CHAPTER 1
INTRODUCTION

With the improvement of living standards and advancement of medical healthcare, modern mankind has been able to enjoy a longer life span compared to his predecessors. According to the *World Population Ageing 2009* report, the life expectancy at birth of the world's population has increased from 46.6 years in the 1950s to 67.6 years in 2010 (United Nations, 2010). It is estimated by year 2050, the proportion of older people (aged 60 and above) would reach 22% of the global population. As more people are surviving into old age, this increases their chances of developing age-related disorders. Ageing is a major risk factor for a myriad of diseases including cancers, stroke, diabetes, heart diseases, arthritis and degenerative brain diseases (Johnson et al., 2005). Advancing age is the greatest risk factor for the incidence of dementia, especially in Alzheimer's disease. The risk for an individual to develop dementia doubles every five years after the age of 65. It is estimated that 35.6 million people are living with dementia globally in year 2010 and the figure is expected to double every 20 years (Alzheimer's Disease International, 2009). The number of dementia patients in Malaysia is projected to increase from 63,000 people in year 2005 to 126,800 people by year 2050 (Alzheimer's Disease International, 2006). In United States, Alzheimer's disease is the 7th leading cause of death with an estimated 5.3 million Americans coping with the disease (Alzheimer's Association, 2010).

Dementia is defined as deterioration of intellectual function and cognitive skills that is of sufficient severity to interfere with social or occupational functioning (Chandra et al., 2006). The impairment of intellectual capability affects memory, learning, orientation, problem-solving, comprehension, language, communication and social skills. Dementia can be caused by various diseases and conditions resulting in damaged brain cells. The common types of dementia are Alzheimer's disease, vascular

dementia, Lewy body dementia, Parkinson's disease and frontotemporal dementia (Alzheimer's Association, 2010). Alzheimer's disease is the most common cause of dementia in the elderly, accounting for an estimated 60-80% of cases. It is characterized by progressive memory loss and other higher cortical functions, in combination with the presence of extracellular senile plaques (β-amyloid) and intracellular neurofibrillary tangles (tau protein) (Wang et al., 2007). Recently, there has been a heightened interest in the study of chronic traumatic encephalopathy (CTE), a neurodegenerative disease caused by repetitive concussions which leads to general cognitive dysfunction and dementia in its final stage of clinical manifestation (McKee et al., 2009). The development of CTE is common in retired professional athletes in contact sports including boxing, football and hockey, with a mean age of onset at 42.8 years (Gavett et al., 2011).

Dementia is a progressively degenerative condition and current medical research has yet to find a cure for it. Nonetheless, patients can seek pharmacological and non-pharmacological interventions to improve their symptoms or delay the progression of the disease. Cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonist are usually prescribed to ameliorate cognitive functions in dementia patients according to their degree of severity. Cholinesterase inhibitors act by augmenting acetylcholine levels in the brain which compensates the losses of cholinergic function. Tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Razadyne®) are drugs of this class. Memantine, marketed under various names including Akatinol® and Namenda®, is an NMDA receptor antagonist which regulates the elevated levels of glutamate that leads to neuronal dysfunction. All these medications pose certain side effects on patients including nausea, vomiting, dizziness, headache, constipation, diarrhoea and confusion (National Institute for Health and Clinical Excellence, 2011).

Age-related neurodegenerative diseases and brain trauma result in the loss of neuronal cells which eventually disrupts normal brain functions. The human central nervous system (CNS) has very limited regenerating capacity after injury and to a certain extent, it is irreparable. However, research studies over the past two decades have revealed the possibility of CNS neuronal regeneration to occur under favourable conditions (Horner & Gage, 2000). The presence of growth-promoting factors at the site of injury enables the neuronal growth cones to detect appropriate environment cues to promote neuronal survival and trigger axonal outgrowth. Hence, *in vitro* neurite outgrowth assay serves as a valuable model for screening potential molecular components for neuronal regeneration.

Neurotrophic growth factors are a group of polypeptides vital for cell survival, proliferation, development and functionality in the nervous system. The neurotrophins, a group of closely related molecules which binds to Trk receptors, consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5) and neurotrophin-6 (NT-6) (Landreth, 2006). In view of the ability of neurotrophins to modulate neuronal survival and axonal growth, these molecules were subsequently tested as therapeutic factors for neurodegenerative disorders and neuronal injuries. Elevated levels of NGF in patients with Alzheimer's disease have enhanced the survival and neurotransmission of cholinergic neurons (Chao et al., 2006).

Herbal remedies may play an indispensable role in the field of traditional medicine across various cultures across the globe. Traditional medicine is still widely practised today especially in developing countries and continues to serve as a primary health care in some countries in Africa and Asia (World Health Organization, 2008). Herbal supplements are widely consumed around the world to enhance health and wellbeing, as well as to prevent modern incurable diseases. In recent years, the

incorporation of functional foods or nutraceuticals in daily diet and nutrition has captured the global interest, with a growing body of scientific studies and reports revealing their physiological benefits in promoting optimal health and reducing the risk of chronic diseases (Liu, 2003). Natural wholesome plant-based foods including vegetables, fruits, herbs, mushrooms and spices are attributed with numerous health benefits yet there remains much to be learned as scientists are continually discovering new and surprising health benefits.

The rising cost of medical healthcare in caring for dementia patients and the elderly population has urged the use of traditional medicinal herbs in promoting mental health and wellbeing especially in Asian countries (Tian, 2005). Several herbal drugs have been widely studied and some successfully developed for the treatment of dementia, especially in Alzheimer's disease. Huperzine A, a potent acetylcholinesterase inhibitor from a traditional Chinese herb *Huperzia serrata*, is a remarkable cognitive enhancer in patients of Alzheimer's disease and is sold as a drug in China (Jiang et al., 2003). *Ginkgo biloba* leaf extract EGb761, a standardized preparation rich in flavonoids and terpenoids, is extensively studied. This extract has shown favourable effects in improving cognitive function in neurodegenerative disorders including Alzheimer's disease. The antioxidative activity of the extract has protected neurons against 6-hydroxydopamine (6-OHDA)-induced damage in Parkinsonism models (Chen et al., 2007).

Apart from medicinal herbs, several culinary ingredients commonly available in gourmet markets including vegetables, fruits, mushrooms and spices are also studied for their potential therapeutic effects in dementia. Curcumin, a polyphenol isolated from turmeric, has exhibited antioxidant and neuroprotective effects on the brain. The widespread usage of turmeric in Indian curry cooking has been a likely cause for the low prevalence of Alzheimer's disease in India, where only a mere 0.7% of the aged

population is affected (Aggarwal et al., 2009). Antioxidant properties of fruits and vegetables have been attributed to the existence of polyphenols, a group of plant secondary metabolites. An increased intake of foods rich in polyphenols such as berries, grapes, pomegranate and tea in one's diet has been associated with a lower risk in various chronic illnesses such as coronary heart diseases (Liu et al., 2001), cancers (Kushi et al., 2006), and Parkinson's disease (American Academy of Neurology, 2011).

Hericium erinaceus (Bull.: Fr.) Pers. is a culinary mushroom reputed for its medicinal values. The discovery of NGF-inducing compounds in *H.erinaceus* has rendered the mushroom to be a popular nutriceutical for the treatment and prevention of dementia (Ma et al., 2010). *Hericium erinaceus* cultivation has been successfully adapted to the tropical climate in Malaysia and there is limited information on this locally adapted species. An earlier study has shown that the locally grown *H.erinaceus* retained its neurotrophic activity and stimulated neurite outgrowth activity *in vitro* (Wong et al., 2007). Furthermore, the mushroom was shown to enhance peripheral nerve regeneration *in vivo* (Wong et al., 2011). The first hypothesis to be tested in this study was that *H.erinaceus* aqueous extract would enhance the stimulation of neurite outgrowth activity of NGF in neuronal cell line NG108-15. The second hypothesis was that the extract would be able to protect neuronal cell under stress conditions and oxidative assaults.

1.1 Objectives

The specific objectives of this study were to:

a) assess the synergistic effect of *H.erinaceus* extract, when combined with commercial NGF, on the neurite outgrowth stimulation activity in NG108-15 cells.
b) investigate the neuroprotective effect of the *H.erinaceus* aqueous extract in neuronal cells under oxidative stress, if any.