UNIVERSITI TEKNOLOGI MARA

IN VITRO EVALUATION OF ANTI-INFLAMMATORY, ANTIOXIDANT, AND ANTI-ACETYLCHOLINESTERASE ACTIVITIES OF APPLE AND DATE VINEGAR EXTRACTS ADDED WITH *CENTELLA ASIATICA*

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ABSTRACT

Neuroinflammation and oxidative stress are major factors of neurodegenerative damage correlated to cognitive impairment in Alzheimer's disease (AD). It results primarily from overproduction of oxidative stress that induce activation of pro-inflammatory cytokines causing to neuronal death. This study investigated the anti-inflammatory, antioxidant, and anti-acetylcholinesterase activities of apple and date vinegar added with *Centella asiatica* in SH-SY5Y neuroblastoma cells. The neuroprotective effect of apple or date vinegar added with various percentage of *C. asiatica* (0, 0.5, 2, 5%) was determined *in vitro*. The methanolic extract of apple vinegar added with *C. asiatica* [AV-CA (2%)] and date vinegar added with *C. asiatica* [DV-CA (2%)] extracts showed potent neuroprotective effect. Both extracts were subjected to liquid-liquid partitioning yielded aqueous (H_2O: AV-CA/DV-CA) and ethyl acetate (EA: AV-CA/DV-CA) at [2% CA] extracts. All of extracts were tested for anti-inflammatory, antioxidant, and anti- acetylcholinesterase activities. Anti-inflammatory response against nitric oxide (NO) was measured in SH-SY5Y neuroblastoma cells. Meanwhile, the intercellular reactive oxygen species ROS inhibition was measured as antioxidant activity and percentage inhibition of acetylcholinesterase (AChE) was measured by using commercially available kit's test. In general, all extracts significantly ($P < 0.05$) inhibited NO production without affecting cell viability. EA-DVCA extract showed better NO production inhibition and induced more attenuation effect on nitrite production with an IC_{50} value of 563.5 ± 0.13 µg/mL. Furthermore, EA: AV-CA extract showed a high percentage ROS inhibition with an IC_{50} value of 17.54 ± 0.40 µg/mL. Strongest AChE inhibition activity was observed in EA: DV-CA (2%) with (IC_{50} = 9.087 µg/mL. It is suggested that the ameliorating effect of EA: DV-CA (2%) on LPS-induced neuroinflammation and memory deficit seems to be attenuated via anti-inflammatory, inhibition of reactive oxygen species and AChE activities.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system (CNS) that is associated with low levels of acetylcholine neurotransmitter and loss of cholinergic cells in the brain (Park et al., 2016). AD is one of the crucial diseases which representing 60-80% of the older patients and it is known as a cognitive neurodegenerative disorder (Rao et al., 2013). There are several pathological features identified in the central nervous system (CNS) of the AD patients such as neurofibrillary tangles, amyloid plaques, neuroinflammation, and disruption of neurotransmitters (Nour et al., 2014). Furthermore, deficits in cholinergic function may hesitate patient's daily activities and delay the advancement of the disease (Rakesh et al., 2016).

Centella asiatica (*C. asiatica*) studied in this research, is one the medicinal plants used for wound healing, skin problems treatment, and brain and nerves cells revitalization (Zhang et al., 2013). The therapeutic action of this medicinal plants is mainly attributed due to the presence of bioactive compounds such as asiaticoside, madecassoside and asiatic acid (Prasad et al., 2014). The utilization of *C. asiatica* in food and beverage has increased due to its properties such as antioxidant, anti-inflammatory, and memory enhancing. It is also an alternative natural medicine especially protection against age-related change in brain antioxidant defence system.

Vinegar has long been part of human diet as food preservative and simple remedies for animals and people. There are many types of raw materials used in the production of vinegar such as grapes, berry and others (Mas et al, 2014). Neuroinflammation in the brain is represented by proliferating of glial cells (mainly astrocytes and microglia) and expression of neurotoxic free radicals as well as expression of neuroinflammation markers (Krause & Müller, 2010). Neuroinflammation has been identified related to AD and may contribute to neuronal injury and hyperphosphorylation (Popp et al., 2017). Inflammation and chronic neuro injury may weakened the brain pathways which usually reduce damage to the synaptic transmission and neural network (Rahimifard et al., 2017).