Medicinal Plants of Northern Thailand for the Treatment of Cognitive Impairment in the Elderly

By: Lisa C. Offringa

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Supervisor: Dr. Michael J. Balick, Ph.D.

Abstract

Dementia is a progressive neurological disease affecting memory and behavior. The diagnosis of dementia is increasing exponentially worldwide and with it the potential risk for a severe social and economic burden of caring for an increasing debilitated elderly population. Cognitive impairment, and especially memory loss, can be the first indication of dementia. This study documents the treatment of cognitive impairment in the elderly by Thai traditional healers in northern Thailand using medicinal plants. Interviews were conducted from 2008-2012 to investigate the etiology of dementia in Thai Traditional Medicine and identify plants used to treat memory loss in the pharmacopeia of northern Thailand. Multi-plant herbal formulas from ancient manuscripts of the *Lanna* Kingdom were obtained from Thai traditional healers. These formulas were analyzed through ethnobotanical inquiry for plant species with potential bioactivity against memory loss in the elderly. Crude extracts of eleven selected plant species were screened through four *in vitro* bioassays to measure their general antioxidant activity, total phenolic content and acetylcholinesterase inhibition activity. Of these eleven species, five plants exhibited high levels of acetylcholinesterase inhibition activity: *Cinnamomum bejolghota* (Buch-Ham.) Sweet, *Dracaena loureiroi* Gagnep., *Diospyros decandra* Lour., *Jasminum sambac* (L.) Aiton., and *Eurycoma longifolia* Jack. One plant, *Cinnamomum bejolghota* demonstrated high

activity in all four *in vitro* bioassays. Three different doses of an ethanol extract of *Cinnamomum bejolghota* were evaluated for their memory enhancing ability on *in vivo* rat behavioral models and enzymatic marker tests on their brain tissue. Results from the Morris Water Maze navigation task showed that the two highest dosages of the extract produced significant memory improvement after two weeks of treatment. Enzymatic marker analyses in three portions of the rats' brains associated with memory formation, the hippocampus, striatum and cortex, showed significant acetylcholinesterase inhibition activity thereby increasing acetylcholine levels in these parts of the brain. This study identified a plant with memory enhancing activity that, with further study, could help to alleviate the suffering of those afflicted with age-related memory decline. Ethnopharmacological studies support the viability of traditional medicine to treat diseases that are relevant in modern society.

To my grandmother, Dorthy Griffith Offringa,

for teaching me to see the natural world with my heart.

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My motivation to realize my dream of obtaining a Ph.D. emanates from a deep love of the natural world, a desire to explore its gifts and a hope to protect it. I often say the lesson of a Ph.D. is not in the subject matter, but in the process. I feel the utmost gratitude for those who encouraged and guided me through this process.

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ethnopharmacological project, and was generous with his knowledge and wisdom as I navigated some challenging situations. Dr. Liesl Jones provided her knowledge of the brain and *in vivo* studies to my work. Her advice on my project was always rational and intelligent. She is an inspirational and accomplished person, and gives sage insight on being a woman scientist. Dr. Raj Kalapatapu contacted Dr. Balick when looking for natural treatments for geriatric psychiatry. His expertise in this area was invaluable to my project design and the interpretation of my results.

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My studies in Thailand spanned over four years and many people there made this possible for me. Dr. Weerachai Nanakorn was my original contact at the Queen Sirikit Botanic Garden, and helped to facilitate my research permits and collaborations. Dr. Konganda Chayamarity was my official collaborator at the Queen Sirikit Botanic Garden. Dr. Santi Wattana assisted me with acquiring my research permits through the National Research Council of Thailand (NRCT), and helped guide me through that process. At the NRCT Miss Pannee Panyawattanaporn from the Office of International Affairs and her assistants, Jeeranan and Surin helped me with my reports and paperwork. Ms. Dussadee Siamhan at Thailand's National Centre for Genetic Engineering and Biotechnology (Biotec) facilitated my short-term training agreement, and provided me with the documentation I need to begin the anthropology portion of my research. Dr. Jintanaporn Wattanathorn, my official collaborator at Khon Kaen University provided me the tools and training for conducting my *in vivo* studies. The students in her laboratory, Namwan, Thipkeaw, Elle, Nanan, Mee, and Sara treated me like family. They helped me in the lab and made sure I enjoyed my time in Khon Kaen. The technicians in her lab assisted me with my research and kept me *jai yen,* or cool hearted, when I would feel stressed. The gratitude I feel towards Ajan Panee Sirisa-ard is infinite. She was my contact at Chaing Mai University, and she introduced me to many of the healers I interviewed. She opened her laboratory to me to conduct my *in vitro* bioassays and treated me like her student, and like family. She exemplifies the kindness found in Thai culture and the *Mettā* of Buddhism. The students in her lab were wonderful: Jum helped me in the laboratory, Jack assisted me with the equipment I needed, and Dr. Wisinee Chanmahasathien advised me on my bioassay techniques.

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CHAPTER 1 – INTRODUCTION AND BACKGROUND

PROJECT DESCRIPTION

This project identified plants used in Thai Traditional Medicine with therapeutic action against the symptoms of cognitive impairment in the elderly. Cognitive impairment, especially severe age-related memory decline, can be an early indicator of dementia. This study applied an ethnopharmacological approach with three parts: anthropological enquiry into Thai Traditional Medicine; collection and botanical identification of plant medicines used to treat age-related cognitive decline; and chemical and pharmacological testing of the plant material to corroborate Thai traditional medical theory. The research conducted for this study resulted in documenting traditional knowledge of medicinal plants and confirmed the relevance of this knowledge for treating health issues in contemporary society.

Thai Traditional Medicine characterizes elderly dementia differently than western medicine. The theoretical foundation of the eastern medical system conflicts with the western construct of symptom-based treatments. This project aimed to elucidate this difference and interpret the memory loss associated with dementia, a western disease construct, using Thai traditional medical philosophies. The knowledge base for this project was from interviews with traditional Thai healers in northern Thailand who are aware of plants they believe can nourish the brain and enhance memory. These healers reported individual plants, as well as plant formulas, used to increase memory and formulas for diseases established as preconditions to a diagnosis of dementia.

The biological activity of the plants in these formulas was investigated using *in vitro* and *in vivo* biological assays. Selected plants, as suggested by the interviewed healers, and formulas

were tested for their potential as acetylcholinesterase inhibitors and anti-oxidizing agents. Acetylcholinesterase is the enzyme that breaks down acetylcholine, an important neurotransmitter for memory formation. By inhibiting the enzyme, there is more of this neurotransmitter for the brain to use to form memories and execute functions based on them (Ringman 2006). Elderly dementia is commonly characterized by the degeneration of brain tissue. Anti-oxidants can help to protect the brain from this degeneration and supplement the increased amount of acetylcholine in the brain (Han et al. 2007). *In vitro* colorimetric bioassays were used to analyze crude extracts of these plants and formulas to determine one plant for testing *in vivo.* Rats treated with an extract of the selected plant were tested on *in vivo* behavioral models and their harvested brain tissue analyzed by enzymatic marker assays. Results from these tests demonstrated the plant extract's memory enhancing ability.

OBJECTIVES, RESEARCH QUESTIONS AND HYPOTHESIS

OBJECTIVES OF RESEARCH

Documenting traditional knowledge is imperative in preserving both biological and cultural diversity (Hofmann 1995; Naranjo 1995). In Thailand, medicinal plant knowledge was transmitted orally through generations of healers or written on palm leaf manuscripts transcribed by monks in the ancient *Lanna* language. Traditional healers in northern Thailand have expressed concern that this information is at risk of disappearing, as the younger generation is not interested in learning about plants and carrying on these traditional practices (Mulholland 1979). Additionally in the Indo-Burma region where I conducted my research, biological diversity is severely threatened by industrial development and environmental degradation (Hirsch 1996).

The objectives of this project were to study and document the medicinal plant knowledge of traditional healers in northern Thailand regarding neurodegenerative memory diseases like dementia, and corroborate its effectiveness using scientific methodology. My partnerships with respected botanical and academic institutions could provide further opportunities for international collaboration between scientists and institutions. This project will contribute to preserving traditional knowledge and medical practices utilizing plants in northern Thailand.

The objectives of this dissertation project were to:

I. Conduct semi-structured interviews to investigate and document knowledge held by traditional healers in northern Thailand regarding medicinal plants used to enhance memory and treat dementia-like disorders.
II. Identify and collect the plants used by traditional healers in northern Thailand for prevention and treatment of the symptoms of cognitive impairment in the elderly.
III. Test selected crude plant extracts on *in vitro* biological assays and *in vivo* on rats using behavioral models and testing their brain tissue for levels of enzymatic markers.
IV. Provide opportunities for international collaboration between the United States and Thailand on an individual and institutional level.

RESEARCH QUESTIONS

The research questions of this study were:

I. How does Thai Traditional Medicine interpret dementia as a disease within its theoretical paradigm?
II. Are there plants in Thai Traditional Medicine that treat memory loss in the elderly population?
III. Can applying modern medical testing techniques substantiate these plants' suggested effect on memory enhancement?

HYPOTHESIS

The hypotheses of this study were:

I. Traditional healers in northern Thailand recognize the symptoms of early elderly cognitive impairment and use medicinal plants to treat this memory decline.

II. Plants identified by Thai traditional healers used to treat cognitive impairment can be tested on *in vitro* bioassays to provide preliminary evidence of efficacy when compared to a negative control and a pharmaceutical positive control.

III. The memory enhancing activity of an ethanol extract of *Cinnamomum bejolghota (*Buch-Ham.) Sweet can be substantiated by testing it on rats in *in vivo* versus an untreated

control group, a group given the liquid vehicle for the plant extract and a conventional pharmaceutical treatment.

RESEARCH OVERVIEW

For this study, I conducted seventeen months of fieldwork around Chiang Mai Province interviewing traditional healers and collecting medicinal plants after receiving my research permits. The National Research Council of Thailand's Foreign Affairs office granted my permits in October of 2009 (Registration number 124/52). Interviews with traditional healers were conducted with the approval of the Institutional Review Board of The Graduate Center at The City University of New York's Office of Research and Sponsored Programs and U.S. Department of Health and Human Services (IRB number: 08-05-1563; PI: Lisa C. Offringa, see Appendix 5 for these documents). I collaborated with the Queen Sirikit Botanic Garden for the fieldwork portion of this research. All fieldwork conducted and data collected followed the Guidelines of Professional Ethics as put forth by the Society for Economic Botany (SEB 1995), The International Society for Ethnobiology (ISE 2008), the Convention on Biological Diversity, and national law of the Kingdom of Thailand with respect to traditional knowledge.

All of the biological assays for this project were conducted in laboratories in Thailand over a period of fourteen months during 2011 and 2012. My research permits did not allow me to bring any plant material back to the United States of America. Therefore, I analyzed the collected plant material at Chiang Mai University's Faculty of Pharmacy where the selected plant species were screened for antioxidant activity, total phenolic content and acetylcholinesterase inhibition. One plant was selected to test on rats *in vivo* at Khon Kaen University's Faculty of Medicine. The rats treated with the plant extract were analyzed using

behavioral models designed to test memory. Additional tests measured acetylcholinesterase activity and free radical scavenging on three parts of the harvest brain tissue of the rats: the hippocampus, the striatum and the cortex. The Ethics Committee of Khon Kaen University, Faculty of Medicine pre-approved the testing protocol.

BACKGROUND

INTRODUCTION

Many pharmaceuticals are derived from the plant kingdom. The basis of these remedies is often ethnobotanical investigation, whether through vigorous research, or by simply observing people, culture or animals (Balick and Cox 1997; Johns, 2000; Homstedt and Bruhn 1995). One of the first plant-based medicines in the western world was *Cinchona* bark from the *Cinchona officinalis* L. tree in South America. This bark became extremely valuable in Spain after it became famous as a potent cure for fever, but only after the Spanish explorers documented its use by the local Andean people (King 1996). Some contemporary remedies also originating from plants are artemesinin from the *Artemesia* species as a common treatment for malaria, *Digitalis purpurea* L. for heart conditions, and *Catharanthus roseus* (L.) G. Don to treat leukemia (Naranjo 1995).

There are numerous other examples of plants used by indigenous peoples sparking the interest of naturalists or researchers, which then led to the "discovery" of a new medicine. The global diversity of plants shows the range of potential chemical molecules and the possibility of bioactivity that is beneficial to humankind (Ramawat et al. 2009). Countries with an enduring culture and wide variety of plants typically have an extensive herbal pharmacopoeia. If research on medicinal plants is performed in an ethical and scientifically valid method then "biodiversity

prospecting can contribute greatly to environmentally sound development" (Walter et al. 1996), and potentially distribute beneficial medical treatment to people worldwide.

This background section provides the foundation for my dissertation study. It offers pertinent information to establish a contextual basis for this thesis. It begins with a brief history of Thai Traditional Medicine and an overview of the literature available on Thai Traditional Medicine (TTM) to illustrate the existing documentation of Thai medical theory and information on medicinal plants used to treat disease. One of the goals of this project is to record the knowledge of traditional healers about Thai Traditional Medicine, specifically focusing on the brain and memory. It is important to know the history of the medical system being studied and what documents have been archived in the past to determine a frame of reference. A description of age-related cognitive decline, dementia, and the most common manifestation of it, Alzheimer's disease follows. The investigation of dementia in the elderly population is rapidly changing, and there are evolving theories to explain the etiology of the disease. These theories are outlined to clarify the symptoms, the disease and how it affects the brain. A discussion is then presented on the prevalence of dementia, primarily Alzheimer's disease, in Thailand and the most common treatments offered to patients by western medical hospitals and doctors. Finally, this chapter finishes with a description of Thailand, the study area of Chiang Mai, the history as the former *Lanna* Kingdom, and the people who inhabitant this region in northern Thailand.

HISTORY OF THAI TRADITIONAL MEDICINE

While there is earlier evidence of plant-based remedies used to treat health conditions, the documentation of Thai Traditional Medicine formally began during the *Sukothai* period between 1238 and 1377. King *Narai* the Great who ruled between 1656-1688, during the *Ayutthaya*

period, organized all of the medicinal plant recipes into a book called the *Tamra Phra Osod Phra Narai* (Archanuparp 1987). Once the capital of Thailand was established in Bangkok in 1782, theories of Thai Traditional Medicine and formulas were engraved on the walls of two temples, but primarily *Wat Phrachettuphon* (*Wat Pho*) where Thai massage is depicted in diagrams of the pressure points on the body, and statues portraying Thai exercise postures (Mulholland 1979).

Western medicine came to Thailand in the late 1800's through the influx of missionaries and medical doctors. The first western hospital opened, and by the early 1900's, the government abolished the practice of Thai Traditional Medicine, as it was not compatible with the new, modern system of western medicine. Traditional healers could not practice and their licensing became unregulated. During this time, Thai Traditional Medicine became the health care system of the poor and uneducated who primarily lived in rural areas (Chokevivat and Chuthaputti 2006). Currently, there is indistinct divide between Thai people who use traditional medicine versus western medicine. People in rural areas do not have access to modern hospitals and rely mainly on traditional medicine practices, but even educated, urban Thai people use home remedies, herbal medicine and ascribe to folklore regarding their health (Mulholland 1979).

A movement toward revitalizing Thai Traditional Medicine started in 1978 when the Thai Ministry of Public Health acted on a request by the World Health Organization to use their traditional medical system and medicinal plants as part of their public health services for their "Health for All" initiative (WHO 1978). Between 1978 and 1999, a joint effort between traditional healers, the Thai government and dedicated non-governmental organizations restored Thai Traditional Medicine. The Thai government officially revived Thai Traditional Medicine in 1999 by the passing of the Thai Traditional Medicine and Local Knowledge Protection and

Promotion Act. The government supported this act by developing a curriculum for teaching and learning TTM, and a way to certify practitioners (National Assembly of Thailand 1999).

This movement toward regulating Thai Traditional Medicine required the field be officially defined. In 1999, the National Assembly of Thailand put forth the following definition:

> "the medical processes dealing with the examination, diagnosis, therapy, treatment, or prevention of diseases, or promotion and rehabilitation of the health of humans or animals, midwifery, Thai massage, as well as the preparation, production of Thai traditional medicines and the making of devices and instruments for medical purposes. All of these are based on the knowledge or textbooks that were passed on and developed from generation to generation."

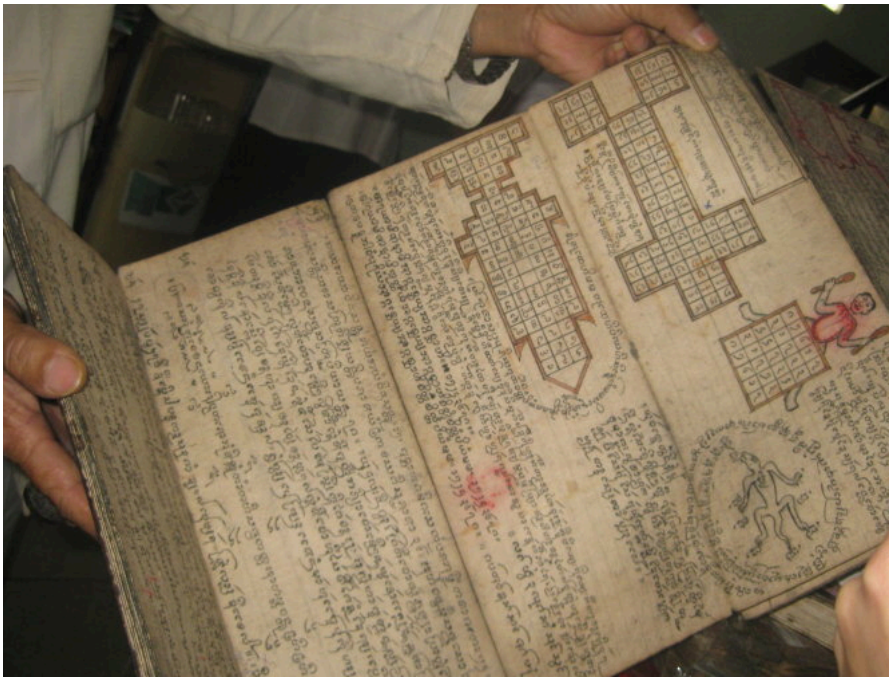
The theories, plant materials and their applications all fall under this definition of Thai Traditional Medicine. A clear description is imperative to protect Thai traditional knowledge and the intellectual property of the Thai people.

The varied history of Thai Traditional Medicine exposes a divide between healers practicing the documented "royal tradition" and the rural folk healers who still keep their formulas secret (Brun and Schumacher 1994). Traditional folk medicine in northern Thailand follows many of the same principles as the "royal tradition", but the infusion of local spiritual beliefs also shape traditional medicine in the North. The local plants used by the Northern folk healers are the most concrete difference in the two traditions.

The influence of Buddhism is apparent in the theory and practice of Thai Traditional Medicine (Subcharoen 2001). The language of Buddhism and the original language of the medical system is *Pali*, which is similar to Sanskrit. *Pali* is an ancient language used for religious purposes by monks and scholars, and is different from the modern Thai script used today. Medical theories were written in *Pali* on the *bap saa*, or manuscripts made on paper from

the pulp of the mulberry tree (*Broussonetia papyrifera* (L.) L'Hér. ex Vent.) (pers. comm. Mor B 2009) or from *Streblus asper* Lour. (Manosroi 2011) (Figure 1.1). Other herbal formulas were written on *bai lan* or "leaf of the *lan* palm tree" (*Corypha lecomtei* Becc. ex Lecomte). These were cut into long strips and incised with *Pali* lettering then wiped with dirt or soot to bring out the letters (Salguero 2003). These *bai lan* and *bap saa* were carried with the monks from India into Thailand and are consulted today for their ancient knowledge. The transmission of medical knowledge in Thailand is through both written and oral communication. The formulas included in the ancient manuscripts could be passed down through the generations, but the theory and practice of medicine was taught from teacher to pupil using the oral tradition. Many times these teachings were held in the monastery as the monks could read *Pali* and were allowed to keep medicines as part of their few belongings (Brun 2003).

Figure 1.1: Ancient manuscript depicting herbal formulas and magic illustrations (Photograph by author).



The influence of Chinese and Ayurvedic medicine on Thai traditional medicine is evident in their similar principles and the significant cross over in utilized plant species (Chokevivat 2007; Mulholland 1979). Religious people from Thailand went to India over 1000 years ago to study a system of medicine similar to Ayurvedic medicine. The first teacher of Thai Traditional Medicine was *Jivaka Komarabhacca* who was sent to India to learn medicine and is also considered the father of Thai Traditional Medicine (Beyer 1907; Brun 2003) (Figure 1.2). Trade with China brought many immigrants to Thailand, which influenced their society and economy. They also brought their knowledge of Traditional Chinese Medicine (Brun and Schumacher 1994). Mulholland (1988) attributes a majority of Thai Traditional Medicine to Ayurveda from India, which emerged about the same time as Traditional Chinese Medicine, and Greek medicine about two and a half thousand years ago. Although in some of the ancient texts it appears these outside influences came after Thai Traditional Medicine was established (Salguero 2003; Mulholland 1988). The true origins of Thai traditional medicine remain a mystery.

Figure 1.2: An altar of a traditional doctor to offer prayers and gifts to *Jivaka*, the "Father Doctor" (Photograph by Mor C).

THE RECORD OF MEDICAL ETHNOBOTANY IN THAILAND

Written information on Thai Traditional Medicine dates back over a thousand years (Sirisa-ard 2010). There are ancient books documenting the theory and uses of medicinal plants in Thai Traditional Medicine. The first of these is the *Tumra Osotpranarai* (B.E. 1450), which was publically published during the *Ayutthaya* period, and is considered the first official text of Thai Traditional Medicine (Chokevivat and Chuthaputti 2005). The first medical textbook published during the reign of King Rama V was the *Phaettayasatsongkro* (The Royal Edition, King Rama V, 1870), which translates into "the study of medicine" and is comprised of three volumes (Mulholland 1979). Other books were later published including a compilation of formulas called the *Tumra Paetsart Sonkhrau Chabub Luang*, and a student textbook the *Tumra Vejasuksa* (Subcharoen 2003). More recently published are books with images and descriptions of Thai medical theory, medicinal plants and their hypothesized bioactivity. The "*Rattanakosin* Pharmacy Ancient Book" (Wuttidharmma, 2007a) and "The Encyclopedia of Medicinal Plants: to help conserve the inherited knowledge" (Wuttidharmma, 2007b) are two of these books. The *Rattanakosin* period begin in 1888 when King *Rama* V reigned over Thailand. These books primarily document the Royal Medicinal Tradition of Thai Traditional Medicine, as it more commonly practiced and regulated than rural medicine.

"The Mekong Exploration Commission Report (1866-1868)" was one of the first documents published in English on plant use in Southeast Asia. The forth volume entitled, "Agriculture and Ethnobotany of the Mekong Basin" was written by Dr. Clovis Thorel. This book examined agricultural practices including soil analysis, climate, crop rotation and irrigation issues, and listed plants used for food, dye, oil, and medicine. The section on medicinal plants is very brief. This section is introduced by stating that studying medicinal plants in this region

would be quite difficult unless one was already familiar with medical theory. He discussed thirteen plants including *Alstonia scholaris* R. Br. for digestion, *Ruta angustifolia* Pers. for women's health and *Antiaris toxicaria* Lesch., which is used as an arrow poison. The book extensively listed the fruits, vegetables and cereal plants grown at that time in the Mekong Basin so it was a valuable collection of agricultural information (Thorel 2001).

Pierce Salguero wrote one of the most accessible books on Thai medical ethnobotany, "A Thai Herbal" (2003). He lived in Bangkok and Chiang Mai for four years, and collected information on Thai Traditional Medicine, primarily on the Royal Medical Tradition, as it is the most accessible for foreigners. His commentary on accessing the knowledge of the rural medicinal traditions is that it is "shrouded in secrecy" and "exceedingly difficult" for foreigners to study (Salguero 2003). His book was a collection of information on Thai Traditional Medical theory, with recipes for medicinal plant formulas and information on the plants contained in the recipes. There is no mention of plants or a plant formula for memory, but a few plants are mentioned for convalescence and for use as nerve tonics.

The late Norman R. Farnsworth, an eminent pharmacognosist and medicinal plant researcher, edited a book with Nuntavan Bunyapraphatsara (1992) on Thai Medicinal Plants for Primary Health Care. This book is a collection of useful medicinal plants commonly used in Thai medicine. Through the Medicinal Plant Information Center at the Faculty of Pharmacy of Mahidol University in Bangkok, the editors compiled information on about sixty plants including a botanical description, the parts used, chemical compounds found in the plant, ethnomedical uses with recipes and toxicity information. It is a reference book, but also can be used as a tool for communities, individuals and doctors to supplement their knowledge when using traditional plant medicine.

In his book, "The Plants and People of the Golden Triangle", Edward F. Anderson (1993) documented over a thousand useful plant species falling into categories like food, medicine, shelter, fibers and bamboos. The chapters deal with these important plant categories, and the appendices list the others by traditional name, scientific name, use, tribe that uses it and herbarium voucher. The challenges of the hill tribe people in terms of their environment and in society are also discussed. This book is an exhaustive look at plant use by the hill tribes of northern Thailand. The plants are listed by general uses, so it was not possible for me to find any plants used specifically for memory in this work.

Finally, Viggo Brun and Trong Schumacher wrote a compendium on Traditional Medicine in northern Thailand (1994). They discussed both the Royal Medical Tradition, and the rural ethnomedicine of northern Thailand. The book focused on disease theory and the plants used to treat the discussed diseases. The appendix is a detailed list of the plants and their uses as they relate to the northern Thailand disease categories. There is an entire section on wind diseases, including some that were introduced to me as potential causes for memory loss, but nothing specific about cognitive impairment (Brun and Schumacher 1994).

Other literature on medical ethnobotany in Thailand focused on investigating the bioactivity and pharmacology of select medicinal plants. Plants were tested for their activity against cancer, inflammation, women's health, gastrointestinal disease and anthelmintic activity (Siriwatanametanon et al. 2010; Sae-wong et al. 2009; Panyaphu et al. 2011; Srithi et al. 2011; Neamsuvan et al. 2012; Atjanasuppat et al. 2009). Only one survey of Thai plants examined their ability to potentially improve memory. Ingkaninan et al. (2003) conducted a survey searching for plants with acetylcholinesterase inhibition activity in traditional and rejuvenating neurotonic remedies from Thailand. This article was used to screen plant names found in the

memory formulas to determine if they were already studied. Otherwise, studies on the use of Thai plants to treat memory have been limited to testing of single plants (Chattipakorn et al. 2007; Limpeanchob et al. 2008; Nakdook et al. 2010; Uabundit et al. 2010). The pharmacopoeia of Thailand is rich and has been documented for centuries. There are plants that have not yet been tested in modern bioassays, but have been in use for thousands of years.

THEORETICAL CONTEXT

This section aims to position this project in a theoretical framework and to clarify the goals of the research. In the United States, many people have an understanding of elderly dementia, but in Thailand, this disease category is less familiar to the general public. Since this project focused on a disease that affects the mind, it was difficult to interpret the underlying causes of the disease within the framework of Thai Traditional Medicine, versus the western medical paradigm. Western medicine, or biomedicine is commonly defined by an evidence based approach and treating specific symptoms, while eastern medical systems, like Chinese or Thai Traditional Medicine, focus on:

> " ...real-life observation, personal experience, the therapeutic encounter, the subjective basis of diagnostic procedures, syndrome differentiation, myriad diagnostic categories, tailoring treatment to individual differences, situation- specific flexibility, attention to time and place, natural healing through balancing bodily processes, qualitative measurement of outcomes, holism, complexity, multiple influences, interconnected processes, interdependent connection between person and macrocosm, the body as an ever-changing nexus of functional processes, and legitimacy grounded in ancient wisdom. (Shea 2006)".

Therefore, it is essential to define the behaviors found in patients with dementia as abnormal within the cultural construct of the Thai people. Charles C. Hughes (1990) in an article on ethnopsychiatry, viewed these symptoms not in terms of pathology, but rather "different

culturally patterned behavior events". To come into any culture for the purpose of research can stretch the boundaries of one's perspective to redefine what is "normal". In Thailand, asking about an elderly person having moments of forgetfulness generated confusion since it is common in many cultures as people age. To try to define where that forgetfulness becomes a symptom of a larger health problem proved to be more difficult. As a foreigner studying in Thailand it was easier to view the causes of dementia through the lens of a western disease paradigm. Yet, the western medicinal template has labeled a group of symptoms as a specific disease that may not be relevant in another geographic place. Nadkarni and Santhouse (2012) argued that many characterized mental disorders of the western medical paradigm do not fit into other countries where the culture is defined so differently. It is important to recognize where our culture sees abnormality and another sees the same behaviors as part of their normal experience. The perception of illness in many rural Thai societies includes the social structure and relationships of the individual therefore disease can disrupt the harmony of the village. Extreme illness removes an individual from their role as a worker, community and family member, and can significantly affect equilibrium in the society (Santasombat 2003).

Initially, this project was going to focus specifically on Alzheimer's disease. In a committee meeting, Rajkumar Kalapatapu, M.D., an expert on dementia, raised the issue that there is a problem in identifying whether the patient being treated had Alzheimer's disease versus any of the other types of dementia (i.e. vascular dementia, Lewy body dementia, etc.). He suggested, since the type of dementia was difficult to diagnose without a complete neurological work up, cognitive impairment become the focus of the project. Cognitive impairment or memory decline, beyond what is expected in an elderly patient, can indicate a progression

towards dementia (Petersen 2004). The Thai healers are somewhat familiar with Alzheimer's disease as a type of dementia, but know about elderly memory loss and treatments to address it.

There has been a renewed interest in the study of traditional medicine in Thailand since the "health for all" World Health Organization initiative in 1978 still change has developed slowly (Archanuparp 1987). There is a visible influence of Western medicine on many of the traditional healers. Some have incorporated modern diagnostic techniques into their practice, while others are interested in treating modern medical problems that Western medicine has been unable to remedy (Santasombat 2003). Research into traditional Thai pharmacopoeia identified a number of plants with potential to treat cognitive disorders such as dementia (Howes & Houghton 2009), and interest in finding herbal medicines to treat dementia is increasing (Perry and Howes 2011). Research into Thai rejuvenating and neurotonic formulas found four plants with acetylcholinesterase inhibition activity (Ingkaninan 2003). Research into formulas to treat memory specifically could provide more remedies that are effective.

Traditional Chinese Medicine (TCM) and Ayurvedic medicine from India have contributed a wealth of knowledge to ethnobotany as a discipline, and as a source of information on medicinal plants (Schultes and Von Reis 1995). Parts of each of these medical systems are woven into the philosophies and traditions of traditional medicine as practiced in Thailand (Mulholland 1988). Ayurvedic medicine and Traditional Chinese Medicine contain plants with bioactivity as cognitive enhancers as found in researched literature. This study found some of these same plants with known memory improving activity in the multi-plant formulas for the nerves and memory in Thai Traditional Medicine like *Piper nigrum* L., *Terminalia chebula* Retz*., Acorus calamus* L*., Centella asiatica* (L.) Urb. and *Curcuma longa* L. (Ingole et al. 2008). *Curcuma longa* L. from Ayurvedic medicine protects the brain against β-amyloid protein

deposits (Park 2002). The known crossover of these two medical systems would suggest the potential for additional similarly active plants in the Thai pharmacopeia.

Numerous studies investigated medicinal plants found in traditional medical systems, like Ayurvedic Medicine from India, Traditional Chinese Medicine, and even the ancient Greek medical system (Mulholland 1979), used in a similar manner as in Thai Traditional Medicine. Adams et al. (2007) studied plants from traditional medicine for age-related brain disorders. Their study included plants from Asia, and most specifically from Traditional Chinese Medicine and Ayurvedic medicine from India. Khan and Balick (2001) identified the bioactivity of numerous plants used in Ayurvedic Medicine. They found three with memory or longevity promoting activity: *Datura metel L.* (Solanaceae) and *Nardostachys jatamansi* (D. Don) DC. (Valerianaceae), *Bacopa monnieri* (L.) Wettst. (Plantaginaceae).

A review of plants used in Traditional Chinese Medicine (TCM), Ayurvedic Medicine from India (AM) and traditional European remedies (EUR) revealed a number of species that have potential for treatment of cognitive impairment and potentially Alzheimer's disease (Dastmalchi et al. 2007; Adams et al. 2007). Some of these plants are potentially also used in Thai Traditional Medicine (Table 1.1).

Table 1.1: A summary of plants with potential for treating the symptoms of dementia from Traditional Chinese Medicine (TCM), Ayurvedic Medicine from India (AM) and traditional European remedies (EUR).

Plant	Family	Use in traditional medicine	Source
Acorus calamus L.	Acoraceae	Memory loss	AM
Angelica archangelica L.	Apiaceae	Cerebral diseases	TCM
Bacopa monnieri (L.) Wettst.	Plantaginaceae	Improve memory and intellect	AM

Biota orientalis (L.) Endl.	Cupressaceae	Relieve mental strain, treat insomnia and amnesia	TCM
Celastrus paniculatus Willd.	Celastraceae	Intelligence promoter, memory enhancer	AM
Centella asiatica (L.) Urb.	Apiaceae	Revitalizing and strengthening nervous function Combat mental and physical exhaustion	AM TCM
Clitoria ternatea L.	Fabeaceae	Brain tonic, promote memory and intellect	AM
Codonopsis pilosula (Franch.) Nannf.	Campanulaceae	Remedy for amnesia, improve circulation, increase vitality	TCM
Coptis chinensis Franch.	Ranunculaceae	Nootropic	TCM
Crocus sativus L.	Iridaceae	Treat disorders of nervous system	TCM
Curcuma longa L.	Zingeberaceae	Anti-aging	AM
Evodia sp.	Rutaceae	Cardiotonic, restorative, analgesic effects, CNS disorders	TCM
Ginkgo biloba L.	Ginkgoaceae	Improvement of memory loss from abnormal blood circulation	TCM
Hypericum perforatum L.	Hypericaceae	Treatment of neurological disorders	EUR
Magnolia officinalis Rehd. & E.H. Wilson	Magnoliaceae	Improving memory	EUR
Piper methysticum G. Forst.	Piperaceae	Enhanced cognitive performance	Micronesia
Polygala tenuifolia Willd.	Polygalaceae	Treatment of amnesia and forgetfulness	TCM
Rheum sp.	Polygonaceae	Treatment of blood stagnation syndrome	TCM
Salvia lavandulifolia Vahl	Lamiaceae	Enhancement of memory	EUR
Salvia miltiorrhiza Bunge	Lamiaceae	Treatment of cardiovascular	TCM

		disorders, insomnia, neurasthenia, inflammation	
Salvia officinalis L.	Lamiaceae	Promoting intellect	EUR
Terminalia chebula Retz.	Combretaceae	Enhance memory, promote longevity	AM
Withania somnifera (L.) Dunal	Solanaceae	Rejuvenative tonics, enhancement of memory and intellect	AM

The translation of dementia from the framework of western medical constructs into Thai Traditional Medicine was a complex undertaking. The perception of dementia within Thai culture is varied, and very much dependent on the distance from the city or urban area, as is the perception and treatment of many diseases. This reason was part of the basis for studying memory specifically, as opposed to focusing on the interaction of symptoms of Alzheimer's disease. Despite the complexity of an investigation of dementia and elderly memory loss as perceived through the lens of Thai Traditional Medicine, the pharmacopeia provided a number of plant-based remedies with potential activity to treat dementia. The blending of knowledge from ancient and powerful medical systems, coupled with the rich biodiversity of Thailand, provided a compelling opportunity for research.

COGNITIVE IMPAIRMENT AND DEMENTIA

Mild Cognitive Impairment (MCI) can be the transition stage between normal aging and dementia. Dementia is a growing health concern and is prevalent in both industrialized and developing nations. One type of dementia, Alzheimer's disease, makes up more than half of the cases of elderly dementia and is the sixth leading cause of death in the US (Alzheimer's

Association 2013). Mild Cognitive Impairment falls on a continuum with normal aging on one end and dementia on the other. Memory impairment in excess of what is considered normal for an age is the most common symptom of MCI while most other cognitive abilities are unimpaired (Petersen 2004). In patients where MCI (non-amnesic) progresses to Alzheimer's disease, memory decline is still the primary complaint (Lopez 2013).

There was no way to determine if the traditional healers were treating a patient who had Mild Cognitive Impairment or dementia verses other issues involiving memory loss. There is a collection of tests given to patients like the Wechsler Memory Scale–Revised, Dementia Rating Scale, Free and Cued Selective Reminding Test, and Auditory Verbal Learning Test (Petersen 1999) and a rating for the degree of dementia (Hanninen 2002) to determine the magnitude of memory impairment in the patient. There are also complex medical assessments to identify biomarkers in the cerebral spinal fluid (Rosen 2013). Neither of these types of screens were available to test the traditional healer's patients, so a loss of memory in the elderly population without a diagnosis will be called cognitive impairment for the purpose of this project.

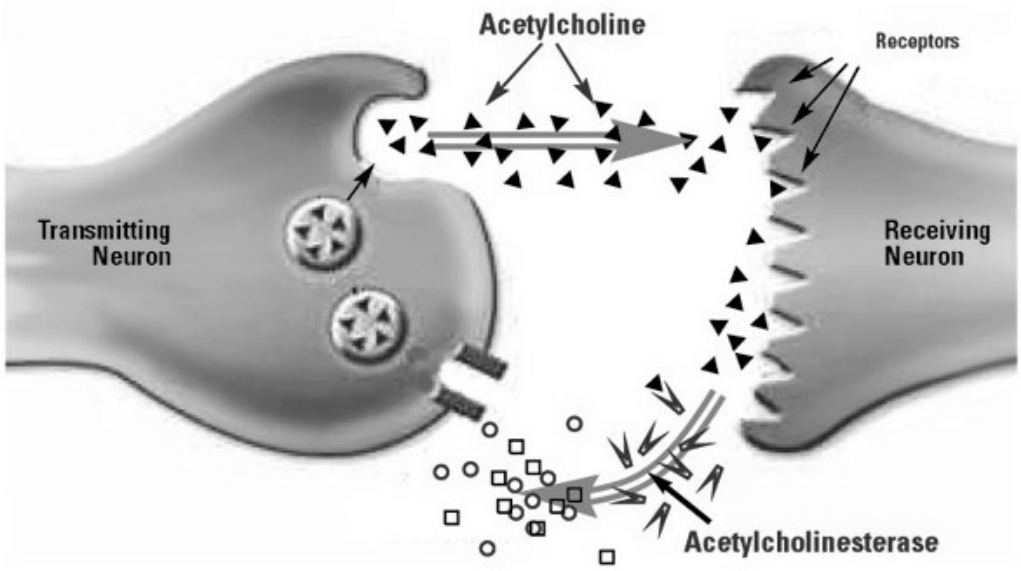
Changing demographics and an extended life expectancy are contributing to growth where the percentage of the population over sixty is increasing. Similar demographic changes present economic and social challenges worldwide. The World Health Organization cites Thailand as having the second largest elderly population in the Southeast Asia region with the expectation of it doubling in the next two decades (Knodel and Chayovan 2008). This growth carries the associated risk of creating a severe social and economic burden of caring for a debilitated elderly population. Of Thai adults ranging from 65-75, 3% of had Alzheimer's disease, compared to 11% in the US (Alzheimer's Association 2013). In "elders" over 60 years old, 48% have memory complaints. This is not an official diagnosis, but notable as a potential

trend, and 21% of them were diagnosed with MCI (Senanarong et al. 2013). Treating dementia in this population is costing millions of dollars, and current research has not presented any promising remedies.

The etiology of dementia related memory loss is not fully understood. There are many theories presented to explain memory loss in the elderly, Mild Cognitive Impairment, Alzheimer's disease and its symptoms. One theory shows a reduction in the neurotransmitter acetylcholine (Ach) in the brain due to its breakdown by acetylcholinesterase (AchE) (Figure 1.3) (Blennow 2004). This reduction contributes to memory loss and cognitive impairment. Some pharmacological therapies address inhibiting acetylcholinesterase to provide the brain with increased levels of acetylcholine (Ringman and Cummings 2006).

The glutamate receptors are also associated with the symptoms of Alzheimer's disease. The N-methyl-D-aspartate (NMDA) group of glutamate receptors are thought to be involved in developing memory and are susceptible to over-excitability which leads to neurotoxicity and cell death. Blocking these receptors can lead to impaired memory (Lee et al. 2002). Treatment with antagonists that have a low to medium affinity for the NMDA receptors have been developed to remedy the symptoms of Alzheimer's disease (Mobius et al. 2004), but were later found to be active in the cholinergic system (Drever et al. 2007).

Figure 1.3: The breakdown of acetylcholine by acetylcholinesterase in the synapses of cerebral neurons (Watkins et al. 1994).



Another theory attributes neurological degeneration to the accumulation of specific β-amyloid protein plaques between cells and tau neurofibrillary tangles within cells that were found post-mortem in the brains of patients with Alzheimer's disease. Amyloid plaques are made of β-amyloid proteins that form from the break down of amyloid precursor protein by two enzymes, β-secretase and γ-secretase. The β-secretase enzyme improperly cleaves the amyloid precursor protein into fragments that stick together and attach to the cell membrane of nerve cells. Substances from outside the cell leak in where the proteins attach causing cell damage and death (Koechling et al. 2010). One therapeutic approach to prevent amyloid plaques is to inhibit the abnormal activity of the secretase enzymes (Bastianetto 2006).

Neurofibrillary tangles of tau protein are an indicator of Alzheimer's type dementia (Ballatore 2007). These tangles result from the improper folding of a hyperphosphorylated tau

protein and prevent it from binding to the microtubules in the nerve cell. This causes the supportive microtubules of the nerve cell to collapse and reduce the cell's ability to communicate with other nerve cells (Hernandez and Avila 2007). The hyperphosphorylated tau protein instead will isolate and make tangles inside the neurons. These tangles disrupt the cytoskeleton of the neuron and its functioning.

A lack of glucose circulating to the brain can increase the buildup of β-amyloid protein plaques. The protein responsible for inducing this buildup of β-amyloid protein plaques is elF2alpha. This protein acts like a switch that activates when the brain is starved of glucose. When there is reduced blood circulation in the body, it results in the reduction of essential glucose for the brain (O'Connor et al. 2008), and the potential for an increase of β-amyloid protein plaque formation. People with high levels of glucose production in their body had changes in glucose metabolism in their brains, which corresponded to increased β-amyloid protein plaques (Shin 2011).

The medial-temporal lobe of the brain is the area responsible for changes in episodic memory indicative of the memory loss associated with Mild Cognitive Impairment. Before the diagnosis of Alzheimer's disease, a reduction of total tissue volume, decreased blood flow, reduced glucose metabolism, and increased β-amyloid plaque deposits are found in the medial-temporal lobe of patients with MCI (Backman 2004). Another study found a prevalence of neurofibrillary tangles composed of improperly folding tau protein in the medial-temporal lobe in patients with MCI, but fewer β-amyloid plaques. This increased occurrence of neurofibrillary tangles in comparison to the appearance of β-amyloid plaques was noteworthy. Peterson (2006) found the brains of patients with MCI had a similar amount of β-amyloid plaques as normal

individuals, so perhaps the plaques specifically were not responsible for their memory impairment.

Patients with cognitive impairment do not yet exhibit all the signs of dementia, but are considered a population at risk, especially if they are diagnosed with Mild Cognitive Impairment (MCI). In a study of patients with MCI, they are similar in age to those with Alzheimer's disease, and were more likely to have the apolipoprotein E ε4 allele than controls, but still less likely to have the aberrant apolipoprotein E ε4 allele than those patients diagnosed with Alzheimer's disease (Grundman 2004). Other biomarkers can be measured to determine the risk for dementia. These are a high concentration of tau proteins and P-tau (tau inclusions) in the brain coupled with a decrease in the Aβ42 protein in the cerebral spinal fluid as found in patients with Alzheimer's disease (Rosen 2013).

The discovery of one commonly prescribed acetylcholinesterase inhibitor, Galanthamine, was from ethnobotanical investigation. Members of the Amaryllidaceae contain Galanthamine, most specifically *Galanthus* sp.. This plant is native to Eastern Europe, and a Bulgarian pharmacologist first noted its use when he observed the plant being rubbed on the forehead to treat nerve pain (Heinrich and Teoh 2004). Locating effective plant therapies for memory loss from the traditional pharmacopoeia of Thailand could help to alleviate the suffering endured by people afflicted with memory-based neurological disorders.

PREVALENCE AND CONVENTIONAL TREATMENT OF DEMENTIA IN THAILAND

Information on the prevalence of cognitive impairment and dementia in Thailand was primarily limited to data on Alzheimer's disease. The occurrence Alzheimer's disease in Thailand is lower than that of the US in most age groups in a study of the elderly population in

the Ayutthaya Province of Central Thailand (Pers. Comm. Dr. Pised, *Suan Prung* Psychiatric Hospital 2008). In adults ranging from 65-75 years of age, 3% of had Alzheimer's disease. Otherwise, of adults in Thailand between 75-85, the prevalence of Alzheimer's disease increased to 10-12% of the elderly population and rose again to 33% in adults between 85-90. After 90 years of age, 45-50% of the elderly population has Alzheimer's disease (Pers. Comm. Dr. Pised, *Suan Prung* Psychiatric Hospital 2008). Senanarong et al. found that 62.6% of Thai elders had the apolipoprotein E ε4 allele, which is an indicator of progression to Alzheimer's disease (2013).

Interviews with neurologists at *Suan Prung* Psychiatric Hospital in Chiang Mai city explored conventional treatment for Alzheimer's disease in Thailand. The treatment for Alzheimer's disease depends on the severity of the symptoms and is very much like treatment in the United States. In its early stages, patients are treated with acetylcholinesterase inhibitors like Rivastigmine and Galanthamine. Other treatment includes remedying other symptoms with anti-psychotic and anti-depressive drugs. Patients with more severe symptoms are treated with Memantine, which acts on the NMDA class of glutamate receptors. Educating the relatives of a patient with Alzheimer's disease was also an important part of the treatment. The relatives could complement drug therapy with "brain training" like asking questions to the patient to help stimulate their brain (Pers. Comm. Dr. Pumin Chalachiva, Neurologist, *Suan Prung* Psychiatric Hospital 2008).

There is an important difference between neurologists in Chiang Mai city recognizing the disease and those outside of the city, in more rural areas. Neurologists at the *Suan Prung* Psychiatric Hospital in Chiang Mai city were very familiar with Alzheimer's disease and listed causes similar to those the United States like acetylcholine depletion and β-amyloid plaques.

Also mentioned were hyperthyroidism and brain tumors as causes of the disease. Diet, exercise and stress management were recommended as a means of prevention (Pers. Comm. Dr. Pumin Chaladriva, *Suan Prung* Psychiatric Hospital 2008).

In rural Thailand, the elderly are primarily cared for by the family. Some of the symptoms of dementia are considered just part of getting old, and are not discussed outside the family. The family gives tonics for the brain and the elderly to the patient. Many times, even a patient with extreme symptoms does not go to the hospital and is completely cared for by the family (Dr. Nopporn, Director, *Mae On* Hospital, pers. comm. 2009).

NATURAL AND CULTURAL HISTORY OF THE STUDY SITE

THAILAND

Thailand is in Southeast Asia (15° 00' N, 100° 00' E), and is bordered by Myanmar (Burma) to the west, Laos and Cambodia to the east and Malaysia to the south. The Gulf of Thailand and the Andaman Sea frame it to the south. Thailand lies at the center of three biologically diverse areas, the Indo-Burmese, Indo-Chinese and Malaysian regions, and shares its flora with these three regions (Bugna and Rambaldi 2001). It has approximately 514,000 square kilometers of very diverse land area marked by mountains to the north, and mangroves and beaches in the south. Thailand has an estimated 15,000 species of flowering plants, 292 species of mammals, 938 species of birds and over 2,000 species of marine fishes (ONEP 2002). Tropical monsoons dominate Thailand's climate, which cycle through the year differently depending on the part of the country. The southern part of the country receives the most rainfall and the northeastern part the least. In most of the country, there are three seasons: rainy, hot and cool. Deciduous forests are found in the lower altitudes, but much of these have disappeared.

The higher altitudes have evergreen broad-leafed forests with native pine trees (Srikosamatara 2004).

The Mekong River borders Thailand on its eastern side and originates in the Tibetan Plateau through China, Myanmar (Burma), Laos, Cambodia and Vietnam. It is the world's twelfth largest river and a major trading route. It is currently under grave environmental threat from already present and planned dams, and is at risk for loss of biodiversity from climate change. Sixteen of the two hundred most biologically diverse areas of the world as defined by the World Wildlife Foundation are found near this river (Blate 2009).

In the early 1960's, the Thai government proposed the "First National Economic Social and Development Plan" which shifted the food production from a domestic, subsistence level, to a high-output and increased yield for export. There were drastic environmental repercussions on the environment and human health. The soil quality was degraded and became depleted of nutrients. To resolve this problem, more chemicals were applied to the land, and it became toxic. This toxicity spread to the farmers who became ill. More land was needed, so the forests were cleared for agriculture by burning them contributing to high levels of air pollution. Unfortunately, this plan for development was not well orchestrated, and the Thailand continues to suffer with these issues. Sustainable agriculture is becoming more prevalent, but not on the scale needed to rectify the already damaging effects of the "First National Economic Social and Development Plan" (Tantemsapya 1997). The other environmental threats throughout Thailand are air pollution from vehicle emissions, water pollution from organic and factory wastes, and wildlife populations threatened by illegal hunting. Thailand's economy is growing intensely. This growth is affecting the environment primarily through resource depletion and pollution. Rapid deforestation, extensive mining and water shortages plague the country, and population

growth and globalization are exacerbating the problems (Hirsch 1996). The decline of the environment in Thailand emphasizes the need to document traditional plant knowledge, and potentially utilize it to work in harmony with land (Gerique 2012).

The Thai were not the original people of their country, but archeological evidence shows that Thailand was inhabited since before 500,000 years ago. The Thai people have populated the area for over 4000 years (London 2008). There are currently about 94 million people in Thailand. The largest city is Bangkok, where about 9 million people reside. Thai is the official language, but there are four distinct dialects throughout the country. Thailand is almost entirely Buddhist (94%). The government is a constitutional monarchy with a Prime Minister as the Head of Government and the King as Head of State. There are currently 132,241 refugees from Burma as of 2007 (United Nations 2008).

CHIANG MAI AND THE *LANNA* KINGDOM

Chiang Mai is one of 76 provinces in Thailand and is located in the northwest part of the country. Chiang Mai is the capital city of the province, with a population of 160,000 people. The principle part of the city, or *muang*, rests between the *Ping* River to the east and a mountain, *Doi Suthep*, to the west. It is a city of great cultural significance and has over three hundred Buddhist temples. The northern part of the country where Chiang Mai is located has three distinct seasons: the rainy season from May to October, the cool season from November to February, and the hot, dry season from February to May. Monsoons control the weather patterns. The winds blow from the southwest, and are wet, during half the year and the northeast, which are dry, during the other half (Anderson 1993).

Figure 1.4: Map of Thailand highlighting Chiang Mai province and city in the northwest (By Hdamm [CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0)], via Wikimedia Commons).

The northwest is mostly mountainous with mixed deciduous forests and deciduous dipterocarp forests. The mixed deciduous forests contain *Tectona grandis* L. f. (teak), *Xylia xylocarpa* Taub. (Burmese Ironwood), *Afzelia* sp. Sm., *Dalbergia* sp. L. f. and bamboo (Bullock et al. 1995). The deciduous ditperocarp forest contains *Dipterocarpus obtusifolius* Teijsm. ex Miq., *D. tuberculatus* Roxb., *Shorea roxburghii* G. Donand, *S. siamensis* Miq. (Anderson 1993). Chiang Mai Province also contains the highest mountain in Thailand, *Doi Inthanon* at an elevation of 2576 meters (8450 feet).

The *Lanna* Kingdom historically encompassed northern Thailand, but also stretched over parts of Myanmar (Burma), China and Laos as a vibrant and successful kingdom. *Lan Na* means "the land of a million rice fields" (Ongsakul 2005). During the Mid-16th Century, the Burmese occupied parts of the *Lanna* Kingdom, and although it escaped their occupation, it was never as strong and was eventually overtaken by the *Chakri* Dynasty in Bangkok (Forbes and Henley 1997). The original inhabitants of the *Lanna* Kingdom were the *Tai Kuean* and the *Tai Lue* from Thailand, and the *Tai Yai* from China. Contemporary Thai people began to populate the area know as *Lanna* in the thirteenth century.

Phaya Mangrai founded Chiang Mai during the 12th century as he was expanding his territories and wanted a capital for his domain (Usavangkul 2005). He founded this "new capital", which was the administrative center of the *Lanna* Kingdom from 1296-1768. The Chiang Mai Chronicles state that the original inhabitants of Chiang Mai were of *Lua* ethnicity. When the Thai people came into the area, they worked together and then blended culturally (Ongsakul 2005). This could be a partial explanation for the distinct spirituality of the people in Chiang Mai.

KHON MUANG

The people of Chiang Mai, or those of the northwestern part of Thailand, refer to themselves as the *Khon Muang*. They are related to the *Lao* of northeast Thailand and Laos, the *Shan* of Burma, the *Tai Lu* of Sipsongpanna in Yunnan, South China, and the *Siamese* of central Thailand. Historically, these people identified with certain areas or *muangs*. The topography of the area made it difficult to communicate and it often took days to travel between one *muang* and another, so the *muangs* became quite distinct from one another. Today, this reference

distinguishes them from the people of central or eastern Thailand and from the ethnic hill tribe people who also inhabit the north. The *Khon Muang* identify as the lowland people of the northern part of Thailand (Forbes and Henley 1997). Most of the interviewed healers proudly declared themselves *Khon Muang* when asked their ethnicity. They were aware of their history, and wanted to be sure to define themselves and their belief system in this way.

Figure 1.5: Map of the former *Lanna* Kingdom in light violet encompassing parts of Thailand, Laos, Southern China and Myanmar (Burma) (By Javierfv1212 (Own work) [CC0], via Wikimedia Commons).

STUDY PRESENTATION AND CHAPTER OVERVIEW

This thesis is organized into seven chapters. The first chapter provides a foundation and the context of this study. It describes the history of the study site, and essential information to understand the theorized etiology of dementia. A complete methodology of the project is provided in chapter two. Chapter 3 describes the principles of Thai Traditional Medicine, how this system of medicine perceives cognitive decline and dementia, and how plants are utilized to treat medical conditions. The ethnomedical results of the fieldwork portion of this study, and the plants used to treat memory impairment in Thai Traditional Medicine are in chapter four. The sections on laboratory testing of the plants are in chapters five and six. Chapter 5 explains the ethnobotanical and *in vitro* screening methodology used to determine the one plant for *in vivo* study. Chapter 6 describes the *in vivo* behavioral experiments on rats and the testing for enzymatic marker levels in the rats' brains after treatment with an extract of the selected plant. Finally, chapter seven provides a summary of the findings of this study and suggestions for further research on this topic.

CONCLUSION

The culture of northern Thailand is rich and enduring. Many *Khon Muang* still honor "the old way", though the new generation fancies technology and modern conveniences. Thai Traditional Medicine reveals the cultural connection to the changing seasons, the land and the food that grows on it. The ancient books like the *Tumra Osotpranarai* and the *Phaettayasatsongkro* speak to keeping balance in the body by aligning the body to the rhythm of the natural world around it. The abundant biodiversity of northern Thailand provides its inhabitants a generous variety of food year round. The ongoing threat to this way of life through

environmental devastation and habitat destruction also puts their medical system, which is dependent on the natural world, at risk. Globalization increases the proliferation of artificial means in dealing with the natural world like processed food, the use of pesticides and air pollution. While they do not know the official etiology of dementia, many traditional doctors in northern Thailand attribute it to the copious amounts of chemicals used in the new, modern world. They believe the escalated prevalence of chronic diseases like cancer, neurological disorders and dementias are caused by an increase in the use of these chemicals and their accumulation in the natural surroundings. One of the primary goals of this research project is to investigate if the ancient system of Thai Traditional Medicine can address the new diseases of today, and to understand if adhering to "the old way" of living can stave off an imminent decline in the health of Thai people.

CHAPTER 2 - FIELDWORK METHODOLOGY

INTRODUCTION

This doctoral dissertation describes a traditional ethnopharmacological project with three aspects: anthropology, botany and chemistry. Each of these aspects utilized different methods for gathering data, yet these methods continuously overlapped throughout the project. Interviews conducted in the early stages of fieldwork determined the depth of knowledge of a particular healer regarding Thai Traditional Medicine and medicinal plants. During the later stages, when selected medicinal plants were screened in the laboratory, a healer who had become a key research participant would be interviewed again to ask about the specific therapeutic characteristics of that particular plant. While these interviews were about different topics, the method of asking questions to obtain information is the same.

The ethnographic data for this study was gathered during semi-structured interviews using an interview questionnaire, and both direct and participant observation (Alexiades 1996). Direct observation allowed me to witness healing sessions without being a part of them, but I was able to participate in the preparation of the remedies and was sometimes treated by the traditional healers I interviewed (participant observation). In the preliminary stages of my research, the interviews focused on selecting traditional healers with knowledge about plants used for the brain and for memory. For this purpose, I used a directed questionnaire seeking information about the healer's background, including information on where they learned about Thai Traditional Medicine and herbal remedies. Specific information on plants was not discussed on the first visit and instead that interview time was used to establish rapport with the healer.

In some cases, it took many interviews to determine if a healer actually had a deep knowledge of medicinal plants, or if they were gifted in another method of healing, like massage, and had only a surface knowledge of plants. One observed cultural characteristic of many healers is the desire to provide an answer, regardless of whether it is correct. In many situations, the healers told me very general or incorrect information, because to say "I don't know" is perceived as a "loss of face" or shows a lack of knowledge in Thai culture. So any answer, even if it is incorrect "saves face" or shows personal knowledge. A number of times the healers said that they would give me information about plants in the following interview. I found the healers who were straightforward about their level of knowledge actually did have information on medicinal plants for memory. In the end, there were some healers who I visited only once as they did not have a deep knowledge of medicinal plants, others about ten times and still others over thirty times. These frequently visited healers became my primary research participants.

SELECTION OF TRADITIONAL HEALERS FOR INTERVIEWS

TYPES OF HEALERS IN THAI TRADITIONAL MEDICINE

Research participants were primarily healers of Thai Traditional Medicine. Thai people call these healers *maws*, and there are usually one or two traditional healers or *maws* in each village. Healers with expertise in medicinal plants were considered specialists and were used as research participants (Alexiades 1996). In this document, I will refer to these specialist research participants as traditional healers. Many of the interviewed healers, especially the men, were previously Buddhist monks and learned about traditional medicine from older monks at the temple. Others learned about it as the knowledge passed through generations of family members. Some received their training from both the temple and their family. Many of the

research participants had formal education in Thai Traditional Medicine. Currently, the Ministry of Public Health licenses many traditional healers, as the profession is becoming more regulated.

There are a number of different specializations for traditional healers in northern Thailand. These range from healers who use physical therapy like massage (*kai bam bad)* to spiritual healers (*pii tii kam bam bad)*, to those who advise on food choices (*ahan bam bad*) and healers that specialize in using medicinal plants (*samunprai bam bad)*. There can be overlap in these types of traditional Thai healers, so some healers are both spiritual healers and use medicinal plants. In addition to traditional healers, there are midwives in Thailand who specialize in pregnancy, childbirth and caring for the mother after the child is born using medicinal plants, prescribed foods and ceremony. The midwife will also ensure the new mother follow the special taboos and postpartum practices. There are specific medical afflictions if the mother does not follow these prescribed regulations in Thai Traditional Medicine.

SELECTION OF PARTICIPATING HEALERS

I met many of my research participants through an introduction by Dr. Panee Sirisa-ard (Ajan Panee), PhD in the Department of Pharmaceutical Sciences at the Faculty of Pharmacy of Chiang Mai University. She is an expert on *Lanna* Traditional Medicine and was my local mentor during my time in Chiang Mai. Without her introduction, many of the healers who agreed to work with me would not have allowed me to interview them at all. The "introduction" is a significant cultural practice in Thailand. Many doors were opened for me because of an introduction, or because of the request of someone in a respected position. By accommodating this request, the person was practicing *kreeng jai*, which is the sense of obligation and politeness found in Thai society (Moore 1992).

Ajan Panee took me to meet some of my key research participants during my first trip to

Thailand in the summer of 2006 (Figure 2.1). When I returned to Thailand to begin my fieldwork in earnest, she again helped me make contact with these healers. She also gave me a list of local healers she had worked with in the past, and selected some of them for me to contact.

Figure 2.1: First visit to a traditional Thai healer with Ajan Panee (photograph by author).

DESCRIPTION OF THE INTERVIEWED TRADITIONAL THAI HEALERS

The healers identified in this dissertation were interviewed at least once, and their traditional knowledge is contained in this document. In the cases where the healer was interviewed only once, it was apparent after the first interview they did not have the mastery of medicinal plants needed for my research. These healers are not included in this list. A healer was interviewed more than once if they had a good understanding of Thai Traditional Medicine or how cognitive impairment and dementia are explained in that context. These healers are described to offer a perspective on the types of training for traditional healers, their differences

and similarities in training and practices (Table 2.1). The names of the interviewed traditional healers are coded to respect their confidentiality.

Five healers became my key research participants. These healers had both the willingness to share with me, and the depth of knowledge I needed for my research. Some of the other healers were interviewed up to five times. I visited the five healers who were my key research participants numerous times, some up to thirty visits.

Table 2.1: Chart of interviewed traditional healers with information on their training and personal characteristics. Key participant healers are underlined.

Name	Age	Sex	Ethnicity	Practices	Training	Additional Information
Mor A	42	Male	Khon muang	Herbal medicine Spiritual healer *Hae*[1]	Grandmother *Pali* manuscripts	
Mor B	66	Male	Khon muang Chinese	Herbal medicine Thai massage	Family Thai Ministry of Health	He is a 9th generation healer and operates his own factory
Mor C	50	Female	Central Thai	Thai massage Herbal medicine	Grandmother Thai Ministry of Health	Teaches Thai massage at her healing center
Mor D	55	Male	Khon muang	Thai massage Herbal medicine	Thai Ministry of Health	Director of healing center in Chiang Mai
Mor E	73	Male	Khon muang	Thai massage "hot foot" massage[2] Herbal medicine	Temple	Healer for over 50 years
Mor F	60	Male	Khon muang	Herbal medicine Spiritual healer	Family	Healer for 45 years
Mor G	51	Male	Khon muang	Massage Herbal Medicine	Temple Learned from *Thai Yai*	Teaches massage Healer for 27 years
Mor H	51	Male	Khon muang	Herbal Medicine	Thai Ministry of Health	Teacher at Thai Massage School Shivagakomarpaj

					Grandmother	(The Old Medicine Hospital) in Chiang Mai
Mor I	75	Male	Khon muang	Herbal medicine Tells horoscopes	Grandfather	Healer for 40 years
Mor J	60	Male	Khon muang	Herbal medicine Spiritual healer Thai massage *Tak sen* massage[3]	Temple Grandfathers (both sides)	Healer for 30 years
Mor K	49	Male	Khon muang	Herbal Medicine Spiritual healer Bone setter "Hot foot" massage[2]	Father Grandfather	Healer for 25 years
Mor L	63	Male	Khon muang	Herbal Medicine	Unknown	Runs an herbal medicine factory
Mor M	58	Male	Khon muang	Herbal Medicine	Grandfather	Healer for 20 years
Mor N	65	Male	Khon muang	Herbal Medicine Scraping[4]	Grandparents	Healer for 35 years
Mor O	Deceased	Male	Unknown	Herbal Medicine	Unknown	Monk at a temple outside of Chiang Mai
Mor P	74	Male	Khon muang	Herbal medicine Fortune teller	Temple Other healers	Healer for 26 years

[1] *Hae* is a practice where a tiger tooth or elephant husk is used to apply herbs to the patients skin.
[2] Hot foot massage is where the healer dips his foot in alcohol, quickly sets it on fire, extinguishes it with water and places it on the body of the patient.
[3] *Tak sen* massage uses an block of wood and a cylindrical piece of wood, which causes a vibration to the *sen* lines in the body.
[4] "Scraping" is where the skin is scraped with a rock to help the disease come out.

METHODS FOR DATA GATHERING

INTERVIEW PROTOCOL

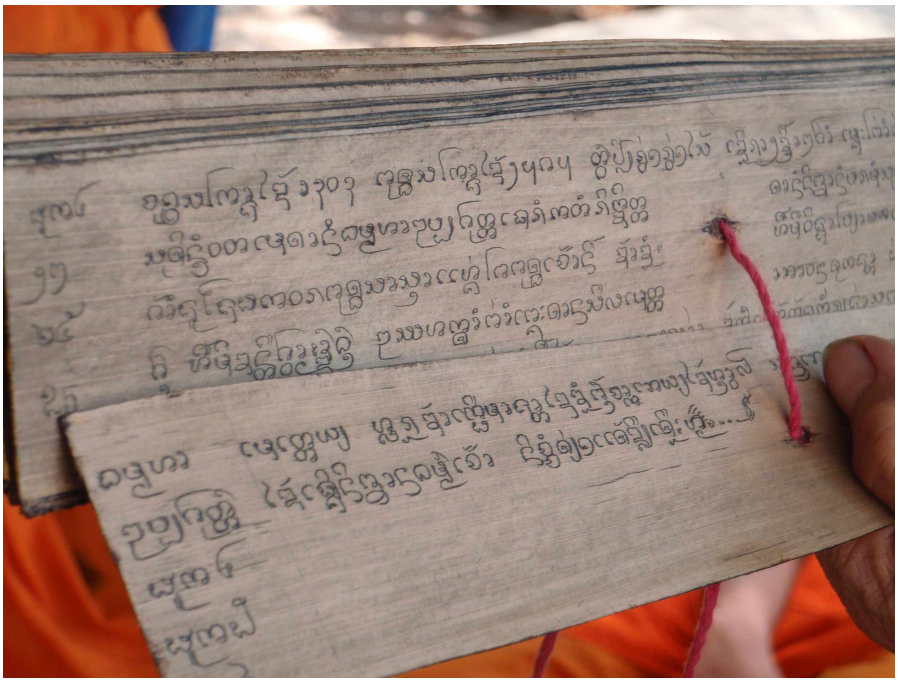
This study focuses on plants used for memory impairment that could lead to general dementia, Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD). It was impossible to identify the type of dementia the plant remedies discussed by the healer were meant to treat;

specifying Alzheimer's Disease or even Mild Cognitive Impairment as the primarily affliction would be inaccurate. Therefore, questions focused on plants to improve memory in general, plants for the elderly who had memory problems, and tonics for the brain. As the disease categories relating to age-related cognitive decline were identified and clarified within the paradigm of Thai Traditional Medicine, there were more questions relating to the specific formulas used to treat these diseases. Some doctors knew about Alzheimer's disease, but not about other type of dementias, and one healer offered a formula specifically for Alzheimer's disease. As this study was a directed ethnopharmacological study, no discussion about other disease categories took place unless there was some relevance to the brain or memory. Healers were asked about herbal remedies for the aforementioned categories and then free-listed the plants used to treat them (Alexiades 1996). Many discussed ancient multi-plant formulas, which were written in *Pali*, the language of the Lanna Kingdom and Buddhism. These formulas were inscribed on palm leaves with a sharp instrument and rubbed with soot for the letters to appear (Figure 2.2). The healers translated these formulas into Northern Thai, and botanists at the Queen Sirikit Botanic Garden or the healers themselves identified the plants contained in them.

Interviews with the healers began with a general explanation of my research and the presentation of my Graduate Center Institutional Review Board (IRB) approval letter written in the Thai language. To secure free prior informed consent, a signature was required, but the first healer I interviewed alerted me that obtaining a signature in this situation was culturally inappropriate. He refused to sign my IRB form and repeatedly stated he would sign it the next time I visited him. He believed that his signature meant I had ownership over his traditional knowledge. He would not sign my IRB letter asking permission to interview him for fear he was signing over all of his knowledge. Instead of a signature, I obtained approval from The Graduate

Center Office of the President for Research and Sponsored Programs Institutional Review Board that required only verbal permission after each healer read the IRB letter describing my project. I audiotaped the healer giving permission for the interview in Thai to document their prior informed consent.

Figure 2.2: A palm leaf manuscript written in the ancient language of *Pali* (Photograph by author).



The interviews were directed by a semi-structured questionnaire approved by the IRB of The Graduate Center at The City University of New York (IRB number: 08-05-1563; PI: Lisa Offringa). The questionnaire contained a number of different types of questions ranging from closed and directed questions, to those that were very open-ended (Alexiades 1996). As I conducted more interviews, the questionnaire was adjusted to account for my understanding of cultural differences in the Thai culture and language. Certain questions were asked during the

first interview, but others were held until subsequent interviews as rapport was established with the healers. Questions about plants and formulas for memory disorders were asked after more general questions about Thai Traditional Medicine and theory on memory loss as described by Thai Traditional Medicine.

During the botanically focused portion of this project, the type of interviews I conducted varied. Initially, I used the questionnaire and only deviated from the questions when the healer offered more information on a specific question. As my work with particular healers continued for many years, the interviews with them became more unstructured. During collecting trips, plants were discussed in field interviews and there were discussions about these plants at the market, or in the healer's home or factory since some of the plants were obtained directly from the healers.

The interviews were audio recorded after the healers freely gave their permission. After I identified my key research participants, the interviews became more informal and I did not record the entire interview. Towards the end of my work, the visits to healer's homes became more like social visits to friends with food and laughter (and sometimes a taste of their homebrewed strawberry wine or *yaa dong*, which is whiskey steeped with herbs). The casual nature of these visits allowed me the opportunity to be present and observe when other villagers would come by for medical or spiritual advising. All interviews used a research assistant to translate from Central and Northern Thai, which was the language of choice for the healers, to English.

With my five key research participants, I asked questions as needed (unstructured interviews) without using a directed questionnaire. Many times, as the healers became more familiar with my project, they understood the information I needed and would just talk about the

topics of my work. In addition, I would conduct interviews on the specific plants to understand the use of the plant in Thai Traditional Medicine to get a better perspective on how the use could translate into western medical concepts. Collecting trips in the forest were perfect for conducting field interviews and asking questions about specific plants. As one of the healers became more familiar with me, he would invite his friends, who are also traditional Thai healers, along on our field trips. These circumstances allowed me to have group interviews, and elicit discourse and debate about plant uses and folk names (group interviews).

Trips to the field were made with the healers to collect plants for my project in addition to plants they needed for their practice. When the trip focused primarily on my project, I was able to conduct field interviews. During other field trips, I was able to observe the healers collecting plants for use in their practice (participant observation)(Figure 2.3).

Figure 2.3: Fieldwork group interview in *Papae* Village, *Lamphun* Province, Thailand (photgrapher: Jiratthitikaan Tumakaew).

All the plants in the memory formulas received from the traditional healers were investigated in online databases (NAPRALERT, PubMed, Scifinder Scholar, etc.) to determine if they had already been tested for antioxidant activity, total phenolic content and acetylcholinesterase activity before bringing them to the laboratory to be studied. If the plant was previously tested, it was removed from the list for *in vitro* screening. I presented the remaining single species names (in Thai) to the healers who provided the formulas in the form of a checklist for them to rank the single plants in the order they would select them to test in the laboratory for memory enhancement activity. The checklists made by the healers were compared, and eleven plants were chosen for *in vitro* screening with the intention of locating one plant for testing on *in vivo* animal behavioral models.

INTERVIEW QUESTIONNAIRE AND DEVELOPMENT

An ethnography is best conducted with a good survey technique and an effective questionnaire (Bernard 2004). The questionnaire used to interview the healers was designed to facilitate semi-structured interviews where the research participant would be asked an open-ended question to allow them to speak for any length of time about whatever they felt pertained to the question (Alexiades 1996). Sometimes these questions would elicit simple, one-word answers, other times they would lead to long stories, which encouraged additional questions. For example, some healers would just state where they were born when asked, while others would talk about where they were born, where their parents were from and what it was like growing up there.

The questionnaire was originally designed utilizing questions from ethnobotanical methods guides (Alexiades 1996; Martin 2004). I also spoke with other students who conducted

similar work, and took the advice of my committee members. As the interview process progressed, the questionnaire was edited as some questions were confusing to the healers. I developed questions during the interviews to address information that was freely offered by some healers and not by others. The final questionnaire is in Appendix 1.

The same questions were asked at the start of each interview, but the discussion would often diverge after a certain level of knowledge was revealed. The questionnaire began with demographic questions about the healer and their practice. The second part of the questionnaire often divided the healers into one of two categories: those who did not know about treating memory loss that can lead to dementia and those that did. These questions addressed the symptoms of cognitive impairment, dementia and Alzheimer's disease, especially memory loss. If the healer was familiar with any of these disease categories or symptoms, then additional questions would be asked to clarify their knowledge and determine if they would be interviewed again about plants they used. If they did not know about memory loss and elderly dementia, then I would use the interview time to learn about Thai Traditional Medicine in general. This approach allowed me to gather necessary information, and at the same time, not cut off the interview and dishearten the healer because they did not possess certain knowledge.

All the healers I interviewed were asked about plant resources and collecting, and about plant management and conservation. These questions would relate specifically to this project if I continued to visit that healer and ask them questions about plants used for memory. If the healer was interviewed only once, these questions would provide me information on the condition of medicinal plants in northern Thailand, and plant resources in general.

Questions about particular plants to treat memory, cognitive impairment and even dementia were asked during the second interview and beyond. Some rapport was established

with the healer before asking about plant formulas to treat memory issues since this information is sometimes considered a guarded secret. One healer provided his formula in its entirety after working with me for over two years. Other formulas that related to memory, for example, to improve blood circulation, or to treat a culturally specific condition that afflicts only women who did not follow the prescribed diet after childbirth, were given more freely.

Questionnaires were to address each formula and the single plants they contained. I asked about the history of the formula and which plants were the most important in the formula. Before the laboratory portion of my work began, a list of plants from the formulas was developed to determine which ten plants to test in the chemical bioassays. Two plants were added to the list of single plants that were tested as it was specifically suggested by more than one healer. One of the plants could not be identified, so it is not reported in the results.

The International Review Board (IRB) approved the orignial interview questions used during this project. Each year these questions were reassessed when I applied for continued IRB approval. The document showing IRB approval for this project is in Appendix 2.

PLANT IDENTIFICATION AND VOUCHER COLLECTION

The medicinal plant samples were collected from the forest, the home gardens of the healers, obtained directly from the healers or purchased at the local herbal market. During the interviews, information on each plant including the folk name, part used, preparation, where the plant could be obtained (forest, garden, market) and any special information on collecting the plant was recorded. I also collected "plant use" information on the activity of each plant as defined by Thai Traditional Medicine. This information helped to bridge the different perspectives between the eastern medical paradigm and the western view of dementia.

For plants collected in the forest and in the healer's home gardens, standard botanical collecting techniques were employed (Alexiades 1996; Martin 2004). Wittaya Pongamornkul, a botanist at the Queen Sirikit Botanic Garden (QSBG), curated the voucher specimen and deposited them in the herbarium of the QSBG. My research permits stipulated that no plant material was to be removed from the Kingdom of Thailand. Therefore, the voucher specimen were photographed and digitized as a record of this work. These files are kept at the Institute of Economic Botany at The New York Botanical Garden (NYBG). A complete list of voucher specimen and collected plant samples are not included in this dissertation as to keep confidential the complete list of species in the multi-plant formulas.

The *Lanna* Kingdom encompassed more than just the current borders of Thailand, and during historical times, includes parts of Laos, China and Myanmar (Burma) therefore some plants listed in the ancient manuscripts are not found in Thailand (Penth 2001). Since this is a contemporary study of Thai Traditional Medicine, the plant material currently used by the healers in their formulas was considered valid for this project. One healer has a factory where he produces his own herbal remedies. Some of the documented plants were obtained directly from him. If he did not have a plant, it was purchased at *Lanna* Herbs in Chiang Mai city, a market commonly used by many healers (Figure 2.4). These plants were identified using standard Thai medicinal manuals and field guides for the area (Wuttidharmma, 2007a, Wuttidharmma, 2007b, Smitinand 2001, McMakin 2001; Pongpangan 1991; Supjareon 1999) and, when available, compared to herbarium specimen (Salick et al. 2006). These names were double checked by presenting the healer with a photograph of the known plant species to confirm the botanical name (Thomas et al. 2007). This reference collection of "market vouchers" was photographed for documentation. The photographs will remain at the Institute of Economic Botany at The

New York Botanical Garden to contribute to the documentation of medicinal plant knowledge in northern Thailand. All scientific names were referenced against the Tropicos website of the Missouri Botanical Garden for accuracy (Tropicos http://www.tropicos.org/).

Figure 2.4: Outside and inside of *Lanna* Herbs store at *Kad Luang* Market, Chiang Mai, Thailand (Photographer: Dr. Jillian De Gezelle).

ETHICS OF ETHNOBOTANICAL RESEARCH

THE PROTECTION OF BIOLOGICAL RESOURCES AND TRADITIONAL KNOWLEDGE

According to the World Health Organization, 80% of people in certain Asian and African countries use traditional medicine as their primary source of healthcare (WHO 2008). These traditional knowledge systems are often mined for information about plants with the potential to treat disease, which can be the beginning of a process where a potential new medicine is found in nature, patented and sold (Kartal 2007). The worldwide use of herbal medicines, many from these traditional medical systems, is in the billions of U.S. dollars. In Western Europe sales from herbal medicine reached five billion dollars for 2003-2004, in China it reached 14 billion dollars in 2005, and in Brazil sales in 2007 were 160 million dollars (WHO 2008).

For some specific medical issues, only a few specialists or traditional herbalists hold information on treatments using medicinal plants, therefore this information is regarded with a high level of sensitivity. These plants can be endemic to the area where they grow, have a specific part that is used and there may be a high demand for them with a potentially low supply (Cunningham 1996). In order to develop marketable herbal products from plant material, more developed countries with fewer natural resources may extract raw biological materials from the less developed countries with considerable biodiversity and traditional plant knowledge. Developing countries have begun to valorize their natural resources, their knowledge for using these resources and the necessity to preserve them (Kartal 2007).

To help regulate the exchange of biological resources, agreements like The Convention on Biological Diversity (CBD, http://www.cbd.int/convention/text) and the Nagoya Protocol on Access to Genetic Resources (http://www.cbd.int/abs/text/default.shtml) were developed. These documents aim to be comprehensive and accessible, so they are of use to local populations who

often are the suppliers of both the biological resources and the knowledge of their use. The Convention on Biological Diversity was adopted in Rio de Janeiro, Brazil in 1992. It is a treaty designed to manage the conservation of biological diversity including genes, species and ecosystems, to promote sustainable use of biological resources, and to direct the "fair and equitable use of benefits" that arise out of the use of these biological resources. It states that the countries, who are the suppliers of biological resources, have "the sovereign right to exploit their own resources according to their environmental policy," and manage and control access to their resources with the least damage to the environment (United Nations Environment Programme 1992). Each state that is party to the CBD must agree to cooperate with each other through international organizations (Article 5), and seek mediation if there is a dispute (Article 27). The Convention for Biological Diversity was ratified in 1993 by many countries, but not the United States (United Nations Environment Programme 1992). The Nagoya Protocol, as an amendment to The Convention for Biological Diversity, was adopted October 29, 2010 and put into force 90 days later. This document addresses access to genetic resources, but also the traditional knowledge associated with those resources. To obtain access to resources and information, prior informed consent must be obtained on levels from securing permits at a national level to acquiring permission from local people. There must be clear information and regulations to follow on obtaining prior informed consent as it relates to biological resources and traditional knowledge (Article 6). The countries or states providing these resources are encouraged to use their benefits for the further conservation of their biocultural diversity (Secretariat of the Convention for Biological Diversity 2011).

PROTECTION OF BIOCULTURAL DIVERSITY IN THAILAND

In 1999, Thailand's legislature passed the "Act on Protection and Promotion of Traditional Thai Medical Intelligence, H.E. 2452" which was signed into law by the current Prime Minister. This law defines Thai Traditional Medicine and proposes the creation of a committee to protect traditional medicine, and manage access to traditional knowledge and biological resources in herbal medicine. It also proposes a process to register traditional knowledge for its protection, and regulates the use and removal of medicinal herbs to guarantee their conservation (Deewised 2011). The "Act on Protection and Promotion of Traditional Thai Medical Intelligence, H.E. 2452" is in Appendix 3.

In this document Thai Traditional Medicine is defined as "the medicinal procedures concerned with examination, diagnosis, therapy, treatment or prevention of, or promotion and rehabilitation of the health of humans or animals, obstetrics, traditional Thai massage, and also includes the production of traditional Thai drugs and the invention of medical devices on the basis knowledge or text that has been passed on from generation to generation" (Royal Thai Government 1999). This definition includes recipes and formulas, and any formal documentation like stone inscriptions, manuscripts written on palm leaf, Thai traditional books, and even statues depicting *ruesi dud ton*, or traditional Thai stretching techniques. Five national traditional Thai books are recognized by the "Act on Protection and Promotion of Traditional Thai Medical Intelligence, H.E. 2452": the *That Pra Narai Inscription* (Palm Leaf) with 81 recipes, the *Inscription Pharmacopoeia of Wat Orasaram* with 203 recipes, the *Medicine Aid Treatise of Rama V*, Vol.1 with 713 recipes, the *Medicine Aid Treatise of Rama V*, Vol.2 with 1,066 recipes and the *Inscription Pharmacopoeia of Wat Po* with 2,022 recipes (Ministry of Public Health 2011). Along with these formal documents, both general and healer's individual recipes on Thai traditional plant knowledge are also protected (Deewised 2011).

DISSERTATION PROJECT ETHICS

Permission was obtained from the National Research Council of Thailand (NRCT) to conduct this research project for the duration of the study period beginning October 2009 through February 2011 (Registration number 124/52). Appendix 4 includes a copy of the research permit permission letter from the National Research Council of Thailand. Provisional permission was received in April 2009 to begin the "social sciences" portion of this research in the form of a six-month Short-Term Training Agreement (No. Biotec 5402/364/2552) through Thailand's National Centre for Genetic Engineering and Biotechnology (Biotec). This agreement is in Appendix 5.

Fieldwork was conducted in collaboration with the Queen Sirikit Botanic Garden (QSBG) with permission from the director, Dr. Konganda Chayamarity. Laboratory research was performed in collaboration with Chiang Mai University Faculty of Pharmacy for the *in vitro* studies, and at Khon Kaen University, Faculty of Medicine for the *in vivo* studies under the supervision of Dr. Jintanaporn Wattanathorn. The research permits obtained from the NRCT for this project had the following stipulations:

1. Research will be conducted under the supervision of Dr. Jintanaporn Wattanathorn of Khon Kaen University and Dr. Konganda Chayamarity the director of the Botanical Garden Organization.
2. Dried samples will be stored in the herbarium of the Botanical Garden Organization.
3. No samples will be taken to be analyzed abroad in the United States.
4. All expenditures which are borne during the survey and analysis in Thailand will be paid by the researcher.

Two extensions were requested and secured from the National Research Council of Thailand (NRCT) for this project. The first was a six-month extension from March 2011 until August 2011 (Registration number 5/54), then again for three months from September 2011 until December 2011 (Registration number 80/2554). Progress reports were submitted to the NRCT every six months during this research period to include a final report of the research results.

After completion of the research at Chiang Mai University, Dr. Wisinee Chanmahasathien and two other students were trained on the modified Ellman's method for acetylcholinesterase inhibition activity (Ellman 1961) using chemicals and enzymes provided by my research. This type of reciprocation is suggested by Gary Martin (2004): "extraction, screening and more sophisticated techniques should be carried out in the local laboratories and with local expertise whenever possible to stimulate capacity building."

All fieldwork conducted and data collected for this project followed the Guidelines of Professional Ethics as put forth by the Society for Economic Botany (1995), the International Society for Ethnobiology (2006), the Convention on Biological Diversity and Nagoya Protocol and national law of the Kingdom of Thailand with respect to traditional knowledge. The International Society for Ethnobiology (ISE) has a very comprehensive Code of Ethics, which is upheld by its members (International Society of Ethnobiology 2006). It is comprised of different sections to address its purpose, suggested principles and practical guidelines. Obtaining prior informed consent requires the researcher to fully communicate his or her research and potential risks to each research participant. It must be in the language of the research participant, and consider cultural context and potential latent misunderstandings. Free Prior Informed Consent was obtained and full disclosure of potential misuse of the research data was explained to all of

the healers who participated in this study, as per the Code of Ethics set forth by the International Society of Ethnobiology (2006).

The Society for Economic Botany (SEB) also has prescribed guidelines which are outlined under four headings calling for responsibility to the public, those individuals or cultures studied, host government and institutions, and to the profession (Society for Economic Botany 1995). The SEB guidelines for professional ethics requires prior informed consent and adherence to local laws, customs and national requirements, both of which were upheld during the duration of this project.

The City University of New York Office of Research and Sponsored Programs through the Institutional Review Board (IRB) with assurance from the U.S. Department of Health and Human Services has approved this project (IRB number: 08-05-1563; PI: Lisa Offringa). I was trained in Responsible Research Conduct and updated the IRB approval for this project yearly to ensure the protocol and permissions were current. The experimental protocol for the animal experiments was approved by the Khon Kaen University Ethics Committee (2011).

CHALLENGES OF FIELDWORK

There were challenges to the research process for my work in Thailand. The first of these was the language. The Thai language is a tonal language, setting it apart from English, which is not. Both the word and the tone must be remembered otherwise no one will understand you. The traditional healers speak the northern Thai dialect, or *kam muang*. It is related to Thai, but sounds distinct from central or Siamese Thai, and uses different words. All of my interviews were conducted in the northern Thai dialect, so there was little need for me to learn the central Thai language for my research.

Gender issues did not affect my research, but there are gender inequalities in Thailand. Based on the Buddhist precepts, women are viewed as inferior. Male monks are considered higher than female nuns, and women cannot touch monks because women are perceived as impure. Women cannot attain the same levels of enlightenment in Buddhism as men because of their impurity (Tanja Trautwein, personal communication, January 3, 2013). In the universities, there was a uniform mix of males and females. For mentors, Ajan Panee and Dr. Jintanaporn assisted me with my research. Both are strong women who are successful in their field demonstrating the culturally progressive nature of science in Thailand.

For this project, all but one of my research participants were men. In many cases, traditional medicine is taught in the monastery to monks and, as mentioned above, monks are customarily men. Some of my key participants were taught about herbal medicine from their family and from the monastery, others just from their family, or just from the monastery. My female key research participant learned about plant medicine from her grandmother, so she was the only example of a matriarchal lineage of traditional plant knowledge.

DATA COLLECTION AND ANALYSIS

Data from my research were transcribed from the audio taped interviews in Microsoft Word© word processing software. The audio taping software used was the Digital Voice Editor Software supplied with the Sony IC Recorder used to tape the interviews. Lists of the medicinal plants in the formulas were managed using Microsoft Excel© software. Initial data analyses of the *in vitro* bioassay results were also analyzed in Microsoft Excel©. The ANOVA and post-hoc analyses of my laboratory research results were analyzed in SPSS Statistics© (2011) software.

CHAPTER 3 - THAI TRADITIONAL MEDICINAL THEORY AND COGNITIVE DECLINE

INTRODUCTION

Traditional medicine in Thailand is an integrative medical system incorporating the environment and the physical body. The primary theories of Thai Traditional Medicine (TTM) reflect the presence of the natural world in the human body, and the maintenance of health within and between both dimensions of life. The holistic approach of Thai Traditional Medicine, and other eastern-based medical systems, perceives the body, mind and spirit as a complete unit, operating in harmony. Disease stems from an imbalance or dissonance in any of these three facets of a person. The "middle way" of Buddhism is representative of the balance found in optimum health (Mulholland 1979) and is reflected in the theories of Thai Traditional Medicine. The individual must also remain in equilibrium with their environment to maintain health. A disconnection from the natural world can manifest in disease. The Thai system of medicine recognizes human beings as emerging from the natural world and not existing separately from it.

This chapter aims to explain Thai Traditional Medicine as it is practiced in northern Thailand, and how healers in this part of the country treat cognitive impairment in the elderly. Many of the foundations of nothern Thai Traditional medicine are the same as in the Royal Tradition that is documented in central Thailand, but differences can be found in both the spirituality and plant use of healers in the north. Spirituality is infused into Thai Traditional Medicine on a number of levels in theory and in practice (Mulholland 1979). Supernatural causes of disease are accepted as an explanation for illness in northern Thailand. Many of the plants used as medicine by northern healers are found in the upland areas characteristic of the northern terrain.

THEORY OF THAI TRADITIONAL MEDICINE

Health in Traditional Thai Medicine (TTM) depends on the balance of the four elements or *tard*: earth (*thaad din)*, water (*thaad naam*), wind/air (*thaad lom*) and fire (*thaad fai*). They represent the four elements of the natural world and living organisms (Ratarasarn 1989; Mulholland 1979). The four elements are "the foundation of the whole body, life and durability – the state of sickness or health of an individual" (Clayton et al. 1994). Their balance and harmony determines good health, and imbalance in the form of excess or deficiency creates illness and disease (Brun 2003).

Serious disease can develop from a lasting imbalance of the elements, or *rok tard*. There are three classifications of disease resulting from an imbalance of the elements: diseases from bile (*di*), diseases from wind (*lom*) and diseases from mucus (*salet*). These three disease categories could be evidence of Thai Traditional Medicine originating from Ayurvedic Medicine from India. Ayurvedic Medicine classifies disease and individuals according to their *dosa*, forming the *tri-dosa* system found in Ayurveda. These *dosas* correlate to the humors of bile, mucus and wind, which are similar to the aforementioned disease categories of Thai Traditional Medicine (Mulholland 1979).

Each part of the physical body is characterized by one of the four elements (Table 3.1). The manifestations in the body of the earth element are the solid organs, and the air, water and fire circulate around them. There are twenty earth organs in the body. Imbalances in the air, water and fire elements can influence the earth element organs, and vice versa. The *sen,* or energetic lines in the body, and the "Nine Natural Passages" are included as parts of the earth element. There are three manifestations of *sen* in the body: tendons and ligaments, blood vessels, and nerves. These are the passages for the elements of water and air (Ratarasarn 1989).

The "Nine Natural Passages" are divided into upper and lower ducts, and are comprised of the two nostrils, the eyes and ears, the mouth, the urinary tract and the anus. There are twelve presentations of the water element in the body. These are liquid like tears, blood, urine and sweat. There are six types of winds in the body: the wind of breathing, the wind in the stomach and intestines, and the heartbeat that moves the wind around the body. There are four kinds of fire. Three of these regulate digestion and metabolism, and one of them manifests as the fire that fuels anger and is released by meditation.

Table 3.1: A description of each element, its position in the body and the result of an imbalance in them (Damrongrachanubhab 1659; Darot 1972).

Earth Element

Issue	Organ (and position)	Imbalance causes:
1.	Hair on head	Pain in the head, hair falling out
2.	Hair on body	Pain on the skin, hair falling out
3.	Nails	Painful, broken nails
4.	Teeth- 20 primary & 32 secondary	Decayed, gum abscess
5.	Skin, cuticle& epidermis	Numb or burning pain
6.	Flesh (including muscles)	Bruising, rash, mole, wart, burning pain
7.	Tendons	Tension over body
8.	Bones (and cartilage)	Painful and brittle
9.	Marrow in bones	Numb
10.	Spleen	Fever
11.	Heart	Bad temper, hungry
12.	Liver	Ulcer in liver, cirrhosis
13.	Fascia	Thirstiness, hemorrhoids
14.	Kidneys	Cystitis, gas in stomach, tiredness
15.	Lungs	Thirstiness, heat in body, asthma
16.	Colon- upper	Diarrhea
17.	Intestine	Burping
18.	Colon- excretion	Colic, vomit, hiccups
19.	Colon –lower	Problems in excretory system, hemorrhoids
20.	Brain and nerves	Deafness, blindness, stiffed tongue, vomit

Water Element

Issue	Organ (and position)		Imbalance causes:
1.	Gallbladder	-liver	Insanity
		-intestine	Fever, jaundice
2.	Phlegm	- throat	Sore throat, thirstiness, asthma
		- chest	Emaciation
		- feces	Blood in feces, diarrhea, hemorrhoids
3.	Pus from wounds and ulcers		Coughing, poor appetite, emaciation
4.	Blood		Fever, blood in urine
5.	Sweat		Coldness in body, tiredness
6.	Fat		Spots on skin, heat
7.	Tears		Eyes diseases
8.	Lymph		Dizziness, yellowish skin & eyes, diarrhea
9.	Saliva		Sore throat
10.	Mucus		Headache, dizziness, runny nose
11.	Marrow		Pain in joints
12.	Urine		Abnormal urine color

Wind Element

Issue	Organ (and position)	Imbalance causes:
1.	Yawn and belch	Heat in the stomach, belch, phlegm
2.	Gas in intestine to anus	Ache all over body
3.	Gas in abdomen	Gastritis, stomachache
4.	Gas in intestine and stomach	Colic, nausea, vomit, poor appetite
5.	Gas in body	Dizziness, pain in spine, poor appetite
6.	Breath	Uncomfortable inhalation

Fire element

Issue	Organ (and position)	Imbalance causes:
1.	Fire for warming body	Coldness in body
2.	Fire for heating body	Heat in body, sweat
3.	Fire for aging body	Decay of human body, deaf
4.	Fire for digestion	Hands and feet ache, gas in stomach, nausea

The causes of imbalance in the elements can originate from behavior. The "middle way" of Buddhism is that of moderation. To develop ways that are extreme, or too far in one direction, can cause disease if practiced regularly. Extreme ways like eating too much or too little food, eating meals at the wrong time, staying in a particular posture too long, lack of sleep,

excessive work, not urinating and defecating regularly, sadness and a tendency towards aggression can cause health problems (Mulholland 1979). These activities must be regulated to maintain good health. The origin of disease can also be attributed to the theory of karma in Buddhism where an individual's predisposition to disease is based on the actions of their past lives (Brun 2003).

Thai Traditional Medicine incorporates Buddhism into the suggestions of maintaining good health. This refers to the practice of *Dhammanamai* for optimum health. It is composed of: *Kayanamai*, or healthy body. To keep a healthy body one should eat good food, especially fruits and vegetables suited to the individual's *tard* or element, and exercise. The Thai traditional stretching exercise is called *ruesi-dud-toni*. It stimulates the flow of blood in the body and massages the *sen* lines. Also the individual should keep a healthy mind, or *Jitanamai,* by practicing meditation, and keep a healthy lifestyle, or *Chevitanamai*, by living on the middle path of Buddhism (Leung-aroon 2005).

Maintaining balance of the four elements is the fundamental basis of health. The foods that are eaten in daily life help to maintain this balance. To maintain good health and balanced elements, eating particular foods are encouraged. Some foods are considered neutral, and others should be avoided, especially during illness. Foods that are considered "hot", like chilies and animal products, should be avoided when a patient has a fever. Instead, the patient should eat foods that are considered "cooling" during this time (Ratarasarn 1989). While some food restrictions seem obvious, like not eating fried food, other food choices depend on the patient and their physical disposition. Food prescriptions should act in agreement with symptoms of a disease, and not act against any medicine the patient is taking (Mulholland 1979). The elements

can be balanced or imbalanced by certain foods, and in some ways, can dictate whether the individual is healthy or ill.

The line between food and medicine is vague in Thai Traditional Medicine and Thai culture in general. Many food recipes contain plants that are medicinal, and some plants are taken as food but also have medicinal qualities. Food is categorized according to the different elements. It is best to eat food corresponding to your element, and therefore keep yourself in balance. The earth element's foods are: jackfruit, papaya, peanuts, banana, beans, squash and melons. The water element's foods are: tamarind fruit, pomello, green mango, star fruit, bitter gourd and lime. The wind element's foods are: pepper, mint, turnips, scallion, ginger, basil, garlic, dill and pepper. The fire element's foods are: mushroom, cabbage, mangosteen, tomato, orange, eggplant and lettuce. By eating the food that corresponds to your element, the body maintains internal equilibrium and balance with the external environment.

Many food choices are dictated by which plants are growing during that season or in the local environment. Traditional healers believe that it is best to eat foods that are growing during each season to help keep the elements balanced in the body, and the body in tune with the cycles of nature (pers. comm. Mor C 2009). The period during the change of seasons can cause illness from the elements failing to adjust (The Bureau of Buddhistic Monastary Affairs 1659). In northern Thailand, there are three seasons (Mulholland 1979).

1. Summer: from the first waning moon of March to the first waning moon of July. It is of fire origin and is from excess strength of the blood.
2. Rainy season: from the first waning moon of July to the first waning moon of November. It is of wind origin and affects circulation.

3. Winter: from the first waning moon of November to the first waning moon of March. It is of water origin and manifests in excess phlegm.

In addition to issues inside the body, disease can be caused by sources outside the body. A change in the body's natural surroundings can cause disease. If someone moves to a new area, their body must adjust to new temperature, water and even air. Their body has developed a personal immunity profile from the place they were born, so a change in environment, can cause illness or expose them to unfamiliar diseases (Ratarasarn 1989). The effect of bacteria, germs or a parasite, infectious diseases (cholera, small pox, etc.), allergies, the wrong food or bad odors are also recognized as the cause of disease. Where the patient lives and the climate where they grew up influences their constitution. In mountainous areas, disease manifests from the fire element. In a warm climate country, disease manifests in imbalance in the water element. In a rainy areas, disease can manifest with problems in the air element, and for people who live near the sea, disease manifests for them in the earth element (Mulholland 1979).

Unique to Thai traditional medicine is the presence of a *mother* in disease. The *mother* is the center of the disease that may have entered the body from the outside, but can also manifest from internal imbalance. In order to cure the disease, the *mother* must be located and destroyed. The *mother* can be destroyed using medicinal plants, or diet restrictions where foods that sustain the *mother* are not eaten and her power diminishes (Brun and Schumacher 1994). The concept of the *mother* can be understood by determining the central cause of the disease, then remove or destroy it to cure the disease (pers. comm. Mor B 2010).

Stress and emotions can cause disease. It is thought that seventy percent of illness comes from the mind, because if the mind is not strong, it will treat the body poorly (pers. comm. Mor H). Everything starts with the mind. Intensely negative emotions can take a toll on health, as

well as decrease resistance to disease and illness from the outside and to cause other health issues to reappear. A negative emotional state can also slow down the healing process of an ill patient (Ratarasarn 1989).

People who have become ill must refrain from certain activities. To practice these activities could result in a slow healing and prolonging of the sickness. These are: don't take a shower, don't use perfume, don't have sex, don't scratch at the body too strongly, don't sleep in the daytime, don't work too hard, don't get angry, don't sleep outside and don't eat oily foods (The Bureau of Buddhistic Monastary Affairs 1659).

Thai Traditional Medical theory states that illness manifests itself differently depending on the age of the patient. There are three periods of a human life:

1. **Childhood** is from birth until sixteen years old. This period is influenced by the water element, with phlegm and blood. Children should not eat sweet, bitter and sour foods. There are two periods of childhood: Newborn to eight years old where illness manifests in phlegm, and then eight to sixteen years old where illness manifests in the blood.
2. **Young adult** is from sixteen to thirty two years of age. This period is influenced by the water and wind elements. Young adults should not eat bitter and sour foods.
3. **Adult** period is from thirty two to sixty four years old. This period is influenced by the wind element. Adults should not eat hot, bitter and sour foods. Anyone over sixty-four years old is elderly and they have illnesses influenced by water, so they have issues with phlegm and sweat (The Bureau of Buddhistic Monestery Affairs 1659; Mulholland 1979).

The elderly become infirm from damage to the four elements from years of use. Some expressions of old age, like hearing and vision loss, bone disorders and memory loss, can

become symptoms of a disease. These types of disease tend to be chronic and repeat after remission (Ratarasarn 1989).

Figure 3.1: Each person also has a main element, or *tard chao ruan*, determined by the month and day of their conception. This chart calculates the *tard chao ruan* (Photograph by author).

A traditional Thai doctor uses his eyes and ears to diagnose a patient (Ratarasarn 1989). There are four categories used to diagnose disease in Thai Traditional Medicine: the history of the patient, the history of the disease, assessing the body and mind, and analyzing the disease symptoms. The patient history involves questions relating to age, place of birth and current residence, habits and occupation, family history and previous illnesses. The history of the disease includes questions about the time of illness, the symptoms and the order they appeared, previous symptoms and any changes in them, and how the disease manifests on a daily basis.

The patient will be examined for skin tone, weight, breathing, heart, lungs, eyes, tongue and then the pulse and blood pressure is taken (Mulholland 1979). Like Traditional Chinese Medicine, the pulse is a diagnosis tool. In Thai Traditional Medicine, there are two pulses in the body. For the upper body, the pulse is in the wrist. For the lower body, the pulse is in the foot (pers. comm. Mor C 2010). The symptoms of the disease are evaluated by asking the patient about their perspiration, temperature, how they are eating and sleeping, and their mental state (Mulholland 1979). The doctor will ask about the bodily discharges of the patient like urine, blood, etc. as they reflect the condition of the internal environment. The doctor must also diagnose the condition of the elements (Ratarasarn 1989). After the disease is identified, then a course of treatment is determined.

There is also a component of illness in Thai Traditional Medicine relating to the time of day. Each day is broken four sections correlating to daytime, and four to night time (Mulholland 1979). The time of day the disease manifests is important to its diagnosis.

Daytime

	Element	**Imbalance**
0600 - 09.00	water	mucus
09.00 - 12.00	water	blood
12.00 - 15.00	water	bile
15.00 - 18.00	air	N/A

Night time

	Element	**Imbalance**
18.00 - 21.00	water	mucus
21.00 - 24.00	water	blood
24.00 - 03.00	water	bile
03.00 - 06.00	air	air

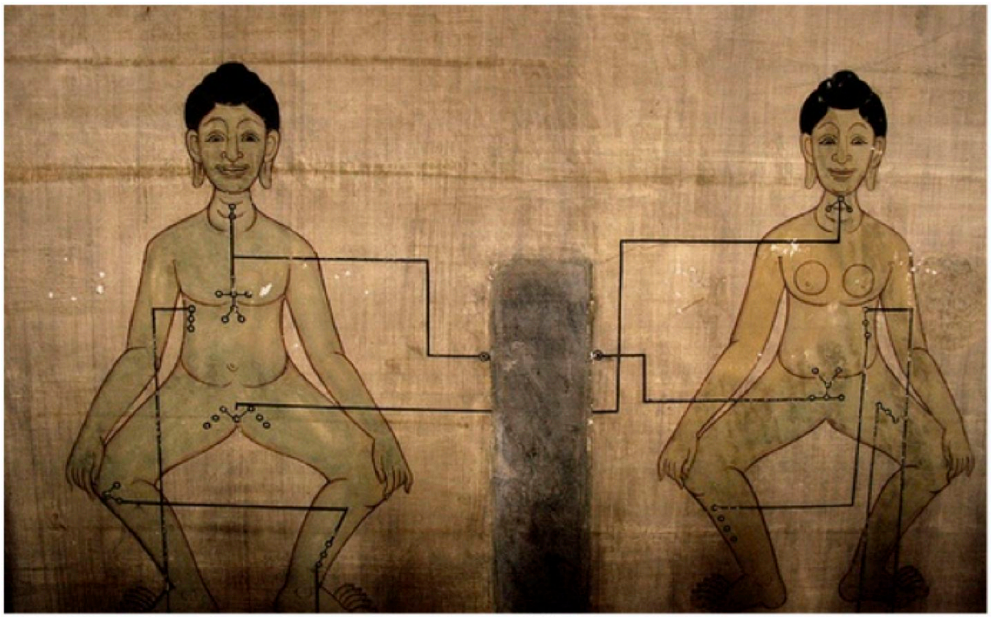
Treatment in Thai Traditional Medicine, like in western medicine, depends on the severity of the disease and symptoms. With minor or mild symptoms, bed rest and home

remedies are used. If the symptoms persist and worsen, or if the illness is severe, then treatments with plant based medicines are used sometimes in combination with massage, food recommendations and meditation. The medicinal plants prescribed to the patient depend on the severity of the disease, the location in the body and the age of the patient. Massage is also used to prevent disease by keeping the elements balanced, and to promote well-being. Medicine, or *yaa*, is applied either externally in the form of baths, herbal steam, poultice, compress, ointment, or internally by inhalant, eye or eardrops, or oral remedies (Ratarasarn 1989). Another type of medicinal plant preparation is scraping a root on rock with water to infuse the water with the medicine in the root (Figure 3.2).

Figure 3.2: Plant parts that are harder, like stems, bark and roots are often scraped or ground against a rock with water to infuse the water with the plant material (Photograph by author).

Thai massage, or *nuad thai*, is a significant part of Thai Traditional Medicine. The teachings of Dr. *Jivaka Kumar Bhaccha*, who was the physician of the Buddha, are the foundation of Thai massage. His teachings imply that there are three parts to the body: the body at the base, the energy in the middle, and the soul at the top. The soul is the most important as it is responsible for knowing, remembering, thinking and relating. Thai massage works at the energetic level of the body. There are 72,000 energy lines in the body, called *sen* lines, but ten main lines, which are worked during a typical Thai massage (Figure 3.3). The purpose of the massage is to align the body structure, restore the organs and body parts, balance and circulate the elements, and stimulate vital energy to prevent disease (pers. comm. Old Medicine Hospital Chiang Mai 2009). Many of these practices were originally performed within the family and villages. During the rice harvest, after working long hours in the fields, family members would massage each other to relieve sore muscles. Soon aromatic and warming plant extracts were used to increase the effectiveness of the massage techniques, and a complex, integrative medical practice began.

Figure 3.3: The *sen* lines of the body used for traditional Thai massage pictured at Wat Pho, Bangkok, Thailand (Photograph by Mor B).



SPIRITUALITY IN THAI TRADITIONAL MEDICINE

Spirituality and supernatural causes of disease have a greater perceived influence on health in the north of Thailand than in the more mainstream medical system of central Thailand. In northern Thailand, the Buddhist religion is mixed with superstition and animism, including a belief in ghosts. Many folk healers in northern Thailand are also spiritual healers. It is common to have a patient come to purchase herbal remedies and after have a ceremony to help create good luck for the patient. The existence of spirits and ghosts, and their influence on humans, can be an explanation for disease, at least initially. In many cases, a healer will perform a ceremony to remove the spiritual cause of disease first, before they investigate other causes. Sometimes a healer will make the medical diagnosis using an ancient drawing or design. This type of medical treatment incorporates myths and legends, which was a valuable way to explore northern Thai Traditional Medicine and the belief system behind its disease categories (Martin 2004).

Supernatural causes of disease, including the effect of a spirit or ancestor's soul, or by the astrology or karma of a patient can be the cause of disease. One healer has a diagnosis tool to determine if ghosts caused the patient's disease (pers. comm. Mor J 2011). He received this tool from a palm leaf manuscript or *bap saa* from his grandfather (Figure 2.4). Interestingly, on top of the turtle is the Hindu deity Krishna possibly indicating India as the origin of the manuscript. The turtle has seven numbers in Thai on and around it. The patient's birthday day determines the number on the turtle and the numbers tell the supernatural cause of the disease.

Figure 3.4: The "turtle fortune to tell about sickness" used to tell the supernatural cause of the disease.

The number corresponding to the supernatural cause of disease:

1	If you pray to the ghosts for something you want, and offer that you will do something in return, then you forget, you will get sick.
2	The spirit of the village, field, orchard or temple has made you sick.
3	The spirit that protects the city has made you sick.
4	One of two different ghosts have come into your body, eaten your organs and made you sick. One ghost is the *pii praii* or the woman who died with a baby inside, the other is the *pii ga yak*, which is the ghost that lives in the forest or an abandon temple.
5	You have done something wrong to the ghosts, especially the ghost of the family or house (*pid pii*), and they will make you sick.
6	If you have a well in the house and it is not covered properly, you become ill from *took sued*.
7	The *pii rai*, a bad spirit, or *pii tdai hong*, the ghost of someone who died in an accident, has made you ill.

To treat these illnesses, the healer must write a protection prayer on paper and form a candle around it. The patient burns the candle and releases the prayer to remove the influence of the spirit. The healer can also speak words or prayers to "give strength to the mind of the patient and to nourish their spirit" (Rajarasarn 1989). This support improves the wellbeing of the patient and assists in their recovery. Similarly, a negative emotional state can cause the patient to become ill or worsen their condition. Meditation is also suggested for the patient to encourage them to focus on their good health, healing and wellbeing.

The spirit of the person or the *quan* can influence their health as well. The *quan* is represented in the different elements in the person. There are thirty-two total *quan* with twenty earth *quan* and twelve *quan* associated with other elements. The *quan* resides in the head in the morning, the chest in the afternoon and the feet at night. These parts of the body where the *quan* resides should be rinsed during the specified time of day to keep the *quan* fresh (pers. comm. Mor C 2010). The *quan* will leave the body if there is a motor vehicle accident. The patient and the healer must go to the place where the accident occurred to perform a ceremony to call the *quan* back. The ceremony involves offering the *quan* foods like fruit and rice, and flowers, all wrapped in a banana leaf to entice the *quan* to return to the body (pers. comm. Mor E 2010).

The *quan* can also have an impact on the memory and cognitive decline. If an elderly person's *quan* leaves them too soon, they will become weak and sick in the body, and forgetful and "crazy in the mind" (pers. comm. Mor E 2010). If this occurs, the healer calls the *quan* back to the person through a similar ceremony. The healer uses a fish net and offers food like fruit and rice, and flowers in banana leaves to ask the *quan* to return to the person. During this ceremony, the *quan* is called back and a white string is tied on the patient's wrist to ensure the spirit stays in the body.

There are aspects of Buddhist spirituality that manifest in Thai Traditional Medicine. In Buddhism, the day of the week someone is born holds great importance. Each day of the week has specific characteristics, and these characteristics are attributed to the person born on that day. The day of the week on which the patient was born can influence their element as each day is affiliated with an element. Monday is the earth element, Tuesday and Thursday are wind, Wednesday and Friday are water, and Saturday and Sunday are fire. Buddhism uses the lunar calendar, and phases of the moon dictate the holy days and festivals. Each festival is not the

same date each year as it would be if Buddhism used the Gregorian calendar. In Thai Traditional Medicine, it is believed that illness becomes worse during the dark moon, and most Buddhist holy days are on full moons, which are considered auspicious.

PLANT USE IN THAI TRADITIONAL MEDICINE

Over seven hundred varieties of medicinal plants are used in Thai Traditional Medicine (Rajarasarn 1989). Of these different varieties, each part of a plant can have different properties for healing. For example, the bark of the tree may treat diarrhea, but the leaves of the same tree may be used as a laxative. The plant parts used in Thai Traditional Medicine are: the vine, the wood, the shoot, the core, the deep root and the shallow root, the bark, the fruit, the flower and the leaf. Sometimes all parts of the plants are used together.

There are four ways to ingest plants to "take herbal medicine" in Thailand (*gin samoon phrai)*. The plants are steeped in water and made into teas, they are put in alcohol and made into a tincture or they are eaten either cooked or raw in food. They can also be scraped into water as already discussed. Some plants must first be processed either by heating them to remove poison, heating them to infuse their properties into a liquid, drying them so they can by made into a powder, or not heating them, but pounding them and squeezing out the juice (Ratarasarn 1989). Plants are also used in the steam bath where they are heated and their vapors inhaled.

Ingesting plants for medicine is to take in the physiological activity of the plant. Thai Traditional Medicine classifies plants into ten tastes: sweet, astringent, bitter, toxic or poison, hot, oily, aromatic and cool, salty, and sour (Gunn 1998) (Table 3.2). Of these, there are three principle tastes to characterize medicinal plants. Plants with a hot taste treat wind disorders, strengthen the elements, and treat fevers. Plants with a cool taste, which can include pollen,

leaves and flowers, treat diseases relating to the fire element and help with poisons. Plants with mild taste treat diseases caused by the water element and help to maintain a healthy heart (Mulholland 1979). The taste of the medicine corresponds with certain parts of the body. The bitter taste is associated with the skin, an astringent taste with the muscles, a salty taste with the tendons; a spicy taste with the bones; a sweet taste with the long intestine; a sour taste with the short intestine; a cool taste with the heart, and an oily taste with the pores (The Bureau of Buddhistic Monastary Affairs 1659).

Table 3.2: Indications of each of the ten tastes in Thai Traditional Medicine.

Taste	**Quality**
Astringent	Used internally as a tonic, digestive and to clean and heal wounds
Sweet	Used for coughing, asthma, thirstiness and exhaustion
Intoxicating	Used to calm pain and treat digestive disorders
Bitter	Used for fever and a vermifuge for children
Spicy	Used internally for digestion and externally as a balm or antispasmodic
Oily	Used internally for rheumatism and externally for the muscles
Cool	Used internally for fatigue, thirst, hunger and lethargy
Salty	Used as a digestive and sore throat
Sour	Used internally as diuretics, expectorants and to ease coughing
Bland	Same uses as sour taste

To treat disease, the healer may first prescribe a general tonic that balances the four elements but focuses primarily on the patient's main element, or *tard chao ruan*. This tonic brings the patient's body into its "normal condition" and allows the patient to have general immunity against disease. The patient is strong when the four elements are in balance. It is important to promote circulation of the elements in the body, especially the water and wind elements, in an effort to balance them. Nourishing the organs strengthens the earth element. The

balance of the elements in the body allows the healer to further address the condition of the patient and determine the actual illness (pers. comm. Mor C 2010).

Each element has a corresponding plant used to strengthen it (Table 3.3). An "elements tonic" is a mix of these five plants in specific amounts with the plant corresponding to the main element of the patient representing the largest proportion. This tonic corresponds to the "5 spices" frequently prescribed group of plants from the ancient legend of the *rsis*. To strengthen the other elements, there is a sequence followed: earth, water, air, fire, and oxygen. For example, to balance the earth element, the healer would measure the powder of 16 parts *diplee* (earth), 8 parts *chaploo* (water), 6 parts *sakan* (air), 4 parts *jettamoon plueng daeng* (fire) and 2 parts *khing* (oxygen). To maintain the balance of all the elements, and remain in good health, mix an equal part of each plant (pers. comm. Mor C 2009).

Table 3.3: The five elements and their compatible plants which when mixed together make a tonic to balance the elements (*n.b.* the healer who explained this to me believed in a fifth element so there are two representations of the air or oxygen element).

Element	**Scientific name**	**Thai name**	**Plant part**
Earth	*Piper chaba* Hunter	*Diplee*	fruit
Water	*Piper sarmentosum* Roxb.	*Chaploo*	leaf/stem
Air	*Piper ribesioides* Wall.	*San kaan*	vine
Fire	*Plumbago indica* L.	*Jettamoon pleung daeng*	root
Oxygen	*Zingiber officinale* Roscoe	*Khing*	rhizome

Most remedies in Thai traditional medicine do not call for a single plant, but instead are complex mixes of a number of plants. These multi-plant formulas address the actual complaint, but also contain other plants that support the action of the main plant ingredients or reduce

unfavorable side effects. Additional plants are added to complement the principle plants, but sometimes also address the specific needs of the individual. Most formulas have at least two to three plants that are the same in the formula. In the plant formula, if one plant is not available a second or third plant could still work, or a different part of the plant is sometimes used (i.e. use bark of tree instead of rind of fruit) (pers. comm. Mor J 2009). Some plants are too strong in a formula and need to be heated first to make them weaker. The plants included in the formulas and their proportions or measurements are often the highly guarded secret of each healer. Many times these plants are the same between healers, but sometimes formulas have a completely different set of plants contained in them. In most cases, these formulas should be taken with alcohol to increase their absorption in the stomach (pers. comm. Mor H 2010).

Specific properties are attributed to each of the plants selected to be included in a treatment formula. As already mentioned, each plant has a characteristic "taste" that classifies it. The taste correlates with the effect of the plant. These qualities are then connected to one of the four elements. The plants specific to that "taste" counter the imbalance of the elements in an ill person. For example, a spicy plant will increase the wind element where it may be lacking, but will decrease the water element. Skin color and blood type will also determine what types of plants the patient should take. For example, a patient with white skin and sweet blood needs plants that are spicy and bitter. One with yellow to white skin with sour blood takes salty tasting plants, and someone with black skin with very salty blood takes plants that are sweet tasting (The Bureau of Buddhistic Monastary Affairs 1659). The combination of plants, their tastes and the elements they relate to, are all considered when an herbal formula is created.

Plant medicines are classified into three groups called the small, large and great classes. This classification helps to clarify the measurements of each plant in the formula and to reduce

confusion if one plant is not available and must be replaced with a similar plant. The "small class" of plants consists of plants with a similar taste, smell or color. These plants may have a similar quality or strengthen the entire formula. The "large class" of plants are groups of plants that are always grouped together. The *Tians*, *Goads* and *Kesorn Tang Haa*, are examples of this class. Plants in the "great class" are the same types of plant but are put into the formulas in varying amount depending if the problem is due to an excess or a deficiency of the element (Mulholland 1979).

Figure 3.5: A multi-plant formula before it is ground into a fine powder in a traditional plant mill (Photograph by author).

GROUPS OF PLANTS USED IN THAI TRADITIONAL MEDICINE

Tians

Healers use this group of plants in combinations of five, seven or nine plants. The seeds are the part used for all the plants. They are used to treat problems associated with wind and are in almost every formula for wind disorders. Each of the plants has similar properties and the specific healer will decide which of the *Tians* to include in the formula. The healers had different uses for them such as: they are good for food poisoning, a tonic for the blood or just for general health to balance the elements, or these plants are used as a heart tonic and can heal issues with the wind element. Healers give this mix of plants to elderly people to take for longevity and to treat dizziness (pers. comm. Mor J, Mor H, Mor F 2010).

Name in Thai	Thai Name	Scientific Name
เทียนแดง	Tian daeng	*Lepidium sativum* L.
เทียนดำ	Tian dam	*Nigella sativa* L.
เทียนข้าวเปลือก	Tian Kao plueak	*Foeniculum vulgare* Mill.
เทียนขาว	Tian kaow	*Cuminum cyminum* L.
เทียนเกล็ดหอย	Tian Kled hoi	*Plantago ovata* Forssk.
เทียนสัตตบุษ	Tian satta boot	*Pimpinella anisum* L.
เทียนตากบ	Tian tdaa kob	*Carum carvi* L.
เทียนตาตั๊กแตน	Tian tdaa tdak ka taen	*Anethum graveolens* L.
เทียนเยาวพานี	Tian yao wa pa ni	*Petroselinum crispum* (Mill.) Fuss

Triplaa

These three fruits are found in many formulas. They are adapted from Ayurvedic medicine and are used together to treat fever and cough, and for good skin (pers. comm. Mor C 2009).

Name in Thai	Thai Name	Scientific Name	Part Used
ลูกมะขามป้อม	Look ma kaam porm	*Phyllanthus emblica* L.	Fruit
ลูกสมอพิเภก	Look sa mor pi pek	*Terminalia bellirica* (Gaertn.) Roxb.	Fruit
สมอไทย	Look sa mor Thai	*Terminalia chebula* Retz.	Fruit

Goads

Each plant in this group of plants has different properties. Some are good for hemorrhoids, others for bones and others for gas. They are all good for memory in general. Again, the healer will decide on their inclusion in a formula (pers. comm. Mor B 2010).

Name in Thai	Thai Name	Scientific Name	Part Used
โกฐชฎามังสี	Kot chada mang sri	*Nardostachys jatamansi* (D. Don) DC.	root
โกฐเชียง	Kot chiang	*Angelica sinensis* (Oliv.) Diels	root
โกฐหัวบัว	Kot hua bua	*Conioselinum univittatum* Turcz.	root
โกฐจุฬาลัมพา	Kot jula lampaa	*Artemisia vulgaris* L.	all parts
โกฐก้านพร้าว	Kot kaan prao	*Picrorhiza kurroa* Royle ex Benth.	root/stem
โกฐเขมา	Kot kamao	*Atractylodes lyrata* Siebold & Zucc.	root
โกฐกระดูก	Kot kra dook	*Saussurea lappa* (Decne.) Sch. Bip.	root
โกฐพุงปลา	Kot Poong plaa/ Look sa mor Thai	*Terminalia chebula* Retz.	seeds
โกฐสอ	Kot sor	*Angelica dahurica* (Fisch.) Benth. & Hook. f.	root

Kesorn Tang Gaow

These nine plants are combined for good memories, and can be individually included in formulas for memory. They are also a heart tonic and can make the mind feel good (pers. comm. Mor B 2010).

Name in Thai	Thai Name	Scientific Name	Part Used
เกสรดอกบัวหลวง	Kesorn do bua luang	*Nelumbo nucifera* Gaertn.	stamen
ดอกมะลิ	Dok mali	*Jasminum sambac* (L.) Aiton	whole flower
ดอกพิกุล	Dok pikun	*Mimusops elengi* L.	whole flower
ดอกบุนนาค	Dok boonnaak	*Mesua ferrea* L.	whole flower
ดอกสารภี	Dok sarapii	*Mammea siamensis* (Miq.) T.Anderson	whole flower
ดอกจำปา	Dok jampaa	*Michelia champaca* L.	whole flower
ดอกกระดังงา	Dok kradong ngaa	*Cananga odorata* (Lam.) Hook. f. & Thomson	whole flower
ดอกลำเจียก	Dok lam jiak	*Pandanus tectorius* Parkinson	whole flower
ดอกลำดวน	Dok lamduan	*Melodorum fruticosum* Lour.	whole flower

Kesorn Tang Haa

These five plants, as taken from the above nine, are used as a heart tonic and to heal wind imbalance (pers. comm. Mor B 2010).

Name in Thai	Thai Name	Scientific Name	Part Used
ดอกบุนนาค	Dok boon naak	*Mimusops elengi* L.	flower
ดอกมะลิ	Dok mali	*Jasminum sambac* (L.) Aiton	flower
ดอกพิกุล	Dok pikul	*Mimusops elengi* L.	flower
ดอกสารภี	Dok sara pii	*Mammea siamensis* (Miq.) T.Anderson	flower
เกสรบัวหลวง	Kesorn bua luang	*Nelumbo nucifera* Gaertn.	stamen

The Five Spices

There is a legend telling of six *rsis*, or holy men, who set out to discover medicines long ago. The first five each found one plant, and the sixth *rsis* decided to mix the five plants together

to make them stronger. This mixture is called the "five spices". It alleviates illness in any of the parts of the body, and improves sleep and appetite (Mulholland 1979).

Name in Thai	Thai Name	Scientific Name	Part Used
ดีปลี	Deeplee	*Piper chaba* Hunter	fruit
สะค้าน	Sakaan	*Piper ribesioides* Wall.	stem
รากช้าพลู	Raakchaplu	*Piper sarmentosum* Roxb.	root
เจตมูลเพลิงแดง	Jet amoon plueng daeng	*Plumbago indica* L.	root
ขิง	Khing	*Zingiber officinale* Roscoe	rhizome

Thai Elixir of Life

This mixture of plants is believed to "prolong life and prevent premature aging" (Mulholland 1979). The plants are from a legend about four *rsis*, who each discovered one of the plants. They are made into an infusion using one part of each plant.

Name in Thai	Thai Name	Scientific Name	Part Used
มะตูมอ่อน	Ma toom on	*Aegle marmelos* (L.) Corrêa	fruit
พิมเสน	Pim sen	*Blumea balsamifera* (L.) DC.	bark
ผักคราดหัวแหวน	Pak krad hua waen	*Spilanthes acmella* (L.) Murray	leaves
บอระเพ็ด	Bor ra ped	*Tinospora crispa* (L.) Hook. f. & Thomson	stem

COGNITIVE IMPAIRMENT IN THAI TRADITIONAL MEDICINE

Wind or air (*lom*) disturbance is the origin of cognitive decline in Thai Traditional Medicine. It is the cause of all conditions affecting the neurological system and brain. In general, the cause of cognitive impairment in the elderly is from an imbalance of all the elements, from overuse, or from a lack of equilibrium specifically affecting the wind element (Ratarasarn 1989). In Thai Traditional Medicine, the wind element draws everything together with the breath and connects it to the root of the individual. If the *lom* moves into the brain and stagnates, it causes imbalance in the body and problems with the mind (pers. comm. Mor J 2010).

There is no word for brain in the *Lanna* language. Instead, they speak of the heart or mind. If there is a brain or nerve problem, then the word to describe it is a problem with *lom* (pers. comm. Mor F 2010). In Thai, the brain is known as the *matthakematthalungkhang*, and it relates to the tissue inside the skull. Its purpose is to control the body functions and manage the five senses, and to govern the, "memory, thought, intelligence, careful consideration and the sense of responsibility" (Mulholland 1979).

There are six important roles of the wind element in the body. First, there is the wind that moves from the bottom to the top (navel to head) that causes coughing, belching and vomiting, and the wind that moves from top to bottom (navel to toe) that causes defecation and urination. There are two types of wind relating to digestion, and they can be sensed when we are hungry or anxious about things. These are the wind inside the digestive system and outside of the digestive system. There is also the wind that makes people move, where too much of it can cause dizziness and ringing in the ears. This wind relates to the cardiovascular system and deals with the movement of blood through the body, which delivers oxygen and nutrients. Finally, there is

the wind for living, the "breath of life". This wind in the body relates to the nervous system, or the "electrical system" of the body (Siri-sard 2010).

The idea of *lom* was difficult to translate into a concept understood within the western medical framework. In Thai Traditional Medicine, it manifests as "air in motion" (Mulholland 1979). Following are some of the beliefs about *lom* from the traditional healers. Of the four elements, wind or *lom* is the most important to the brain. It controls the "main line" made up of the spinal cord, brain, nerves and circulatory system. *Lom* provides the energy that controls the heartbeat and nerves, and regulates thoughts, emotions and the conscious mind. An imbalance of the wind element reduces flow and circulation in the body. It can cause stagnation in the movement of the body and of things through the body. It mobilizes the body, provides life and stimulates all other elements. Without *lom*, it is believed that life ends very quickly (pers. comm. Mor E, Mor J, Mor H 2010).

If the wind or *lom* in the body is not strong enough to move the blood, then disease can manifest. Respiration and the heart beat moves *lom* through the body. The *lom* travels through the body through the circulatory system, regulates digestion and travels up the spine into the brain. The *lom* moves with the blood through the brain, heart and stomach, and circulates through the *sen* lines, and through the bones (Ratarasarn 1989). The *lom* follows the blood, so in western terms it is thought of as the oxygen that is attached to the red blood cells and travels with the blood. The idea that *lom* is oxygen is a very narrow perspective as viewed by Thai traditional healers.

In Thai Traditional Medicine, adults over thirty-two are prone to disease caused by *lom* imbalances resulting in dizziness, twitching and headaches. One healer expressed that if there is too much wind moving the blood, the patient can have a stroke or go crazy. If there is not

enough wind moving the blood, the memory is bad and the patient is tired. Any imbalance, either too much or too little, can be problematic (pers. comm. Mor B 2010). If there is too much *lom* in the brain, it can build up pressure, similar to high blood pressure. This type of *lom* build up can cause a stroke (pers. comm. Mor F 2010).

Along with the circulation of *lom* in the body, a reduced blood flow to the brain can cause memory impairment. For many years, health practitioners world-wide believed *Ginkgo biloba* L. (Ginkgoaceae) improved memory by increasing the blood flow to the brain. Krieglstein et al. (1986) found that extracts of *Ginkgo biloba* did improve local cerebral blood flow, but its effects on enhancing memory were never confirmed.

According to another healer, there are two main types of *lom* in the body. There is the *lom gon yaab,* which is the wind from digestion, and there is the *long gong la eid*, which is the wind in the heart. When people are over fifty years old, the *lom gon yaab* can become problematic (pers. comm. Mor F 2010). In the elderly, when the *long gong la eid* is not well the heart and heartbeat are compromised and there can be dizziness in the head. If it is not treated and becomes more severe, it can lead to dementia. Therefore, a patient with memory loss may also have problems with heart disease. Research by Jennings et al. (2005) confirmed this theory when they found that hypertension, when not managed, can cause memory impairment.

Both epilepsy and migraine headaches are thought to be generated from an imbalance or stagnation of wind or *lom* in the body. Epilepsy occurs when the wind in the body is unbalanced, but a different type of wind causes dementia. Migraine headaches are also from a different type of wind than the one that causes dementia. These headaches happen because the wind is stuck in the head like in *lom mar ren kroot* (see section on culturally specific diseases).

In addition to overuse and imbalance, the circulation of *lom* in the body carries with it any toxins that are present in the air or ingested in food. It is believed these toxins travel up to the brain and affect its functioning. The accumulation of toxins in the body from the environment is one rationale used by the traditional healers to explain the increase in the prevalence of cognitive impairment in the elderly. A number of healers believe the increasing number of patients with dementia and other chronic diseases is due to the accumulation of toxins in the environment. They prescribe taking plants that detoxify the body to help prevent these types of diseases (pers. comm. Mor B, Mor J, Mor H 2010).

Eating foods that are good for you and restricting specific foods that are harmful is prevalent throughout Thai Traditional Medicine. Bad food can cause disturbances in both the *lom*, and the blood, which can affect the functioning of the brain. While *lom* is the cause of memory disturbances, the brain is considered an earth organ. Food is also of the earth, so food choices are particularly important when considering the effect on the brain. "Bad food causes *lom* disturbances" (pers. comm. Mor H)

Lifestyle issues in the form of "stress" can contribute to memory loss as people age. If a person works too much, or has many problems like family problems, job difficulties, money problems or problems in their community, it can cause memory issues later in life. The wind occurring from stress and tension contribute to dementia. This belief speaks to the recognition of the connection between the mind and body in Thai Traditional Medicine.

TREATMENT OF COGNITIVE DECLINE IN THAI TRADITIONAL MEDICINE

To treat cognitive decline, the healer must first determine if the cause of the illness is from bad spirits, or a lost spirit (*quan*). Supernatural causes of disease are a prevalent belief in

Thai Traditional Medicine as it is practiced in northern Thailand. If the healer believes the patient has lost their *quan*, then a ceremony must be performed to call the *quan* back. If the illness is from bad spirits, a spell is written down then wrapped in a candle, and given to the patient. If the illness is not gone, then the healer will give them an herbal formula to help their memory. Memory loss could also result from a problem in their mind, like an irrational fear or anxiety (pers. comm. Mor H 2010).

Food is part of the prevention and treatment of all disease in Thai Traditional Medicine, including memory disorders. Greasy foods can cause a build-up of fat in the veins, which prevents the flow of blood and therefore *lom*. Eating spicy, warming foods like ginger, pepper and garlic, and also bitter and sour foods can strengthen the wind element. The elderly, regardless of their mental state, are given specific foods. For example, the elderly are encouraged to eat foods that have a bitter taste like *fa thalaii* or *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae). This plant increases the appetite, and increase strength. The elderly are given foods that are easy to digest like pumpkin, soft greens, bananas and papaya (pers. comm. Mor C 2010).

Plants used to treat cognitive decline have specific characteristics. In Thai Traditional Medicine, plant remedies are classified as light weight versus heavy weight in addition to their characterization by taste, temperature, season and quality. For healing the brain, light weight plants are recommended, for example, using flowers in a formula as opposed to roots (pers. comm. Mor H 2010). Different plant parts are used in Thai Traditional Medicine, but one group of plants used in many of the formulas for memory is the *Kesorn Tang Gaow,* which is comprised of nine plants, primarily flowers, often prescribed together as part of another formula.

There are two types of medicines suggested for the nervous system. The first are stimulants for the heart and nerves. These are to increase energy and make the body stronger and are given to patients who are recovering from other illnesses. One example of this type of plant is tea (*Camellia sinensis* (L.) Kuntze, Theaceae). The second type calms the nerves and bring them back into balance if they are over stimulated. These plants can induce sleep and stimulate the appetite like *Rauvolfia serpentina* (L.) Benth. ex Kurz, (Apocynaceae) (pers. comm. Mor J 2010).

Figure 3.6: Leaves and flowers of *ra yorm noi* or **ระย่อมน้อย** (*Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae)) (Photograph by author).

The herbal steam bath allows some medicinal plants to be inhaled and taken in through the skin. In addition to the typical plants used in the steam bath like tamarind leaves (*Tamarindus indica* L.), camphor (*Cinnamomum camphora* (L.) J. Presl) and kaffir lime leaves

(*Citrus hystrix* DC.), warming plants such as *plai* (*Zingiber cassumunar* Roxb.), and ginger (*Zingiber officinale* Roscoe) are added for the elderly. The herbal steam bath also encourages blood circulation in the body, and blood to the brain, which is important in keeping the brain healthy (pers. comm. Mor C 2009).

Figure 3.7: Fresh plants chopped and prepared for the steam bath. The basket is placed over the vent where the steam is released to infuse the aromatic properties of the plants into the air (Photograph by author).

MEDICINAL PLANT MANAGEMENT AND PLANT HARVESTING PRACTICES

Historically, plant remedies are gathered from the forests or taken from the healer's garden. One healer would ask the rural hill tribe villages to collect the plants for him and then he dries them himself (pers. comm. Mor B 2010). This method guarantees the plants are identified

correctly and contain no pesticides. Doctors with close proximity to a city can buy many of the plants they need from the market, and some doctors who produce their remedies on a larger scale, have their plant material shipped from Bangkok where there is a huge demand for medicinal plants primarily from south east Asia.

A hospital in *Mae On* focuses on treatment for HIV positive patients using traditional medicine to treat people at all stages of the disease. They use plants collected by a local person who knows about the herbs. They tell the collectors which plants to collect, and the collector knows when they are in bloom, and when they will be large or small in their growth pattern. The hospital buys the plants from these local collectors then the raw material is processed into products for the patients (Mae On Hospital, personal communication, 10-13-08).

When harvesting, the healer should take the whole plant and it should be picked when it is just starting to flower. The time of the moon is not important, but the day of the week has significance for harvesting. On Thursday, plants from the north should be harvested; on Friday, plants from the west should be harvested; on Sunday, plants from the east should be harvested and from the south on Tuesday. The fruit, flowers and leaves should be harvested during the rainy season. During the dry season, the root and bark is harvested, and during the cold season, small, whole plants can be harvested in addition to the fruit, flowers, leaves and seeds. It is best to harvest plants in the wild as they have different chemicals and weather influences (pers. comm. Mor J and Mor F 2010).

The best time of the year to collect the plants depended on the part of the plant used and the weather during that time of the year. The rhizome is best collected from November to January during the cool season, and the bark is collected at the end of the hot season (April/May). Collecting the leaves, flowers and fruit depends on the plant and the age of the

plant, but the chemicals in the leaves concentrate after the rainy season (Wittaya Pongamornkul, pers. comm. 11-9-2010).

CONCLUSION

Thai Traditional Medicine provides a route to optimum health and long life through a combination of principles on how to live. These are to eat healthy food, stretch and exercise, exist in harmony with the surrounding natural environment, maintain a peaceful mind and address disease when it occurs. The last period of life, when an individual is considered elderly, has prescribed foods, behaviors and medicines appropriate to preserve vitality and encourage longevity. It is anticipated that some faculties will not remain strong as people approach the end of their life. The body does not work as well, and bits of information fall from the mind. Forgetfulness outside of what is typical for an elderly person is a manifestation of imbalance, whether it is from their present circumstances, or from past lifestyle choices. In these situations, there are medicinal plants available to remedy the mind and improve memory.

Dementia, and its most prevalent manifestation Alzheimer's disease, is a relatively new disease concept when compared to the development of the theories and therapeutics of Thai Traditional Medicine. Since there were multi-plant formulas for forgetfulness found in the ancient manuscripts from the *Lanna* Kingdom, and knowledge of medicinal plants from long ago, memory loss in old age is not a new problem. Contemporary contributions to deteriorating health like pesticide use and processes foods may be exacerbating health issues. The application of the principles of Thai Traditional Medicine, in addition to using memory enhancing plant formulas, could potentially abate the escalating rates of dementia worldwide.

CHAPTER 4 - THE MEDICAL ETHNOBOTANY OF TREATING COGNITIVE IMPAIRMENT IN THE ELDERLY OF NORTHERN THAILAND

INTRODUCTION

The foundation of good health in Thai Traditional medicine is in the balance of the four elements. Like many eastern medical systems, disease is caused by an excess or deficiency in any of these elements and body systems. In Thai Traditional Medicine, age-related cognitive decline is caused by a persistent imbalance in the wind element. When treating memory decline, the goal of the health practitioner is to provide guidance bringing the body systems back into balance, especially for an aberrant element, and restore health through the use of food, physical manipulation like massage or acupuncture, and lifestyle suggestions. Traditional healers also frequently utilize medicinal plant species to balance the body's elements and treat disease when it occurs.

In Thai Traditional Medicine, herbal remedies are in the form of multi-plant formulas containing species with compounds, which can act against the causes of disease. These formulas will also include plants to balance the body systems or support the more bioactive plants. Many times the supporting plants are in many multi-plant formulas regardless of the purpose of that formula. The herbal formula, by containing many different plants, treats the entire system of the body (pers. comm. Mor H 2011). Plant based formulas are used to treat cognitive impairment after its underlying cause is determined by the healer. Occasionally, a single plant to improve memory function is used, but in Thai Traditional Medicine, like Ayurvedic and Traditional Chinese Medicine, most remedies are a mixture of plants (Fabricant 2001).

The aim of this study was to identify plant species used by Thai traditional healers to treat cognitive decline and memory impairment in the elderly population. During interviews with traditional healers, the underlying cause of cognitive impairment was identified, and herbal remedies in the form of multi-plant formulas, and sometimes individual plants, were discussed. Most disorders involving the brain and its functions are cause by an imbalance of the wind element. Since the etiology of these disorders is the same, the overlap of shared species in the multi-plant formulas was investigated.

METHODOLOGY

A total of 70 interviews were conducted with Thai traditional healers over three years of fieldwork in northern Thailand. The herbal formulas presented in this chapter were obtained from five primary research participants with a particularly deep knowledge of medical plants for memory. Some of these formulas were from the healer's experience and others were documented in the palm leaf manuscripts, and then adapted to serve their specific purpose: to nourish the brain and improve memory. There exist formulas used for disorders that could be associated with cognitive decline like formulas for the nerves, for long life or for giving strength to the elderly. Formulas for ailments that could cause memory problems, but not specific for memory issues, were also investigated. These formulas were for diseases found only in Thailand, were culturally specific, and did not often translate directly into western medical constructs, but were pertinent to the study of memory loss in Thai Traditional Medicine.

Five multi-plant herbal formulas to treat memory were obtained from four interviewed traditional healers. These formulas were of primary importance to this study as they relate specifically to cognitive decline in the elderly. Many plants listed in these multi- herb formulas

already have documented bioactivity in the brain, nervous system and on memory. An exhaustive search was performed on all the plants listed in these formulas to determine if the plants contained in them were already tested in the bioassays selected for this project. Both NAPRALERT and SciFinder Scholar were investigated for the plants already screened in the selected bioassays. These plants were excluded from the list of testable single plants for laboratory screening, but are listed below to identify plants in the memory formulas with the desired bioactivity.

The multi-plant herbal formulas received from traditional healers were analysed for the single plant species included and analyzed for overlap of plant species in the formulas. Each formula received from the traditional healers will briefly be discussed to include the origin of the formula, and the some of plant species in each formula that have already been tested on bioassays relating to memory. The Jaccard Similarity Coefficient is frequently used to analyze the presence of plant species in two ecological habitats by comparing the similarity of sample sets (Jaccard 1901):

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}$$

For this study, the Jaccard Similarity Coefficient was utilized to quantify the overlap within formulas for the same condition and between the types of formulas.

RESULTS AND DISCUSSION

FORMULAS FOR MEMORY

Mor O Nerves Formula

This formula came from a temple outside Chiang Mai where an elderly monk recorded his knowledge on plants for the brain (Sundharathummo 1984). The formula contains ten plants. Two plants in this formula that were previously researched for memory enhancement are *Zingiber offinale* Roscoe (khing haeng/ **ขิง**) (Hanumanthachar and Parle 2006) and *Piper nigrum* L. (prik Thai **พริกไทย**) (Ingkaninan et al. 2003).

Good Memory Formulas from Mor H

Two formulas for memory came from Mor H, and both were received from his grandmother. The first formula contains seventeen plants and includes the group of nine plants known as the *Kesorn Tang Gaow*. The second formula consists of nine plants. Both formulas included *Cannabis sativa* L. *or C. indica* Lam. (gun chaa/ **กัญชา**), which was not added to the prepared formula when it was tested in the laboratory. A number of other plants with previously demonstrated memory enhancement were included in both formulas. The leaf of *Centella asiatica* (L.) Urb was shown to have a nootropic effect on young male mice in both behavioral studies and studies on acetylcholine levels in the brain (Sulochana et al. 2005). When included in a multi-plant formula from Chinese Traditional Medicine, *Acorus calamus* L. (waan nam/ **ว่านน้ำ**) was found to help with scopolamine induced memory impairment in mice (Nishiyama 1994). Both *Stephania rotunda* Lour. (bor ra ped poong chang/ **บอระเพ็ดพุงช้าง**) and *Alpinia galanga* (L.) Willd. (khaa/ **หัวข่า**), which are included in these formulas, exhibited

aceylcholinesterase inhibition activity when tested *in vitro* (Vinutha 2007; Ingkaninan 2006). Four of the flowers in the *Kesorn Tang Gaow* including *Mammea siamensis* (Miq.) T.Anderson (dok sarapii/ **ดอกสารภ**), *Michelia champaca* L. (dok jampaa/ **ดอกจำปา**), *Mimusops elengi* L. (dok pikun/ **ดอกพิกุล**) and *Nelumbo nucifera* Gaertn. (kesorn do bua luang/ **เกสรดอกบัวหลวง**) were tested for acetylcholinesterase inhibition activity (Ingkaninan 2003).

Forgetful Formula from Mor F

In the old language of *Pali*, this formula is called the "Forgetful Formula". This formula came from the ancient knowledge that traveled with Buddhism from India and Nepal, and was recorded on the *bap saa*, the *Lanna* notebooks made from mulberry paper (pers. comm. Mor F 2010). This formula was not tested in the laboratory, as it was not possible to locate many of the plants.

This formula contains from eight to nine plants. Mor F expressed that a multi-plant formula is more effective because using the plants together makes them stronger (2011). Of them, *Zingiber officinale* Roscoe (khing / **ขิง**) was found to have memory enhancing, or nootropic activity, in mice (Hanumanthachar and Parle 2006). *Alpinia galanga* (L.) Willd. (khaa/ **หัวข่า**) was tested for aceylcholinesterase inhibition activity on *in vitro* tests, but not found to have strong activity (Vinutha 2007).

Brain and Nerve Tonic from Mor J

Mor J received this formula from the medicinal manuscripts of his grandfather. The plants in this formula can be taken together as a treatment or separately to be a tonic for the brain. Some of these plants are known to have psychoactive properties. Coffee and tea are used

as mental stimulants worldwide, and both *Rauvolfia serpentina* (L.) Benth. ex Kurz and *Cannabis sativa* L. are known to effect the mind.

Included in this formula were plants with proven bioactivity against memory impairment. *Camellia sinensis* (L.) Kuntze (chaa lae miang or fermented tea/ **เมี่ยง**) inhibited both acetylcholinesterase and butyrylcholinesterase *in vitro*, both of which are implicated in dementia-like disorders (Okello et al. 2004). *Senna alata* (L.) Roxb. (choom hed Thai/ **ชุมเห็ดไทย**) was found to ameliorate impairment in cognition from scopolamine injections in behavioral mice models (Kim et al. 2007), and *Strychnos nux-vomica* L. (sa laeng jai/ **แสลงใจ**) also has memory enhancement properties when tested on *in vivo* models (Robinson 1968).

Mor B's Alzheimer's Disease Formula

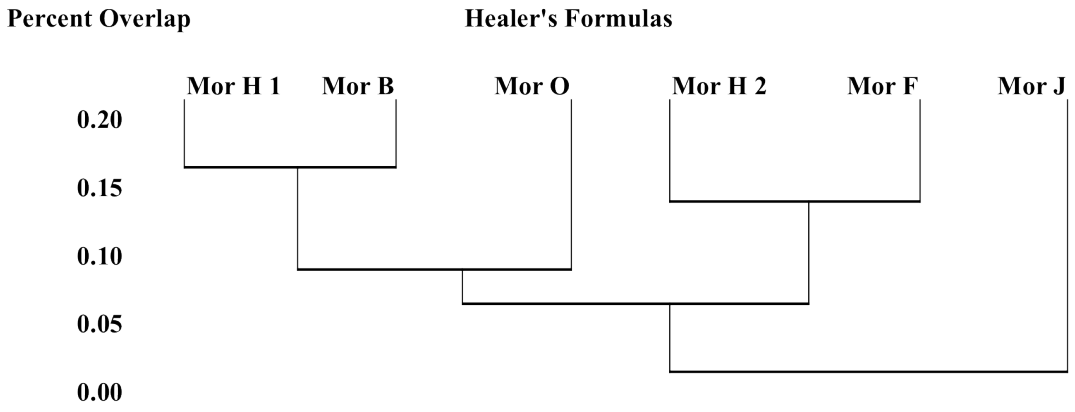
This formula is believed to treat and prevent Alzheimer's disease specifically, and was the only formula received for this specific disease. Mor B said he treated someone with Alzheimer's disease using this formula, and their condition improved after taking the formula (2010). This formula consists of twenty-three plants, many of which have documented cognitive enhancing activity. Ingkaninan et al. (2003) tested the acetylcholinesterase inhibition of a number of Thai plants on *in vitro* bioassays similar to the one employed in this study. This formula contained six of those plants: *Mammea siamensis* (Miq.) T. Anderson (dok saripee/ **ดอกสารภี**), *Mimusops elengi* L. (dok pi guen/ **ดอกพิกุล**), *Nelumbo nucifera* Gaertn. (kesorn do bua luang/ **เกสรดอกบัวหลวง**), *Piper nigrum* L. (prik Thai/ **พริกไทย**), *Plumbago indica* L. (jettamoon plueang daeng/ **เจตมูลเพลิงแดง**), and *Terminalia bellirica* (Gaertn.) Roxb. (samor pipek/ **สมอพิเภก**). None of these plants showed significant activity in the inhibition bioassay.

Zingiber officinale Roscoe (khing/ ขิง) (Hanumanthachar and Parle 2006), which is in many formulas, demonstrated memory enhancing activity as a nootropic. *Myristica fragrans* Houtt. (both the seed (nutmeg) and aril (mace) are used in Thai Traditional Medicine) and the fruit of *Terminalia chebula* Retz. (somor Thai/ สมอไทย) also showed acetylcholinesterase inhibition activity (Dhingra 2006; Vinutha 2007).

Overlap in Memory Formulas

The formula from Mor B to treat Alzheimer's disease and the first "good memory" formula from Mor H contained 15% of the same plants. The second "good memory" formula from Mor H and Mor F's "Forgetful formula" shared about 14% of the same species. Mor O's "nerve formula" overlapped with these formulas in about 8% of the species. The total overlap in species within all of the formulas for memory is about 3% (Figure 4.1).

Figure 4.1: Percent overlap between the healer's formulas relating to memory loss in the elderly population by calculating the Jaccard Similarity Coefficient.



FORMULAS RELATING TO LONGEVITY OR STRENGTH IN OLD AGE

Longevity Formulas

This project focuses on plants used to combat cognitive decline in the elderly, which is a potential precursor to dementia. Dementia primary affects the elderly, although there is a growing population of younger people (under 65) troubled with memory loss and confusion (McMurtray 2006). When asked about plants to treat memory loss, some healers offered herbal formulas to promote strength and longevity for the elderly.

Longevity Formula from Mor A

This formula is one of the longest formulas including twenty-nine plants. Many of them contain compounds found to remedy memory loss like *Alpinia galanga* (L.) Willd. (khaa/ **หัวข่า**), *Centella asiatica* (L.) Urb (bua bok/ **บัวบก**), and *Piper nigrum* L. (prik Thai **พริกไทย**). *Curcuma longa* Linn. (ka min chun/ **ขมิ้นชัน**) is a well known as an herbal treatment for memory loss. Pyrzanoska et al. (2010) demonstrated this activity on male rats in both behavioral evaluations and neurotransmitter measurements primarily in the hippocampus.

Longevity Formula of Mor J

Mor J had three formulas relating to longevity. Two formulas are called Long Life (both with 5 plants), and the other the Longevity Formula (8 plants). His formulas contained fewer plants and can be taken daily as a tonic. These formulas will be analyzed together. The only plant that overlaps in these three formulas is *Piper nigrum* L. (prik Thai **พริกไทย**), which is found in two of the three formulas. They all contain honey, which is often used to provide energy to the elderly and make them feel "fresh" (pers. comm. Mor J 2010).

These formulas contain the previously discussed *Myristica fragrans* Houtt. (look jan/ **ดอกจันทน์**), specifically the seed or nutmeg, and *Curcuma longa* Linn. (ka min chun/ **ขมิ้นชัน**) (Pyrzanoska et al. 2010). This formula contains *Glycyrrhiza glabra* L. (cha aim taid/ **ชะเอมเทศ**). Dhingra et al. (2004) found the roots and rhizomes of this plant to reverse memory damage from scopolamine and diazepam in mice because of increased acetylcholinergic activity in the brain.

Mor E Longevity Formula

Mor E had no formula to increase memory, but he uses his formula for longevity instead because it integrates the body, which will help the mind. For longevity, he recommends plants with a bitter taste like *bor ra ped* or **บอระเพ็ด** (*Tinospora crispa* (L.) Hook. f. & Thomson), which is taken for long life and given to the elderly. In the past, it was believed to have special properties for longevity. He said, "If you eat the amount you can fit around your waist each day you will live a long time". His formula contained eight plant species including *Piper nigrum* L. (prik Thai **พริกไทย**).

Mor I's Formula for Giving Strength to the Elderly

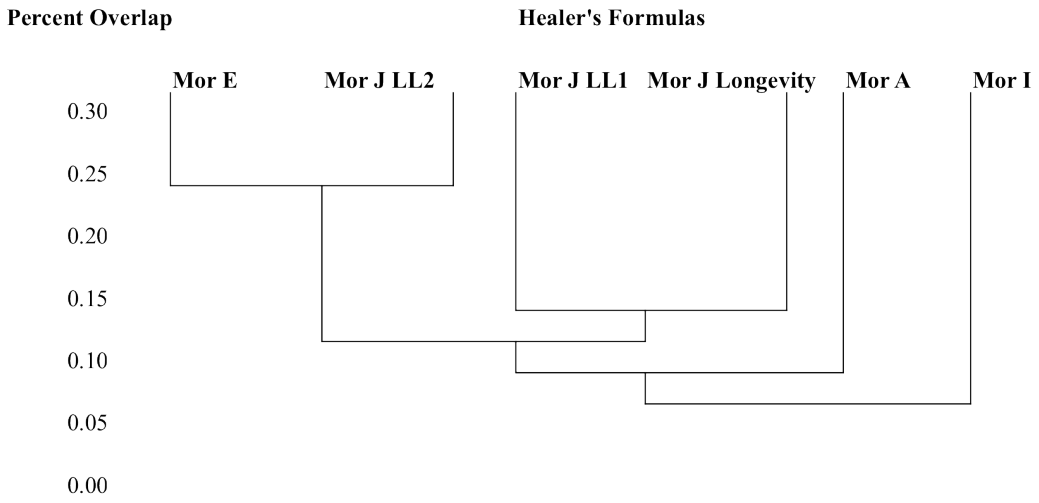
This formula was from the doctor's "masters", primarily different monks he studied with in the temple where he learned Thai Traditional Medicine. He then developed his own formula from this knowledge. This nine plant formula incorporates two of the "five spices" (*Piper chaba* Hunter (deeplee/**ดีปลี**) and *Piper ribesioides* Wall. (sakaan/ **สะค้าน**), one of the four medicines from the "Thai elixir of life", (*Aegle marmelos* (L.) Corrêa (ma toom on/ **มะตูมอ่อน**), and two

of the *Kesorn Tang Gaow:* (*Mesua ferrea* L. (dok boonnaak/**ดอกบุนนาค**) and *Mammea siamensis* (Miq.) T. Anderson (dok sarapii/ **ดอกสารภี**).

Overlap in Formulas for Long Life and Strength in the Elderly

The formulas from Mor J (Formula 2 for Long Life) and Mor E's longevity formula contained about 25% of the same plant species. Mor J's Longevity formula contained 15% of the same species as his first formula for Long Life. These four formulas contained 13% of the same species with a total overlap in all formulas for long life and strength in the elderly of about 9% (Figure 4.2).

Figure 4.2: Percent overlap between the healer's formulas relating to long live, longevity and strength in the elderly population by calculating the Jaccard Similarity Coefficient (LL = Long Life).



CULTURALLY SPECIFIC DISEASES

The following three diseases are not found in western medicine, but are well known in Thai Traditional Medicine. The primary cause of all of them is "wind disturbance". In Thai Traditional Medicine, an imbalance of wind is one of the reasons the elderly develop dementia or have a decline in memory functioning (pers. comm. Mor J 2009). These diseases were discussed during interviews with the traditional healers, and formulas to treat them are used as a comparison to those used to treat memory specifically.

Maha Sannibad

Theory and Symptoms

Maha Sannibad is called the "combined disease". It manifests when diseases of bile, wind and mucus all occur together, and usually when the weather is changing (Mulholland 1979). One healer said *Maha Sannibad* is a disease related to the wind element that primarily manifests in the blood, respiratory system and the gall bladder. The symptoms can be serious if left untreated; "if the wind leaves your body through the eyes, the patient will go blind; through the ears, the patient will go deaf. The patient will have a high fever and their skin will be red. If the patient is not healed of the disease, their body will shake out of control (similar to epilepsy)" (pers. comm. Mor I 2009). One healer said the patient will become unconscious from not treating severe symptoms, like a bad fever, and that is *Maha Sannibad* (pers. comm. Mor E 2010). Twenty-one different symptoms define *Maha Sannibad* as a disease. Only one formula was received to treat *Maha Sannibad*. It will be compared with other formulas for "wind disturbance" in a later section of this chapter.

Treatment of *Maha Sannibad*

Mor E's Formula for *Sannibad*

This formula has eighteen ingredients. Half of the ingredients are the *Tians.* They are a group of nine plants used in many Thai formulas for issues with unstable wind in the body, used as a heart tonic and given to the elderly for strength (Mor J 2010). An additional plant found in this formula is *Zingiber cassumuna* Roxb. (plai/ ไพล). *Plai* is a very commonly used plant in Thai Traditional Medicine. It is added to the herbal steam bath for its aromatic and healing properties (Mor C 2010). The essential oil of *plai* was studied for its acetylcholinesterase and butyrylcholinesterase inhibition, but it did not have significant *in vitro* activity (Wantida 2010).

Lom Mar Reng Kroot

Theory and Symptoms

Lom mar reng kroot, caused by an unstable or stagnant wind in the body, is the source of migraine headaches (pers. comm. Mor F 2010). Similar to *Maha Sannibad*, if the wind becomes volatile in the eyes and it is not treated, the patient could go blind. If it is in the ears and untreated, the patient could go deaf. If wind is unbalanced in the brain, it causes *lom mar reng kroot* (pers. comm. Mor P 2011).

There were two formulas obtained for treatment of *Lom Mar Reng Kroot.* They overlapped only 4% in shared species according to the Jaccard Similarity Coefficient. This small similarity coeffiecent could be due to the difference in the number of plants contained in the formulas. Mor H's formula contained ten plants where Mor J's formula contained forty-seven plants.

Treatment of *Lom Mar Reng Kroot*

Mor H's Formula for *Lom Mar Reng Kroot*

This formula contains ten plants and medicinal or rock salt. It contains four plants already discussed for their properties relating to memory: *Acorus calamus* L. (waan nam/ **ว่านน้ำ**) (Nishiyama 1994), *Curcuma longa* L. (ka min chun/ **ขมิ้นชัน**) (Pyrzanoska et al. 2010), *Zingiber officinale* Roscoe (khing / **ขิง**) (Hanumanthachar and Parle 2006), and *Piper nigrum* L. (prik Thai **พริกไทย**) (Ingkaninan et al. 2003). *Citrus hystrix* DC. (pew ma kroot/ **มะกรูด**), was previously tested with *plai* for acetylcholinesterase and butyrylcholinesterase activity but no significant activity was found (Wantida 2010).

Mor J's Formula for *Lom Mar Reng Kroot*

This is the longest formula containing forty-seven plants. The majority of the plants are from the commonly prescribed groups of plants like the *Tians* (all), *Goads* (all), and the *Kesorn Tang Haa*. The *Kesorn Tang Haa* are five of the nine *Kesorn Tang Gaow*, which are selectively used for a heart tonic and to heal *lom* (Mor B 2010). *Myristica fragrans* Houtt. (both the seed (nutmeg), and aril (mace) are used in this formula) were found to have acetylcholinesterase inhibition activity (Dhingra 2006). *Cyperus rotundus* L. (new moo/ **หญ้านิ่วหมู**) is in this formula. It was studied for acetylcholinesterase inhibition, but no significant activity was found (Ingkaninan 2003).

Lom Pid Duan

Theory and Symptoms

Thai tradition stipulates certain behaviors and taboos for a woman postpartum. The mother must stay inside the house for one month, hence the name in Thai, *yuu duan* or "stay month". During this month, she must remain warm, to keep her blood circulation flowing, by sitting next to a fire in a room with her child after delivery. This tradition is called *yu fi*, or mother roasting. The woman is not to bathe in or drink cold water as she has lost a good deal of blood during childbirth, and the cold water will cause the blood in her body to stagnate. The "bad blood" could stay in her body and cause her to be sickly, to have a weak stomach, and become cold easily. The new mother's food is restricted for ten days after giving birth. She can eat only rice with salt (pers. comm. Mor C 2010).

After ten days, she can eat healthy foods with lots of special herbs, including warming plants like ginger (*Zingiber officinale* Roscoe). *Curcuma xanthorrhiza* D. Dietr. is a common plant used for post-partum care of the mother. This plant encourages the womb to regain its normal shape after childbirth, helps regulate the menstrual cycle and prevents uterine cancer (pers. comm. Mor C 2009). New mothers are also encouraged to sweat in the steam bath where specific plants are added to help their condition like *plai* (*Zingiber cassumunar* Roxb.), lemongrass (*Cymbopogon sp.* Spreng.), kaffir lime (*Citrus hystrix* DC.), tamarind leaves (*Tamarindus indica* L.), *Acacia catechu* (L. f.) Willd., *Acorus calamus* L.*, Curcuma xanthorrhiza* D. Dietr.*, Cryptolepis buchanani* Schult., and camphor (*Cinnamomum camphora* (L.) J. Presl) (pers. comm. Mor J 2009). Plants associated with a spicy taste like *khing* (*Zingiber*

officinale), *chaploo* (*Piper sarmentosum*) and *diplee* (*Piper chaba*) are often added to the food of the new mother (pers. comm. Mor A 2010).

Two formulas were obtained to treat *Lom Pid Duean.* These formulas had 35% of the same plant species in them. This formula is believed to treat memory impairment in elderly women, and potentially estrogen imbalance since it is for women only. A formula to treat two symptoms could have more similar plant species than formulas to treat only one symptom. There was also considerable overlap in the use of the "*tians".* Five of the nine *Tians* are included in both formulas: *Lepidium sativum* L. (tian daeng/ **เทียนแดง**), *Nigella sativa* L. (tian dam/ **เทียนดำ**), *Foeniculum vulgare* Mill. (tian kao plueak/ **เทียนข้าวเปลือก**), *Cuminum cyminum* L. (tian kaow/ **เทียนขาว**), and *Anethum graveolens* L. (tian tdaa tdak ka taen/ **เทียนตาตั๊กแตน**).

Treatment of *Lom Pid Duean*

Mor I's Formula for *Lom Pid Duean*

This formula contains seventeen plants. One of the *Kesorn Tang Gaow* is in it, but it is one not included in the *Kesorn Tang Haa*: *Mesua ferrea* L. (dok boonnaak/ **ดอกบุนนาค**). This flower is good as a brain and heart tonic, to balance the elements, and nourish strength (pers. comm. Mor I 2010). The formula contains three of the "five spices": *Piper chaba* Hunter (deeplee/**ดีปลี**), *Plumbago indica* L. (jetta moon plueang daeng/ **เจตมูลเพลิงแดง**) and *Zingiber officinale* Roscoe (khing / **ขิง**).

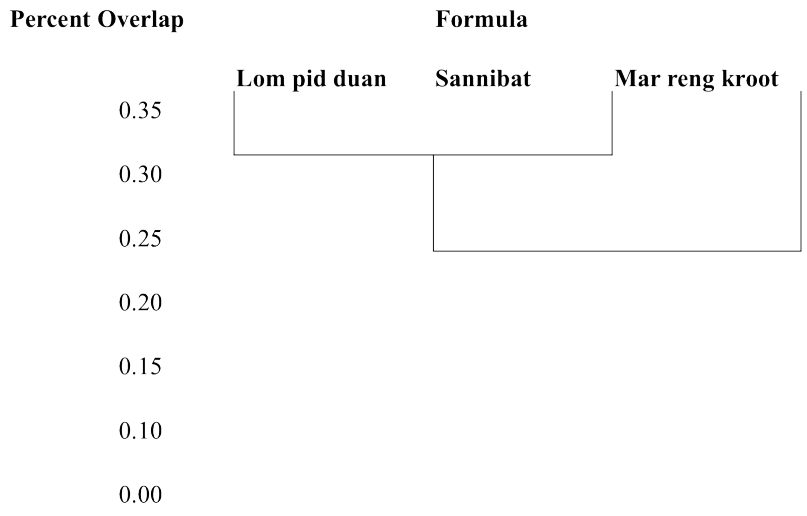
Mor E's Formula for *Lom Pid Duean*

This formula consists of fifteen plants, and is almost entirely comprised of commonly prescribed groups of plants, or plants that are often grouped together for a similar purpose. This formula contains all of the *Kesorn Tang Haa*. One of the "five spices", *Piper chaba* Hunter (deeplee/**ดีปลี**), is in this formula. Three plants in this formula that have already been mentioned for their memory enhancing properties are *Zingiber cassumuna* Roxb. (plai/ **ไพล**)(Wantida 2010), *Piper nigrum* L. (prik Thai **พริกไทย**) (Ingkaninan et al. 2003), and *Acorus calamus* L. (Waan nam/ **ว่านน้ำ**) (Nishiyama 1994).

Overlap in Species Included in the Formulas

These culturally specific disease categories all have the same etiology of wind disturbance and many of the plant species within the formulas are the same. The formulas for *Lom Pid Duan* and *Maha Sannibad* overlap in 31% of the species contained in them. The formulas for *Lom Pid Duan* overlaps with the formulas for *Lom Mar Reng Kroot* in 26% of the species, and the formulas for *Maha Sannibad* and *Lom Mar Reng Kroot* overlap in 21% of the species (Figure 4.3). This overlap is expected since the underlying cause of these diseases is considered the same.

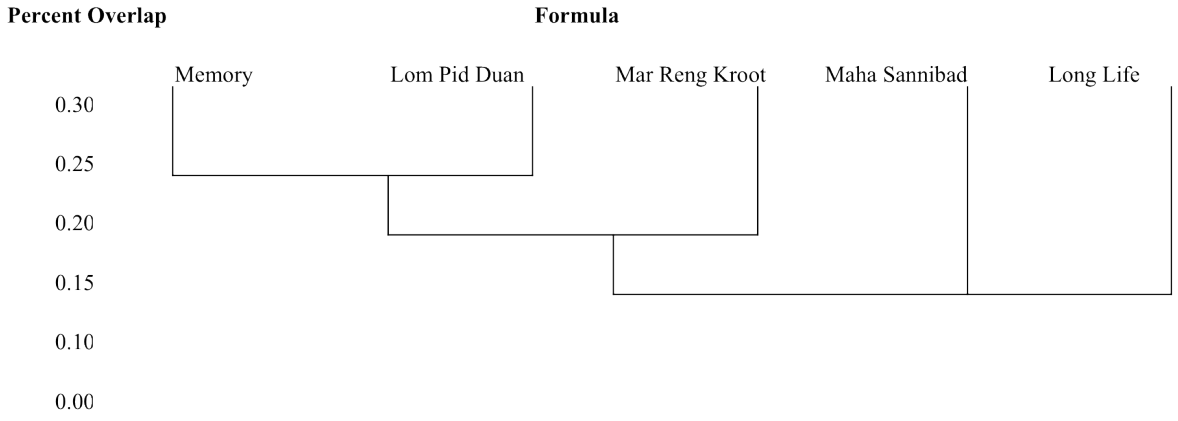
Figure 4.3: Percent overlap between the healer's formulas relating to wind disturbance as a common cause of the disease by calculating the Jaccard Similarity Coefficient.



There was notable overlap in all of the formulas received from the traditional healers when they were compared using the Jaccard Similarity Coefficient. Other single plants, which were already tested for memory enhancing or nootropic effects, are recurring in many of the formulas. Some of these plants, like *Curcuma longa*, come from Ayurvedic Medicine, while others are unique to Thai Traditional Medicine. The plants in the memory formulas, when combined, have substantial overlap with the formulas for the other disease categories. The memory formulas when compared to other formulas for etiology in wind disturbance, excluding longevity and strength formulas, overlapped 28% in shared plant species. All of the formulas contained at least one, and some shared all, of the "five spices". Formulas for memory, *lom mar reng kroot* and *lom pid duan* all contained the same *Kesorn Tang Gaow* and they shared *Myristica fragrans*, which is a known psychoactive and an acetylcholinesterase inhibitor (Weiss 1960; Dhingra 2006). The memory, longevity and *lom mar reng kroot* formulas share the *Triplaa* formula, which is a standard balancing formula in Ayurveda. The overlap in these

formulas demonstrates their common etiology in Thai Traditional Medicine and indicates their potential success in treating disease originating from the imbalance of *lom* (Figure 4.4).

Figure 4.4: Percent overlap between the healer's formulas relating to wind disturbance, long life and memory by calculating the Jaccard Similarity Coefficient.



There was also overlap found in plants used to treat memory and other disorders involving wind disturbance in Thai Traditional Medicine, and plants used for similar ailments in both Traditional Chinese Medicine and Ayurvedic Medicine. In Traditional Chinese Medicine, *Angelica dahurica* (Fisch.) Benth. & Hook. f. (Apiaceae) specifically, and plants from the genera *Acorus* and *Atractylodes* was found beneficial to patients who had dementia following the occurrence of a stroke (Adams 2007). In Ayurvedic medicine, there are a number of formulas using plants commonly used in Thai Traditional Medicine. *Acorus calamus L.* (Araceae), *Piper longum* L. (Piperaceae), *Centella asiatica* (L.) Urb. (Apiaceae), and *Glycyrrhiza glabra* L. (Fabaceae), which were included in some formulas for memory and the brain. Also, *Terminalia chebula* Retz. (Combretaceae) and *Nardostachys jatamansi* (D. Don) DC. (Caprifoliaceae), which are both part of the *Goads,* are used in Ayurveda to promote memory and intellect, and

prolong life (Clayton et al. 1994). They are also commonly used in Thai Traditional Medicine for a similar effect. Finally, some genera were found to have memory promoting activity in both Ayurvedic medicine and TTM, these are: *Butea* (Fabaceae) (Ingkaninan 2003) and *Terminalia* (Combretaceae).

One plant from Thailand, the leaves of *Michelia champaca* L. (Magnoliaceae), treats brain disorders in Thai Traditional Medicine (Clayton et al. 1994). Healers suggested this plant as good for memory. It is included in the *Kesorn Tang Gaow* which is a formula given for the same reason. When asked about this plant, it was suggested to use the leaves for nerve disease and the seeds to balance the elements, and for dizziness, but it was not suggested as a plant to test in the laboratory (pers. comm. Mor J 2010). A number of plants found in the multi- herb formulas were from the Zingiberaceae family. *Curcuma longa* L. has psychoactive effects. The rhizome is used as a brain tonic and for mental debility. The rhizome of *Curcuma zedoaria* (Christm.) Roscoe and *Zingiber officinale* Roscoe are both used as a stimulant (Jain and Shukla 1996).

PLANT FREQUENCY IN ALL FORMULAS

Species frequently incorporated in these formulas can indicate their widespread distribution within the study area, or indicate a plant family with many known medicinal plants. The botanical families most represented in all of the formulas received were: Apiaceae (13), Zingiberaceae (9), Asteraceae (6), and Piperaceae (5). Euphorbiaceae, Lauraceae and Fabaceae were all represented four times. Arecaceae, Combertaceae, Lamiacea, Menispermaceae, Plumbaginaceae, Poaceae, and Rutaceae were represented three times. At the genus level, *Piper* was represented five times, *Cinnamomum* four times, and *Angelica* and *Terminalia* three times.

Thai Traditional Medicine uses a number of spices as medicinal remedies. Many of these spices are found in the Apiaceae family including *Pimpinella anisum* L.*, Carum carvi* L.*, Anethum graveolens* L.*, Petroselinum crispum* (Mill.) Fuss*, Foeniculum vulgare* Mill.*,* and *Cuminum cyminum* L. The frequently mentioned *Tians* are all spice plants that can be traced back to origins in Ayurvedic medicine from India. The second most represented family, Zingiberaceae, is widely distributed in Southeast Asia, and Thailand particularly. This prevalence depicts the extensive distribution of the family in the study area. Many Zingiberacous plants are also used as medicine as they have abundant secondary compounds. Asteraceae, the third most represented family, is the largest botanical family, so its frequent appearance in the formulas is indicative of its representation worldwide.

The family Piperaceae and the genus *Piper* were both highly represented in the data. These groups have a pan tropical distribution, and are prevalent in Southeast Asia. The genus *Piper* is a very large genus and has some well-know species with medicinal properties like *Piper methysticum* G. Forst. (Kava), and *Piper betle* L. (Betel leaf). Many species in the genus *Cinnamomum* are from Southeast Asia and contain known medicinal properties. For example, camphor, an ingredient in medicines and incense, comes from the tree *Cinnamomum camphora* (L.) J. Presl. Common cinnamon (*Cinnamomum zeylanicum* Blume*)* can help regulate blood sugar in the body. *Cinnamomum cassia* (L.) C. Presl (*Ob choei*) has medicinal properties similar to that of common cinnamon. Often times there is an overlap in medicinal compounds within the same genus (Dugoua 2007).

FORMULA ADDITIONS: HONEY

Many formulas contain honey, or can be taken with honey when the plant parts are ground into a powder. Two healers added it to their longevity formulas. One says, "It has a sweet and cool taste. It helps certain recipes for medicine taste better and it makes you feel fresh" (Pers. comm. Mor E 2010). Honey is "stronger" if it is wild honey produced from the nectar of many types of flowers. This type of honey is usually gathered from the mountains around the north of Thailand (Pers. comm. Mor J 2010). The bottles of honey are "sealed" with some bee's wax and sometimes a whole bee to preserve the honey.

SINGLE PLANTS USED FOR MEMORY

While most botanical health treatments are administered in multi-plant formulas in Thai Traditional Medicine, occasionally single plant remedies were discussed. These plant species are also included in the herbal formulas, but are believed to contain potent bioactivity when used individually.

Table 4.1: Table of single plants suggested to treat cognitive decline and enhance memory including plant name, part used, effect and source.

Thai Name	Scientific Name	Family	Part Used	Effect	Healer Source
Gan chaa	*Cannabis sativa* Linn., *C. indica* Lamk.	Cannabaceae	Flowers Leaves/ flowers	-poison and drunken taste - increase appetite -strength tonic -good for nerves - too much addictive - brain and nerve tonic - feel "fresh" -small dosage - better mixed with other plants	RPAB* Mor H Mor J Mor E
Ma led gaa	*Coffea arabica* L.	Rubiaceae	Seeds	- bitter taste	RPAB*

fae				-tonic for nerves and heart -strength tonic	
Ma led choom hed Thai	*Cassia tora* L.	Fabeaceae	Seeds	-drunk and bitter taste - soporific -balance blood pressure - detoxifier	RPAB*
Raak ra yom	*Rauvolfia serpentina* (L.) Benth. Ex kurz.	Apocynaceae	Roots	- bitter taste -nerve tonic -soporific and appetite enhancing -blood purifier	RPAB*
Lampong dam	*Datura metel* Linn.	Solanaceae	Seeds	-poison and drunk taste -tonic for brain and nerves - low dosage -drunk and poison taste -small dosage	RPAB* Mor E
Ma led sa laeng jai	*Strychnos nux-vomica* L.	Loganiaceae	Seeds	- poison and drunk taste -heart and nerve tonic -appetite enhancing -low dosage - brain and nerve tonic -small dosage	RPAB* Mor E
Jan kaow	*Diospyros decandra* Lour.	Ebenaceae	Bark	- bitter and sweet taste -balances body heat -good for lungs and liver	RPAB*
Waan naam	*Acorus calamus* L.	Acoraceae	Rhizome	-tonic for brain, nerves and memory	Mor E
Booraped	*Tinospora crispa* Beumee ex K.Heyne	Menispermaceae	Vine		Mor H
Kesorn bua luang	*Nelumbo nucifera* (Gaertn.)	Nelumbonaceae	Stamens/ Seeds		Mor H Mor A
Diplee	*Piper chaba* Hunter	Piperaceae		-heats the body -helps organs with earth and fire	Mor B
	Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.	Apocynaceae		-increases heat in body which is good for memory	Mor B

*The *Rattanakosin* Pharmacy Ancient Book - Herbs for the Nerves (Wuttidhamma 2007b)

INFORMATION ON THE PLANTS FROM THE INTERVIEWS WITH TRADITIONAL HEALERS

During the interviews, the traditional doctors were asked about each plant in the formula. They told me information on each plant so their activity in Thai Traditional Medicine could be understood. This list of plants is in Appendix 6.

TREATMENT OF *CANNABIS* SP. FOR STUDY PROJECT

Many of the interviewed traditional doctors suggested the use of *Cannabis sativa* L. or *C. indica* Lam. (Cannabaceae - flowers (*gan chaa* in Thai)) to treat memory loss. It was the first ingredient in many of the formulas, and the healers suggested it for use as an individual plant as well. Their suggestions are as follows: the patient can use it as a nerve tonic, to help make them hungry and to sleep well. In the proper amount, it is good for the nerves and the brain. If too much is taken or it is taken too frequently, then the patient can become lazy and paranoid (pers. comm. Mor H, Mor J, Mor E 2010).

Despite its proposed activity and the frequency it was mentioned by the traditional healers, it could not be used for this project because of its legal status. For testing in the laboratory, it was not tested as a single plant and was removed from the multi- plant formulas. These formulas are from long ago, before the government regulated plants and plant compounds. The bioactivity of *Cannabis sativa* L. has been well documented, but the synergistic activity of it with the other plants in the formulas is not known.

Cannabis sativa has historically been used for its intoxicating properties, as a medicine and for ceremonies. Its legal status in many countries limits its scientific research, but there have been some studies to document its effects on the brain. In regards to this project, there is research investigating its action on the part of the brain used for memory and the implications of

these effects on dementia. Miller and Branconnier (1983) reviewed all of the studies on *Cannabis* and memory. They concluded that *C. sativa* affected the limbic system (where the hippocampus, a vital component for memory formation and retrieval, is located) similarly to patients with damage in the region in terms of memory loss. Compounds found in *Cannabis*, primarily Δ9-tetrahydrocannabinol, affect the activity of the neurons found in the septal-hippocampal pathway, which is partially responsible for learning and memory. These results support the commonly held view that *Cannabis* use negatively affects memory.

Conversely, Jiang et al. (2005) found that treatment with a synthetic cannabinoid generated neuronal cells in the hippocampus of rats. The resulting neurogenesis in this part of the brain showed a positive affect on learning and memory when the rats were tested in a behavioral model, and upon inspection of their brain tissue, the density of neurons in this area increased. This study shows the positive effect of cannabinoids on learning and memory.

Another study found that Δ9-tetrahydrocannabinol (THC) inhibits the activity of acetylcholineserase (AchE) and prevents its influence on amyloid β -peptide (Aβ) aggregation (Eubanks et al. 2006). This activity was tested through modeling enzyme-compound interactions, *in vitro* testing of AchE inhibition activity and AchE influenced creation of the β-amyloid plaques that are characteristic of Alzheimer's type dementia. My project specifically investigates plants with acetylcholinesterase enzymatic activity to remedy the effects of memory loss due to a deficiency in acetylcholine that can arise with age. The results of Eubanks et al. (2006) are especially pertinent as they address AchE inhibition specifically. Wolf et al. (2010) found that mice treated with exogenous cannabinoids THC and CBD had altered memory functioning and neurogenesis. The mice fed Δ9-tetrahydrocannabinol (THC) showed a decrease in learning and memory in the Morris Water Maze (Morris 1981) test when compared to a

control group. The Morris Water Maze is one of the *in vivo* behavioral models used in this project. The cannabidiol (CBD) fed mice did not have impaired learning and memory, and showed an increase in neurogenesis not present in the control or THC fed groups.

These opposing views on the affect of *Cannabis* on the brain do not provide support to explain the inclusion of this plant in the multi-plant formulas to help with memory disorders. *Cannabis*, like many other medicinal plants, has a number of bioactive compounds. Two well-known cannabinoids are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Both compounds have a neuroprotective affect on brain tissue (Hayakawa et al. 2007). Most of the aforementioned studies examined THC as the primary contributor to the investigated bioactivity as opposed to CBD.. Furthermore, the mechanisms by which the compound acts on the brain and memory loss are very different. Additional research is needed to thoroughly explain how the variety of cannabinoids found in *Cannabis* act on the brain, influence memory loss and potentially have therapeutic action against dementia.

CONCLUSION

Thai Traditional Medicine identifies the cause of all diseases affecting the memory and the brain as having a common etiology in "wind disturbance". Interviewed healers presented multi-plant formulas to address memory loss, age-related cognitive decline and to increase longevity. Some of the plants in these formulas have already been researched using scientific methodology, and were proven to enhance cognition. Novel plants species for testing were identified through investigating herbal formulas specifically used to treat cognitive impairment using an ethnobotanical filtering technique, and by relying on the traditional knowledge of the interviewed healers. These plant species may show bioactivity in *in vitro* screening, and have

potential to treat patients with memory decline. Throughout history, humans have utilized the natural world to better their circumstances. Cultures using traditional medical systems actively possess and benefit from their knowledge of plants. Ethnopharmacological research can potentially uncover plants and plant compounds to treat these diseases which plague mankind.

CHAPTER 5 - SCREENING FOR PLANTS IN HERBAL FORMULAS FROM TRADITIONAL DOCTORS OF NORTHERN THAILAND TO TREAT COGNITIVE IMPAIRMENT

INTRODUCTION

Cognitive impairment can be an early indication of dementia manifesting as memory loss beyond what is considered normal (Petersen 2004). Alzheimer's Disease, the most common type of dementia, is the sixth leading cause of death in the U.S. today (Alzheimer's Association 2012). In patients where cognitive impairment progresses to Alzheimer's disease, memory decline is still the primary complaint (Lopez 2013). The incidence of dementia type memory disorders will increase exponentially in coming decades with the potential for an associated financial and societal cost.

There are currently a number of theories to explain the etiology of age-related memory disorders. The abundance of the neurotransmitter acetylcholine in the brain has long been associated with memory formation. This proliferation of this neurotransmitter decreases with age and can result in age-related cognitive decline. Acetylcholinesterase is the enzyme responsible for breaking down acetylcholine in the synapses of cerebral neurons, therefore by inhibiting this enzyme, more of the neurotransmitter is available to the neurons and memory formation is improved (Ringman 2006). Doctors prescribe acetylcholinesterase inhibitors to prevent the progression of cognitive impairment to dementia (Snyder and Facchiano 2012).

While not considered the primary cause of dementia, oxidative stress can contribute to neurological degeneration (Markesbery 1997; Giasson 2002; Sayre 2001). High levels of oxidative stress markers found in the post-mortem brains of patients diagnosed with Mild Cognitive Impairment, a diagnosed precursor syndrome to dementia, suggest that oxidative

damage could be responsible for some of the cognitive decline leading to elderly dementia (Keller et al. 2005). Dietary polyphenols are specific antioxidant compounds found in many plants. They prevent oxidative stress by modifying the process where free radicals damage the body's macromolecules (Han et al. 2007). Krikorian et al. (2010) found that adding blueberry juice, a fruit known to contain high levels of antioxidants, to the diet of older adults improved their memory function after a twelve-week period.

Plant compounds have historically been a starting point for modern medicine and through ethnobotanical inquiry can continue to provide novel chemical compounds (Balick and Cox 1997; Johns, 2000; Homstedt and Bruhn 1995). Research investigating plant use in traditional medical systems identified a number of plants with potential to treat the memory decline associated with dementia (Howes & Houghton 2009). *Lanna* ethnomedicine, as practiced in the province of Chiang Mai in northern Thailand, is a combination of traditional Thai medicine, which originated in central Thailand, and animistic beliefs found in the more rural areas of the north (Chokevivat & Chuthaputti 2005).

The present study aims to screen a list of plants developed from ethnobotanically guided interviews with northern Thai traditional healers. The goal of the study is to identify a single plant with the most potential to treat age-related memory decline. Multi-plant herbal formulas obtained from traditional healers will also be tested as a means to corroborate their medical knowledge. Using modern *in vitro* biological assays, eleven plant species from the pharmacopeia of northern Thailand were screened for their antioxidant activity, total phenolic content and acetylcholinesterase inhibition in four assays. The healers will be provided with these results to use in their medical practice and to assist them in the treatment of cognitive decline in their communities.

MATERIALS AND METHODS

INTERVIEWS WITH TRADITIONAL HEALERS

Seventy-five interviews with twenty different traditional healers around Chiang Mai Province were conducted between the years 2008 and 2011. These interviews focused primarily on plant remedies and herbal formulas with the potential to treat age-related memory loss. The Graduate Center of The City University of New York granted Institutional Review Board approval for these interviews (IRB #08-05-1563; PI: Lisa C. Offringa) and research participants gave audio-recorded oral informed consent before participating in interviews.

PLANT SELECTION AND MATERIALS

Interviews identified seventy different species of plants used to treat memory loss. Most of these plants were a part of multi-plant formulas that prescribed specific amounts of each plant to be included. Each plant was researched to verify that the proposed *in vitro* bioassays had not been conducted on them. The databases used for this research included SciFinder Scholar, NAPRALERT and PubMed. Plant species previously tested for acetylcholinesterase inhibition activity, or in *in vivo* memory models, were excluded from the list of prospective plants. Five traditional healers ranked the remaining thirty plant names (in the Thai language) from the original list of plants for memory. The healers ranked the top plants they would test in the laboratory. The healers understood that testing the plants in the laboratory would confirm their biological activity against memory loss, therefore their ranking is considered valid. The eleven plants recommended the most frequently by the healers where then processed into crude extracts and tested for antioxidant and acetylcholinesterase inhibition activity.

Selected plants and plant formulas were collected from the forest, the healer's home gardens or received directly from the healers (Table 5.1). Botanists from the Queen Sirikit Botanic Garden in Chiang Mai, Thailand identified the plant material to species. As specified by my research permit stipulations, voucher specimens for the collected plants have been deposited in the herbarium of the Queen Sirikit Botanic Garden. Voucher specimens have been photographed as a record of this project, and the files stored at the Institute of Economic Botany at The New York Botanical Garden.

Table 5.1: Single plant species tested in *in vitro* bioassays for general antioxidant activity, total phenolic content and acetylcholinesterase inhibition activity.

Scientific name	**Family**	**Name in Thai**	**Voucher**
Angelica dahurica (Fisch.) Benth. & Hook. f. L.	Apiaceae	โกฐสอขาว	LCOM63
Cinnamomum bejolghota (Buch-Ham.) Sweet	Lauraceae	อบเชยไทย	LCOI17
Aquilaria agallocha Roxb.	Thymelaeaceae	ไม้กฤษณา	LCOP23
Tinospora crispa (L.) Hook. f. & Thomson	Menispermaceae	บอระเพ็ด	LCOI9
Diospyros decandra Lour.	Ebenaceae	จันทน์ขาว	LCOM6
Dracaena loureiroi (Lour.) Gagnep.	Asparagaceae	จันทน์แดง	LCOM5
Eurycoma longifolia Jack	Simaroubaceae	ปลาไหลเผือก	LCOI9
Leonurus sibiricus L.	Lamiaceae	กัญชาเทศ	LCOPT22
Gymnopetalum cochinchinense (Lour.) Kurz	Cucurbitaceae	ลูกกระดอม	LCOC9
Nigella sativa L.	Ranunculaceae	เทียนดำ	LCOM64
Jasminum sambac (L.) Aiton	Oleaceae	ดอกมะลิ	LCOCD9

PREPARATION OF EXTRACTS

Plant material was processed in a traditional plant mill by grinding it to a fine powder. As suggested by the traditional healers, 50 g of powdered plant material for each single plant species was used. Extractions were performed at room temperature. For each plant, the powder (50 g) was extracted with 150 ml of ethanol (95%) for 24 hours as indicated by the traditional method. The extraction process was completed by 2 h in a temperature-controlled shaker. The

extracts were filtered and concentrated *in vacuo* at 40°C. The residue was reconstituted with 95% ethanol to a standard concentration. Extracts were stored at -20°C in brown glass bottles until used.

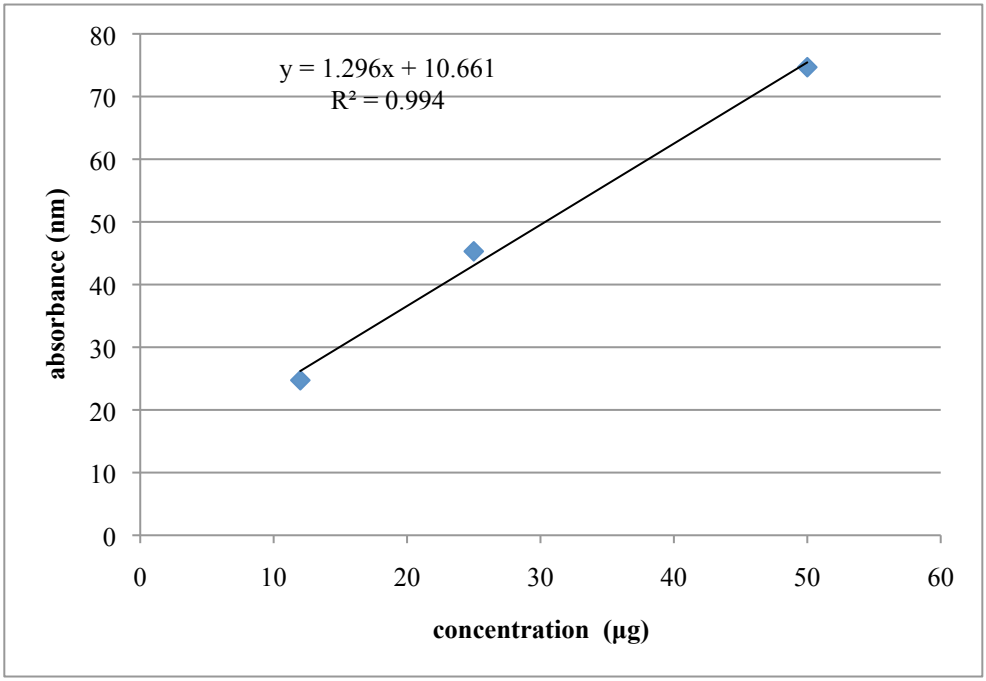
IN VITRO EXPERIMENTAL PROTOCOL

All laboratory procedures and *in vitro* bioassays were conducted by the author at the Faculty of Pharmacy of Chiang Mai University in Chiang Mai, Thailand.

DPPH (1,1-DIPHENYL-2-PICRYHYDROZYL) FREE-RADICAL SCAVENGING ASSAY

The DPPH assay was modified from Yamaguchi et al (1988). Five dilutions of each extract were made using 95% ethanol and ranged from 500 µl to 1 µl. For each dilution, 50 µl was added to selected wells. To wells on half the plate, 150 µl of 400 µM DPPH solution (2,2-diphenyl-1-picrylhydrazyl, Sigma, Germany) was added. The other half of the plate did not contain DPPH solution to control for the color of the extract dilutions. The plate was incubated for 30 min at 37°C before it was read at 515 nm (DTX880 multimode detector, Beckman Coulter). The experiment was performed in duplicate. Percent inhibition was calculated using a negative control group of 95% ethanol. The IC_{50} score is the concentration of the extract needed to scavenge 50% of DPPH free radicals. Gallic Acid dilutions from 12.5 µg to 100 µg were used as a reference ($y=1.296x +10.661$; $R^2=0.994$) (Figure 5.1).

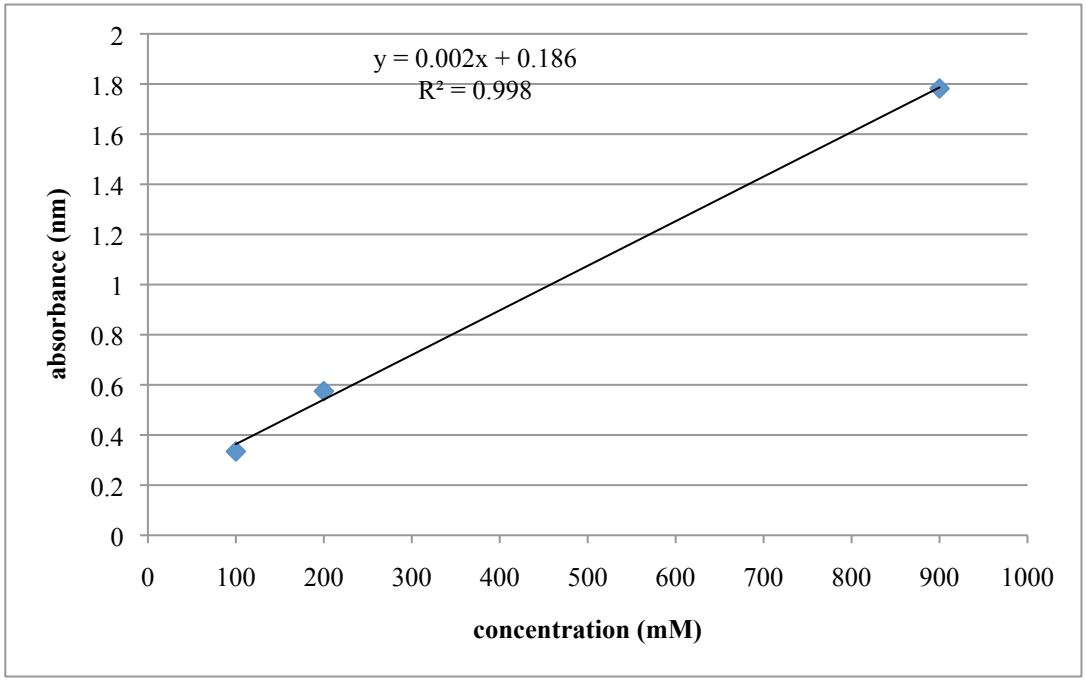
Figure 5.1: Standard curve of galllic acid for DPPH (1,1-diphenyl-2-picryhydrozyl) free-radical scavenging assay.



FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996). Each extract (50 µl) in five incremental dilutions from 30 to 500 ug/ml were added to wells containing 150 µl of FRAP reagent, mixed well, and the absorbance measured at 595 nm for 5 minutes by a microplate reader (DTX880 multimode detector, Beckman Coulter). Each experiment was performed in triplicate. Measurements are made in milligram gallic acid equivilants per gram dry weight (DW) of plant sample (Y=0.002X+0.186; R^2 = 0.998) (Figure 5.2).

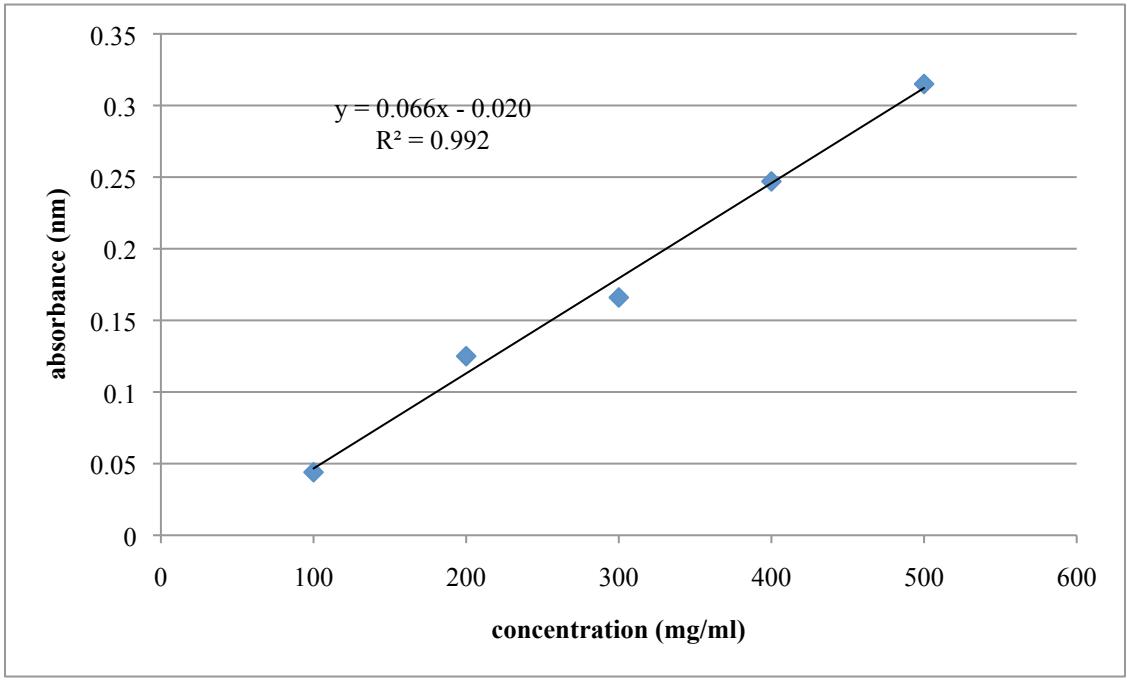
Figure 5.2: Standard curve of gallic acid for ferric reducing antioxidant power (FRAP) assay.



TOTAL PHENOLIC CONTENT (TPC) BY THE FOLIN-CIOCALTEU METHOD

A modified Folin-Ciocalteu method measured the total phenolic content (Reynertson et al. 2008). A sample or gallic acid (100 µl) and 1 ml Folin-Ciocalteu reagent (2N, diluted ten times) was mixed in test tubes and incubated for 5 min at room temperature before 1ml of 10% Na_2CO_3 solution was added. After 90 minutes at room temperature, the mixtures were poured into 5 ml glass cuvettes and measured at 765 nm (UV-Vis Shimadzu UV-2950 Japan). Each sample was measured in triplicate. TPC is expressed in gallic acid equivalents (GAE) in milligram per gram of dry weight ($Y=0.066X-0.020$; $R^2=0.992$) (Figure 5.3).

Figure 5.3: Standard curve of gallic acid for the total phenolic content (TPC) by the Folin-Ciocalteu Method.

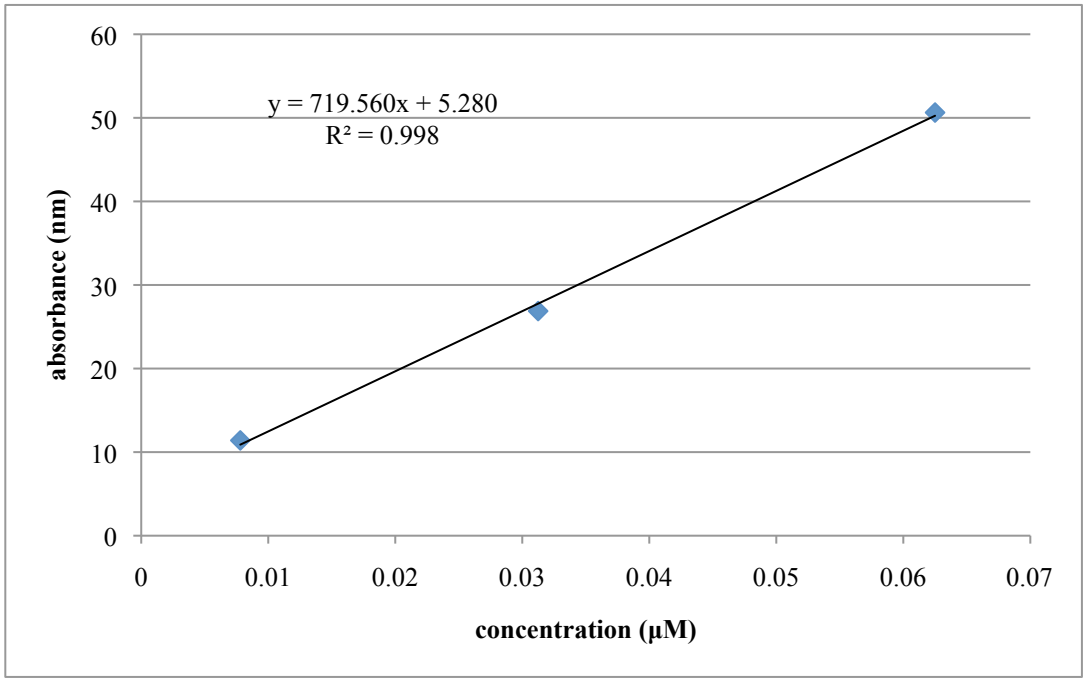


ACETYLCHOLINESTERASE INHIBITION ASSAY

A modified Ellman's method measured the acetylcholinesterase inhibitory activity of the plant extracts. The hydrolysis of acetylthiocholine by the enzyme, acetylcholinesterase, reacts with DTNB to create a color that can be measured at 405 nm (Ellman et al. 1961; Rhee et al. 2001). Samples were diluted to range from 0.1 µg/ml to 2 mg/ml before 25 µl of each dilution was added to the wells of the microplate. To half the wells, 25 µl of 15 mM acetylthiocholine iodide in water, and 125 µl DTNB in 50 mM Tris-HCl, pH 8, with 0.1M NaCl and 0.02 M $MgCl_2$. 6 H_20. The other half contained only the sample to account for the color of the extracts. The plates were read at 405 nm in a DTX880 multimode detector, Beckman Coulter. The enzyme (25 µl of 0.22 U/ml) was added and the plates were read again. Spontaneous hydrolysis was corrected for by subtracting the pre-enzyme reading from the post-enzyme reading. The

percent inhibition was calculated by comparing these rates to 95% ethanol used as the negative control. Galanthamine hydrobromide (Sigma-Aldrich) in MeOH (10 dilutions from 5mM to 0.1μM) was used to define the detection limit ($y = 719.560x + 5.280$; $R^2 = 0.998$) (Figure 5.4).

Figure 5.4: Standard curve of galanthamine in MeOH for acetylcholinesterase inhibition assay.

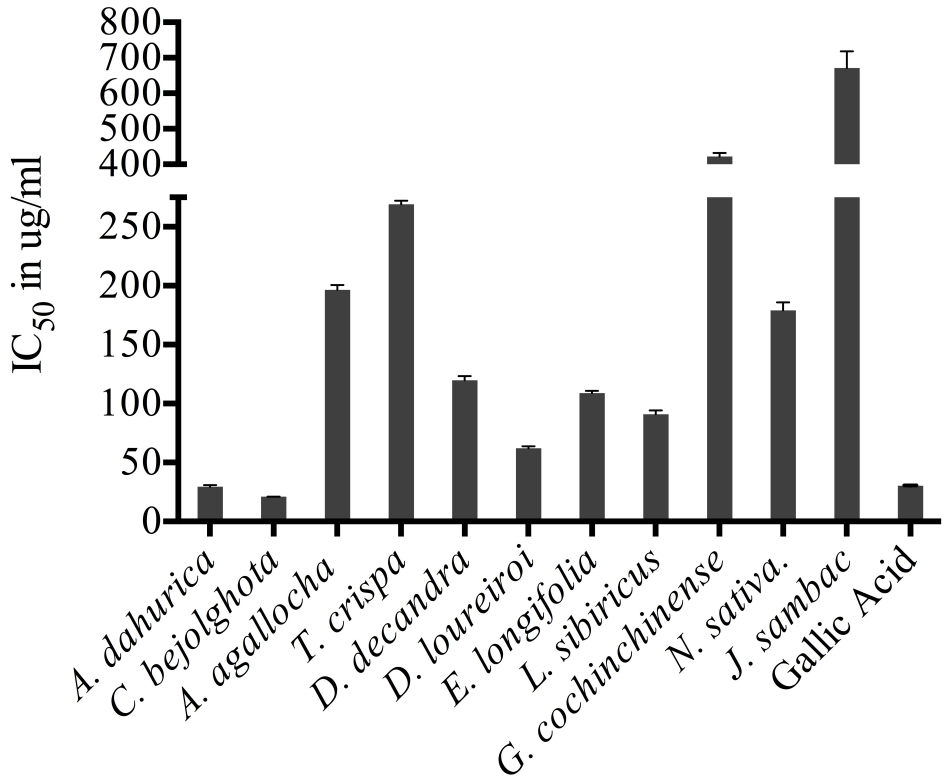


RESULTS

DPPH (1,1-DIPHENYL-2-PICRYHYDROZYL) FREE-RADICAL SCAVENGING ASSAY

The results of the DPPH assay determined two plants with strong antiradical scavenging activity. These were *Cinnamomum bejolghota* >*Angelica dahurica.* Moderate activity was found in *Dracaena loureiroi* > *Leonurus sibiricus.* Other species tested showed low or no activity (Table 5.2). Results are shown as the IC_{50} value in microgram per milliliter (Figure 5.5).

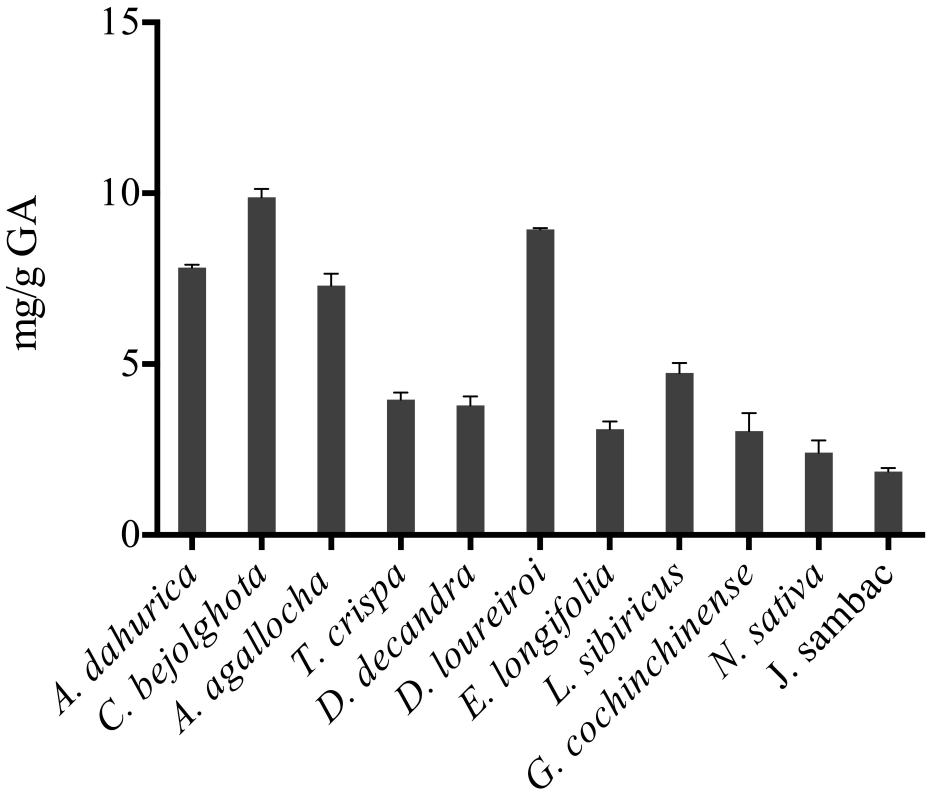
Figure 5.5: DPPH free-radical scavenging activity of the tested plant species showing the half maximal inhibitory concentration in micrograms per milliliter.



FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The FRAP results were divided into five categories ranging from low activity (n=4) to very high activity (n=3). The two plants in the very high activity range were *Cinnamomum bejolghota* > *Dracaena loureiroi*. The results are shown in milligrams per gram of gallic acid (Table 5.2, Figure 5.6).

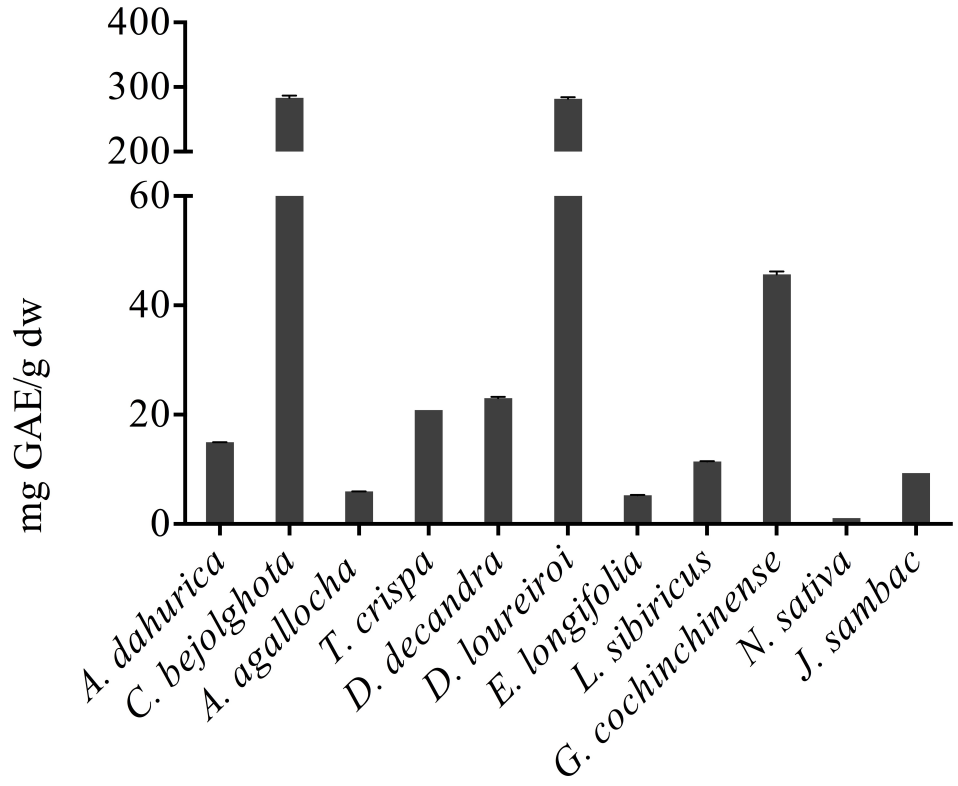
Figure 5.6: Ferric Reducing Antioxidant Power (FRAP) in mg per gram of gallic acid of the selected plant species.



TOTAL PHENOLIC CONTENT

The total phenolic content of most of the tested plants fell in the range of 1.03 to 45.66 mg GAE/g, except for two plants. *Dracaena loureiroi* and *Cinnamomum bejolghota* had a high phenolic content of 281.99 and 283.48 mg GAE/g respectively. The results are shown in milligram of gallic acid equivalents per gram of the dry weight of plant material (Table 5.2, Figure 5.7).

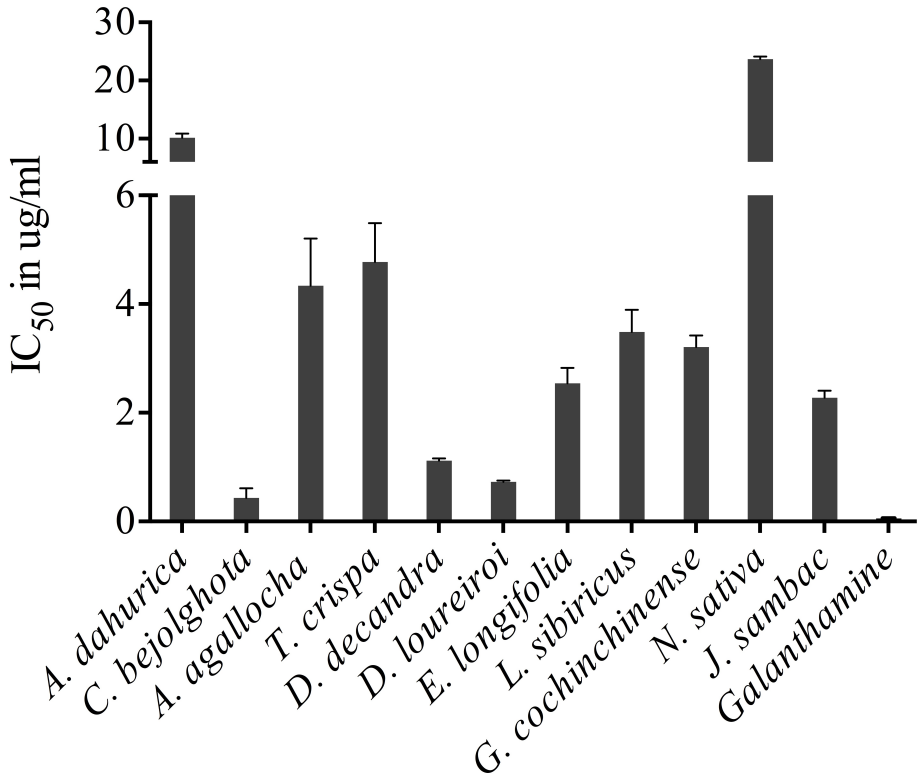
Figure 5.7: Total phonolic content of the selected plant species in milligrams of gallic acid equivalents per gram of dry plant weight.



ACETYLCHOLINESTERASE INHIBITION ASSAY

Five of the eleven single plants tested were found to have significantly high acetylcholinesterase inhibition activity (Tukey's post-hoc comparison at P value < 0.05) when compared to galanthamine. These plants are *Cinnamomum bejolghota* > *Dracaena loureiroi* >*Diospyros decandra* > *Jasminum sambac* > *Eurycoma longifolia*. The results are shown as the IC_{50} value in micrograms per milliliter (Figure 5.8, Table 5.2).

Figure 5.8: The IC_{50} in micrograms per milliliter for the acetylcholinesterase inhibition activity of the selected plant species.



DISCUSSION AND CONCLUSION

The ethnomedical filter utilized in this study successfully identified plants with high activity in the selected *in vitro* bioassays. Since the plants tested were selected specifically for their potential to have biological activity against memory loss, the results of the acetylcholinesterase inhibition assay are most pertinent. The tests for general antioxidant activity and total phenolic content are supporting evidence for the efficacy of the selected plants against age-related memory decline.

As a general measure of antioxidant activity, the DPPH and FRAP assays identified similar plants (Figures 5.5 & 5.6, Table 5.2). The plants *Cinnamomum bejolghota* and *Dracaena*

loureiri both exhibited significant activity in both tests. In addition to these plants, the DPPH test also specified *Angelica dahurica* and *Leonurus sibiricus* as containing free radical scavenging activity. A Tukey's post-hoc test for homogeneity demonstrated that these plants were significantly similar ($\alpha = 0.05$). In a Pearson's correlation, the results of the DPPH and FRAP assay showed a relatively strong negative correlation with each other (r= -0.673). Similarly, both *Cinnamomum bejolghota* and *Dracaena loureiroi* contained a large quantity of phenolic compounds (Table 5.2). These results indicate that the phenolic compounds could be responsible for their antioxidant activity.

Table 5.2: Summary of *in vitro* activity testing eleven Thai plants for antioxidant (DPPH, FRAP and TPC) and acetycholinesterase inhbition.

Plant Species	**DPPH**	**FRAP**	**FCR**	**AchE**
	(IC_{50} in ug/ml)	**(mg/g GA)**	**(mg GAE/g dw)**	**(IC_{50} in ug/ml)**
Angelica dahurica	29.64± 1.78	7.82 ± 0.08	14.92 ± 0.03	10.16 ± 0.71
Cinnamomum bejolghota	20.98± 0.07	9.88 ± 0.25	281.77 ± 3.42	0.44 ± 0.18
Aquilaria agallocha	196.40± 4.35	7.30 ± 0.35	5.87 ± 0.01	4.34 ± 0.87
Tinospora crispa	269.17± 3.01	3.96 ± 0.21	20.878 ± 0	4.77 ± 0.71
Diospyros decandra	119.91± 3.48	3.79 ± 0.26	22.91 ± 0.22	1.12 ± 0.04
Dracaena loureiroi	62.22± 1.47	8.94 ± 0.03	280.27 ± 2.52	0.73 ± 0.02
Eurycoma longifolia	108.95± 1.89	3.10 ± 0.22	8.21 ± 0.09	2.54 ± 0.29
Leonurus sibiricus	90.89± 3.32	4.70 ± 0.29	10.53 ± 0.01	3.49 ± 0.41
Gymnopetalum cochinchinense	422.16± 9.60	3.04 ± 0.52	45.66 ± 0.53	3.21 ± 0.21
Nigella sativa	179.25± 6.57	2.41 ± 0.35	1.02 ±0	23.60 ± 1.47
Jasminum sambac	670.97± 46.88	1.86 ± 0.10	9.30 ± 0	2.27 ± 1.06
Gallic acid				
Galanthamine				0.059 ± 0.004

The standard for the acetylcholinesterase inhibition assay was galathamine because it is a plant-based treatment. The origin of galantamine is from traditional knowledge found in Eastern Europe by a Bulgarian pharmacologist when he observed *Galanthus nivalis* L. (Amarylilidaceae)

being rubbed on the forehead to treat nerve pain (Heinrich 2004). Five of the eleven selected single plants compared significantly with galanthamine in the acetylcholinesterase inhbition assay in a Tukey's post-hoc test of homogeneity, and *Cinnamomum bejolghota* ranked the highest in this assay. The Cinnamon species displayed an IC_{50} score of 0.44 ± 0.18 μg/ml, while galanthamine's IC_{50} was 10 times more potent (0.059 ± 0.004 μg/ml). Galathamine, while still a plant-based substance, is an isolated compound. The *Cinnamomum bejolghota* was tested as a crude extract, which could account for the ten fold difference in enzyme inhibition activity. In a Pearson's correlation, the results of the acetylcholinesterase inhibition assay did not show a relationship with the DPPH test of antioxidant activity, and a very low correlation coefficient with the FRAP assay (r=-0.304) and the total phenolic content (r=-0.327). While antioxidans have been implicated in dementia related memory loss (Giasson 2002), there is not evidence to suggest they can inhibit neurotransmitters in the brain.

This study identified a number of plants with potential to improve the symptoms of age-related cognitive decline. The plant scoring the highest in all of the *in vitro* assays was *Cinnamomum bejolghota*. This plant was selected for continued testing on an *in vivo* rat model. This research demonstrates the success of ethnobotanical screening methods to isolate plant species from a traditional pharmacopeia with potential to treat chronic, contemporary diseases.

VALIDATION OF THAI TRADITIONAL MEDICAL KNOWLEDGE BY SCIENTIFIC INVESTIGATION

INTRODUCTION

The five multi-plant herbal formulas used to generate the list of single plants investigated in the *in vitro* biological assays were tested to corroborate the traditional medical knowledge of the interviewed healers. These results will be returned to them in a document to provide them with the scientific evidence verifying their medical expertise, and potentially offer them a means to treat elderly patients with memory loss in the future.

METHODOLOGY

The testing of the five formulas was performed simultaneously to the testing of the single plant extracts. The methodology and experimental protocol is the same.

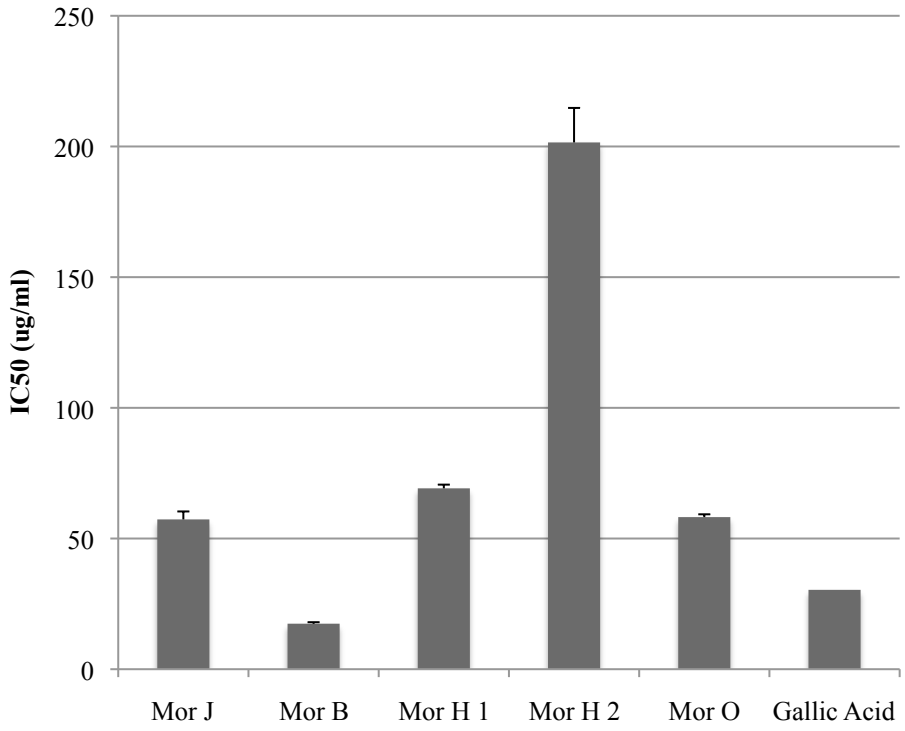
RESULTS

DPPH (1,1-diphenyl-2-picryhydrozyl) Free-Radical Scavenging Assay

The DPPH bioassay tests the general antioxidant activity found in the formula extract. Since it is an inhibition assay, a lower score indicates stronger activity. In this test, the formula from Mor B showed the strongest general antioxidant activity. This formula contained the plant selected for *in vivo* testing.

Figure 5.9: The DPPH free-radical scavenging assay results from the tests of the multi-plant herbal formulas.

Healer	Score (IC_{50} in ug/ml)
Mor J	57.308 ± 3.05
Mor B	17.410 ± 0.58
Mor H 1	69.198 ± 1.42
Mor H 2	201.543 ± 13.20
Mor O	58.193 ± 1.08

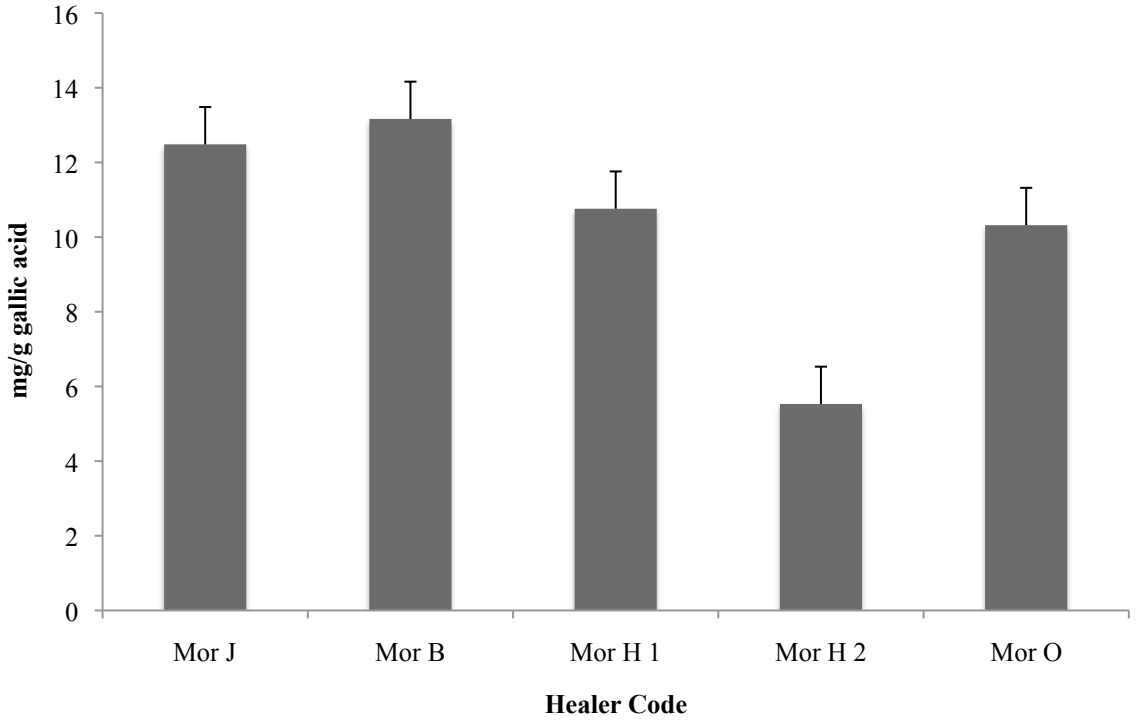


FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

This bioassay also tests general antioxidant activity, but it is not an inhibition assay, so a higher score is desired. Again, Mor B's formula showed the strongest activity. These results are in agreement with the results from the DPPH test.

Figure 5.10: The ferric reducing antioxidant power (FRAP) assay results from the test of the multi-plant herbal formulas.

Healer	Score (mg/g gallic acid)
Mor J	12.484 ± 0.13
Mor B	13.163 ± 0.53
Mor H 1	10.759 ± 0.55
Mor H 2	5.530 ± 0.27
Mor O	10.319 ± 0.44

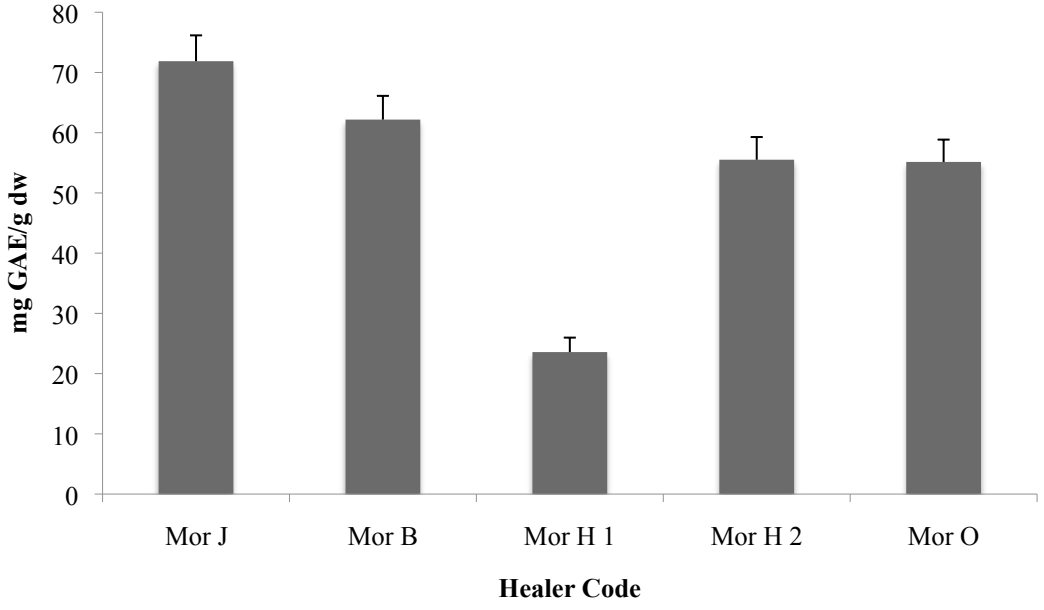


TOTAL PHENOLIC CONTENT (TPC) BY THE FOLIN-CIOCALTEU METHOD

This bioassay tests for phenolic compounds, which are a specific type of anti-oxidant. These compounds protect the neurons in the brain against oxidative stress (Han et al. 2007). This bioassay is not based on inhibition, so a high score is desirable. In this assay, the formula obtained from Mor J showed the highest activity. This formula scored the second highest in the DPPH and FRAP bioassays, so these results could indicate that the strong general antioxidant activity the two assays measuring general antioxidant activity are because of a large quantity of phenolic compounds in the plants that comprise this formula.

Figure 5.11: The total phenolic content assay results from the tests of the multi-plant herbal formulas.

Healer	Score (mg GAE/g dw)
Mor J	71.871 ± 4.29
Mor B	62.175 ± 3.94
Mor H 1	23.5752 ± 2.40
Mor H 2	55.512 ± 3.77
Mor O	55.145 ± 3.71

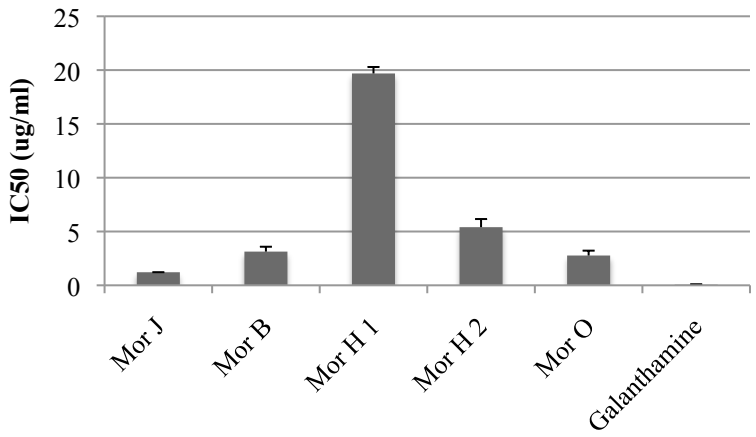


ACETYLCHOLINESTERASE INHIBITION ASSAY

This bioassay measures the inhibition of the enzyme that breaks down acetylcholine in the brain. It is an inhibition assay, so a lower number is more desirable. The bioactivity of this assay does not correlate with that of the other three assays. The results of this assay are the most pertinent as they relate to acetylcholine levels in the brain. Acetylcholine is responsible for memory and learning. In this assay, Mor J's formula showed the most inhibition, and therefore the strongest desired activity. Mor O's formula closely followed, then Mor B and Mor H's second formula. Mor H's first formula showed very little bioactivity in this assay, but could have activity on memory and learning using a different mechanism of action.

Figure 5.12: The acetylcholinesterase inhibition assay results from the tests of the multi-plant herbal formulas.

Healer	Score (IC_{50} in ug/ml)
Mor J	1.214 ± 0.01
Mor B	3.1318 ± 0.45
Mor H 1	19.685 ± 0.60
Mor H 2	5.402 ± 0.76
Mor O	2.768 ± 0.45
Galanthamine	0.059 ± 0.04



CONCLUSION

A Pearson's correlation showed a strong negative correlation between the results of the DPPH and FRAP assays, and between the total phenolic content and acetylcholinesterase inhibition assays. The DPPH and FRAP assays both measure general antioxidant activity, but only one measures inhibition, so a strong negative correlation is expected, and reinforces the validity of the test. Any correlation between the total phenolic content and acetylcholinesterase inhibition assay has no relevance as they measure different bioactivity. There were no other relationships between the scores of the other bioassays.

These tests help to legitimize Thai Traditional Medicine as useful for treating memory decline from dementia. These formulas are from manuscripts from ancient times long before many contemporary diseases were a problem. Perhaps it is because humans currently live longer than before, or it could be from our lifestyle or the toxins in our environment accumulating in the body and causing chronic disease like cancer, dementia, multiple sclerosis and lupus. These results show that ancient Thai knowledge can treat the memory loss in the elderly, and potentially be applicable to other contemporary health problems.

CHAPTER 6 - IN VIVO COGNITIVE ENHANCING EFFECTS OF AN ETHANOL EXTRACT OF *CINNAMOMUM BEJOLGHOTA* (BUCH.-HAM.) SWEET

INTRODUCTION

Dementia is a progressive, debilitating disease affecting memory and behavior primarily in the elderly population. Mild Cognitive Impairment (MCI) is a diagnosable transition stage between normal aging and dementia. Age-related memory decline can be observed before diagnosis with MCI and can be the first indication of dementia. In patients diagnosed with MCI, memory functioning is comparable to patients diagnosed with Alzheimer's disease and the probability for continued progression toward dementia is significantly increased (Petersen 1999; Petersen 2004). Most people experience cognitive decline as they age, about 1 in 100 experience no deterioration of mental functioning, but the annual rate of people detected with MCI is about 10-15% in the US (Farias et al. 2009).

Acetylcholinesterase inhibitors are often used to treat mild to moderate dementia (Snyder and Facchiano 2012). In the aging brain there is a reduced amount of acetylcholine causing a decrease in learning and memory functions as seen in patients with age-related memory loss. The enzyme acetylcholinesterase breaks down acetylcholine in the brain. By inhibiting this enzyme, more acetlycholine is available to the brain and, more specifically, in the parts of the brain responsible for memory like the hippocampus, striatum and cortex (Ringman 2006). Additional theories to explain dementia focus on β- amyloid protein plaques in the brain (Citron 2010). Acetylcholinesterase has been found to co-localize with β- amyloid protein deposits and

potentially aid in their formation (Alveraz 1997). Inhibiting this enzyme could have a two fold positive effect on age-related cognitive impairment.

Oxidative stress is believed to cause some of the memory degeneration occurring in patients with cognitive impairment (Giasson 2002). Levels of oxidative markers were higher in patients with Mild Cognitive Impairment and Alzheimer's disease, then in normal, healthy control subjects (Lopez et al. 2103). Dietary supplementation of known antioxidant fruits, like blueberries, was found to improve memory function in older adults and potentially prevent neurodegeneration (Krikorian et al. 2010).

In northern Thailand, traditional folk healers have plants they use to treat memory conditions, tonics for aging brains and formulas specifically for dementia. Five multi-plant formulas were obtained through ethnobotanical fieldwork and filtered for useful single plants. These eleven plants were screened using four *in vitro* bioassays measuring antioxidant activity and acetylcholinesterase inhibition to determine one plant for further testing. *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Lauraceae) scored highest on all the bioassays and was selected for testing on *in vivo* animal models. *Cinnamomum bejolghota* is distributed throughout Southeast Asia, south China, India and Nepal (efloras, Flora of China 2013). In Thai Traditional Medicine, it is used as a brain and heart tonic, it helps the patient think quickly and clears the brain (pers. comm.). The purpose of this study was to determine if an ethanolic extract of *C. bejolghota* would enhance the memory of rats tested in behavioral memory models and subsequent enzymatic marker testing on their brain tissue.

MATERIALS AND METHODS

The experimental methodology for animal testing was adapted from Wattanathorn et al. (2011), Hawiset et al. (2011), and Wattanathorn et al. (2012).

PLANT MATERIAL AND EXTRACT PREPARATION

Plant material was collected from the Lamphun Province of northwestern Thailand and was identified by Wittaya Pongamornkul of the Queen Sirikit Botanic Garden in Chiang Mai, Thailand (Voucher # LCOI14). The harvested tree bark was dried in the sun and ground in a traditional herb mill to a powder. The interviewed traditional doctors suggested an alcohol extraction method for the single plants tested (IRB # 08-05-1563; PI: Lisa C. Offringa). The powder (50 g) was added to 150 ml of 95% ethanol for 24 h. The extraction process was completed by 2 h in a temperature-controlled shaker. The extract was filtered under vacuum and dried in the rotary evaporator at 40°C. The residue was reconstituted with 95% ethanol to a 10 mg/ml concentration. The extract was dried down via lyophilization and mixed with the liquid vehicle to use in the rat studies.

ANIMALS

Adult female Wistar rats (8 weeks old) were obtained from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. The animals weighed between 220-290 grams at the beginning of the experiment, 8 were housed per cage in standard metal cages at 22 ± 2° C on a 10:14 h light /dark cycle and given access to food and water *ad libitum*. The rats were treated according to the internationally accepted principles for laboratory use and care of the European Community (EEC directive of 1986; 86/609/EEC). The experiments were performed

to minimize animal suffering. The Institutional Ethics Committee of Khon Kaen University pre-approved all experimental protocol.

DRUGS

The standard drug in this study was Donepezil hydrochloride (Aricept) (Pfizer Pharmaceuticals Inc.). All other chemicals were purchased from Sigma Aldrich.

EXPERIMENTAL PROTOCOL AND DRUG ADMINISTRATION

The animals were randomly divided into 6 groups (n=8 animals/group). They were given the treatment via gavage once daily at the same time each day, except for the control group, which was not given either the extract or vehicle during the treatment period. The author was responsible for administering the treatments via gavage.

I: Control group: no treatment given.

II: Vehicle group: animals were given 0.5 ml propylene glycol.

III: Positive control group: Rats were treated with donepezil hydrochloride (Aricept) dissolved in a vehicle of propylene glycol and administered via gavage at dose of 1 mg/kg BW.

IV-VI: Experimental groups. Rats were treated with the Cinnamon extract of 50, 100 or 200 mg/kg BW dissolved in propylene glycol.

The dosages used on the rats were determined using their body weight while accounting for toxicity issues (Ulbricht et al. 2011). Based on previous studies, up to 6 g of a cinnamon extract can safely be taken orally daily for up to six weeks (Khan et al. 2003). Using the body surface area (BSA) normalization method, this would be 1 g in rats, which is much higher than the dosage given per day to each animal (Reagan-Shaw 2008).

All rats, except those in the control group, were given the same volume of substance for 14 days. They were tested using the Morris Water Maze (Morris 1981) and observed for stereotypical locomotor behavior after the first treatment, then again at 7 and 14 days. After the 14 day test, the rats were given their final treatment of the plant extract, then injected with 0.03 g: 10 ml of 90% scopolamine hydrochloride to induce cognitive impairment (Appenroth 2010). The rats were tested for escape latency in the Morris Water Maze. The following day, the rats were tested for retention time in the Morris Water Maze. These tests were conducted to determine the memory enhancing potential of the extract.

After the behavioral experiments were complete, the rats were anesthetized with 50 mg/kg sodium thiopental and sacrificed via an intraperitoneal injection of 50 mg/kg BW of pentobarbital sodium. The hippocampus, striatum and cortex were isolated from the remaining brain tissue. Laboratory technicians and members of Dr. Jintanaporn's lab sacrificed the rats and isolated the brain tissue. These portions were homogenized separately in 1.5 ml phosphate buffered saline (PBS) and centrifuged at 3000 g for 15 min at 4°C. The homogenates of the three brain tissues were tested to determine acetylcholinesterase (AChE) activity and measure oxidative scavenger enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) level. The author was responsible for homogenizing and testing the brain tissue in the bioassays.

Morris Water Maze Test

The Morris Water Maze test measures spatial learning and memory (Morris 1981, Morris et al. 1982). The water maze is a metal pool (170 cm in diameter × 58 cm tall) filled with tap water (25°C, 40 cm deep). The pool is divided into 4 quadrants (NE, NW, SE, and SW) by two

invisible lines crossing the center of the pool. Colored shapes such as a red circle, a blue square, black moon and yellow star marked each quadrant. A removable platform was placed just below the water level in the center of one quadrant and remained there throughout the training of each rat. The water surface was covered with non-toxic white powder during the testing period so the rats were unable to see the platform. During training, the rats memorize the platform location in relation to the environmental cues available, primarily the shapes placed in the quadrants of the pool. Each rat was gently placed in the water facing the wall of the pool in one of the quadrants along the perimeter of the pool. The release quadrant was the same for training and testing. The animal was allowed to swim until it located and climbed onto the platform. During the training session, the rat was gently placed on the platform when it could not locate it after 60 seconds. The animal remained on the platform for 15 seconds and then was removed from the pool during both training and testing.

During testing, the time for animals to climb on the hidden platform was recorded as escape latency. In order to determine the capability of the animals to retrieve and retain information, the rat was tested on its ability to remember the location of the platform after 24 hours. The platform was removed and the rat released into the pool. The time the rat spent in the area where the platform was previously located was recorded as retention time. After each trial, the animal was quickly dried with a towel before being returned to the cage. If the extract is found to enhance spatial memory, the escape latency of the rats will be reduced and the retention time increased (Morris 1981). All rats were tested within 30 minutes of receiving the treatment. The author and laboratory technicians tested the rats in the behavioral models.

Spontaneous Locomotive Activity

All rats were observed for spontaneous locomotion behaviors such at rearing, licking and grooming in an open field test (Joshua et al. 2010). This test acts as a control for a false positive result in the behavioral experiments to show that the desired activity is from the plant extract and not stress. A platform 80 cm in diameter with an opaque wall 30 cm high was used as the open field. The rats were placed in the center of the platform and locomotive activities were tallied for 5 min.

ENZYME LEVEL BIOASSAYS

Protein Estimation

Protein was measured for all brain samples using a modified version of Lowry et al. (1951). Bovine serum albumin (BSA) was used as a standard and measured in the range of 2mg/ml to 20mg/ml.

Acetylcholinesterase Inhibition Activity in Brain Tissue

An acetylcholinesterase inhibition assay was performed using the colorimetric method of Ellman et al. (1961) with minor modifications. The reaction mixture was 200 µL of phosphate buffer (0.1 M, pH 8.0), 10 µL of 0.01 M dithiobisnitrobenzoic acid (DTNB) and 20 µL of the hippocampus, striatum and cortex homogenates. The substrate acetylthiocholine iodide (0.075 M) was then added. A change in absorbance was recorded every 2 min for 5 min at 415 nm by a microplate reader (UV-1601, Shimadzu). The enzymatic activity was expressed as micromoles hydrolyzed per min per gram of protein (µmol/min g protein).

FREE-RADICAL SCAVENGING ENZYMES ASSAYS

Assay for Catalase (CAT) Activity

Catalase is an enzyme responsible for converting harmful hydrogen peroxide to water and oxygen thereby preventing the potential oxidation of the fatty acids in the brain. Catalase activity was measured by the photometric method described by Goldblith and Proctor (1950). The supernatant was screened for catalase activity by observing the rate of disappearance of H_2O_2 by recording the decrease in absorbance at 515 nm. The catalase activity was expressed as μ mol H_2O_2 /min/mg protein.

Assay for Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was measured using the technique of Kakkar et al. (1984) based on the inhibition of the rate of reduction of cytochrome C by the superoxide radical, which was observed at 550 nm. The superoxide dismutase solution was used as a standard for enzyme activity. One unit of activity will be defined as the amount of enzyme necessary to inhibit the rate of reduction of cytochrome C by 50 % in the coupled system using xanthine-xanthine oxidase at pH 7.8.

Assay for Glutathione Peroxidase (GPx) Activity

The glutathione peroxidase activity was determined by the method of Flohe & Gumler (1984). The activity was measured indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydrogen peroxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease is

directly proportional to the glutathione peroxidase activity. The glutathione peroxidase solution was used as a standard enzyme activity. The standard curve was plotted as the rate of A340nm per minute against the GPx activity. The data was reported in units of GPx per mg of protein.

Determination of Malondialdehyde (MDA) Levels

The level of malondialdehyde in the brain tissue supernatant was determined by measuring the accumulation of thiobarbituric acid reactive substances (TBARSs). This test was performed according to the method of Ohkawa et al. (1979).

Statistical Analysis

Data are expressed as mean ± S.E.M. and were analyzed by one-way ANOVA. Significance was determined by a Tukey's post-hoc test (LSD). The results were considered statistically significant at P-value < 0.05.

RESULTS

MORRIS WATER MAZE TEST

After 14 days, there was a significant difference (P-value < 0.01) in the time it took for the rats to escape (escape latency) between the groups given only the vehicle treatment and those given the 100 and 200 mg/kg BW dosage. As expected, the rats given the standard drug, Aricept, also showed a significantly reduced escape time.

Retention time is measured the day after the test of escape latency. It demonstrates the rat's memory of the quadrant where the platform was located by measuring the time in seconds the rat spends in that quadrant. After the first treatment, the group given 100 mg/kg BW of the

extract showed a significantly improved retention time when compared with both the control and vehicle groups. After 14 days, all treatment groups showed a significant difference to the vehicle group. The 100 mg/kg dosage group also showed improvement when compared to the control group (Figures 6.1 & 6.2).

Figure 6.1: Morris Water Maze test of escape latency after 14 days of treatment. The 100 and 200 mg/kg BW dosages were found to be significant against the vehicle (P-value < 0.01).

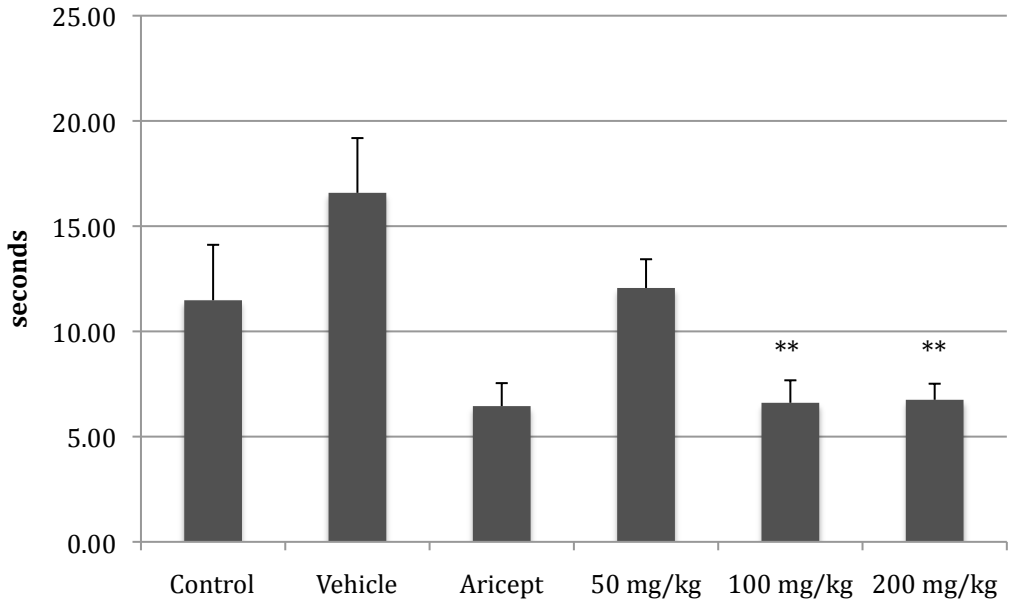
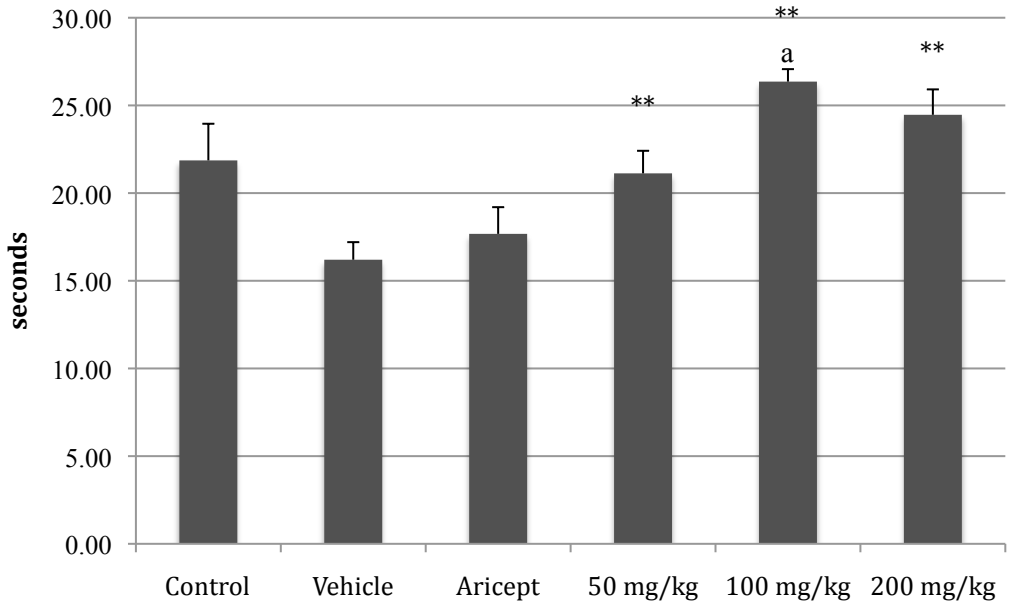


Figure 6.2: Morris Water Maze test of retention time after 14 days of treatment. The 50, 100 and 200 mg/kg BW dosages were found significant against vehicle (P-value < 0.01). The 100 mg/kg BW dosage was significant against the control group (P-value < 0.05).



SPONTANEOUS LOCOMOTIVE ACTIVITY

After a single dose of the extract in 100mg/kg BW treatment group, there was an increase in rearing and licking behavior. No significant changes in any locomotive behaviors were found after 7 and 14 days of treatment.

PROTEIN ESTIMATION

Protein was estimated for the supernatant of the hippocampus, striatum and cortex of each rat. The calculated amount was given in mg/ml, and used to compute acetylcholinesterase activity and the enzymatic levels in the brain tissues.

ACETYLCHOLINESTERASE INHIBITION ACTIVITY IN BRAIN TISSUE

Significant acetylcholinesterase activity was found in all tested parts of the brain. In the hippocampus, the 100 mg/kg BW treatment group showed significant inhibition of the enzyme when compared to the vehicle. The 50 mg/kg BW group demonstrated activity in the striatum as compared to both the vehicle and the control groups. In the cortex, both the 100 mg/kg BW and the 200 mg/kg BW treatment groups were significant when compared to the control group (Table 1 & Figures 6.3-6.5).

Table 6.1: The acetylcholinesterase inhibition activity found in the hippocampus, striatum and cortex of the brains of rats treated with an extract of *Cinnamomum bejolghota* for 14 days (asterisks at significant results).

Acetylcholinesterase Inhibition in umol/min.g. protein			
Group	**Hippocampus**	**Striatum**	**Cortex**
Control	15.475 ± 2.61	15.65 ± 3.966	16.101 ± 4.09
Vehicle	16.508 ± 4.28	14.129 ± 2.61	15.181 ± 1.92
Aricept	* 9.190 ± 2.18	* 5.506 ± 1.99	* 9.371 ± 0.85
50 mg	10.564 ± 1.15	* 6.739 ± 2.36	* 9.567 ± 1.29
100 mg	* 9.180 ± 1.27	13.887 ± 2.29	* 9.651 ± 2.13
200 mg	11.712 ± 1.45	11.198 ± 1.18	16.142 ± 1.05

Figure 6.3: Acetylchoinesterase inhibition in the hippocampus of rats treated with an extract of *Cinnamomum bejolghota* for 14 days. The 100 mg/kg BW dosage and Aricept were significant against the vehicle (P-value < 0.05).

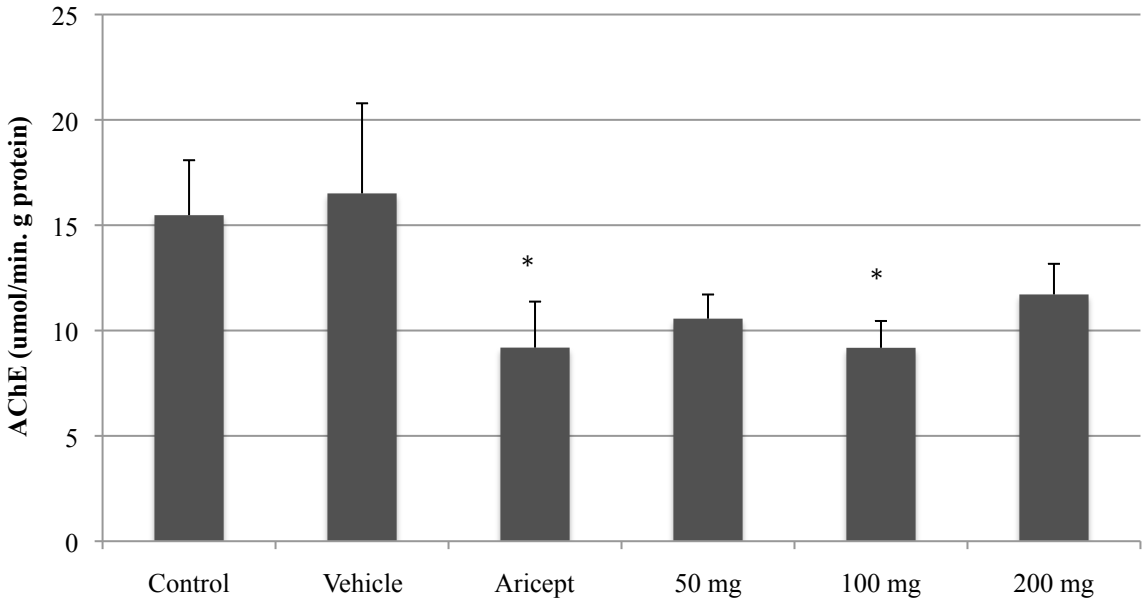


Figure 6.4: Acetylchoinesterase inhibition in the striatum of rats treated with an extract of *Cinnamomum bejolghota* for 14 days. Both Aricept and the 50 mg/kg BW dosage were significant against the vehicle and the control (P-value < 0.05).

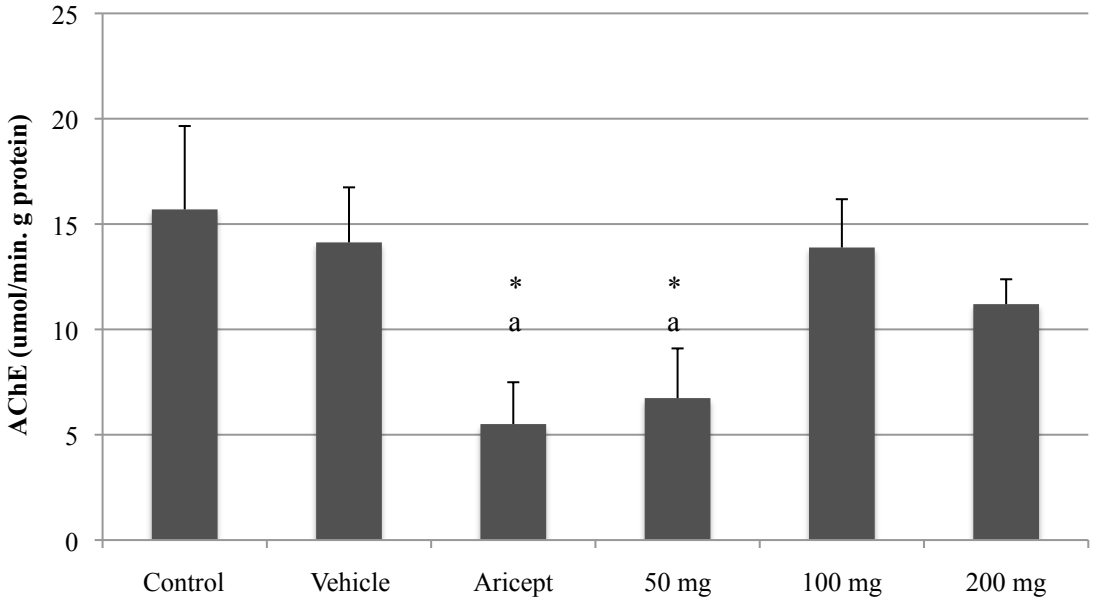
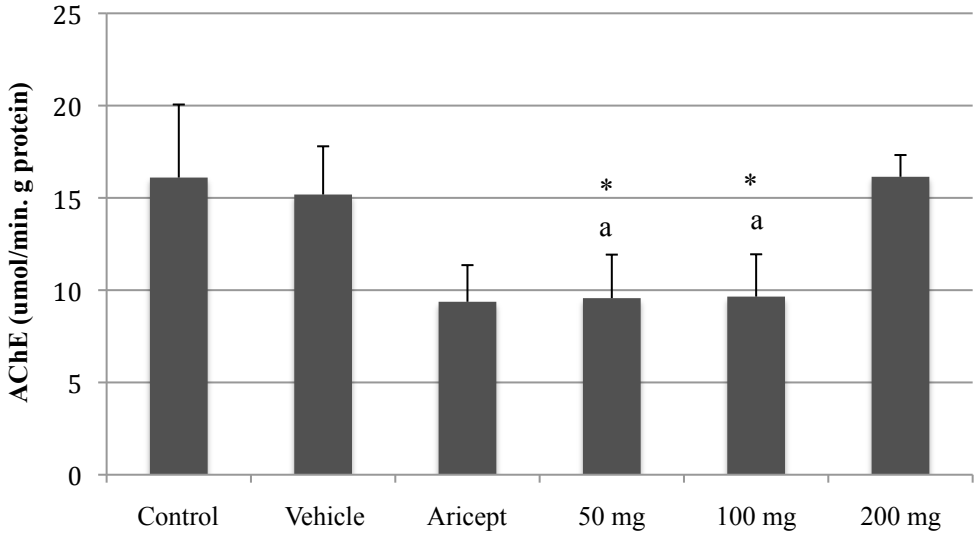


Figure 6.5: Acetylchoinesterase inhibition in the cortex of rats treated with an extract of *Cinnamomum bejolghota* for 14 days. The 50 and 100 mg/kg BW dosage, and Aricept were significant against the vehicle and the control (P-value < 0.05).



FREE-RADICAL SCAVENGING ENZYME ASSAYS

Evidence of free radical scavenging activity was present in some of the brain tissue. The striatum brain supernatant of the rats in the 100mg/kg BW group exhibited high levels of catalase activity. Significant glutathione peroxidase activity was found in the hippocampus of the 100mg/kg BW treatment group as compared to the vehicle and control. Low malondialdehyde levels were found in the striatum of the 100mg/kg BW groups when compared to the control group. Superoxide dismutase (SOD) activity was not found in any of the assayed brain tissue (Table 6.2 and Figures 6.6-6.8).

Table 6.2: Free-radical scavenging enzymes assays showing levels of oxidative markers in the hippocampus, striatum and cortex of rats treated with an extract of *Cinnamomum bejolghota* for 14 days (asterisks at significant results).

Malondialdehyde (MDA) Level in nmol/mg protein			
Group	**Hippocampus**	**Striatum**	**Cortex**
Control	0.056 ± 0.01	0.063 ± 0.01	0.090 ± 0.01
Vehicle	0.053 ± 0.01	0.067 ± 0.01	0.083 ± 0.01
Aricept	0.038 ± 0.01	0.057 ± 0.01	0.063 ± 0.01
50 mg	0.048 ± 0.01	* 0.028 ± 0.01	0.048 ± 0.01
100 mg	0.044 ± 0.01	0.060 ± 0.01	0.066 ± 0.00
200 mg	0.059 ± 0.01	0.061 ± 0.00	0.051 ± 0.00

Superoxide Dismutase (SOD) in u/mg.protein			
Group	**Hippocampus**	**Striatum**	**Cortex**
Control	4.139 ± 0.92	5.464 ± 0.73	10.313 ± 1.69
Vehicle	5.132 ± 0.42	5.040 ± 0.45	7.669 ± 2.40
Aricept	5.055 ± 0.62	5.240 ± 0.61	6.177 ± 1.14
50 mg	5.118 ± 1.00	4.197 ± 0.62	* 4.795 ± 1.15
100 mg	5.023 ± 0.56	5.482 ± 0.90	* 4.121 ± 0.63
200 mg	4.250 ± 0.39	5.210 ± 0.67	6.904 ± 2.94

Glutathione Peroxidase (GPx) in units of GPx per mg of protein			
Group	**Hippocampus**	**Striatum**	**Cortex**
Control	11.705 ± 1.31	8.990 ± 1.58	10.100 ± 1.07
Vehicle	12.857 ± 1.77	9.150 ± 1.53	9.899 ± 1.86
Aricept	11.824 ± 2.22	11.291 ± 1.04	10.487 ± 1.68
50 mg	* 20.837 ± 2.97	8.973 ± 1.18	11.753 ± 2.31
100 mg	14.431 ± 1.57	11.836 ± 0.94	12.006 ± 2.54
200 mg	10.403 ± 1.00	8.710 ± 1.10	8.897 ± 1.83

Catalase (CAT) in µ mol H2O2 /min/mg protein			
Group	**Hippocampus**	**Striatum**	**Cortex**
Control	16.293 ± 2.26	18.222 ± 2.05	25.403 ± 6.30
Vehicle	16.995 ± 3.24	16.270 ± 1.73	25.921 ± 5.49
Aricept	17.098 ± 3.71	15.973 ± 3.61	24.0901 ± 2.02
50 mg	19.250 ± 2.34	16.805 ± 2.39	32.085 ± 3.88
100 mg	17.139 ± 2.50	22.91 ± 2.15	27.867 ± 3.55
200 mg	16.613 ± 2.43	16.562 ± 3.31	23.940 ± 3.94

DISCUSSION

Memory formation and retrieval is a complex process involving different parts of the brain. The hippocampus, as part of the limbic system, is the area of the brain responsible for episodic and, as part of a larger medial temporal lobe memory system, declarative memory. Declarative memory is responsible for the recall of facts and events, and episodic memory encodes and storages spatial representations of such events (Good 2002). Procedural memory of trial-and-error learning and motor patterns based on learning are controlled by the striatum (Doeller et al. 2008). The striatum and pre-frontal cortex work together to produce visual working memories (Voyte 2010) and to coordinate executive functioning (Buckner 2004). Parts of the cortex are also responsible for memory formation. Damage to the cortex was found to impair performance in the Morris Water Maze (D'Hooge & De Deyn 2001). These parts of the brain interact during learning, so it is important to measure all areas to get a comprehensive perspective on memory processes in the brain. Together they provide a cognitive map of the spatial relationship between the available visual cues and memory based decision-making in the Morris Water Maze (Good 2002). Measurements of enzymatic activity in these areas can help to clarify their involvement in memory and learning, and the effect of the Cinnamon extract on these portions of the brain.

MORRIS WATER MAZE TEST

The Morris Water Maze tests spatial learning and memory, and can investigate cognitive decline in rats similar to that of dementia in humans (D'Hooge & De Deyn 2001). The results of this study indicate that rats treated with at least the 100 mg/kg BW dose of the cinnamon extract have improved performance in both measurements of the Morris Water Maze: retention time and

escape latency. The rats given the middle and high dosage (100 and 200 mg/kg BW) exhibited an improvement in escape latency against the vehicle comparable to the pharmaceutical control. Retention time improved on all dosages of the cinnamon extract against the vehicle, and at 100 mg/kg BW against the control. These results are compelling because the treatment is a crude plant extract as opposed to an isolated pharmaceutical compound.

Scopolamine, as a means to mimic memory loss, should affect Morris Water Maze performance during the acquisition phase, or training of the rat, as opposed to affecting its recall during performance or testing (D'Hooge & De Deyn 2001). The results show no neuroprotective affect of the extract on the rat's brains and no improvement on either the escape latency or the retention time after the injection of scopolamine despite treatment with the Cinnamon extract (Figure 6.1 & Figure 6.2).

SPONTANEOUS LOCOMOTIVE ACTIVITY

While there was evidence of the extract affecting spontaneous locomotive activity after a single dose, there was no significant change in normal behaviors after 14 days of treatment. This test supports that the results are not due to a false positive.

ACETYLCHOLINESTERASE INHIBITION ACTIVITY IN BRAIN TISSUE

Memory, learning and recall all have a foundation in acetylcholine activity and its abundance in the neural synapses of the brain (D'Hooge & De Deyn 2001). Lowered levels of acetylcholinesterase, the enzyme that breaks down acetylcholine, were measured in all three homogenates, which results in an increase of acetylcholine available to these parts of the brain. This, in turn, can help maximize learning and memory potential. In the hippocampus and cortex,

the 100 mg/kg BW dosage was the most effective and was the threshold dose for improvement. The 200 mg/kg BW dose was not effective. The 50mg/kg dose showed the greatest activity in the striatum where neurons may be more sensitive to the enzyme inhibition (Figures 6.3, 6.4 & 6.5).

FREE-RADICAL SCAVENGING ENZYME ASSAYS

Oxidative stress has been associated with cognitive impairment and its tendency to develop into dementia (Torres et al. 2011). Serum levels of markers of oxidative stress were measured to determine if the Cinnamon extract provided protection against the effects of lipid oxidation in the brain tissue. An increase in oxidative stress could be due to escalated levels of acetylcholinesterase activity. The chemical breakdown of acetylcholine could be producing oxidizing agents, like nitric oxide, a known radical gas produced in neuronal functions (McCarty 2006). A high level of catalase activity was found in the striatum of the brain tissue in the rats receiving the middle dosage of the Cinnamon extract (100 mg/kg BW). The low malondialdehyde levels found in the striatum of the 100 mg/kg BW groups compared to the control group indicates that this portion of the brain was protected from increased oxidation by the extract. Malondialdehyde is a byproduct of oxidative activity therefore high levels of it are evidence of oxidative stress in the brain. Significant glutathione peroxidase activity was found in the hippocampus supernatant of the middle dosage (100 mg/kg BW).

The areas of the brain were not similarly affected by the cinnamon extract in all of the tests. The striatum showed a lower threshold for activity in the acetylcholinesterease inhibition assay potentially because of the neurochemistry of the cells in that part of the brain. The oxidative stress markers were also most active in the striatum, which could be attributed to the

increase in acetylcholine activity in this part of the brain. The cortex and the hippocampus were affected by the middle dose of the extract yet there was little antioxidant activity found in either of these parts. These three parts of the brain are coordinated for memory and learning, but the connections between them are complex and therefore, these results are difficult to interpret as they apply to the prevention of memory decline (Figures 6.6, 6.7 & 6.8).

CONCLUSION

The results show the crude extract of *Cinnamomum bejolghota* to have cognitive enhancing activity in the selected *in vivo* rat behavioral models and subsequent acetylcholinesterase inhibition assay of brain tissue. This plant extract could potentially remedy the memory decline associated with cognitive impairment in the elderly. The precise bioactivity of the chemical compounds in the crude extract is unknown and merits further investigation. The specific compounds responsible for the effect of the extract could be explored using activity-guided fractionation to determine the compound responsible for the effects of *Cinnamomum bejolghota*. Additional toxicology testing is needed prior to human clinical trials.

Figure 6.6: MDA (malondialdehyde) levels after treatment for 14 days with an extract of *Cinnamomum bejolghota*. The 50 mg/kg BW dosage were significant against both the vehicle and the control in the striatum (P-value < 0.05).

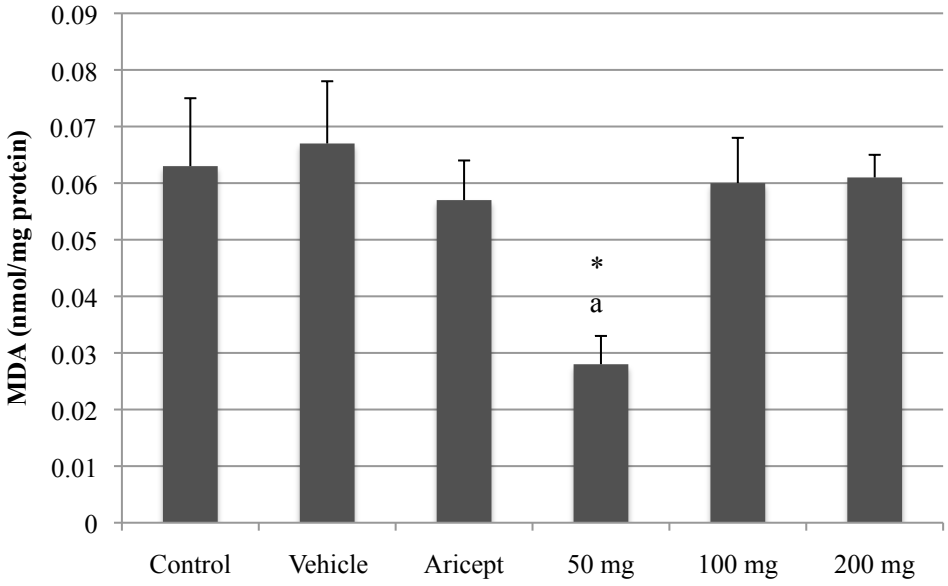


Figure 6.7: SOD (superoxide dismutase) levels in the cortex of rats treated for 14 days with an extract of *Cinnamomum bejolghota*. The 50 and 100 mg/kg BW dosages were significant against the vehicle.

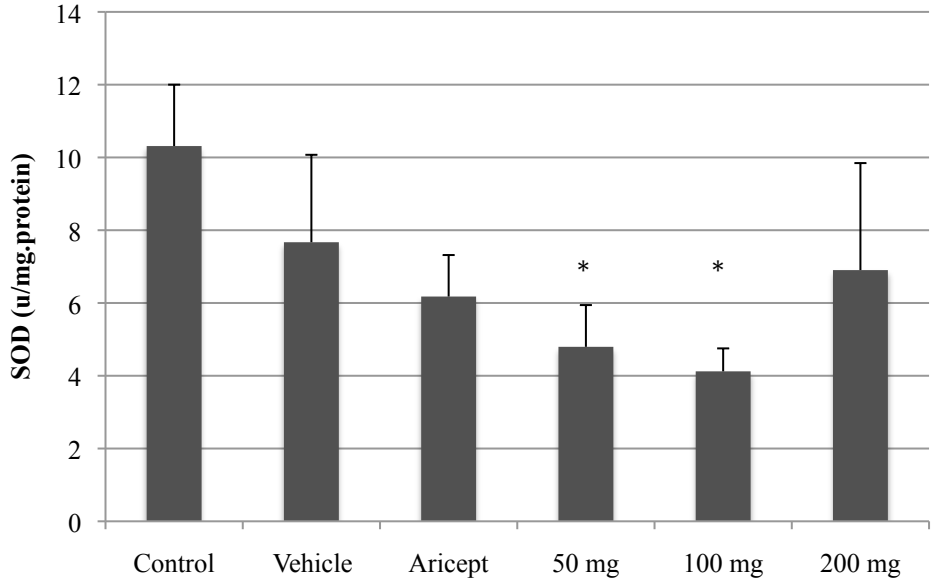
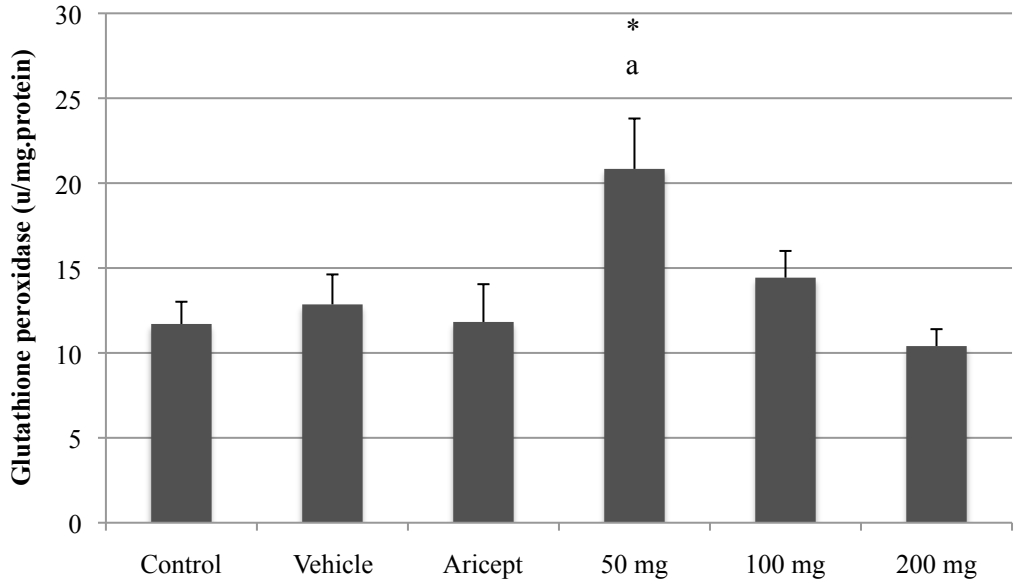


Figure 6.8: Glutathione levels in the hippocampus of rats treated for 14 days with an extract of *Cinnamomum bejolghota*. The 50 mg/kg BW dosage against both the vehicle and the control was significant.



CINNAMOMUM BEJOLGHOTA (BUCH.-HAM.) SWEET (LAURACEAE)

Synonyms
Laurus bejolghota Buchanan-Hamilton, Trans. Linn. Soc. London 13: 559. 1822; *Cinnamomum obtusifolium* (Roxburgh) Nees; *L. obtusifolia* Roxburgh.

TAXONOMIC TREATMENT

Worldwide there are almost 2,800 species of plants in the family Lauraceae mostly growing in tropical areas. Their center of diversity is in Southeast Asia, where they are found in Burma, Laos, Thailand, Vietnam, China, India, Nepal, Bhutan and Bangladesh (Tropicos 2013). This family is also distributed in South America, Madagascar, and even temperate areas like North America, Japan, Europe, New Zealand and Chile. The Lauraceae are divided into two tribes, which are distinctly classified by their inflorescence, floral morphology and wood anatomy. Important species in this family are avocados (*Persea americana*), cinnamon (*Cinnamomum zeylancium*) and bay leaf (*Laurus nobilis*). Members of this family are also used, and sometimes exploited, for timber and essential oils (Madrinan 2004).

Cinnamomum bejolghota are small to large trees up to about 25 m tall with green scented bark. They have opposite branches with sub opposite leaves. The leave blades are oblong and leathery about 12-30 by 4-9 cm. The lateral veins rise off the leaf base. The panicled inflorescence has many flowers with slightly hairy pedicels. The flowers are yellow and measure about 6 mm with a six lobed grey perianth, nine stamens and an oblong ovary with a slender style and discoid stigma. The fruit is ellipsoid and green when fresh. It flowers in March and April and fruits in May through July (Flora of China 2008). The first type specimen is stored at The Royal Botanic Garden of Edinburgh (Figure 6.9).

Figure 6.9: Type specimen of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Lauraceae) from the Royal Botanic Garden Edinburgh collected from Bhutan (Grierson and Long 1879).

ETHNOBOTANICAL USES

Cinnamon is a commonly consumed spice and was one of the first spices documented. It was recorded in the Bible, and there are historical references to Egyptian times. Cinnamon is made from grinding the dried bark of the *Cinnamomum zeylanicum* tree, which grows primarily in Sri Lanka. The meaning in Greek of Cinnamon is "sweet wood" referring to its aromatic wood and flavorful bark. *Cinnamomum bejolghota* specifically is used as a spice and sold in the markets of Assam, in Northern India. It is used to flavor a local beverage that is like beer (Deori

2007; Baruah 1997). It is used to treat health complaints like colds and coughs, toothaches, liver and gall stone issues and as a mouthwash (Rao 1981). The wood is soft and finely grained. The powered bark is also used for a spice by people in Hainan, China (Flora of China 2008).

PREVIOUS STUDIES

Previous studies identified a connection between cinnamon and dementia, but these are based on different mechanisms of action than this research proposes. Taupathies, or a disfunction in the processes of the tau protein in neurons, have become a defining characteristic of Alzheimer's like dementias. The tau proteins, which typically aid in microtubule organization and promote functioning of the cytoskeleton, instead clump together and form tangles inside the neurons. Research on *Cinnamomum zeylanicum* found that an extract of the bark inhibited the accumulation of these proteins into tangles and disassembled tangles that were already formed (Peterson 2009).

The majority of research on Cinnamon as a nutraceutical relates to its use in stabilizing blood sugar for diabetes. Adisakwattana et al. (2011) examine four species of the genus *Cinnamomum* on intestinal enzymes associated with the digestion of starches and therefore changes to blood sugar. The results found that the different species of Cinnamon had varying action on specific enzymes, but that there were dramatic inhibitory affects on all the enzymes. A phytochemical analysis on phenolic compounds, flavonoids and condensed tannins showed the Thai cinnamon, *Cinnamomum bejolghota*, to have the highest content of these compounds of the four species.

Research by Amin and Abd El-Twab (2009) compared the effect of atorvastatine, a common drug for high cholesterol levels in the blood, and cinnamon on parameters relating to

lipid levels in body. They examined the antioxidant effects of *Cinnamomum zeylanicum* on a number of oxidative stress markers. They found that the strong antioxidant affect of the oral cinnamon treatment was because of its high phenolic content, a conclusion also supported by this research.

Cinnamon has a beneficial effect on insulin sensitivity, explaining its use for diabetes. It also reduces the production of cholesterol in neurons, which in turn reduces the production of β-amyloid from the amyloid precursor protein (McCarty 2006). When the amyloid precursor protein is broken down, it forms the characteristic plaques in the brains of patients with Alzheimer's disease (Alzheimer's Association 2008). Interestingly, this effect relates to the research by Amin and Abd El-Twab (2009) on the effect of cinnamon on lowering blood cholesterol levels. Increased levels of cholesterol will promote β- amyloid production; so inhibiting cholesterol can prevent the production of β- amyloid plaques (McCarty 2006).

Additional research by Frydman-Marom et al. (2011) showed that an aqueous extract of Cinnamon bark affected the formation of β- amyloid from amyloid precursor protein, disrupted the formation of β- amyloid into plaques and influenced aberrant behavior in transgenic fly and mice models of Alzheimer's disease. After the extract was given to the flies, their locomotor abilities and longevity was improved, and the β- amyloid plaques in their brains diminished. The formation of abnormal β- amyloid protein and plaque formation in the brains of the mice was reduced, and their performance in the Object Recognition Test improved.

This research project provided evidence of a Cinnamon extract inhibiting acetylcholinesterase both *in vitro* on colorometric chemical bioassays, *in vivo* on rat behavioral models, and in enzymatic tests of their brain tissue. Inhibiting the breakdown of acetylcholine in the brain provides neurons with an increase level of this useful neurotransmitter, which aids in

memory formation. The aforementioned research finds Cinnamon bark useful for other causes of dementia, especially the taupathies and formation of β- amyloid plaques, but using a different approach. The apparent twofold activity of Cinnamon bark could potentially ameliorate the memory loss in the elderly if further research is conducted.

TOXICITY

Cinnamon bark is classified as generally recognized as safe (GRAS) by the Food and Drug Administration (Ulbricht 2011). A dosage of up to 6 grams taken orally over a short duration was found not to cause any toxic reactions (Khan et al. 2003), but there are other contraindications with specific medications (Ulbricht 2011).

There is some concern regarding the quantity of coumarin in certain Cinnamon species, as coumarin can cause hepatotoxicity (Su et al. 1999). Testing for this compound in the bark of the plant used for medicinal purposes could alleviate concern for ingesting high levels of coumarin. Some people have an allergic reaction when exposed to plants in the Lauraceae family (Ulbricht 2011). Treatment with medicinal plants from this family would be inappropriate for these individuals.

Dosages have been determined for a number of medicinal uses for cinnamon species. As an antioxidant, an aqueous extract of the bark was administered to patients in doses of 250 mg two times a day for twelve weeks (Roussel et al. 2009). Cinnamon lozenges were given to patients with HIV to treat candidiasis eight times daily for one week with no adverse effects (Quale et al. 1996). To treat diabetes, one to six grams of cinnamon was given orally for forty days without adverse reactions (Blevins et al. 2007). In a clinical trial to treat a Helicobacter pylori infection, eighty milligrams of a cinnamon extract was taken daily for four weeks (Nir et

al. 2000). These studies show that treatment with cinnamon for extended periods does not produce harmful effects in human clinical trials.

The bark of *Cinnamomum bejolghota* was processed into an ethanolic extract as suggested by traditional healers (pers. comm. Mor H 2011). In tests on mice, an ethanolic extract of C. zeylanicum bark showed no acute or chronic toxicity when the mice were treated orally with the solution (Tremblay and Avon 2008). In development studies of Drosophila melanogaster, there was no mutagenic activity displayed with on *in vitro* studies when an ethanolic extract was used (Ungsurungsie et al. 1984).

Additional toxicological studies would need to be conducted to determine if *Cinnamomum bejolghota* has a particular chemistry that could cause adverse reactions in humans after extended treatment. These studies would need to address the preparation method (i.e. water versus ethanolic extraction), dosage levels and type of treatment (i.e. oral versus topical). If bioactivity guided isolation was conducted on the bark of *Cinnamomum bejolghota,* these toxicological tests would need to be repeated using only the isolated compound.

CHAPTER 7 - CONCLUSION

INTRODUCTION

This study investigated the anthropological, botanical and pharmacological aspects of treating cognitive decline in the elderly using medicinal plants from northern Thailand. Interviews with traditional healers explored the concepts of cognitive impairment in the elderly and dementia within the cultural construct of Thai Traditional Medicine. Further dialogue with traditional healers uncovered plant based treatments for memory disorders, longevity and culturally based diseases related to memory loss. The plants in these ancient Thai therapeutic formulas were collected or purchased to reflect the practices of contemporary Thai traditional healers. The plant names were subsequently confirmed for botanical identification.

These formulas, which contain a unique combination of numerous plants, were tested in both *in vitro* and *in vivo* laboratory experiments to substantiate Thai medicinal theory. The plants from these formulas were researched to determine if they were previously studied, then additional interviews determined eleven single plants that were most promising for laboratory testing. Crude ethanol extracts were made from the selected plants and multi-plant formulas as indicated by the traditional healers. Three antioxidant bioassays and one bioassay measuring acetylcholine were performed on five of the memory formulas and eleven single plant extracts. Testing the memory formulas will provide the results of the bioassays to the traditional healers for them to use in their practice, and to corroborate the bioactivity of these ancient plant preparations.

Screening the single plant extracts identified one plant, *Cinnamomum bejolghota*, with high antioxidant activity and compounds that increase the level of the neurotransmitter

acetylcholine in the brain. Forty-eight rats were treated with an extract of *Cinnamomum bejolghota* for fourteen days. The rats were assessed using *in vivo* behavioral models to determine the extract's potential memory enhancing activity. Brain homogenate of the treated rats was further examined for levels of oxidative stress markers and acetylcholine by measuring inhibition of acetylcholinesterase. Results from the *in vivo* and enzymatic tests demonstrated the extract of *Cinnamomum bejolghota* to have memory enhancing cognitive effects.

The results of this project display the potential of the ancient Thai medical system to include treatments for modern day afflictions like debilitating neurological disorders. During this study, the medical theory and plant use of Northern Thai traditional healers was scientifically analyzed and documented, providing a lasting written record of what is primarily an oral tradition. The official collaborations and unofficial friendships that were formed during my research could provide additional study opportunities for other students. The results from this work can initiate further investigation of *Cinnamomum bejolghota* by scientists as a means to utilize and protect their traditional knowledge.

KEY FINDINGS

The significant research conclusions are discussed in this section. These findings are categorized under the three areas explored during this study.

ANTHROPOLOGICAL

There is a resurgence of the practice of Thai Traditional Medicine throughout Thailand, and especially in northern Thailand, as traditional healers are now formally licensed by the Ministry of Health.

Thai people have a strong sense of national pride. They delight in believing the Thai way is the best way. Still, many modern Thai people prefer western medicine to their own traditional medical system. In the past, Thai Traditional Medicine was perceived as archaic and used only by the poor. The Thai government perpetuated these ideas when they stopped teaching Thai Traditional Medicine in medical schools because it was considered too theoretical and subjective compared to western medicine (Chokevivat 2003).

In 1978, the World Health Organization encouraged the Thai government to regulate and integrate Thai Traditional Medicine into their national healthcare system. The current worldwide interest in spa and massage services, especially Thai massage, cultivated additional interest by the government and Thai people to reconsider their medical heritage. This movement ushered in a more widely utilized and accepted traditional medical system (Chokevivat 2003). The Ministry of Health licenses traditional healers, and there is a formal educational path in place for those individuals who want to study Thai Traditional Medicine. The government established Good Manufacturing Practices (GMP) for herbal remedies and regulates access to Thai traditional knowledge by non-Thai people (Department of Medical Services 1993; Mistry of Public Health 2002; Ministerial Regulation on the Organization of the Department for Development of Thai Traditional and Alternative Medicine 2002). Many practitioners of Thai Traditional Medicine support individuals studying traditional medicine and help them to navigate the regulatory process. A *farang,* or non-Thai person, is not allowed to formally learn about Thai Traditional Medicine or become licensed, except for Thai massage.

My experience accessing information on Thai Traditional Medicine was varied. The government was diligent in granting their permission for me to research medicinal plants in

Thailand. It was a long and complex process to obtain research permits as a foreigner, and they had strict stipulations when I did receive them. Some traditional healers were skeptical about my research and questions, while others were delighted that a *farang* was interested in Thai Traditional Medicine and openly shared their knowledge. These two very different reactions both display the reverence Thai people have for their culture and heritage.

Thai Traditional Medicinal healers recognize elderly dementia as a disease. They expressed concern regarding the potential growth of this health issue in the future, and care for the elderly with this disorder.

The rate of dementia is rising worldwide in both developed and developing nations, yet the care of individuals with the disease in most developing countries has not been formally established (George 2010). Through extensive interviews with Thai Traditional healers it was apparent they were aware of dementia as a disease category and, as some healers expressed, potentially a disease with ramifications in the cultural, social and economic arenas. Some healers treated patients with dementia, while others treated elderly people with memory problems. "Same, same but different" as they say in Thailand. Those who were aware of the disease, or had personal experience treating someone with it, were aware of the diagnostic spectrum of the disease and how debilitating it can eventually become.

In Thailand, western medical care of an individual with dementia in an urban area is similar to that in the United States (Pers. Comm. Dr. Pumin Chalachiva, Neurologist, Suan Prung Psychiatric Hospital, 2008). The care of a patient in a rural area is much different. These individuals are cared for in the home, as many people do not have access to modern medical

facilities, or they cannot afford that level of care. The family tends to the elderly person and may bring them to see the local "folk" healer in the village.

According to the traditional healers I interviewed, there are more elderly people with dementia, and forgetfulness in the elderly is becoming increasingly common. Even healers who not were key research participants, who did not know about medicinal plants to treat this condition, did know of people with dementia in their community. Many of my key research participants anticipate an increase in the prevalence of dementia in Thailand. They believe one of the causes of the illness is toxicity from chemicals in the natural environment. The chemicals are accumulating in the environment and in people's bodies possibly causing diseases like cancer and dementia. It is thought that the use of pesticides and an increase in the consumption of processed foods are both contributing to the toxicity of the people and their environment. With no visible end of these dangerous practices, an escalating frequency of chronic disease will potentially follow.

There are medicinal plants used by Thai traditional healers to treat memory disorders and prevent cognitive impairment in the elderly.

Traditional medicine in Thailand follows the more holistic perspective of many eastern style medical systems. The symptom-based approach of western medicine, where a medication treats a specific symptom is not as prevalent and instead, the entire body is treated and the symptoms are just the indication the body is out of balance. In my research, plant use was primarily indicated in the form of multi-plant herbal formulas. These formulas offered treatment both for the symptoms of the disease and contained plants to promote balance in the entire body.

When asked about plants used for memory disorders in the elderly, many of the traditional healers recommended formulas for imbalance of the *lom* or wind in the body, which is considered the underlying cause of dementia in Thai Traditional Medicine. Some healers had formulas specifically for memory, but these also contained plants to address the imbalance in the body that caused memory impairment. Other healers provided tonics for the brain and nervous system, and formulas for long life and longevity. Some single plants were suggested to treat memory decline as a symptom, but these were discussed only when I specifically asked about them.

The healers obtained the multi-plant formulas either from their ancestors or from ancient manuscripts that were records of traditional medical theory and remedies. While dementia is not an entirely modern disease category, the first documented and named case of it was at the start of the 20th century when Alois Alzheimer described his first case of Alzheimer's disease (Maurer 1997). The ancient manuscripts containing the formulas for memory impairment are much older.

This project demonstrated the relevance of the formulas in these documents to potentially treat more contemporary disease categories like dementia. The plants in this project were tested on bioassays related to one theory on the etiology of dementia, acetylcholinesterase inhibition to increase acetylcholine in the synapses of the neurons in the brain. This theory was among the first to explain memory loss, and acetylcholine is known to be involved in memory formation (Sudha 1995). Testing the same plants and plant formulas on bioassays addressing other causes of dementia, like the build up of tau neurofibrillary tangles within neuron cells, would be notable as this cause is specific to dementia, and not just memory.

Thai Traditional Medicine believes that disregarding culturally sanctioned postpartum behaviors can be the cause of memory decline in the female elderly population.

Traditionally in Thailand a woman who has just given birth should remain inside for one month to recover her strength and tend to her newborn child. If she does not observe this tradition, she can be vulnerable to specific diseases when she gets older (See section on *lom pid duan* in chapter 4). I learned about *lom pid duan* when investigating gender differences regarding the prevalence of dementia in the elderly population. Some healers believe women get theses symptoms more often because many of them do not "do their month" after childbirth. The incidence of this disorder is increasing as Thai people are aligning themselves with more western ways and women are having babies in hospitals or continuing with their career soon after giving birth.

Additionally, food plays an important role in Thai culture. Many times when you meet a Thai person they will ask if you have eaten, and if you have, then what did you eat. Outside of the urban areas that provide easy access to processed food, there is an emphasis on eating fresh food, eating with the season and not eating certain foods at certain times. Eating these "wrong foods" can contribute to illness by imbalancing the elements. They believe chronic diseases like dementia can be caused by consistently imbalanced elements. Eating the proper foods, as promoted by classic Thai culture, can help to prevent these types of diseases.

BOTANICAL

Many of the plants described in the ancient Lanna manuscripts on medicine are not found in northern Thailand. Therefore, in contemporary Thai Traditional Medicine, most folk healers do not harvest all of the medicinal plants that they use in their practice.

The *Lanna* Kingdom was thriving during the 13th to 18th century. It included northern Thailand and parts of Myanmar (Burma), Laos and Southern China (Penth 2001). Many of the multi-plant formulas received from the traditional healers were from manuscripts writing during this time. This knowledge has been passed down through families or temples, and these documents are still consulted for their information on medicinal plants. Some of the plants contained in these formulas came from northern Thailand, while others can be found only in the surrounding area that comprised the *Lanna* Kingdom.

There were healers who collected many of the plants used in their practice, while there were others who purchased the plants they use at the local market. A shop in Chiang Mai city called *Lanna* Herb*s* was the trusted source of hard to find medicinal plants, and many healers would purchase their plants here. One healer managed his own factory that manufactured capsules of his formulas. He ordered many of his plants in bulk from neighboring countries, and was the source of some of my sample vouchers for plants that could not be found in the forest or home gardens for collecting. One formula I received could not be tested in the laboratory because some of the plants in the formula could not be found. The plants in this formula could not be collected, purchased or obtained through the healer with the factory. One of the plants in the formula was so obscure it was only familiar to a few of the traditional healers who were my

key research participants. This unidentifiable plant was thought to grow only in Northwest Myanmar (Burma) and glow green at night.

The recent revitalization of Thai Traditional Medicine could be one reason these plants are currently purchased. Perhaps the persistence of these practices will encourage more plants to be grown locally and allow access to fresh plant material. The plants found in the ancient *Lanna* manuscripts do not all grow in northern Thailand, so the healers cannot easily access them. Distributors like *Lanna* Herbs allow traditional healers to procure the plants they need to maintain their practice and prolong the customs of Thai Traditional Medicine.

PHARMACOLOGICAL

Screening medicinal plants through ethnobotanically guided interviews with traditional healers is advantageous in determining the plants with the most potential for the desired bioactivity.

Ayurvedic and Traditional Chinese Medicine are considered two of the most powerful ancient medical traditions in the world. Thai Traditional Medicine draws on the theories and plant use of both of these medical systems (Mulholland 1979). Thailand's proximity to both countries, and the movement of people and religion between them, contributed to its integrative approach to health (Chokevivat 2003). Herbal formulas received from Thai traditional healers contained plants familiar to both Ayurvedic medicine and Traditional Chinese Medicine, many of which had been previously researched for antioxidant and acetylcholinesterase activity. In this study, the plants listed in the memory formulas were first screened to identify those that had already been tested for the two types of activity investigated in this study. The remaining plants were discussed with the five traditional healers, who were my key research participants, to

determine which plant species would be the most appropriate for testing in the laboratory. Asking the healers to rank the plants from one to ten, eleven single plants were chosen for testing on the four *in vitro* models.

In many ethnomedical studies, the most frequently mentioned plants by the traditional healers often determine which plants have the most potential to show the desired bioactivity (Hoffman and Gallaher 2007). In medical systems that use multi-plant formulas, this method can be incorrect. Some frequently mentioned plants are included in the formula because they are adjunctive plants. These plants can balance out the formula, strengthen the plants with the most bioactivity, or reduce the potential side effects associated with certain plants. These plants are found in many formulas, so they do not define the bioactivity of the formula, and therefore should not be a plant selected to test in the laboratory. In Thai Traditional Medicine, the "large class" of plants in a formula are established groups of plants with a determined bioactivity that are included in a number of formulas. One example of these "large class" plant formulas are the *Tians.* The plants in these groups are not unique, and therefore, should not be tested, but occur frequently in plant-based recipes. By presenting a list of plants to the traditional healers to select the ones they expect to have the strongest activity, then ranking the plants according to their selection, I was able to actively utilize their expertise. This choice of methodology was successful in identifying a number of plants with the ability to increase levels of acetylcholine, an important neurotransmitter for memory formation in the brain.

In vitro biological testing can provide traditional healers with validation of their traditional plant knowledge to use in their practice and within their community.

The selected plants were screening through *in vitro* bioassays to determine a single plant for the *in vivo* testing portion of this project. The multi-plant formulas were tested to evaluate the medicinal plants used by the traditional healers and support their practice. It is difficult to ascertain which plant is contributing the majority of the desired bioactivity when testing multi-plant formulas. There may be synergistic affects between the plants in a formula that requires extensive pair-wise testing of the plants in the formula to resolve (pers. comm. Dr. Brad Bennett 2010). Evaluating the plants contained in the formulas by interviewing the traditional healers on each plant was a valid method to determine which plants to test in the bioassays. The complete formulas were tested to determine their efficacy and usefulness as remedies for memory disorders. Using specific *in vitro* bioassays can demonstrate bioactivity by assessing only one mechanism of action (Houghton et al. 2007). *In vitro* testing cannot provide information on toxicity or show the potency of the remedy on the human body, but since the formulas are comprised of traditionally used medicinal plants, it is accepted they are safe for consumption.

The traditional healers who told me their formulas were grateful and excited to have them tested in a modern chemistry laboratory. They knew my collaborating professor in Chiang Mai, and trusted her expertise, so they were confident of my work. The results of the *in vitro* testing of the formulas will be returned to the traditional healers in a document for them to use in their medical practice. This document provides them with a summary of the interpretation of dementia in Thai Traditional Medicine, and the efficacy of their formulas and selected plants as established by the *in vitro* bioassays. These documents can be useful to the traditional healers and their community. I noticed in my research that while many healers knew of plants believed to enhance memory and potentially treat patients with dementia, they did not have a methodology for treating people with these conditions. Documenting the application of these

plants for memory decline can potentially contribute to promoting traditional medical knowledge and the use of medicinal plants.

Testing selected plant extracts in appropriate in vitro bioassays can confirm which plant(s) contains the largest quantity of active compounds or strongest activity to obtain the desired biological effect.

The use of *in vitro* bioassays to correlate traditional plant knowledge with the biological activity of plants is common, but somewhat inaccurate considering the complexity of the human body (Houghton et al. 2007; Gertsch 2009). Examining the activity of the plant *in vivo* is preferred, but not always possible, and until human studies are performed, also not completely accurate. Of all the organ systems in the human body, bioactivity in the brain is the most difficult to analyze because of its intricate network of connections. This study of plants used to enhance memory was limited by the availability of bioassays to test all the brain structures involved in the memory process, and the current knowledge of the etiology of age-related memory decline. This research tested acetylcholinesterase inhibition, which is only one chemical phenomenon used to explain memory processes in the brain. Therefore, the use of *in vitro* bioassays for this study was a screening technique to determine one plant for testing *in vivo*, and a means to provide scientific evidence on the traditional knowledge of the interviewed healers.

The traditional healers provided a number of formulas used to treat cognitive impairment, or enhance memory. All of the plants in the formulas were screened to determine which plants had previously been tested on the bioassays used in this project. The healers were provided the

remaining plant names for them to rank which single plants they believed would treat cognitive impairment in the elderly most effectively. This methodology provided an ethnobotanical filter before *in vitro* testing. The remaining ten plants, and two additional suggestions, were investigated for their antioxidant and acetylcholinesterase inhibition activity in the pharmacy department's laboratory. The results were decisive. One plant performed the best in all of the bioassays. Other plants exhibited the desired activity, and some had almost no bioactivity at all. The plants showing no bioactivity are potentially active against memory decline, but may have different mechanisms of action not measured by the bioassays selected for this research. This study, and the chosen bioassays, did identify one plant with high antioxidant and acetylcholinesterase inhibition activity to evaluate *in vivo* on rats.

Cinnamomum bejolghota has memory enhancing activity demonstrated by in vivo rat behavioral models and enzymatic testing on their brain tissue.

Cinnamomum bejolghota was one of the species in a multi-plant herbal formula I received to treat memory decline in the elderly. After an ethobotanical and *in vitro* screening, the extract was examined for its memory enhancing effect on rats in the Morris Water Maze, a commonly used experiment to test memory and learning. The rats' brains were harvested after two weeks of treatment, and their hippocampus, striatum and cortex were homogenized and tested for enzyme marker levels. These tests measured acetylcholinesterase inhibition, and markers for oxidation like catalase activity, superoxide dismutase activity, glutathione peroxidase activity, and levels of malondialdehyde. The extract of *Cinnamomum bejolghota*

displayed significant memory enhancing activity in the rat behavioral models and strong acetylcholinesterase inhibition in all three parts of the brain.

FUTURE STUDIES

This study identified plants with acetylcholinesterase activity using an ethnobotanical screening method and *in vitro* bioassay testing, and confirmed one plant with memory enhancing effects using *in vivo* behavioral and enzymatic marker tests on rats. These results support using ethnobotanically guided interviews and screening by traditional healers as a justifiable methodology to locate plants with a desired bioactivity. The traditional healers chose the plants tested in the bioassays selected for this project. During numerous interviews, they gained a comprehensive understanding of the objectives of this project. Therefore, their choice of plant species for testing was derived from their traditional knowledge and of the eleven plants tested, five plants demonstrated high activity in the selected assays. The plants showing little bioactivity in these assays could potentially operate using a different mechanism of action in the brain than what was tested in this study. Further research could analyze these species on bioassays that examine a different neurotransmitter, or look at other theories such as the proliferation tau neurofibrillary tangles or β-amyloid protein plaques in the brain that are characteristic of Alzheimer's disease specifically.

Cinnamomum bejolghota could potentially be utilized as a remedy for the memory dysfunction found in age-related cognitive decline, or as a supplement to enhance memory in healthy individuals. Further testing would be required before either of these prospects would be possible, especially to resolve any potential toxicity issues. The active chemical compound in the plant could be identified and isolated using various types of chromatography. It should be

considered that some chemicals in plants may work synergistically with other compounds in the plant, so isolating a single active compound might not identify full potency. It is possible that combining it with the original multi-plant herbal formula and testing the entire formula *in vivo* could increase its effectiveness. The plant extract would require additional testing for toxicity issues, and then could be tested on human subjects for its effect on the complete body system.

There were significant obstacles to conducting medicinal ethnobotany research in Thailand. The permitting requirements are stringent and lengthy, but necessary to protect the intellectual property and genetic resources of the country. For this reason, it would be advantageous that further research into the bioactivity of *Cinnamomum bejolghota* be performed under the supervision of a Thai organization. This would ensure protection for the traditional knowledge of the healers, and the biocultural resources of Thailand.

Many people in Thailand, especially those living in rural or remote areas, do not have access to western medical hospitals, or clinics. They define medicine by the plants around them that are used as remedies when someone in the village falls ill, or those plants used by the local traditional healers. For these people, the plants discussed in this thesis are their only option for treatment when an elderly member of their village becomes forgetful, or starts to behave strangely. Remarkably, many of the Thai people I spoke with, non-specialists and even friends, knew about some of the plants I was studying. There is great wealth of "common knowledge" of medicinal plants in Thailand. With the increase in globalization and influx of western ways, I believe this knowledge will quickly diminish. There is a movement in the traditional medicine community to help the next generation of healers become educated and pass the requisite exams for licensing, since the Ministry of Health in Thailand both encourages and requires this documentation. Still, the practice of Thai Traditional Medicine in Thailand is strong. Every

pharmacy and market sells some type of traditional remedy, whether for skin whitening (which is very popular), or for common illnesses. Therapeutic massage is offered on almost every street in Chiang Mai, and many use aromatic herbs as part of the massage. Traditional medicine is part of the national identity and heritage of Thailand, and while the tradition is vibrant, the knowledge is eroding and its documentation only carried out by healers and monks.

CONCLUSION

It is with great hope that this study adds to the knowledge of medicinal plants in Thailand for memory impairment. Research is one way to document and therefore protect the traditional plant knowledge of Thai healers. As globalization continues to erode "the old way" in Thailand, it is the responsibility of the younger generation, Thai and *farang,* to perpetuate these practices. The prevalence of chronic disease worldwide is increasing exponentially and at a frightening rate. New diseases are being discovered, and old diseases are again becoming common problems. While modern technology provides some treatment for these illnesses, there is wisdom in the time-honored practices of traditional medicine. Eastern philosophies, like those represented in Thai Traditional Medicine, suggest balance as the key to health and to life. Returning to balance, in the body and on the planet, could have a powerful effect on humanity.

Appendices

Appendix 1: Interview questionnaire

Interview questions

Village and Province of interview: Date:

Profile of healer

Gender:

Age (approximate):

Where were you born?

Where you raised there?

How long have you lived in this community?

What is your ethnicity?

What languages do you speak? What is your primary language?

Are you married?

How many children do you have?

What kind of healer are you?

How long have you been a healer?

Is this your full or part time occupation?

Who do you usually treat (family, friends, community)?

How many people a day do you treat?

Who taught you about being a healer?

Where did you learn to be a healer?

Do you have any other roles in the community?

Do you use plants in your practice?

Do you use other method of healing in your practice?

Do you get medications from other places (hospitals, stores)?

Non-specific remedies for the central nervous system

Do you use plants or plant mixtures as tonics/formulas for the nerves?

Do you use plants or plant mixtures as tonics/formulas for the brain?

What plants or plant mixtures do you use to treat memory loss?

Do you treat many elderly people?

Are there any tonics/ formulas used specifically for the elderly?

Are their any tonics/ formulas for rejuvenation?

Are their any tonics/ formulas for longevity?

Do you use plants or plant mixtures to treat psychosis in elderly patients?

Do you treat elderly people for "rot the brain"?

Questions relating to Alzheimer's disease

Have you heard of Alzheimer's disease?

Practitioners familiar with Alzheimer's disease

What are some of the symptoms of Alzheimer's disease?

What is the cause of these symptoms?

Is there a local name for these types of symptoms?

Are there more men or women who get this disease?

Is a special tonic or plant remedy given to females that exhibit these symptoms?

Are you finding an increase in the number of patients with Alzheimer's disease more recently?

Practitioners not familiar with Alzheimer's disease

(Language problems - forget words or substitute unusual words)
How do you treat an elderly person who forgets what words to use or uses strange or unusual words instead of the correct ones?

(Disorientation to time and space - getting lost in a familiar area)
How do you treat an elderly person who gets confused about where they are, or the time of day or year?

How do you treat an elderly person who gets lost in an area that should be familiar?

(Misplacing things - putting things in unusual places) How do you treat an elderly person who misplaces common things or puts them in strange places?

(Poor or decreased judgment) How do you treat an elderly person who behaves in a way that is unusual for them?

(Changes in mood or behavior- confused, suspicious, fearful or dependent) How do you treat an elderly person who is acting differently than usual? For example, confused about whom their children or relatives are, or being scared or suspicious of their family members?

(Difficulty with complex tasks) How do you treat an elderly person who cannot complete tasks that used to be easy for them?

(Loss of initiative) How do you treat an elderly person who has lost their motivation to do things they used to enjoy?

(Complete inability to care for self) How do you care for elderly people who can no longer take care of themselves?

What is the cause of these symptoms?

Is there a local name for these types of symptoms?

Are you finding an increase in the number of patients with these symptoms more recently?

Is a special tonic or plant remedy given to females that exhibit these symptoms?

Questions relating to preventing Alzheimer's disease

What do you recommend to prevent Alzheimer's disease or the symptoms of it?

What do you recommend to prevent other chronic diseases like cancer or cardio-vascular disease?

What treatment or plant/ plant mixtures do you use to increase blood circulation in the body?

What treatment or plant/ plant mixtures do you use to decrease inflammation in the body?

What plants or plant mixtures do you use to detoxify the body or blood?

Plant Resources

Where do you get the plants that you use?

Who collects the plants?

How did you learn about collecting plants?

Are there special tools that you use to collect plants?

Do you find that plants collected in different places (i.e. in the wild vs. in the garden) have different properties?

Are the plants collected during a certain time of the day, month or year?

Are plant treatments given during a certain time of the day, month or year?

Plant Management and Conservation

Are your medicinal plant resources managed by anyone besides you?

Is this plant found in many places?

Is this plant native to Thailand? To this area?

Have you had to use any other plants because the plant you needed was no longer available?

Plant specifics (these questions will be asked regarding each plant that is mentioned for the above symptoms)

What do you call this plant?

How do you recognize this plant?

Is there more than one form of this plant?

What parts are collected? What parts are used?

How much of this plant is collected?

Do you harvest the plant at a certain time in its life cycle?

How long does it take for the plant to get to this point in its life cycle?

Is this plant collected during a certain time of the day, month or year?
Is this plant easy to find? Has it become more difficult to find?

Is this plant native to Thailand? To this area? Is it found in many places?

Does this plant need to be used fresh or can it be stored?

How do you store this plant and how long can it be used after it has been stored?

How much of the used part is measured for the remedy?

How is the used part processed?

Are there other plants you use with this one to help it work?

How is the remedy administered?

How long is the remedy administered in this manner?

What is the reaction to this plant remedy?

How long does it take to see this reaction?

Appendix 2: Institutional Review Board documents

Office of the Vice President for Research and Sponsored Programs
Committee on the Protection of Human Subjects

The Graduate School and University Center
The City University of New York
365 Fifth Avenue
New York, NY 10016-4309
TEL 212.817.7523 FAX 212.817.1629

TO: Ms. Lisa Offringa
Biology

FROM: Richard G. Schwartz, Ph.D. RGS
Graduate Center IRB

SUBJECT: IRB Approval (Expedited Review)

STUDY: **08-05-1563 Medicinal Plants in Northern Thailand Used for the Elderly**

DATE: July 5, 2011

The Graduate Center IRB has approved the above study involving humans as research subjects. This study was Approved - Expedited Category: 7 - based on 45CFR46.

IRB Number: **08-05-1563** This number is a Graduate Center IRB number that should be used on all consent forms and correspondence.

Approval Date: **July 5, 2011**
Expiration Date: **July 4, 2012**

THIS APPROVAL IS FOR A PERIOD OF ONE-YEAR OR LESS. YOU SHOULD RECEIVE A COURTESY RENEWAL NOTICE BEFORE THE EXPIRATION OF THIS PROJECT'S APPROVAL. HOWEVER, IT IS YOUR RESPONSIBILITY TO INSURE THAT AN APPLICATION FOR CONTINUING REVIEW APPROVAL HAS BEEN SUBMITTED BEFORE THE EXPIRATION DATE NOTED ABOVE. IF YOU DO NOT RECEIVE APPROVAL BEFORE THE EXPIRATION DATE, ALL STUDY ACTIVITIES MUST STOP UNTIL YOU RECEIVE A NEW APPROVAL LETTER. THERE WILL BE NO EXCEPTIONS. IN ADDITION, YOU ARE REQUIRED TO SUBMIT A FINAL REPORT OF FINDINGS AT THE COMPLETION OF THE PROJECT.

Consent Form: All research subjects must use the approved and stamped consent form. You are responsible for maintaining signed consent forms for each research subject for a period of at least three years after study completion.

Mandatory Reporting to the IRB: The principal investigator must report, within five business days, any serious problem, adverse effect, or outcome that occurs with frequency or degree of severity greater than that anticipated. In addition, the principal investigator must report any event or series of events that prompt the temporary or permanent suspension of a research project involving human subjects or any deviations

from the approved protocol.

Amendments/Modifications: All amendments/modifications of protocols involving human subjects must have prior IRB approval, except those involving the prevention of immediate harm to a subject. Amendments/modifications for the prevention of immediate harm to a subject must be reported within 24 hours to the IRB.

Stipulations: None.

If you have any questions, please do not hesitate to contact Kay Powell in the IRB Office at 212-817-7525.

Good luck on your project.

cc: Michael Balick Ph.D.
Biology

Sign the Verification Statement below. Return the original signed copy of this memo to the IRB Office and retain a copy for your records. The IRB Office must receive a copy of the signed verification statement before research may begin.

VERIFICATION:

BY SIGNING BELOW, I ACKNOWLEDGE THAT I HAVE RECEIVED THIS APPROVAL AND AM AWARE OF, AND AGREE TO ABIDE BY, ALL OF ITS STIPULATIONS IN ORDER TO MAINTAIN ACTIVE APPROVAL STATUS, INCLUDING TIMELY SUBMISSION OF CONTINUING REVIEW APPLICATIONS AND PROPOSED PROTOCOL MODIFICATIONS, AS WELL AS PROMPT REPORTING OF ADVERSE EVENTS, SERIOUS UNANTICIPATED PROBLEMS, AND PROTOCOL DEVIATIONS. I AM AWARE THAT IT IS MY RESPONSIBILITY TO BE KNOWLEDGEABLE OF ALL FEDERAL, STATE AND UNIVERSITY REGULATIONS REGARDING HUMAN SUBJECTS RESEARCH INCLUDING CUNY'S FEDERALWIDE ASSURANCE (FWA) WITH THE DEPARTMENT OF HEALTH AND HUMAN SERVICES OFFICE OF HUMAN RESEARCH PROTECTIONS.

Signature of Principal Investigator Date

Signature of Faculty Advisor for Student Research Date

Doctoral Program in Biology

The Graduate Center
The City University of New York
365 Fifth Avenue
New York City, New York 10016-4309

แบบข้อมูลการค้นคว้า: พืชที่ใช้ทำยาสำหรับผู้สูงอายุในภาคเหนือของประเทศไทย

นักค้นคว้า: Lisa Offringa
นักศึกษาปริญญาเอก
City University of New York/ The New York Botanical Garden

จุดประสงค์ของการค้นคว้า: จุดประสงค์ของการค้นคว้าครั้งนี้คือ บันทึกการใช้ประโยชน์ ของพืชที่ใช้ทำยา (สมุนไพร) และภูมิปัญญาพื้นบ้านเกี่ยวกับการใช้ยาดังกล่าว จุดมุ่งหมายหลัก ของการค้นคว้าครั้งนี้คือ การศึกษาพืช(สมุนไพร) ยาบำรุง เพื่อรักษาผู้สูงอายุ กับภาวะ ความจำเสื่อม และอาการที่เกี่ยวข้อง รวมทั้ง วิธีการเตรียมยา และสัดส่วนของการใช้ยา ซึ่งตัวอย่างของพืช (สมุนไพร) ที่ศึกษา จะถูกเก็บเกี่ยว เพื่อการศึกษาเพิ่มเติมในห้องทดลองในประเทศไทย การค้นคว้าครั้งนี้ เป็นการค้นคว้าสำหรับดุษฎีนิพนธ์ และผลของการค้นคว้า จะถูกนำมาใช้เพื่อการศึกษา เท่านั้น

วิธีการค้นคว้า: ดิฉันจะสัมภาษณ์หมอยาพื้นบ้าน ๒๐ ท่านผ่านนักแปลภาษา การสัมภาษณ์หมอยาพื้นบ้านแต่ละท่าน อาจใช้เวลาถึงครึ่งวัน ซึ่งหากท่านอนุญาต ดิฉันจะขอบันทึกเสียงสัมภาษณ์ โดยใช้เครื่องบันทึกเทป วิธีนี้จะช่วยให้การสัมภาษณ์มีประสิทธิภาพมากขึ้น และยังช่วยประหยัดเวลาใน การสัมภาษณ์ด้วย หากท่านไม่อนุญาตให้ดิฉันบันทึกเสียง ดิฉันจะใช้การจดบันทึกแทน

ข้อมูลลับเฉพาะ: ท่านสามารถร้องขอให้เก็บรักษาการสัมภาษณ์นี้เป็นความลับ ในกรณีนี้ ชื่อ และ ลักษณะ ของท่านจะไม่ถูกเอ่ยใช้ในการศึกษา

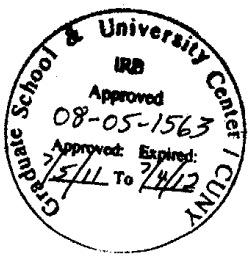
ข้อมูลและผลของการค้นคว้า: บันทึกของการสัมภาษณ์ทั้งหมดจะถูกเก็บรักษาไว้ใน เครื่องคอมพิวเตอร์ส่วนตัวของดิฉัน

และถูกป้องกันด้วยรหัสลับเฉพาะ ที่มีดิฉันเท่านั้นที่รู้ ดิฉันสามารถสั่งพิมพ์ ข้อมูลของการครั้งนี้ได้
หากท่านต้องการสำเนาของการศึกษา กรุณาให้ที่อยู่ของท่านไว้กับดิฉัน

ผลประโยชน์ และความเสี่ยงของการให้สัมภาษณ์: ผลประโยชน์ของการเข้าร่วมในการศึกษาครั้งนี้ คือมีส่วนช่วย
ในการบันทึกความรู้พื้นบ้าน ของการใช้พืชที่นำมาใช้ทำยา (สมุนไพร) การเก็บรักษา ความรู้นี้จะช่วยรักษาความรู้
ให้คงอยู่ต่อไปและยังช่วยกระตุ้นให้รู้ถึงประโยชน์และการใช้พืชสมุนไพร รวมไปถึงการนำความรู้นั้นมาใช้
การเข้าร่วมในการศึกษาครั้งนี้ไม่มีความเสี่ยงใดใด

ผู้เข้าร่วม: ผู้เข้าร่วมการศึกษานี้ เข้าร่วมด้วยความยินยอม และเต็มใจเท่านั้น ท่านสามารถเลือกที่จะไม่ตอบคำถามใดก็ได้
หรือหยุดการสัมภาษณ์เมื่อใดก็ได้ หากท่านมีข้อข้องใจ หรือมีคำถามเกี่ยวกับการค้นคว้าครั้งนี้ ท่านสามารถติดต่อ
ดิฉันโดยตรงได้ที่ ๑-๕๑๕-๔๒๕-๘๗๓๙ (ประเทศสหรัฐอเมริกา) หรือโดยอีเมลล์ที่ loffringa@gc.cuny.edu
หรือติดต่อผ่านทางอาจารย์ ที่ปรึกษาของดิฉัน Dr. Michael Balick ที่ ๑-๗๑๘-๘๑๗-๘๗๖๓ (ประเทศสหรัฐอเมริกา) หรือโดย
อีเมลล์ที่ mbalick@nybg.org หรือหากท่านมี คำถาม หรือข้อข้องใจ เกี่ยวกับสิทธิ์ของผู้เข้าร่วม ในการศึกษาในครั้งนี้
ท่านสามารถติดต่อ คุณ Kay Powell ผู้บริหาร IRB, The Graduate Center, City University of New York หมายเลขโทรศัพท์
๑-๒๑๒-๘๑๗-๗๕๒๕ (ประเทศสหรัฐอเมริกา) หรืออีเมลล์ที่ kpowell@gc.cuny.edu

หากท่านต้องการมีส่วนร่วมในการศึกษาในครั้งนี้กรุณาระบุว่าท่านสมัครใจทุกประการที่จะมีส่วนร่วม
ดิฉันจะให้สำเนาเอกสาร ๑ ชุด ของแบบยินยอมนี้กับท่านเพื่อเก็บรักษา



Doctoral Program in Biology

The Graduate Center
The City University of New York
365 Fifth Avenue
New York City, New York 10016-4309

Research Consent Form: Medicinal plants in Northern Thailand used for the elderly.

Researcher: Lisa Offringa
Doctoral Student
City University of New York/The New York Botanical Garden

Purpose of Research: The purpose of this research is to document the use of medicinal plants and traditional knowledge about them. The primary aim of this research is to study plants and tonics that are used for the elderly to treat conditions of memory loss and behavioral issues. The preparation method and dosage of these plant remedies will also be investigated. Some the identified plant species may return with me to a chemistry laboratory in New York City to be analyzed. This research is for my doctoral dissertation thesis and the results of my study will be used for academic purposes.

Methods: I will be interviewing 20 healers using a translator. The interview may take up to half of a day. I will record the interviews by audiotape with your permission. This method offers an accurate and quick record of the interview. If you do not chose to be recorded, I can hand write your responses.

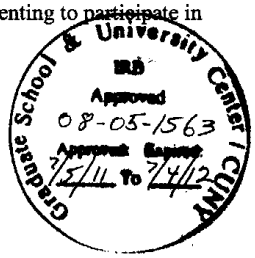
Credit/Confidentiality: You may request to remain confidential. In this case, your name and personal characteristics will not be associated with this study.

Data and Results: All of the records of these interviews will be stored on my personal computer. It is protected by a password only I know. I may publish the data and results of this study. If you would like a copy of the study, please provide me with your address.

Benefits and Risks: The benefit to participating in this study is to aid in documenting traditional knowledge about plant use for medical conditions. Archiving this knowledge will lead to its persistence and raise awareness the use of medicinal plants. There are no known risks for participating in this study.

Participation: Participation in this study is entirely voluntary. You may chose to not answer certain questions or end the interview at any time. If you have any questions about this research, you can contact me at (415) 425-8739 in the US or by e-mail at loffringa@gc.cuny.edu , or my advisor Dr. Michael Balick at (718) 817-8763 or mbalick@nybg.org . If you have questions about your rights as a participant in this study, you can contact Kay Powell, IRB Administrator, The Graduate Center/City University of New York, (212) 817-7525, kpowell@gc.cuny.edu.

If you would like to participate in this study, please state that you are freely consenting to participate in this study. I will give you a copy of this form for you to keep.



Appendix 3: The Act on Protection and Promotion of Traditional Thai Medical Intelligence, H.E. 2452

Tentative translation

ACT ON PROTECTION AND PROMOTION OF TRADITIONAL THAI MEDICINAL INTELLIGENCE, B.E. 2542

BHUMIBOL ADULYADEJ REX.

Given on the 19th Day of November, B.E. 2542

Being the 54th Year of the Present Reign.

His Majesty King Bhumibol Adulyadej is graciously pleased to proclaim that:

Whereas it is expedient to have a law on protection and promotion of traditional Thai medicinal intelligence;

This Act contains certain provisions that restrict the rights and liberties of the people, which are permissible under section 29, along with section 35, section 48 and section 50 of the Constitution of the Kingdom of Thailand;

Be it, therefore, enacted by His Majesty the King, by and with the advice and consent of the National Assembly, as follows:

Section 1. This Act shall be called the "Act on Promotion and Protection of Traditional Thai Medicinal Intelligence, B.E. 2542."

Section 2. This Act shall come into force as from 180 days after the date of its publication in the Government Gazette.

Section 3. In this Act,

"traditional Thai medicinal intelligence" means the basic knowledge and capability concerned with traditional Thai medicine;

"traditional Thai medicine" means the medicinal procedures concerned with examination, diagnosis, therapy, treatment or prevention of, or promotion and rehabilitation of the health of humans or animals, obstetrics, traditional Thai massage, and also includes the production of traditional Thai drugs and the invention of medical devices, on the basis of knowledge or text that has been passed on from generation to generation;

"text on traditional Thai medicine" means technical knowledge concerned with traditional Thai medicine which has been written or recorded in Thai books, palm leaf, stone inscription or other materials or that have not been recorded but passed on from generation to generation;

"Thai traditional drugs" means medicines obtained directly from herbs or derived from mixture, blended or transformed herbs, and also includes Thai traditional drugs under laws on drugs;

"formula on traditional Thai drugs" means a formula stated as the production process and ingredients which contain Thai traditional drugs, no matter what form the ingredients are;

"herbs" means plants, animals, bacteria, minerals, extracts of plants or animals used, or transformed or mixed or blended as drugs or food for diagnosis, therapy, treatment or prevention of diseases, or for promotion of the health of humans or animals. The meaning also includes the origin or the areas in which the products exist;

"controlled herbs" means herbs that have been announced as controlled herbs under the Ministerial Notification;

"conservation areas" means national reserved forests and other conservation areas for reservation and protection of natural resources, as stated by law;

"original extracts" means natural extracts that have not been mixed or new substances obtained from addition of molecules under scientific procedures;

"rough transformation" means the mixture, blend or transformation of herbs or the quality of herbs in order to obtain substances as a whole by original procedures and newly developed procedures without differentiating the pure ingredients of different substances;

"successor of formula on traditional Thai drugs or text on traditional Thai medicine" means persons who have been passed on with the text on traditional Thai medicine or formulas on traditional Thai drugs from the discoverer, the improver, or the developer of the substance, or those who have learnt from generation to generation from the discoverer, the improver or the developer of the substance, or those who were given the above mentioned from others;

"right holder" means those who have registered their intellectual property rights on traditional Thai medical intelligence under this Act;

"sales" means selling, disposition, distribution or exchange and also includes possession for sales;

"export" means to bring or export out of the country.

"transform" means to dispense or transform, or change the quality of the herbs;

"members" means the members of the Committee on Protection and Promotion of Traditional Thai Medicinal Intelligence;

"Committee" means the Committee on Protection and Promotion of Traditional Thai Medicinal Intelligence;

"licensing authority" means the Permanent Secretary or person assigned by the Permanent Secretary;

"registrar" means the central registrar or the provincial registrar, as the case may be;

"competent officials" means the licensing authority, the registrar, and the person appointed for the execution of this Act by the Minister;

"Permanent Secretary" means the Permanent Secretary of the Ministry of Public Health;

"Minister" means the Minister having charge and control of the execution of this Act.

Section 4. The Minister of Public Health shall have charge and control of the execution of this Act and shall have the authority to appoint competent officials, issue Ministerial Regulations to specify fees not exceeding the rate annexed to this Act, and outline other activities, including issuance of rules and notifications for the execution of this Act.

The Ministerial Regulations, Rules and Notifications shall come into force upon their publication in the Government Gazette.

CHAPTER 1
The Committee on Protection and Promotion of Tradition Thai Medicinal Intelligence

Section 5. There shall be the Committee on Protection and Promotion of Traditional Thai Medicinal Intelligence comprising of the Permanent Secretary of the Ministry of Public Health as chairman, the Director-General of the Medical Services Department, Director-General of the Intellectual Property Rights Department, Director-General of the Livestock Department, Director-General of the Forestry Department, Director-General of the Department of Agriculture, Director-General of the Medical Sciences Department, Secretary-General of the Food and Drug Administration, Secretary-General of the Environment Policy and Planning Office, and Director of the Medical Registration Division as members *ex officio*, and the equal number of the qualified members appointed by the Minister, on the basis of selection from the practitioners, those with knowledge, capability and experience in traditional Thai medicine, the production or sales of traditional Thai drugs and plantation or transformation of herbs. The Director of the Institute for Traditional Thai Medicine shall be a member and secretary of the Committee.

The selection of the qualified members under the first paragraph shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

Section 6. The Committee shall have the following power and duties to:

(1) provide consultation or advice to the Minister in issuing the Ministerial Regulations, Rules or Notifications under this Act;

(2) promote and develop the use of intellectual traditional Thai medicine and herbs;

(3) outline measures to strengthen stability and coordination between government agencies, state enterprises, communities and non government organizations on issues concerned with protection and promotion of intellectuals on traditional Thai medicine and herbs;

(4) approve the withdrawal of registrations of intellectual property rights on traditional Thai medicine under section 39 (3);

(5) consider the appeal against the order or decision of the registrar or the licensing authority under this Act;

(6) lay down rules concerned with standards and procedures on lodging an appeal and consideration of appeal, registration of intellectual property rights on traditional Thai medicine, the administration and arrangement of benefits and expenses of the fund, and works concerned with protection and promotion of intellectuals on traditional Thai medicine and herbs;

(7) perform other activities as stated in this Act or under other laws as the authority of the Committee;

(8) perform other activities as assigned by the Minister.

Section 7. The qualified members appointed by the Minister shall hold office for a term of two years as from the day of the appointment. In case the qualified members vacate office before the end of their term subject to section 5 paragraph two, the Minister may appoint another person with similar qualifications as the qualified member, and the appointee shall hold office for the remaining term of the member he or she replaces.

The qualified members who vacates office at the expiration of term may be re-appointed.

Section 8. Apart from vacating office at the expiration of term under section 7, the qualified members appointed by the Minister shall vacate office upon :

(1) deaths;

(2) resignation;

(3) being a bankrupt;

(4) being incompetent or quasi-incompetent person;

(5) being removed by the Minister for the reason of negligence, or dishonest to the duty, or indulge in unacceptable behavior;

(6) being imprisoned by a final judgement, except for an offence committed through negligence or petty offence;

(7) termination from being a practitioner in case the person was appointed under that criteria.

Section 9. At the meetings of the Committee, the presence of not less than one half of the total number of members is required to constitute a quorum.

If the chairman of the Committee is unable to attend any of the committee meeting sessions, the members shall select one among. Them selves to preside over meeting.

The decision of the meeting shall be by majority of votes. Each member shall have one vote. In case of an equality of votes, the person presiding over the meeting shall have an additional vote as the casting vote.

Section 10. The Committee shall have the power to appoint a sub-committee to consider or carry out any particular work as assigned by the Committee.

Section 9 shall apply *mutatis mutandis* the meeting of the sub-committee.

Section 11. In carrying out their duties under this Act, the Committee and sub-committee shall have the power to order any person to give or submit any evidence to supplement their considerations.

Section 12. There shall be the Institute for Traditional Thai Medicine under the Office of the Permanent Secretary, the Ministry of Public Health, having the authority to carry out duties concerned with protection and promotion of education, training, research, studies and development of intelligence on traditional Thai medicine and herbs and shall also be responsible for the administrative and technical works of the Committee.

Section 13. The Director of the Institute for Traditional Thai Medicine shall be the central registrar and the provincial health chiefs are the provincial registrars.

CHAPTER 2
Protection and Promotion of Intelligence on Traditional Thai Medicine

Section 14. The intellectual property rights on traditional Thai medicine to be protected under this Act shall be the right to intellectual property over the formula of traditional Thai drugs and text on traditional Thai medicine.

Section 15. The Institute for Traditional Thai Medicine shall be responsible for compiling information on traditional Thai medical intelligence concerned with formulas of traditional Thai drugs and text on traditional Thai medicine from throughout the country, for registration.

The registration of intellectual property rights on formulas of traditional Thai drugs and text on traditional Thai medicine under paragraph one shall be in accordance with the Rules issued by the Committee.

Section 16. There shall be three types of traditional Thai medicinal intellectual property rights as follows:

(1) the national formula of traditional Thai drugs or the national text on traditional Thai Medicine;

(2) general formula of traditional Thai drugs or general traditional Thai medicine document; and

(3) personal formula of traditional Thai drugs or personal text on traditional Thai medicine.

Section 17. The Minister has the power to notify that formulas on traditional Thai drugs or text on traditional Thai medicine that is of benefit, or has special medical or public health value, as the national formula on traditional Thai drug, or the national text on traditional Thai medicine, as the case may be.

The Notification under paragraph one shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

Section 18. The Minister has the power to notify the formulas of traditional Thai drugs or text on traditional Thai medicine that have been widely

used or whose intellectual property protection has expired under section 33, as general formula of traditional Thai drugs or general text on traditional Thai medicine, as the case may be.

The Notification issued under paragraph one shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

Section 19. Whoever wishes to use the national traditional Thai drugs for registration and permission for production of drugs according to the Drug Law, or wishes to use it for research on improvement or development of new drug formulas for commercial benefit, or wish to research the national text on traditional Thai Drugs for development and improvement for commercial benefit, shall forward their application to obtain benefits and pay fees and the remuneration for making use there of to the licensing authority.

The application and permission, limitations to the rights and the remuneration shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial regulation.

Section 20. Personal formula of traditional Thai drugs or personal text on traditional Thai medicine under section 16(3) may be registered for protection of intellectual property rights and may be promoted according to the provisions of this Act by applying for registration to the registrar.

The application for registration of intellectual property rights on traditional Thai medicine under paragraph one shall be in accordance with the rules, procedure and conditions prescribed in Ministerial Regulation.

Section 21. The persons who have the right to register for protection of intellectual property rights according to section 20 must be of Thai nationality and shall have the following qualifications:

(1) being an inventor of the formula on traditional Thai drugs or text on traditional Thai medicine;

(2) being an improver or developer of formula on traditional Thai drugs or text on traditional Thai medicine; or

(3) being an inheritor of the formula on traditional Thai drugs or text on traditional Thai medicine.

Section 22. Registration for protection of intellectual property rights on traditional Thai medicine is prohibited if the registrar is of the opinion that:

(1) the drug formula belongs to the national formula on traditional Thai drugs, or national text on traditional Thai medicine, or is a general formula on traditional Thai drug, or general text on traditional Thai medicine, or

(2) the drug formula is a personal formula on traditional Thai drug that has been developed on non medical basis like the use of extracts of plants, animals or micro organisms that have not be obtained from natural extracts or the transformation that is not considered rough transformation.

Section 23. Any application for registration of intellectual property rights on traditional Thai medicine that is not made according to the requirements prescribed in the Ministerial Regulation issued under section 20 paragraph two be called for changes by the registrar within 30 days beginning from the date the applicant receives the request for changes.

If the applicant does not comply with the request of the registrar within the specified period mentioned in paragraph one, the registration shall be revoked.

Section 24. After examining the application, if the registrar is of the opinion that the applicant is qualified under section 21 and the application therefor is not prohibited under section 22, the registrar shall, without delay, announce the application at the Registration Office and at the office of the local administration organizations.

Section 25. In case many people jointly apply for registration on protection of intellectual property rights traditional Thai medicine, the registrar shall specify a date for inquiry and inform all the applicants.

Under the inquiry to be carried out according to paragraph one, the registrar may request any applicant for further questioning, more information or documents. After the inquiry has been made and the Permanent Secretary has made the decision, the registrar shall notify all the applicants the decision thereof the registrar.

The inquiry and consideration on the rulings shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

Section 26. In case there is a separate application for protection of intellectual property rights on the same traditional Thai medicine by different applicants, the person who forwarded the application first shall have the right to register. If the application is forwarded at the same time, on the same date, the applicants shall have to decide on whether they would settle with any one person having full right over the registration or that they would jointly have equal rights within the time period specified by the registrar. If the case shall not be settled within the period specified by the registrar, the parties shall bring the case to the court within 90 days as from the date on which the registrar's specified time period ends. If the case is not take to court within the specified time period, the application for registration shall be revoked.

Section 27. If after examining the application for registration, the registrar is of the opinion that the applicant is not qualified according to section 21 and the intellectual property rights on traditional Thai medicine applied for is prohibited under section 22, the registrar shall refuse the registration and issue a letter to inform the applicant within 30 days from the date on which the refusal was made.

Section 28. In case the applicant for registration appeals against the ruling of the registrar according to section 27, and if the Committee rules that the rulings of the registrar is incorrect, the registrar shall proceed with the application process.

Section 29. After making publication on the application forwarded for registration according to section 24, people who feel they have the right over the intellectual property rights on traditional Thai medicine shall forward their objection to the registrar and provide evidence within 60 days from the date on which the publication was made under section 24.

Section 30. Before making the ruling, the registrar shall request the applicant and the person who object the registration to explain or provide evidence to supplement the consideration.

When the registrar has ruled on the application, a letter shall be issued to inform the applicant and the person who objected the application on the ruling of the registrar and the reasons thereof within 30 days from the ruling date.

Section 31. In case there is no objection as mentioned under section 29, or in case there is an objector and a final ruling has been made to enable the applicant or the objector have the right to register, the registrar shall issue an order authorizing the registration of protection of intellectual property rights on traditional Thai medicine to the applicant or the objector.

Once the order for registration of intellectual property rights on traditional Thai medicine has been issued under paragraph one, the registrar shall issue a letter to inform the applicant or the objector of the application and ask for payment of fees for registration within 30 days from the date the letter is received. If the applicant for registration or the objector does not pay the fees within the time period specified, the registration will be revoked.

The registration certificate for intellectual property rights on traditional Thai medicine shall be in the form as prescribed in the Ministerial Regulation.

Section 32. In case the Permanent Secretary rules according to Section 25 (2) that many people jointly have the right to register, or in case many people apply for registration of the same product and have joint ownership of the intellectual property rights on traditional Thai medicine, or a final ruling on joint ownership of the intellectual property rights on traditional Thai medicine is made by the court in accordance with section 26, the concerned parties should have equal rights for registration over protection of intellectual property rights on traditional Thai medicine.

In the joint registration on protection of intellectual property rights on traditional Thai medicine, the concerned parties shall jointly outline a memorandum as to how they would jointly share the registration rights and forward it to the registrar along with the registration.

Section 33. The right to intellectual property on traditional Thai medicine under this section should be valid for a life time of the bearer of the registration and extend for another 50 years from time the owner of the registration has deceased.

In case there is joint ownership of the registration right under section 32, the intellectual property rights on traditional Thai medicine shall be valid for a life time of the bearer and extended for another 50 years from the date on which the last joint owner of the registration deceases.

At the end of the period specified under paragraph one or two the Minister shall publish in the Government Gazette, specifying the formula on traditional Thai drug or text on traditional Thai medicine as a general formula on traditional Thai drug or general text on traditional Thai medicine in accordance with to section 16 (2), as the case may be

Section 34. The right holder would have the sole ownership on the production of the drug and have sole right over the research, distribution, improvement or development of formulas on traditional Thai drugs or intellectual property rights of traditional Thai medicine under the registered text on traditional Thai medicine.

The provisions of paragraph one shall not apply to:

(1) any act that is of benefit for studies, findings, tests or research according to the regulation specified by the Minister; or

(2) preparation of specific drugs according to prescription of holders of registration certificate on traditional Thai medicine, or

(3) production of drugs for household use or production of drugs by state hospitals or government or state agencies, for use in state hospitals, or the use of text on traditional Thai medicine for benefits in treatment of patients in state hospitals, provided that it shall be in accordance with the Rules issued by the Minister.

Section 35. The intellectual property right on traditional Thai medicine under this section shall not be transferred to others, except for the case in which it is passed on by succession.

The successor on traditional Thai medicine through inheritance according to paragraph one must forward their request for registration to the registrar within 2 years from the date the right holder deceases.

In case there is no request for registration of rights on traditional Thai medicine passed on by inheritance within the time period specified in paragraph two the right to be protected under this Act shall be terminated and section 33 (3) shall apply herewith *mutatis mutandis*.

Section 36. The right holder may permit any person to use their right under section 34.

The permit for use of the right under paragraph one shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

Section 37. The registrar has the right to revoke the registration of intellectual property rights on traditional Thai medicine under the following cases:

(1) the right holder misuses the right against the public order and good morals;

(2) the right holder violates or does not comply with the conditions or limitations outlined by the registrar in registration of the intellectual property rights on that traditional Thai medicine; or

(3) the right holder exercised the right that may cause severe damage to the registered intellectual property on traditional Thai medicine.

Section 38. The concerned party or the public prosecutor may file complaints with the court to revoke registration over intellectual property right on traditional Thai medicine that had been registered in contrary to unfairly under section 21 or section 22.

Section 39. Before revoking the registration of intellectual property rights on traditional Thai medicine under section 37, the registrar shall conduct an inquiry and inform the right holder, so that the person can provide an explanation within 30 days from the acknowledgement date.

In conducting an inquiry under paragraph one, the registrar may permit the concerned parties explain or provide evidence required for consideration.

After having conducted an inquiry if the registrar is of the opinion that the registration of the intellectual property rights on traditional Thai medicine shall be revoked, the registrar shall seek approval from the Committee. After the Committee has approved the revoke the registration of intellectual property rights on traditional Thai medicine, the registrar shall order the revoke and issue a letter stating the decision made and the reasons therefor to the right holder within 30 days from the date on which the order was issued by the registrar.

Section 40. The right holder whose registration has been revoked under section 39 may re-apply for a registration in accordance with section 20 after the expiration of one yeas from the date the registration of intellectual property rights on traditional Thai medicine has been revoked.

Section 41. In case the person permitted with the intellectual property right on traditional Thai medicine exercise their rights against public order or good morals or violate or not comply with the conditions specified in the Ministerial Regulation issued under section 36 paragraph two or exercise their rights which may cause serious damage to intellectual property right on traditional Thai medicine that has been registered, the registrar shall have the power to revoke the promission to the intellectual property rights on the traditional Thai medicine.

The revoke of the permission to the intellectual property right on traditional Thai medicine under this section shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

Section 42. Before ordering the revoke on the permission of intellectual property rights on traditional Thai medicine under section 41, the registrar shall issue a letter to inform the right holder on traditional Thai medicine to give a statement within 15 days from the date on which the letter from the registrar has been received, and section 39 paragraph two shall apply herewith *mutatis mutandis* .

When the registrar issues an order revoking the permission of intellectual property right on traditional Thai medicine, a letter shall be issued to inform the right holder and the person to whom the permission of intellectual property rights of traditional Thai medicine has been granted within 30 days from the date of the issuance of the revoking order.

Section 43. Persons with the nationality of other nations who agree to permit persons with Thai nationality to have the protection of intellectual property rights on traditional Thai medicine may seek registration of intellectual property rights protection on the local traditional medicine in their country under this Act.

The application for registration, the issuance of a certificate of registration, and the revocation of the registration under paragragh one shall be in accordance with the rules, procedure and conditions prescribed the Ministerial Regulation.

CHAPTER 3
Protection of Herbs

Section 44. For the purpose of herbs protection, the Minister, with the advice of the Committee, shall have the power to issue a Notification in the Government Gazette to specify the kind, characteristic, type, and names of herbs that are of study and research value, or have important economic significance, or may become extinct, as controlled herbs.

Section 45. For the purpose of the protection of controlled herbs, the Minister shall, with the advice of the Committee, notify in the Government Gazette:

(1) specifying the amount or quantity of controlled herbs under possession, to make use of, under care, under conservation or under transportation which needs to be informed the registrar;

(2) specifying rules, procedure and conditions in informing under (1);

(3) specifying rules, procedure and conditions on the possession, to make use of, under care, under conservation or for transportation of controlled herbs;

(4) specifying rules, procedure and conditions on studying and researching the controlled herbs;

(5) specifying rules, procedure and conditions on exporting controlled herbs for commercial and noncommercial purposes, or distribution or transformation of controlled herbs for commercial purposes;

(6) specifying other requirements to conserve, prevent or prohibit or reduce dangers or damage that may be caused to controlled herbs.

Section 46. No person shall research or export controlled herbs or sell or transform them for commercial purposes, unless a licence has been obtained from the licensing anthority.

The application for a license and the issuance thereof under paragraph one shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

The licence issued under paragraph one shall be valid up to 31 December of the third year as from the year in which the licence is issued.

Section 47. The licence issued under section 46 shall cover the employees or representative of the licensee.

It shall be deemed that the act of the employee or representative of the licensee under paragraph one is the act of the licensee unless the licence can prove that the act has been committed without his or her knowledge or beyond his or her control.

Section 48. The provisions of section 46 shall not apply to research on controlled herbs which have been carried out by state agencies, but such agencies must inform the registrar and comply with the rules, procedure and conditions prescribed by the Minister under section 45(4).

Section 49. The application for renewal of a licence issued under section 46 shall be made by the licensee before the expiration of the licence, and upon such application, the licensee can continue his or her business until the licensing authority refuses the renewal thereof.

The application for renewal of a licence and the permission thereof shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

Section 50. In case the licence issued under section 46 is lost or destroyed, the licensee may apply for a licence substitute within 30 days from the date of knowledge of the loss or destruction.

The application for and the issuance of the licence substitute shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

Section 51. The person who possesses controlled herbs beyond the amount or quantity notified by the Minister under section 45(1) on the date on which the Notification on controlled herbs is made in the Government Gazette, shall inform the registrar of the possession of controlled herbs as required under this Act, within the period of time specified in the Notification.

Section 52. In case any lecensee under section 46 does not comply with this Act or the Ministerial Regulations, Rules or Notifications issued under this Act, the licensing authority shall have the power to suspend the licence for not more than 90 days each time.

The licensee whose licence has been suspended must to stop all the performances permitted under the licence and, within the suspension period, no application for other licence under this Act shall be made.

Section 53. The licensing anthority shall have the power to revoke the order suspending the licence before the period specified, when satisfied that the licensee whose the licence has been revoked has complied with the provisions of this Act or the Ministerial Regulations, Rules or Notifications issued under this Act.

Section 54. If the licensee under section 46 does not comply with the provisions of this Act or Ministerial Regulations, Rules or Notifications issued under this Act and indulges in severe fault, the licensing authority shall have the power revoke the licence.

The licensee whose the licence has been revoked must stop all the performances permitted under the licence and shall not apply for any other licence under this Act until a period of two years, from the date the licence is revoked has elapsed and the licensing authortiy may issue such other licence as he or she deems appropriate.

Section 55. The order to suspend the licence under section 52 and the order to revoke the licence under section 54 shall be made in writing and notified the licensee. In case the licensee can not be found or the licensee refuse to accept the document, the order shall be posted at a conspicuous areas that can be easily seen at the place specified in the licence and it is deemed that the licensee has acknowledged the order from the date on which the order has been posted.

Section 56. The licensee whose the licence has been revoked under section 54 may sell the controlled herbs under their possession to other licensees or other person who the licensing authority deems appropriate, within 60 days from the date the notice is acknowledged. In case an appeal is made, the days shall be counted from the date on which the decision is made by the Committee, unless the licensing authority has extended the time period, but it shall not more than 60 days.

Section 57. For the purpose of conserving herbs and the areas from where the herbs naturally originate in the ecological system, or has biological diversity, or may have been easily effected by humans, in areas which have been specified as conservation areas, the Minister, with advice of the Committee, shall outline a plan entitled "Plan for Conservation of Herbs" to be forwarded to the Council of Ministers for approval.

Plans for conservation of herbs according to paragraph one may be made as a short term plan, medium term plan or long term plan according to appropriateness and has to include work plans and procedures leading to the following matters:

(1) stipulation of conditions in permitting a person to enter conservation areas as specified under the law on such matter for the compliance of the government agency concerned in order to conserve natural resources or the value of herbs or not to effect the natural, ecological system, or biological diversity in the areas where the herbs originated;

(2) laying down of procedures in handling particularly with the areas from where the herbs originated and also outlining the duties and responsibilities of the government agency concerned, for maximum benefit in coordination, in order to bring about efficiency in conserving the nature, the ecological system, biological diversity and the value of the herbs in that area;

(3) survey and research on herbs and the areas from where they originate for benefits in outlining procedures to conserve herbs and the place of their origin;

(4) the inspection, follow-up and analysis in entering into the conservation areas for benefits in carrying out the works as planned and enforcement of the laws concerned.

Plans for conservation of herbs under paragraph one shall be published in the Government Gazette.

Section 58. For benefits in outlining plans for conservation of herbs under section 57, the competent officials shall have the power to enter the specified conservation areas in order to inspect and research the herbs and the areas from where the herbs originate. This shall have to be done with support and coordination from other concerned state authorities, as specified by Rules prescribed by the Council of Ministers.

Section 59. After the publication of the plan on conservation of herbs under section 57, the protection and management of conservation area shall be carried out according to the plan on conservation of herbs and the law on such matter.

Section 60. If any conservation area is inappropriately managed or has problems concerned with inappropriate conservation of herbs, or has caused destruction of herbs, or areas in which the herbs originated, leading to severe crisis which needs to be tackled immediately and the state authorities concerned do not have the authority to do so under the law, or can not solve the problem, the Minister, with the advice of the Committee shall propose the Council of Ministers to permit the Ministry of Public Health to take action in conservation as outlined under the Plan for Conservation of Herbs as stated under section 57, as necessary and appropriate, to control and solve the problems.

Section 61. In case any area in which the herbs originated have natural ecology system or biological diversity that may be destroyed or may be easily effected by humans or the entry into the area for use of herbs has caused risk to extinction or degradation or reduction of species of herbs, or the state aims at increasing public involvement in the management, development and making use of herbs in the area, and the area has not been notified as conservation area, the Minister, with the advice of the Committee, has the power to issue Ministerial Regulation notifying the area as a herb conservation area.

The land within the specified area shall to be notified as herb conservation area under paragraph one shall not be the area under the right

ownership or possession under the Land Code of individuals who are not the state entities.

The Ministerial Regulation under paragraph one shall have a plan indicating the land area specified as herb conservation area attached therewith.

Section 62. In issuing a Ministerial Regulation according to section 61, at least one or many of the following protection measures shall be outlined in the Ministerial Regulation:

(1) The use of herb should maintain nature and value of the herb, or should not effect the natural ecology or biological diversity in the area;

(2) Any act that may be of danger or cause effects that could lead to change in the ecology system within the area, or biological diversity, or effect the value of the herbs, shall be prohibited;

(3) The imposition of specific management measures for those areas, along with outlining duties and areas of responsibility of the state, for effective cooperation and coordination in working towards conservation of natural resources and maintaining the value of the herbs, or the ecological system or biological diversity within the area.

(4) Outlining of other conservation measures as seen necessary and appropriate with the environment in the area.

Section 63. In the herb conservation areas, no person shall own or possess land, or plant or construct anything, or cut, clear, burn, or destroy trees or other plants, or destroy biological diversity or the ecology system, or carry out digging of minerals, stones, soil or change in the water ways or any act that results in pollution or causes flood or running dry of water, stream, canals, ponds, or causes danger to herbs, except for the act that is done to administer the area with the objective of conserving herbs or to make use of herbs, with permission from the licensing authority.

The application for and the issuance of the permit under paragraph one shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

The permit issued under paragraph one shall be valid up to 31 December of the third year after the year in which the permit is issued.

Section 49 and section 50, and section 52 up to section 55 shall apply *mutatis mutandis* to the renewal of permit, application for the substitute, suspension and revocation of the permit issued under paragraph one.

Section 64. In order to support the participation of the private sector in conservation, promotion and development of herbs, the owner or the possessor of the land from where the herbs originate or the land used for plantation of the herbs have the right to register the land with the registrar, in order to obtain assistance or support under this Act.

The obtainment of registration, the issuance of certificate of registration and the revocation of the registration shall be in accordance with the rules, procedure and conditions prescribed in the Ministerial Regulation.

Section 65. The owner or the possessor of the land that has been registered under section 64 has the right to obtain assistance or support as prescribed in the Rules issued by the Minister.

CHAPTER 4
Conservation

Section 66. In case the registrar rejects the application for registration of intellectual property rights on traditional Thai medicine under section 27, the person who seeks registration has the right to appeal the order with the Committee within 30 days from the date on which the order was received from the registrar.

Section 67. In case the registrar has made a decision on the right of the applicant over the registration for intellectual property rights under section 30 paragraph two, the applicant or the objector, as the case may be, has the right to appeal to the Committee within 30 days, from the date of receiving of the decision of the registrar.

Section 68. In case the registrar issues an order revoking the registration on intellectual property rights on traditional Thai medicine as stated

under section 39 paragraph three, the right holder has the right to appeal the order to the Minister within 30 days from the date on which the notice has been received from the registrar.

The decision of the Minister shall be final.

The appeal under paragraph one shall not entail a stay of the execution the order to revoke the registration of intellectual property rights on traditional Thai medicine.

Section 69. In case the registrar issues an order to revoke the permission to exercise the intellectual property rights on traditional Thai medicine under section 41, the person who has the right to exercise the rights thereof may appeal the order to the Committee within 30 days from the date the notice of the registrar has been received.

The appeal under paragraph one shall not entail a stay of the execution of the revoke order on permission to exercise the intellectual property rights on traditional Thai medicine.

Section 70. In case the licensing authority orders the suspension of the permit under section 52 or orders the revocation of the permit under section 54, the person whose permit has been suspended or revoked has the right to appeal the order to the Committee within 30 days from the date the notice from the registrar has been received.

The appeal under paragraph one shall not entail a stay of the execution of the suspension or revocation of the permit.

Section 71. The decisions of the Committee under section 66, section 67, section 69 and section 70 shall be final.

Section 72. The appeal and procedure to consider the appeal shall be in accordance with the rules and procedure prescribed in the Ministerial Regulation.

CHAPTER 5
Competent Officials

Section 73. In carrying out their duties, the competent officials shall have the power to:

(1) enter any place during the working hours to inspect and control of the execution of this Act;

(2) inspect any place or vehicles between the time when the Sun rises until the Sun sets or during working hours, in case there is suspicion that this Act has been violated, and in case there is a reasonable ground to believe that the delay in issuing search warrant may lead to removal or hide or destruction of documents or products concerned with the commission of offence, and if the inspection during the specified time is not complete, it can be continued;

(3) confiscate or take into custody the documents or products concerned with violation of this Act, as evidence in taking action against the case;

(4) request for any person for questioning or submitting documents or evidence in case there is a reasonable ground to believe that the statements, documents or evidence is of benefit to find or use as evidence in proving the violation of this Act;

(5) request any person to move out of the herb conservation area or avoid any act in violation of section 63.

In carrying out their duties in accordance with paragraph one, the concerned parties shall facilitate the competent officials as necessary.

Section 74. In carrying out their duties, the competent officials must produce their identity cards for identification.

The identity cards of the competent officials shall be in the Form as prescribed by the Minister by publication it in the Government Gazette.

Section 75. In carrying out their duties under this Act, the competent officials shall be the officials under the Penal Code.

CHAPTER 6
Fund on Traditional Thai Medicine Intelligence

Section 76. A fund entitled "Fund on Traditional Thai Medicine Intelligence" shall be set-up in the Office of the Permanent Secretary, Ministry of

Public Health, as a revolving fund for expenses concerned with works on conservation and promotion of intelligence on traditional Thai medicine.

The fund shall include the following money and property;

(1) subsidy from the state;

(2) money or property received from the private sector from within and outside the country, foreign governments or international organizations;

(3) interests and benefits obtained from the fund.

(4) other earnings obtained from the functioning of the fund.

The earnings of the fund shall not be considered as earnings that must be remitted to the Finance Ministry under the law on the treasury balance and the law on the budgetary procedures.

The Office of the Permanent Secretary, Ministry of Public Health is responsible for maintaining the money and property of the fund, and shall withdraw the money in the fund in accordance with the provisions of this Act.

The administration, management of benefits and use of money in the fund shall be in accordance with the rules prescribed by the Committee, with the approval of the Finance Ministry.

CHAPTER 7
Penalties

Section 77. Any person who violates the orders of the Committee or Sub-committee appointed under section 11 together with section 6(5) or order of the registrar under section 39 paragraph two or the order of the competent officials under section 73(4) shall be liable to imprisonment for a term of not more than one month, or a fine of not more than 2,000 baht, or both.

Section 78. Any person who violates section 19, section 46, section 52 paragraph two, section 54 paragraph two, or section 63 paragraph one or does not comply with the orders of the competent officials under section 73(5) shall be liable to imprisonment for a term of not more than one year, or a fine of not more than 20,000 baht, or both.

Section 79. Any person who violates section 51 shall be liable to imprisonment for a term of not more than six months, or a fine of not more than 10,000 baht, or both.

Section 80. Any person who violates the protection measures specified under the Ministerial Regulation issued under section 62(2) shall be liable to imprisonment for a term of not more than two years, or a fine of not more than 40,000 baht, or both.

Section 81. Any person who does not facilitate the competent officials under section 73 paragraph two shall be liable to a fine of not more than 2,000 baht.

Section 82. In case the wrongdoer who has to be liable under this Act is a juristic person, the managing director, manager, or representative of the juristic person shall be liable to the penalty imposed thereto, unless they can prove that the act of the juristic person have been committed without their knowledge or consent.

Countersigned by:

Chuan Leekpai

Prime Minister.

RATE OF FEES

(1)	Licence to research on the controlled herbs or the renewal thereof	10,000	baht each
(2)	Licence for sales, export or transformation of controlled herbs for commercial purpose or the renewal thereof	20,000	baht each
(3)	Permit to administer the herbs control area or make use of herbs therein for commercial purpose or the renewal thereof	10,000	baht each
(4)	Substitute of the licence or permit under (1) (2) or (3)	100	baht each
(5)	Certificate of registration of land of herbs origin	1,000	baht each
(6)	Substitute of the certificate of registration of land of herbs origin	100	baht each
(7)	Permit for making use of formula of the national traditional Thai drugs or national traditional Thai medicine under section 19	20,000	baht each
(8)	Objection of the registration of intellectual property rights on traditional Thai medicine	500	baht each
(9)	Certificate of registration of intellectual property rights on traditional Thai medicine	1,000	baht each
(10)	Substitute of the registration certificate of intellectual property rights on traditional Thai medicine	100	baht each
(11)	Application form for a licence or permit or the renewal thereof, or for the registration, under this Act	500	baht each

Appendix 4: National Research Council of Thailand letter of permission

สำนักงานคณะกรรมการวิจัยแห่งชาติ
196 ถนนพหลโยธิน เขตจตุจักร
กรุงเทพฯ 10900
โทรศัพท์ 0-2579-2690,0-2579-2285
โทรสาร 0-2561-3049
Website: www. nrct-foreignresearcher.org

NATIONAL RESEARCH COUNCIL
196 PHAHOLYOTHIN RD., CHATUCHAK,
BANGKOK 10900, THAILAND
Telephone 0-2579-2690, 0-2579-2285
Fax (66) 2561-3049
Email: webmaster@nrct-foreignresearcher.org

No. 0002.3/ 7037

2 October B.E. 2552 (2009)

Dear Miss Offringa,

We are pleased to inform you that the Office of the National Research Council of Thailand (NRCT) has permitted you to conduct the research on "Medical Plants of Northern Thailand for the Prevention and Treatment of Mild Cognitive Impairment in the Elderly" from October 2009 – February 2011. In this regard, you have to conform to the following conditions.

1. Conduct the research under the supervision of Dr. Jintanaporn Wattanathorn, Khon Kaen University and Dr. Kongkanda Chayamarith, director of the Botanical Garden Organization.
2. Store dried samples in the herbarium of the Botanical Garden Organization.
3. Not get any samples to be analyzed abroad.
4. Pay all expenditure which is borned during survey and analysis in Thailand.

According to our immigration law, you are advised to apply for non-immigrant visa (RS) before leaving for Thailand. Furthermore, you are required to report to the Office of International Affairs, NRCT within seven days after your arrival in Thailand in order to pay a deposit of 10,000 baht for guaranteeing submission of the research report to NRCT, then obtain concerned documents.

We look forward to welcoming you.

Sincerely yours,

Mr. Krithawat Nophakeepongse
Deputy Secretary - General
For Secretary - General

Miss Lisa Offringa
Nakornping Condominium, Room 808
Juristic 2 Rajchaphrunk Road
Amphur Maung
Chiang Mai 50300

Appendix 5: Short-Term Training Agreement for Biotec

BIOTEC
a member of NSTDA

No. BIOTEC 5402/364/2552

March 9 , 2009

Ms. Lisa Offringa
City University of New York
The New York Botanical Garden
Kazimiroff Blvd. & 200 Street
Bronx, New York 10458 USA.

Dear Ms. Lisa Offringa,

I am pleased to inform you that the National Center for Genetic Engineering and Biotechnology (BIOTEC), a government agency under the auspice of the National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology, Thailand, has already approved your research training during March 15 – September 14, 2009. You will be participating in the project entitled "Preliminary Study on the Medicinal Plant used by Traditional Healer's Using Social Science Method" under the supervision of Dr. Vanicha Vichai at the Bioassay Laboratory, Bioresources Technology Research Unit.

If you need any further information, please contact Ms. Wanravee Chanto at email address: wanravee@biotec.or.th.

We look forward to welcoming you to BIOTEC.

Sincerely yours,

D. Siamhan

Ms. Dussadee Siamhan
Deputy Executive Director
Acting Executive Director
National Center for Genetic Engineering and Biotechnology

Research Unit Coordination Division
Tel : (66-2) 564 6700
Fax: (66-2) 564 6707

ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ
สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ
113 อุทยานวิทยาศาสตร์ประเทศไทย ถนนพหลโยธิน ตำบลคลองหนึ่ง อำเภอคลองหลวง
จังหวัดปทุมธานี 12120 โทรศัพท์ 0 2564 6700 โทรสาร 0 256 6701-5

National Center for Genetic Engineering and Biotechnology
National Science and Technology Development Agency
113 Thailand Science Park, Phahonyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand.
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BIOTEC
a member of NSTDA

NATIONAL CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

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Tel: (662) 564-6700 Fax: (662) 564-6707

ST 5/2009

Short-Term Training Agreement

This Short-Term Training Agreement (hereinafter referred to as **"Agreement"**) is made and entered into on March 9th, 2009 (hereinafter referred to as **"the Effective Date"**), by and between **National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency**, represented by Ms. Dussadee Slamhan, Deputy Executive Director, Acting Executive Director of National Center for Genetic Engineering and Biotechnology, located at 113 Thailand Science Park, Phahonyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand (hereinafter referred to as **"BIOTEC"**) and **Ms. Lisa Offringa**, passport number 420741168, a student from City University of New York, the New York Botanical Garden, Kazimiroff Blvd. & 200 Street Bronx, New York 10458 USA., residing at City University of New York, the New York Botanical Garden, Kazimiroff Blvd. & 200 Street Bronx, New York 10458 USA. (hereinafter referred to as the **"Recipient"**).

BIOTEC and the Recipient agree on the followings:

1. The Recipient shall be trained at Bioassay Laboratory, Bioresources Technology Research Unit (hereinafter referred to as the **"The Laboratory"**) under the supervision of Vanicha Vichai, Ph.D. (hereinafter referred to as the **"The Laboratory Supervisor"**), during 15 March -14 September, 2009 (hereinafter referred to as the **"term of the training"**).
2. BIOTEC shall not be responsible for any allowance of the Recipient during the term of her training.
3. The Recipient shall make her best effort to complete her training as stipulated in the scope of work of Appendix A.
4. The Recipient can access research facility at the Laboratory only as permitted by the Laboratory Supervisor. The Recipient shall not remove any biological resources from BIOTEC's premises without BIOTEC's written consent. The Recipient will be responsible for the cost of repair or replacement of the Laboratory equipment, should the damage is caused by the Recipient.
5. During the term of the training, the Recipient may get access to BIOTEC's confidential information, including but not limited to proprietary technical, experimental, and business information (hereinafter referred to as the **"Confidential Information"**). The Recipient shall not disclose the Confidential Information without prior written approval from BIOTEC. This obligation for BIOTEC's Confidential Information shall continue for ten (10) years after the end of this Agreement, but shall not apply to any specific portion of the Confidential Information that is or becomes available to the public generally, and/or that is allowed by BIOTEC under this Agreement. The Recipient also agrees to avoid using the Confidential Information commercially or for her own or third party purposes without BIOTEC's written consent.

6. BIOTEC allows the Recipient to disclose the Confidential Information if and only if the Recipient agree to comply with the restriction below:

   (1) the Confidential Information disclosure is limited only for the Recipient's research result under this Agreement;

   (2) the Recipient may disclose the Confidential Information only in the Recipient's report, however, the Recipient must obtain written approval from BIOTEC before submitting such report;

   (3) the Recipient must notify the university where the report will be submitted of the sole ownership and Confidential Information of BIOTEC over the Recipient's research result disclosed in the Recipient's report.

7. Any creative ideas, developments, and inventions, whether patentable or not, developed or conceived by the Recipient as a result of this training will be the property of BIOTEC. The Recipient agrees to disclose all such ideas, developments and inventions to BIOTEC. In the event BIOTEC decides that these matters are important enough to merit patent, copyright, or other legal protection, the Recipient agrees to fully cooperate, if asked, in the prosecution of such matters. The Recipient agrees to assign completely to BIOTEC all of her rights in such matters, without further payment to the Recipient by BIOTEC.

8. During the term of the training, should BIOTEC consider the Recipient to be unsuitable for this training, BIOTEC retains the right to terminate this Agreement before the date indicated with seven (7) days advance notice to the Recipient.

9. Notwithstanding, the expiration under Clause 1 and the termination of this Agreement under Clause 8 shall not affect the work already in progress under this Agreement and the provisions of Clause 5, 6, and 7.

10. During the term of training at the Laboratory, the Recipient agrees to comply with the safety regulations of BIOTEC and suggestion of the Laboratory Supervisor.

11. This Agreement shall be governed by the laws of the Kingdom of Thailand.

12. The report made by the Recipient must be endorsed by the Laboratory Supervisor and must be submitted by September 10th, 2009 to BIOTEC Attn: Ms. Surang Pajaiprasert, 113 Phahonyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand.

BIOTEC

Ms. Lisa Offringa

IN WITNESS WHEREOF, This Agreement shall be effective as of the Effective Date and is executed in two identical counterparts which are equally valid.

By : D. Siamhan ..... (BIOTEC)

(Ms. Dussadee Siamhan)
Deputy Executive Director
Acting Executive Director of BIOTEC

By : ..... (Recipient)

(Ms. Lisa Offringa)
Student

Witness : .....

(Lily Eurwilaichitr, Ph.D.)
Acting Director
Bioresources Technology Unit

Witness : .....

(Vanicha Vichai, Ph.D.)
Researcher of BIOTEC

Appendix A

Scope of Work

For Ms. Lisa Offringa

Research Title: "Preliminary Study on the Medicinal Plant used by Traditional Healer's Using Social Science Method"

The specific objective of this project is as follows:

- To preliminary study and document the medicinal plant knowledge used by the traditional healer's in Northern Thailand using the social science method of semi-structured interviews

Duration 6 months (March 15-14 September, 2009)

Supervisors:

Vanicha Vichai, Ph.D

Work Location:

Bioassay Laboratory, Bioresources Technology Research Unit

Recipient Responsibility:

The recipient agrees to devote full time performing the duties as set forth Research Objectives under this Agreement, the best results according to the recipient knowledge and ability. The recipient shall perform service within the time and accordance with scope of work and the other terms and condition of the Agreement.

Appendix 6: List of plants from the herbal formulas and their properties

The follow is a collection of some of the plants found in the multi-plant formulas for memory or diseases related to memory. Included in this list are plants that have been formally researched and their bioactivity published. The plants are listed alphabetically by Latin binomial to include the family name. The traditional healer's names have been coded.

***Acorus calamus* L. (Acoraceae)**

-It is used as a brain tonic (Mor H).

- The rhizome is used to release "bad" blood after the period (Mor H).

- It is used as a heart tonic and to heal *lom* (Mor E).

***Aegle marmelos* (L.) Corrêa (Rutaceae)**

- It is used to balance the elements and it is good for strength (Mor J).

- It is good for longevity, to clean the blood and as a skin tonic (Mor E).

***Alpinia galanga* (L.) Willd. (Zingiberaceae)**

- The rhizome is good for *lom* in the brain for people over 32 (Mor H).

-It is used as a heart tonic and to release wind in the body and brain (Mor B).

-It is used as a heart tonic and will help wind that moves up to the head. It helps to balance the wind and the blood (Mor F).

-It is used to nourish the wind element and for joint pain (Mor J).

***Angelica dahurica* (Fisch.) Benth. & Hook. f. (Apiaceae)**

- It is good for tendons, hiccups and coughs. It is good for the heart and to treat a fever with shivers (Mor J).

***Angelica sinensis* (Oliv.) Diels (Apiaceae)**

-It is used to treat cough and fever (Mor J).

***Apium graveolens* L. (Apiaceae)**

-It is used as an elements tonic, to help digestion and balance heat in the body (Mor J).

***Artemisia vulgaris* L. (Apiaceae)**

- It is used to treat fever, chicken pox and a cold (Mor J).

***Atractylodes lyrata* Siebold & Zucc. (Asteraceae)**

- It is used to treat a cough and asthma, and to heal a wound in the mouth (Mor J).

***Blumea balsamifera* (L.) DC. (Asteraceae)**

- It is used a tonic for the heart and when a patient feels dizzy (Mor J).

***Camellia sinensis* (L.) Kuntze (Theaceae)**

- When fermented as *miang*, it is used to wake up brain and good for the nerves.

***Cannabis sativa* L. (Cannabaceae)**

- It is used as a nerve tonic, to help make the patient hungry and sleep well. If the patient uses this plant too much they can become lazy and paranoid (Mor J).
- It is good for the nerves in the brain (Mor H).
- In the proper amount, it can wake up the nerves and brain and put the patient in a good mood (Mor J).

***Carum carvi* L. (Apiaceae)**

- It is used to heal the stomach, expel wind in the intestines, and to balance the elements (Mor J).

***Carum copticum* (L.) Benth. & Hook. f. (Apiaceae)**

- It is used to expel wind, as a tonic for the elements, to nourish the kidney and as a heart tonic. It can also be used to make the mind feel good (Mor J).

***Cinnamomum bejolghota* (Buch.-Ham.) Sweet (Lauraceae)**

- It is used as a brain and heart tonic, helps sooth muscle pain and will keep the brain "fresh" (Mor B).
- It is used for brain nourishment, as a heart and nerve tonic, helps you to think fast and makes the brain clear. It expels the wind from the brain and intestines, and makes the brain tissue healthy (Mor B).
-It is used to nourish the blood, liver, kidney and headache. Given to nourish the wind element and increase strength (Mor J).

-It is used as an elements tonic, a heart tonic, to improve strength, for *lom* disorders, headaches, to release the wind in the body and for *sannibad* (Mor J).

***Citrus hystrix* DC. (Rutaceae)**

- The leaf is used in foods to get rid of a bad smell in the food. It is used to expel wind. The fruit is used in *yaa dong* to expel wind and treat paralysis (Mor J).

***Coffea arabica* L. (Rubiaceae)**

-It is used as a nerve tonic, but only in small amounts (Mor J).

-It can be used to wake up the brain and nerves, but only in small amounts (Mor J).

***Cuminum cyminum* L. (Apiaceae)**

- It is used to expel wind, mucus and treat kidney stones (Mor J).

***Curcuma longa* L. (Zingiberaceae)**

- It is an antioxidant (Mor J).

***Cyperus rotundus* L. (Cyperaceae)**

- It has a hot and spicy taste. It is used for longevity and to balance the elements (Mor E).

***Datura metel* L. (Solanaceae)**

- The flowers and leaves when burned can heal problems with the sinuses. The seeds can be used to heal rashes and wounds. The roasted seeds are good from psychosis and mental problems like *sannibad baa* (Mor J).

- It can be used to wake up the nerves and brain if used in the proper amount (Mor J).

***Diospyros decandra* Lour. (Ebenaceae)**

-It is used because it is good for the brain (Mor C).

-It is used to treat fever and poisoning. It can give strength to the body (Mor J).

***Foeniculum vulgare* Mill. (Apiaceae)**

- It is used to expel wind, increase strength and treat a weak pulse. It is used to treat sleepwalking, sleeptalking and nightmares (Mor J).

***Lepidium sativum* L. (Brassicaceae)**

- It is used to expel wind and mucus, balance the bile, and it will purify the blood. It is used to promote lactation (Mor J).

***Mammea siamensis* (Miq.) T. Anderson (Clusiaceae)**

- It is good for strength and used as a tonic for the heart. It will make you feel fresh (Mor I).

***Mesua ferrea* L. (Calophyllaceae)**

- It is used as a brain and heart tonic (Mor B).

- It has a spicy taste. It can be used to balance the elements and nourish strength. It is a tonic for the blood and skin (Mor I).

***Mimusops elengi* L. (Sapotaceae)**

- It is used as a brain and heart tonic (Mor B).

***Myristica fragrans* Houtt. (Myristicaceae)**

- It is used as a heart and strength tonic. It is a very important ingredient in many tonics (Mor J).

***Nardostachys jatamansi* (D. Don) DC. (Caprifoliaceae)**

- It is used to expel worms, get rid of poison, heal wounds and rid the body of bad blood (female) (Mor J).

***Nigella sativa* L. (Ranunculaceae)**

- It is used to treat headache, dizziness and disorders of the wind element (Mor B).

- It is used to treat disorders of the wind element (Mor F).

-It is used for problems with *lom*, for body detoxification and for women when in the month after they give birth (Mor J).

-It is used to expel wind and mucus. It is used to nourish the blood (Mor J).

***Petroselinum crispum* (Mill.) Fuss (Apiaceae)**

- It is used to spread out the wind in the body, break up mucus and treat a stomachache (Mor J).

***Picrorhiza kurroa* Royle ex Benth. (Scrophulariaceae)**

- It is used to treat fever, hiccups and asthma (Mor J).

***Pimpinella anisum* L. (Apiaceae)**

-It is used to treat pregnant women if they eat the wrong food, and helps with hiccups and asthma (Mor J).

***Piper chaba* Hunter (Piperaceae)**

- Used as a tonic to balance all of the elements (Mor H).

-It has a spicy taste and is good for *lom* and for blood circulation to the brain. It will help you sweat and will balance the elements (Mor I).

- It is good for the wind in the body and balances the earth element. It is used for paralysis and to balance the earth elements. Also, it is used to treat epilepsy and increase hunger (Mor J).

- Use it as an elements and body tonic. It can help to repair the cells of the body (Mor E).

***Piper nigrum* L. (Piperaceae)**

-It is used to release the wind in the body. It also helps to reduce phlegm and will cause you to sweat (Mor H).

- It is used to release sweat, expel wind and treat paralysis. It also strengthens formulas if added to them (Mor J).

- It was found to be a bioavailability enhancer (NatPro 2009).

- It has a hot and spicy taste. It helps the circulation of blood and *lom* in the body. It will make you sweat (Mor E).

***Piper ribesioides* Wall. (Piperaceae)**

- It is used to expel wind and balance the elements (Mor J).

***Piper sarmentosum* Roxb. (Piperaceae)**

- It is used primarily for the water element, but will balance all of the elements (Mor J).

***Plumbago indica* L. (Plumbaginaceae)**

- Helps to warm the body and connect the fire element though the *sen* lines (Mor B).

- It is used to nourish the fire element (Mor J).

- It has a spicy taste and can be used to balance the elements and nourish strength (Mor I).

- It is used to treat the fire element by helping with digestion and metabolism during aging. It is a blood tonic and a tonic for all the elements (Mor J).

- It purifies the blood and helps digestion. It also helps circulation of blood and *lom* (Mor E).

***Rauvolfia serpentina* (L.) Benth. ex Kurz (Apocynaceae)**

-It is used to treat fever, diabetes and as a diuretic. It nourishes the heart and nervous system (Mor J).

-The root is used to help the nerves and brain, treat diabetes and high blood pressure (Mor J).

***Saussurea lappa* (Decne.) Sch. Bip.**

- It is used to nourish the bones, and expel wind and mucus (Mor J).

***Stephania rotunda* Lour. (Menispermaceae)**

- It is used to increase strength, nourish the blood, and balance the wind and fire element. It is not to be taken if pregnant (Mor J).

***Strychnos nux-vomica* L. (Loganiaceae)**

-It is used to nourish the nerves, treat dizziness and increase strength. The seeds are poisonous, so use only a few for treatment (Mor J).

-The seeds can be used to wake up the nerves and brain, but only a small amount (Mor J).

***Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae)**

-It is used to kill bacteria in the mouth, to release wind in the stomach and treat gastritis (Mor B).

***Terminalia belirica* Wall. (Combretaceae)**

- It is used to treat cough and excrete from the body (Mor J).

***Terminalia chebula* Retz. (Combretaceae)**

- It is used to treat diarrhea and stomachache, fever and cough (Mor J).
- It has an astringent taste and so it helps with excretion (Mor J).

***Tiliacora triandra* Diels (Menispermaceae)**

- It is used to treat cancer, high blood pressure, a hangover, joint pain, diabetes and for detoxification of the body (Mor J).

- The root is used to treat a fever and to fight against poison. The leaf is good to treat joint pain (Mor J).

***Tinospora crispa* Beumee ex K.Heyne (Menispermaceae)**

- It is used to heal a fever, and for strength and longevity (Mor J).
- It is a longevity tonic and can make you hungry. It can heal malaria and fever, and can be used on animals (Mor E).

***Zingiber cassumunar* Roxb. (Zingiberaceae)**

-It is used to heal asthma and to nourish the nerves. It also releases the menstruation in women so it should not be used during pregnancy, but after giving birth it can be used to shrink the uterus (Mor H).
- It has a hot and spicy taste. It is very useful for the northern people's way of life. It can be used for babies until they are elderly. For babies, it can be rubbed on their stomach if they have a stomachache. For the elderly, it is good to heal joint and bone pain. It is used in women before they give birth and while they are in their month (Mor E).

***Zingiber officinale* Roscoe (Zingiberaceae)**

- It is used to treat wind disorders (Mor F).
- It has a hot and spicy taste. It is used to balance the wind in the body (Mor I).
- It is used to expel wind and treat a patient who feels dizzy. It will treat stomachache, asthma, and cough. It will help the wind flow throughout the body (Mor J).
- It has a hot and spicy taste. It is used to heal *lom* and move out phlegm (Mor E).

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