer’s disease. **Neurochemistry International.** 53(5): 103-111.

Papandreou, M. A., Dimakopoulou, A., Linardaki, Z. I., Cordopatis, P., Klimis-Zacas, D., Margarity, M. and Lamari, F. N. (2009). Effect of a polyphenol-rich wild

blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. **Behavioural Brain Research.** 198(2): 352-358.

Pardo Andreu, G. L. P., Maurmann, N., Reolon, G. K., de Farias, C. B., Schwartsmann, G., Delgado, R. and Roesler, R. (2010). Mangiferin, a naturally occurring glucoxilxanthone improves long-term object recognition memory in rats. **European Journal of Pharmacology.** 635(1-3): 124-128.

Pasqua, G., Monacelli, B., Mulinacci, N., Rinaldi, S., Giaccherini, C., Innocenti, M. and Vinceri, F. F. (2009). The effect of growth regulators and sucrose on anthocyanin production in *Camptotheca acuminata* cell cultures. **Plant Physiology and Biochemistry.** 43(3): 293-298.

Paxion, G. and Watson, C. (2010). **The rat brain in sterreotaxic coordinates.** California Elsevier.

Pedraza-Chaverri, J., Reyes-Fermin, L. M., Nolasco-Amaya, E. G., Orozco-Ibarra, M., Medina-Campos, O. N., Gonzalez-Cuahutencos, O., Rivero-Cruz, I. and Mata, R. (2009). ROS scavenging capacity and neuroprotective effect of α-mangostin against 3-nitropropionic acid in cerebellar granule neurons. **Experimental and Toxicologic Pathology.** 61(5): 491-501.

Perez-Perez, E. and Rodriguez-Malaver, A. J. (2005). Antioxidant capacity of Kraft black liquor from the pulp and paper industry. **Journal of Environmental Biology.** 26(3): 603-608.

Placzek, A. N., Zhang, T. A. and Dani, J. A. (2009). Nicotinic mechanisms influencing synaptic plasticity in the hippocampus. **Acta Pharmacologica Sinica.** 30(6): 752-760.

Pothitirat, W., Chomnawang, M. T., Supabphol, R. and Gritsanapan, W. (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. **Fitoterapia.** 80(7): 442-447.

Sarkaki, A., Farbood, Y. and Badavi, M. (2007). The effect of grape seed extract (GSE) on spatial memory in aged male rats. **Pakistan Journal of Medical Sciences.** 23(4): 561-565.

Schmitt-Schillig, S., Schaffer, S., Weber, C. C., Eckert, G. P. and Muller, W. E. (2005). Flavonoids and the aging brain. **Journal of Physiology and Pharmacology.** 56(1): 23-36.

Sethi, P., Jyoti, A., Hussain, E. and Sharma, D. (2009). Curcumin attenuates aluminium-induced functional neurotoxicity in rats. **Pharmacology, Biochemistry and Behavior.** 93(1): 31-39.

Singleton, V. L. and Rossi, J. A. Jr. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. **American Journal Enology and Viticulture.** 16(3): 144-158.

Socci, D. J., Crandall, B. M. and Arendash, G. W. (1995). Chronic antioxidant treatment improves the cognitive performance of aged rats. **Brain Research.** 693(1-2): 88-94.

Spencer, J. P. E. (2009). Flavonoids and brain health: multiple effects underpinned by common mechanisms. **Genes & Nutrition.** 4(4): 243-250.

Stanojevic, L., Stankovic, M., Nikolic, V., Nikolic, L., Ristic, D., Canadanovic-Brunet, J. and Tumbus, V. (2009). Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. **Sensor**. 9(7): 5702-5714.

Suksamrarn, S., Suwannapoch, N., Phakhodee, W., Thanuhiranlert, J., Ratananukul, P., Chimnol, N. and Suksamrarn, A. (2003). Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. **Chemical & Pharmaceutical Bulletin.** 51(7): 857-859.

Sunderland, T., Tariot, P. N., Cohen, R. M., Weingartner, H., Mueller, E. A. and Murphy, D. L. (1987). Anticholinergic sensitivity in patients with dementia of the Alzheimer type and age-matched controls: a dose-response study. **Archives of General Psychiatry.** 44(5): 418-426.

Thimmappa, S., Anekonda, P. and Reddy, H. (2005). Can herbs provide a new generation of drugs for treating Alzheimer's disease? **Brain Research Reviews.** 50(2): 361-376.

Transdermscop. (2013). Transderm Scop patch prescribing information [on-line]. Available: .http://www.transdermscop.com/prescribing-information.htm.

Uabundit, U., Wattanathorn, J., Mucimapura, S. and Ingkaninan, K. (2010). Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease model. **Journal of Ethnopharmacology.** 127(1): 26-31.

Veerendra Kumar, M. H. and Gupta, Y. K. (2002). Effect of different extracts of *Centella asiatica* on cognition and markers of oxidative stress in rats. **Journal of Ethnopharmacology.** 79(2): 253-260.

Vinutha, B., Prashanth, D., Salma, K., Sreeja, S. L., Pratiti, D., Padmaja, R., Radhika, S., Amit, A., Venkateshwarlu, K. and Deepak, M. (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. **Journal of Ethnopharmacology.** 109(2): 359-363.

Wang, L. H. and Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. **Cancer Letters.** 269(2): 281-290.

Weecharangsan, W., Opanasopit, P., Sukma, M., Ngawhirunpat, T., Sotanaphun, U. and Siripong, P. (2006). Antioxidative and neuroprotective activities of 37 extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.). **Medical Principles and Practice.** 15(4): 281-287.

Weinreb, O., Mandel, S., Amit, T. and Youdim, M. B. H. (2004). Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases**. Journal of Nutritional Biochemistry.** 15(9): 506-516.

White, P. F., Tang, J., Song, D., Coleman, J. E., Wender, R. H., Oqunnaike, B., Slonninsky, A., Kapu, R., Shah, M. and Webb, T. (2007). Transdermal Scopolamine: An Alternative to Ondansetron and Droperidol for the Prevention of Postoperative and Postdischarge Emetic. **Symptoms. Anesthesia and Analgesia.** 104(1): 92-96.

Wikipedia. (2013). **Cerebral cortex** [on-line]. Available: http://en.wikipedia.org/wiki/Cerebral_cortex.

Wikipedia. (2013). **Scopolamine** [on-line]. Available: http://en.wikipedia.org/wiki/Scopolamine.

Wikipedia. (2013). **Vitamin E** [on-line]. Available: http://en.wikipedia.org/wiki/Vitamin_E.

Williams, P., Ongsakul, M., Proudfoot, J., Croft, K. and Beilin, L. (1994). Mangostin inhibits the oxidative modification of human low density lipoprotein. **Free Radical Research.** 23(2): 175-184.

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. **Progress in Neurobiology.** 37: 475-524.

Woolf, N. J. (1996). Global and serial neurons form a hierarchically-arranged interface proposed to underlie learning and cognition. **Neuroscience.** 74(3): 625-651.

World Health Organization. (2010). **Dementia** [On-line]. Available: http://www.who.int/mediacentre/factsheets/fs362/en/.

Xu, J., Rong, S., Xie, B., Sun, Z., Zhang, L., Wu, H., Yao, P., Zhang, Y. and Liu, L. (2009). Procyanidins extracted from the lotus seed pod ameliorate scopolamine-induced memory impairment in mice. **Phytotherapy Research.** 23(12): 1742-1747.

Yang, W., Shim, K., Choi, M., Park, S., Choi, B., Chang, M. and Park, S. (2008). Novel effects of *Nelumbo nucifera* rhizome extract on memory and neurogenesis in the dentate gyrus of the rat hippocampus. **Neuroscience Letter.** 443: 104-107.

Youdin, K. A. and Joseph, J. A. (2001). A possible emerging role of phytochemicals in improving age-related neurological dysfunction a multiplicity of effects. **Free Radical Biology & Medicine.** 38(6): 583-594.

Youdin, K. A., Martin, A. and Joseph, J. A. (2000). Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. **Free Radical Biology & Medicine.** 29(1): 51-60.

Yu, L., Zhao, M., Yang, B., Zhao, Q. and Jiang, Y. (2007). Phenolics from hull of Garcinia mangostana fruit and their antioxidant activities. **Food Chemistry.** 104: 176-181.

Zadernowski, Z., Czaplicki, S. and Naczk, M. (2009). Phenolic acid profiles of mangosteen fruits (Garcinia mangostana). **Food Chemistry.** 112(3): 685-689.

Zemishlany, Z. and Thorne, A. E. (1991). Anticholinergic challenge and cognitive functions: a comparison between young and elderly normal subjects. **The Israel Journal of Psychiatry and Related Science.** 28(3): 32-41.

Zhang, L., Xu, J. Q., Rong, S., Xie, B. J., Sun, Z. D., Zhang, Y. J. and Liu, L. G. (2009). The mixture of procyanidins extracted from the lotus seed pod and 38 bilobalide ameliorates scopolamine-induced memory impairment in mice. **Neuroscience Bulletin.** 25(4): 203-208.

Zubenko, G. S., Moossy, J., Martinez, A. J., Rao, G. R., Kopp, U. and Hanin, I. (1989). A brain regional analysis of morphologic and cholinergic abnormalities in Alzheimer's disease. **Archives of Neurology.** 46(6): 634-8.

APPENDICES

APPENDIX A

PREPARATION OF TISSUE HOMOGENATE

PREPARATION OF TISSUE HOMOGENATE

Chemicals:

- Di-sodium hydrogen phosphate anhydrous (Na_2HPO_4, BDH Ltd., UK)
- Sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4.H_2O$, BDH Ltd., UK),
- Triton-X ($C_{34}H_{62}O_{11}$, Acros, New Jersey, USA)
- Sodium hydroxide anhydrous pellets (NaOH, Carlo Erba Reagents, France),
- Hydrochloric acid (HCl, Carlo Erba Reagents, France)

Solutions:

1 M HCl

Stock solutions: 37% HCl

Preparation for 1 M HCl: mixed 9.85 ml of 37% HCl with DDD water.

1 M NaOH

Preparation: dissolved 3.997 g of NaOH in DDD water and then adjusted the final volume to 100 ml

1 M phosphate buffer solution, pH 7.4

Dissolved 10.65 g of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4; BDH Ltd., UK) and 3.968 g of sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4.H_2O$; BDH) in 80 ml of double deionized distilled (DDD) water.

This solution was stirred on a hot plate magnetic stirrer (VELP Scientifica, Europe) at 50 to 60 °C for 1 h, left to be cool down to room temperature and then adjusted volume to 100 ml with DDD water in a volumetric flask. This solution was adjusted to pH 7.4 with 1 M HCl or 1 M NaOH.

0.1 M phosphate buffer solution, pH 7.4 (500 ml)

Mixed 50 ml of 1 M phosphate buffer (pH 7.4) with DDD water and then adjusted the final volume to 500 ml. This solution was then adjusted to pH 7.4 with 1 M HCl or 1 M NaOH

0.1 M phosphate buffer solution containing 1% Triton-X 100, pH 7.4 (1000 ml)

Mixed 10 ml of Triton-X 100 with 0.1 M phosphate buffer solution and then adjusted the final volume to 1000 ml. This solution was then adjusted to pH 7.4 with 1 M HCl or 1 M NaOH.

The preparation of tissue homogenates was performed by modified method of Chattipakorn et al. (2007) and Papandreou et al. (2009). The brain tissues were weighed and then homogenized (Ultrasonic, Sonic & Material, Inc, USA) with ice cold 10% of 0.1 M phosphate buffer (pH 7.4) containing 1% Triton-X 100 at 15,000 rpm for 15 min at 4 °C. The brain homogenates were stored at -80 °C.

APPENDIX B

ASSAY FOR ACETYLCHOLINESTERASE ACTIVITY

ASSAY FOR ACETYLCHOLINESTERASE ACTIVITY

Chemicals:

- Di-sodium hydrogen phosphate anhydrous (Na_2HPO_4, BDH Ltd., UK)
- Sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4.H_2O$, BDH Ltd., UK),
- 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB: $C_{14}H_8N_2O_8S_2$, Sigma-Aldrich; St. Louis, USA)
- Acetylthiocholine iodide (ATCI, Sigma-Aldrich; St. Louis, USA)

Solutions:

0.1 M phosphate buffer solution, pH 7.4 (500 ml)

Stock solution: 1.0 M phosphate buffer solution, pH 7.4

Preparation of 0.1 M phosphate buffer solution (pH 7.4): mixed 50 ml of 1 M phosphate buffer (pH 7.4) with DDD water and then adjusted the final volume to 500 ml. This solution was then adjusted to pH 7.4 with 1 M HCl or 1 M NaOH.

0.1 mM 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), (300 ml)

Preparation of stock solution (1.0 mM DTNB, 50 ml): dissolved 1.98 g of DTNB in 0.1 M phosphate buffer solution (pH 7.4) and then adjusted the final volume to 50 ml.

Preparation of 0.1 mM DTNB: mixed 3 ml of stock with 0.1 M phosphate buffer solution (pH 7.4) and then adjusted the final volume to 300 ml.

1 mM acetylthiocholine iodide (ATCI), (30 ml)

Preparation of stock solution (1.0 mM DTNB, 30 ml): dissolved 0.86 g of ATCI in 1.0 M phosphate buffer solution pH (pH 7.4) and then adjusted the final volume to 30 ml.

Preparation of 0.1 mM ATCI: mixed 0.3 ml of stock with 0.1 M phosphate buffer solution pH (pH 7.4) and then adjusted the final volume to 30 ml.

Procedures:

Determination of AChE activity was based on colorimetric method adapted from the methods of Ellman et al. (1961), Chattipakorn et al. (2007) and Nakdook et al. (2010). The assay was performed in 96-well plate. The mixture consisted of:

- 50 µl of brain homogenates,
- 25 µl of 0.1 M phosphate buffer (pH 7.4),
- 125 µl of 0.1 mM 5,5′-dithiobis (2-nitrobenzoic acid),
- 25 µl of 1 mM acetylthiocholine iodide,

The absorbance of the assay mixtures was measured by Benchmark Plus microplate Spectrophotometer (Benchmark Plus, BIO-RAD, Japan) at wavelength of 405 nm and monitored over period of 6 min. All determinations were performed in triplicate. AChE activity was assessed by following formula:

$$R = [\Delta A / (1.36 \times 10^4)] \times [1 / (Co \times \text{mg of protein})]$$

$$R = \Delta A / [1.36 \times 10^4 \times (50/225) \times \text{mg of protein}]$$

R = rate of enzyme activity (expressed as mole of acetylcholine iodide hydrolyzed/ minute/ mg of protein),

ΔA = the change in absorbance per minute,

Co = original concentration of tissue (mg/ml) 50/225 is volume correction 1.36×10^4 is the extinction coefficient of the yellow product.

APPENDIX C

DETERMINATION OF PROTEIN

DETERMINATION OF PROTEIN

The measurement of protein was adapted from the method of Lowry et al. (1951).

Chemicals:

- Copper sulfate ($CuSO_4.5H_2O$, Sigma-Aldrich; St. Louis, USA)
- Sodium tartate ($Na_2C_4H_4O_6.2H_2O$, Sigma-Aldrich; St. Louis, USA)
- Sodium azide (BD, Ltd., UK),
- Sodium hydroxide (NaOH, Sigma-Aldrich; St. Louis, USA),
- Sodium carbonate (Na_2CO_3, Sigma-Aldrich; St. Louis, USA)
- Sodium dodecyl sulfate (SDS: $C_{12}H_{25}NaO_4S$, BDH, Ltd., UK)
- Folin-Ciocatteau reagent (Merck Millipore, Germany)
- Bovine Serum Albumin (BSA, Sigma-Aldrich; St. Louis, USA)

Reagents:

1. Solution A:

- Copper sulfate 0.15 g
- Sodium tartate 0.30 g
- Sodium azide 0.05 g

Dissolved these chemical in DDD water and made up a final volume to 250 ml and stored in refrigerator.

2. Solution B:

- Sodium hydroxide 2 .0 g
- Sodium carbonate 8.0 g
- Sodium dodecyl sulfate 1.0 g

Dissolved these chemicals in DDD water, adjust the final volume to 250 ml, and stored at room temperature.

3. Solution C:

Diluted the 2 N Folin-Ciocatteau reagent with DDD water (1:20). Stored in an amber bottle at room temperature.

4. Standard protein solution:

Dissolved bovine serum albumin (BSA) with DDD water (0, 250, 500, 1000, 2000 and 4000 µg/ml).

Procedures:

1. Pipetted 30 µl aliquots of samples and standards into triplicate well of flat-bottom 96 well plate (Biofil., Japan).

2. Freshly mixed solution A and solution B (1:3). Added 100 µl of this mixed solution to each well and incubated at room temperature for 60 minutes.

3. Added 150 µl of solution C in into triplicate well plate

Pipetted the solution into each tube as followed:

	Sample (µl)	Standard (µl)	Bank (µl)
Standard	-	30	-
Sample	30	-	-
A+B	100	100	100
C	150	150	150
0.1 M PB	-	-	30

PB = phosphate buffer solution, pH 7.4

4. Mixed and incubated at room temperature for 30 minutes.

5. Read optical density (O.D) at 650 nm by Benchmark Plus Microplate Spectrophotometer (Benchmark Plus, BIO-RAD, Japan).

Calculation:

$$\Delta A_{650\ nm}\ \text{Standard} = A_{650\ nm}\ \text{Std} - A_{650}\ \text{Blank}$$

Plotted the $\Delta A_{650\ nm}$ standard againt protein concentration on the standard graph.

$$\Delta A_{650\ nm}\ \text{Sample} = A_{650\ nm}\ \text{Sample} - A_{650}\ \text{Blank}$$

Determined the mg protein from the standard curve.

CURRICULUM VITAE

Name	Miss NAIYANA NONTAMART
Date of Birth	16 July 1983
Place of Birth	Kalasin, Thailand
Education	
2008- Present	M.Sc. (Environmental Biology) Suranaree University of Technology, Nakon Ratchasima, Thailand.
2002-2005	B.Sc. (Animal Production Technology) Suranaree University of Technology, Nakon Ratchasima, Thailand.
Work Experience	
2008- Present	Teaching Assistant in School of Biology, Suranaree University of Technology, Nakon Ratchasima, Thailand.