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NEUROPROTECTIVE EFFECT OF *CAULERPA LENTILLIFERA* EXTRACTS AGAINST LIPOPOLYSACCHARIDE-INDUCED NEUROINFLAMMATION IN RAW 264.7 MACROPHAGES AND SK-N-SH NEUROBLASTOMA CELLS

AIMAN BIN HAMLI

MSc

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ABSTRACT

Current existing therapies against Alzheimer's disease (AD) relieve only the clinical symptoms but fail to halt the neurodegenerative disease progression. This raises the need for alternative neuroprotective agents that can prevent AD. *Caulerpa lentillifera* J. Agardh appears to be useful against the neuroinflammation-driven AD given its antioxidant and anti-inflammation properties. To date, no study has reported *C. lentillifera's* neuroprotective potential against neuroinflammation. Hence, this study screened four *C. lentillifera extracts* for their anti-oxidative, acetylcholinesterase (AChE) inhibitory and anti-inflammatory potentials using the high-performance thin layer chromatography (HPTLC). To this end, freeze-dried *C. lentillifera* was extracted using sequential liquid-liquid partitioning with methanol, hexane, chloroform and ethyl acetate. HPTLC analyses found all four *C. lentillifera* extracts to exhibit varying degrees of free radical scavenging activities. Amongst the extracts, the *C. lentillifera* chloroform extract (CLCE) exhibited the highest free radical scavenging activity (6.135 µg/ 1 mg GAE). However, none of the extracts exhibited detectable AChE inhibitory activity. All *C. lentillifera* extracts exhibited varying degrees of COX-1 inhibitory activities, with the *C. lentillifera* ethyl acetate extract (CLEAE) demonstrated the highest COX-1 inhibitory activity (9,613.62 µg/ 1 mg SAE). Qualitative test for phenols indicated the presence of phenolic compounds in all *C. lentillifera* extracts. The HPTLC-based $FeCl_3$ assay confirmed and found CLCE to contain the highest total phenolic content (40.79 µg/ 1 mg GAE). NMR phytochemical profiling revealed that the *C. lentillifera* methanolic extract (CLME) contained sugar, sterols and amino acids; the *C. lentillifera* hexane extract (CLHE) contained fatty acids, sterols and olefinic compounds, CLCE contained palmitic acids, polysaccharides and amino acids; CLEAE contained polysaccharides, terpenoids and phenolic compounds. The present study went on to examine the neuroprotective potentials of the four *C. lentillifera* extracts and unravel the underlying mechanisms through oxidative stress, inflammatory and apoptotic markers in LPS-induced RAW 264.7 macrophages and SK-N-SH neuroblastoma cells. With regards to LPS-induced RAW 264.7 macrophages, all four *C. lentillifera* extracts significantly reduced ROS production, with CLCE and CLHE being the two most potent extracts that significantly reduced ROS production ($p < 0.05$) by 79.8% and 63.8%, respectively when compared to $control_{LPS}$. All four *C. lentillifera* extracts also significantly reduced NO production, with CLCE being the most potent extract that significantly reduced NO production ($p < 0.05$) by 80.1% when compared to $control_{LPS}$. Nevertheless, only CLCE resulted in significant reduction of Caspase-3 activity ($p < 0.05$; -29.6%) when compared to $control_{LPS}$. Whilst only CLCE and CLHE significantly upregulated NOS2 and NRF2 expression, only CLCE significantly upregulated NOS3 expression. With regards to LPS-induced SK-N-SH neuroblastoma cells, all four *C. lentillifera* extracts significantly reduced ROS production, with CLHE emerged as the most potent extract that significantly reduced ROS production ($p < 0.05$) by 72.7% when compared to $control_{LPS}$. All four *C. lentillifera* extracts also significantly reduced Caspase activity, with CLHE and CLCE emerged as the two most potent extracts that significantly reduced Caspase-3 activity ($p < 0.05$) by 48.9% and 45.3%, respectively. However, none of the extracts resulted in significant changes to NOS2, NOS3 and NRF2 expressions. The present findings implied the potentials of the *C. lentillifera* extracts (especially CLCE and CLHE) as natural neuroprotective agents against the neuroinflammation-driven AD.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that constitutes 60–70% of dementia cases worldwide. In fact, AD is affecting over 50 million people, with its prevalence expected to triple to 152 million by 2050 (Kanik et al., 2024; Tahami Monfared et al., 2022). This escalating prevalence is closely linked to the growing ageing population who are increasingly susceptible to cognitive decline and neurodegenerative diseases like AD. The global population aged 65 years and above is projected to rise from 10% in 2022 to 16% in 2050, with more than twice as many older persons as children under five (United Nations Department of Economic and Social Affairs, 2022). The increased dementia prevalence and care needs certainly incur high global societal costs, which was estimated at US$1.313 trillion in 2019 (Wimo et al., 2023) and is expected to rise in the future (Lastuka et al., 2024).

AD is defined by two core hallmarks, amyloid-β (Aβ) plaques and tau protein tangles, both of which collectively contribute to neuronal death and cognitive decline (Kulshreshtha & Piplani, 2016; Pákáski & Kálmán, 2008). Its pathogenesis is largely driven by neuroinflammation, which is orchestrated by a complex interplay between neurons, microglia, astrocytes, and infiltrating macrophages. In fact, current evidence implies that neuroinflammation is not merely a consequence of Aβ and tau pathology, but may also act as an initiating factor of AD (Weifeng Zhang et al., 2023). Basically, dying neurons release damage-associated molecular patterns (DAMP), which activate microglia and peripheral macrophages through receptors such as Toll-like receptors (TLR) and receptor for advanced glycation end products (RAGE), triggering the release of pro-inflammatory cytokines (e.g., IL-1β, TNF-α, IL-6) and reactive oxygen species (ROS) (Mammana et al., 2018; Zhang et al., 2017). This inflammatory cascade would exacerbate neuronal damage via the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Mitogen-Activated Protein Kinase (MAPK) pathways, leading to synaptic dysfunction, tau hyperphosphorylation and neurodegeneration (Weifeng Zhang et al., 2023). Peripheral macrophages which infiltrate the brain would