EFFECTS OF *BACOPA MONNIERI* EXTRACT AND ITS MICROCAPSULES ON IMPROVING COGNITIVE FUNCTIONS IN A LIPOPOLYSACCHARIDE-INDUCED DEMENTIA IN *VIVO* MODEL

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MASTER OF SCIENCE
IN
HEALTH AND BIOMEDICAL ANALYTICS

SCHOOL OF HEALTH SCIENCE
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THIS THESIS IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HEALTH AND BIOMEDICAL ANALYTICS

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MAE FAH LUANG UNIVERSITY
2025

THESIS APPROVAL

MAE FAH LUANG UNIVERSITY

FOR

MASTER OF SCIENCE IN HEALTH AND BIOMEDICAL ANALYTICS

Thesis Title: Effects of *Bacopa monnieri* Extract and Its Microcapsules on Improving Cognitive Functions in a Lipopolysaccharide-induced Dementia *In Vivo* Model

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ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to all who contributed to the completion of this thesis. I am deeply grateful to my advisor, Assoc. Prof. Sasitorn Chusri, for her invaluable guidance, insightful advice, and expert comments throughout this study, and to my co-advisor, Prof. Shang Yazhen, for his thoughtful suggestions and unwavering support.

I am also thankful to Prof. Shang Yazhen's team (Chengde Medical University, China), Prof. Sharida Fakurazi's team (Universiti Putra Malaysia), and Asst. Prof. Thammarat Kaewmanee's team (Prince of Songkla University, Thailand) for their guidance and support throughout this research.

My gratitude extends to Mae Fah Luang University for thesis support and research presentation grant, the exchange programs scholarship, and project funding (No. 672A05017), as well as to the Health and Biomedical Analytics program, School of Health Science, for providing essential tools, equipment, and research facilities.

Finally, I wish to express my sincere gratitude to my family, partner, and friends for their encouragement and support. I also acknowledge, with deep appreciation, those whose contributions are not specifically mentioned here but have played a significant role in my academic journey.

Sasithon Aunsorn

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ABSTRACT

Bacopa monnieri (BM) is a traditional medicinal herb with neuroprotective and cognitive-enhancing properties, but poor stability and bioavailability limit its practical application. In this study, the effects of BM extract and its microencapsulated formulation (BM110-180) were evaluated in a lipopolysaccharide (LPS)-induced dementia rat model. The BM extract contained high levels of key bioactive compounds, including phenolics and flavonoids, and showed potent antioxidant activity through single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. Safety assessment in *C. elegans* confirmed that the extract had no toxicity or adverse effects on feeding behavior. BM110-180 was prepared through spray-drying with maltodextrin and unripe banana flour to improve stability, protect bioactive compounds, and potentially enhance bioavailability. In LPS-treated rats, both BM extract and BM110-180 improved cognitive functions, including spatial learning, memory, and exploratory behavior, while reducing behavioral disturbances associated with neuroinflammation. These findings suggest that BM extract and its microencapsulated form are effective neuroprotective interventions, with microencapsulation providing additional benefits for preserving bioactivity and enhancing therapeutic potential in dementia-related cognitive deficits.

Keywords: *Bacopa monnieri*, Microencapsulation, Neuroprotective Effect, Cognitive Function, Antioxidant Activity

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ABBREVIATIONS AND SYMBOLS

5-HT	5-hydroxytryptamine
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ABTS	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADI	Alzheimer’s Disease International
Arc	Activity-regulated cytoskeleton-associated protein
AUC	Area under curve
AVLT	Rey auditory verbal learning test
Aβ	Amyloid beta
BBB	Blood-brain barrier
BChE	Butyrylcholinesterase
BDNF	Brain-derived neurotrophic factor
BM	*Bacopa minnieri*
C. elegans	*Caenorhabditis elegans*
CA	Cornu ammonis
CAE	Catechin equivalents
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CBD	Cognitive demand battery test
CESD-10	Center for epidemiologic studies depression scale
CI	Carr’s index
CNS	Central nervous system
COX	Cyclooxygenase
DA	Dopamine
DALYs	Disability-adjusted life years
DG	Dentate gyrus

ABBREVIATIONS AND SYMBOLS

DM2	Diabetes mellitus type 2
DOPAC	Dihydroxyphenylacetic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl)
DSST	Digit Symbol Substitution Test
E. coli	Escherichia coli
EDTA	Thylenediaminetetraacetic acid
EPM	Elevated Plus Maze
ERP	Event-Related Potential
ESI	Electrospray ionization
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GFAP	Glial fibrillary acidic protein
GMS	Glyceryl monostearate
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione-S-transferase
HPLC	High-performance liquid chromatography
HR	Hausner ratio
i.c.	Intracardiac injection
i.c.v.	Intracerebroventricular injection
i.p.	Intraperitoneal injection
IACUC	Institutional Animal Care and Use Committee
IFN	Interferon
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IT	Inspection Time
L•	Lipid radicals
LC-MS	Liquid chromatography-mass spectrometry

ABBREVIATIONS AND SYMBOLS

LPS	Lipopolysaccharide
MCA	Metal chelating activity
MCI	Mild cognitive impairment
MCP	Monocyte chemoattractant protein3,4-
MDA	Methylenedioxyamphetamine
MMSE	Mini-mental state examination
MUS-1	Muscarinic M1
MW	Molecular weights
MWM	Morris water maze
N/A	Not available
NFTs	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOR	Novel Object recognition
OD	Optical density
ORAC	Oxygen radical antioxidant capacity
p.o.	Oral administration
PD	Parkinson's disease
PICO	Specialized framework of population, intervention, comparison, and outcome
RAM	Radial arm maze
ROO•	Peroxyl radicals
ROS	Reactive oxygen species
RT	Retention time
RVIP	Rapid visual information processing task
s.c.	Subcutaneous injection
SDAT	Senile dementia of the Alzheimer type
SLNs	Solid lipid nanoparticles
SOD	Superoxide dismutase

ABBREVIATIONS AND SYMBOLS

SOD	Superoxide dismutase
SPF	Specific pathogen free
SRT	Spatial recognition task
STAI	State-trait anxiety inventory
STZ	Streptozotocin
TBARS	Thiobarbituric acid reactive substances
TE	Trolox equivalents
TFC	Total flavonoid content
TNF	Tumor necrosis factor
TPC	Total phenolic content
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
WHO	World Health Organization
WMS	Wechsler memory scale
WSI	Water solubility index

CHAPTER 1

INTRODUCTION

1.1 Rationale and Background

Dementia is a progressive neurodegenerative disorder characterized by a decline in memory, reasoning, and other cognitive functions that interfere with daily living (Kumar et al., 2025). It represents a growing global health concern, with more than 57 million people living with dementia in 2021 and nearly 10 million new cases reported each year (World Health Organization, 2025). The prevalence is particularly high in the Asia–Pacific region due to population aging and lifestyle-related risk factors (Alzheimer's Disease International, 2014). Dementia imposes a substantial healthcare and economic burden and significantly affects the quality of life of patients and their caregivers (Tay et al., 2024). Alzheimer's disease (AD) is the most common form, accounting for approximately 60-80% of dementia cases (Alzheimer's Disease International, 2021). AD presents with both neurological and neuropsychiatric symptoms, such as depression, apathy, aggression, and psychosis (Pless et al., 2023), as well as impairments in learning, problem-solving, memory, and information processing (Morley et al., 2015).

The complex pathogenesis of dementia involves multiple interrelated mechanisms, including amyloid-beta (Aβ) aggregation, tau hyperphosphorylation, oxidative stress, and neuroinflammation (Kamatham et al., 2024). Neuroinflammation plays a major role as both a response to pathological protein accumulation and a driver of neuronal injury and cognitive decline, particularly in AD (Zhang et al., 2023). Chronic activation of microglia and astrocytes triggers the release of pro-inflammatory cytokines and reactive oxygen species, resulting in neuronal damage (Deng et al., 2024). Recently, increasing evidence has highlighted the gut–brain axis as a key modulator of neurodegenerative processes. Gut dysbiosis can compromise intestinal and blood–brain barrier (BBB) integrity, allowing lipopolysaccharide (LPS) which is a component of Gram-negative bacterial membranes and other microbial metabolites to

enter circulation, promoting systemic inflammation and microglial activation that lead to cognitive decline (Saji et al., 2022). Thus, systemic inflammation and gut microbiota imbalance are important mechanisms linking peripheral immune activation to central neurodegeneration.

Despite advances in biomedical research, there remains no curative treatment for dementia, and current pharmacological therapies mainly aim to manage symptoms and slow disease progression (Dementia Australia, 2025; Zhang et al., 2024). Medications for cognitive symptoms in AD primarily include cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine (Singh et al., 2024). Non-pharmacological therapies such as dietary interventions, physical exercise, meditation, computerized cognitive training, and sleep optimization have shown benefits for memory, attention, and executive function (Livingston et al., 2020; Sachdeva et al., 2015). However, the limited efficacy and side effects of available drugs have led to increasing interest in complementary and alternative approaches, particularly herbal medicines and natural antioxidants (May et al., 2009). These herbal medicines, rich in phytochemicals such as flavonoids, phenolics, and terpenoids, exhibit neuroprotective, antioxidant, and anti-inflammatory effects through modulation of multiple signaling pathways (Cave et al., 2023; Liu et al., 2021).

Among herbal medicines, *Bacopa monnieri* (BM) has been widely used in traditional Ayurvedic medicine for its memory-enhancing and neuroprotective effects (Uabundit et al., 2010). It is a key component in many Ayurvedic formulations used to treat memory loss, anxiety, poor cognition, and lack of concentration (Nemetchek et al., 2017). BM contains bioactive compounds including mainly bacosides, flavonoids, and phenolics which are contribute to its antioxidant and anti-inflammatory activities (Fatima et al., 2022). Preclinical and clinical studies have shown that BM enhances learning, memory, and cognitive function by modulating neurotransmitter activity, reducing oxidative stress, and suppressing neuroinflammatory mediators (Gościniak et al., 2025; Valotto Neto et al., 2024). However, its therapeutic potential is limited by poor water solubility, low bioavailability, and degradation from environmental factors such as heat and moisture (Phrompittayarat et al., 2008; Gohel et al., 2016; Thakkar et al., 2017).

Microencapsulation technology offers a promising strategy to enhance the efficacy of BM. It involves enclosing bioactive compounds within microcapsules (1-1000 μm) composed of core and wall layers (Lengyel et al., 2019; Chaturvedi & Sharma, 2024). Various techniques, such as spray-drying, coacervation, and liposomal encapsulation, can be employed depending on the properties of the herbal extract (Panagiotakopoulos & Nasopoulou, 2024). This approach improves compound stability, protects sensitive molecules from degradation, masks unpleasant taste, enhances solubility, and allows controlled release and targeted delivery (Peanparkdee et al., 2016). By improving intestinal absorption and prolonging biological activity, microencapsulation can increase the therapeutic effectiveness of herbal extracts and ensure more consistent neuroprotective effects *in vivo* (Grgić et al., 2020; Pudziuvelyte et al., 2019).

However, limited studies have compared the effects of BM extract and its microencapsulated form in models of inflammation-induced cognitive impairment. Understanding these differences may provide valuable insights into formulation-based enhancement of herbal efficacy. Therefore, this study aims to investigate the effects of BM extract and its microcapsules on cognitive function in an LPS-induced dementia rat model. The findings may contribute to the development of functional foods or nutraceuticals that support brain health and mitigate neuroinflammation-related cognitive decline.

1.2 Objectives

1.2.1 To produce and characterize the microencapsulation of *Bacopa monnieri*.

1.2.2 To assess the *in vitro* antioxidant activity of *Bacopa monnieri* extract and its microcapsules.

1.2.3 To evaluate the effects of *Bacopa monnieri* extract and its microcapsules on cognitive function and associated neuroprotective effects in a lipopolysaccharide-induced dementia rat model.

1.3 Study Scope

This study was designed to comprehensively evaluate the phytochemical profile, antioxidant activity, safety, and cognitive-enhancing potential of BM extract and its microencapsulated formulation. The BM was analyzed for total phenolic and flavonoid contents, antioxidant activity, and phytochemical composition. The extract's safety was then assessed through toxicity evaluation, and its cognitive-enhancing effects were investigated *in vivo*. Subsequently, BM extract was microencapsulated, and the resulting microcapsules were subjected to physicochemical characterization. The total phenolic and flavonoid contents, antioxidant activity, and phytochemical compounds of the microcapsules were determined to assess the retention of bioactive compounds following encapsulation. Finally, the cognitive-enhancing effects of the microencapsulated formulation were evaluated, providing a systematic comparison between the crude extract and its microencapsulated form.

1.4 Expected Outcomes

1.4.1 The *Bacopa monnieri* microcapsules have stable particle size, morphology, and physicochemical properties.

1.4.2 The *Bacopa monnieri* extract and its microcapsules have significant *in vitro* antioxidant activity, indicating neuroprotective potential.

1.4.3 The *Bacopa monnieri* extract and its microcapsules improve behavioral performance, indicating improvement in cognitive function in LPS-induced dementia rats.

1.5 Conceptual Framework

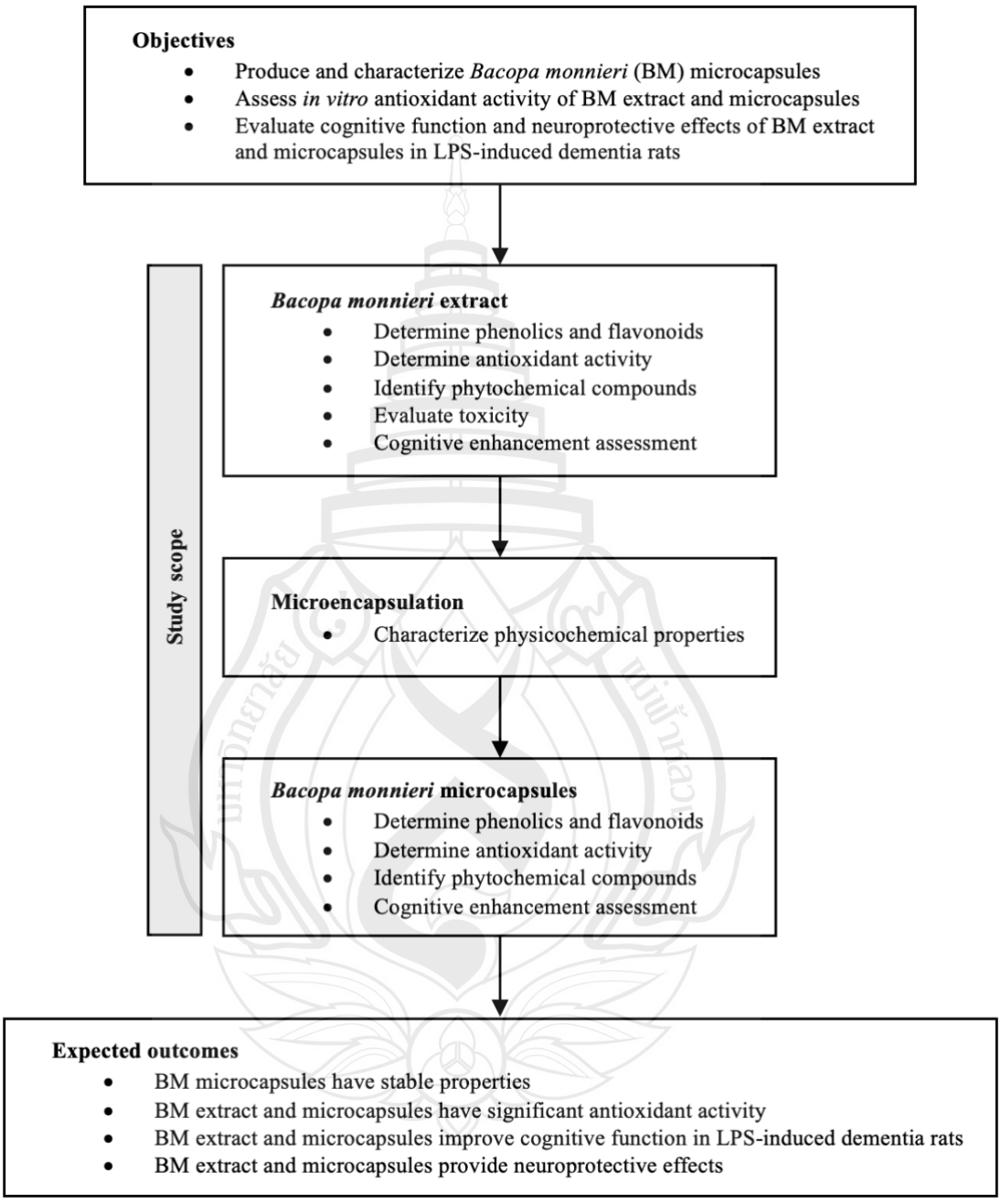

Figure 1.1 Conceptual framework

CHAPTER 2

LITERATURE REVIEW

2.1 Dementia and Cognitive Impairment: An Overview

2.1.1 Pathogenesis of Dementia

Dementia is a progressive neurodegenerative disorder characterized by a decline in memory, learning ability, and cognitive functions severe enough to interfere with daily activities (Kumar et al., 2025). The most common type of dementia is AD , which accounts for approximately 60-80% of all dementia cases, followed by vascular dementia, Lewy body dementia, and frontotemporal dementia (Alzheimer's Disease International, 2021). Although each subtype present distinct pathological hallmarks, they share common mechanisms, including neuronal loss, synaptic dysfunction, and chronic neuroinflammation, which collectively impair neural communication and cognitive decline (Gadhave et al., 2024).

The pathology of AD is multifactorial, involving several interrelated processes. The primary pathological features include the accumulation of Aβ proteins and the formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau proteins (Cai et al., 2022). Aβ aggregation leads to the formation of insoluble amyloid fibrils that cluster into extracellular plaques, disrupting synaptic signaling within neural circuits. This aggregation process activates intracellular kinases, resulting in tau hyperphosphorylation and destabilization of microtubules. The consequent formation of NFTs impairs axonal transport and neuronal communication, eventually leading to neuronal apoptosis (Tiwari et al., 2019). Furthermore, these protein aggregates activate microglia and astrocytes, inducing the release of pro-inflammatory cytokines that contribute to neuroinflammation, synaptic loss, and cognitive impairment observed in AD (Novoa et al., 2022).

Neuroinflammation refers to an inflammatory response within the central nervous system (CNS) triggered by pathological stimuli such as infection, trauma, ischemia, or toxins, and primarily involves innate immune cells like microglia and astrocytes (Leng & Edison, 2021). Microglia and brain macrophages play key roles in clearing pathogens, dead cells, protein aggregates, and toxins (Colonna & Butovsky, 2017). However, chronic microglial activation that stimulated by interferon-γ (IFN-γ) from immune cells (Ottum et al., 2015), LPS from gram-negative bacteria including gut microbes (Kim et al., 2021), and accumulated Aβ and tau proteins that promotes the release of pro-inflammatory mediators such as interleukin-1β (IL-1β), IL-6, IL-12, IL-18, tumor necrosis factor-α (TNF-α), nitric oxide (NO), and reactive oxygen species (ROS). These mediators contribute to synaptic damage and neuronal death, ultimately leading to neurodegeneration and cognitive decline (Wu & Eisel, 2023; Zhao et al., 2019).

Astrocytes, star-shaped glial cells distributed throughout the brain and spinal cord, play essential roles in maintaining synaptic transmission and supporting neuronal networks. They provide biochemical support to endothelial cells of the blood–brain barrier (BBB), supply nutrients to neurons, and aid in neural tissue repair (Cai et al., 2017; Heneka et al., 2015). When activated by pro-inflammatory cytokines released from microglia such as IL-1α, TNF-α, and complement component C1q, astrocytes further secrete TNF-α, IL-1β, IL-6, NO, and ROS, exacerbating neuronal injury and apoptosis (Phillips et al., 2014; Wu & Eisel, 2023). Hence, the reciprocal activation between microglia and astrocytes amplifies neuroinflammatory cascades, contributing to progressive neurodegeneration and cognitive impairment in AD (Lecca et al., 2022) (Figure 2.1).

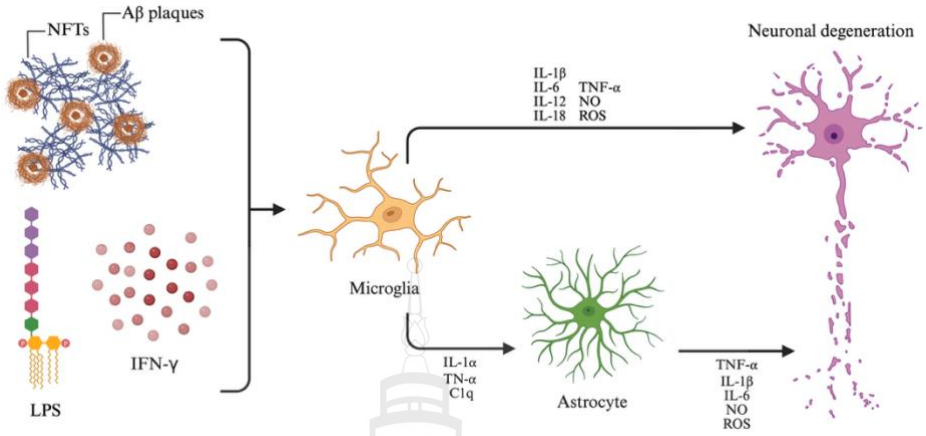

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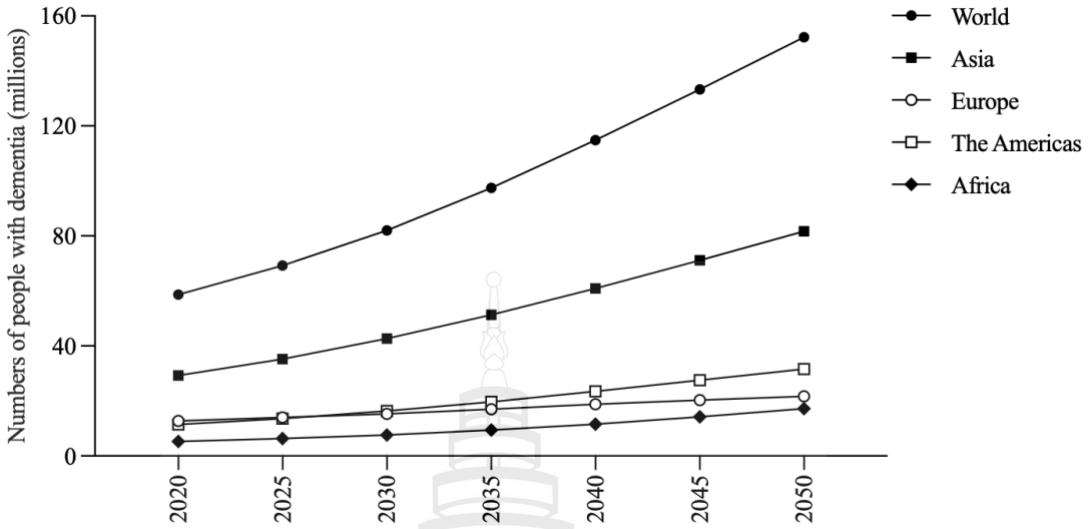
Figure 2.1 Neuropathological features of Alzheimer's disease and dementia

Elevated LPS levels have been detected in both the plasma and brains of AD patients, suggesting a strong association between chronic endotoxemia and neurodegeneration (Brown & Heneka, 2024). Increased plasma LPS levels are also linked to cognitive decline, small vessel brain disease, altered gut microbial metabolite profiles, and certain dietary patterns, indicating that LPS may serve as a potential risk marker for cognitive impairment and vascular brain changes (Saji et al., 2022). Mechanistically, LPS binds to Toll-like receptor 4 (TLR4) on microglial membranes, activating the NF-κB signaling pathway and promoting the release of pro-inflammatory cytokines (Romo et al., 2024). Prolonged exposure to LPS induces neuronal injury, memory deficits, and behavioral alterations which are hallmarks of dementia-like pathology. Therefore, LPS-induced neuroinflammation is widely employed as an experimental model to replicate the inflammatory component of dementia *in vivo* (da Silva et al., 2024; Skrzypczak-Wiercioch & Sałat, 2022).

2.1.2 Prevalence and Healthcare Burden Related to Dementia

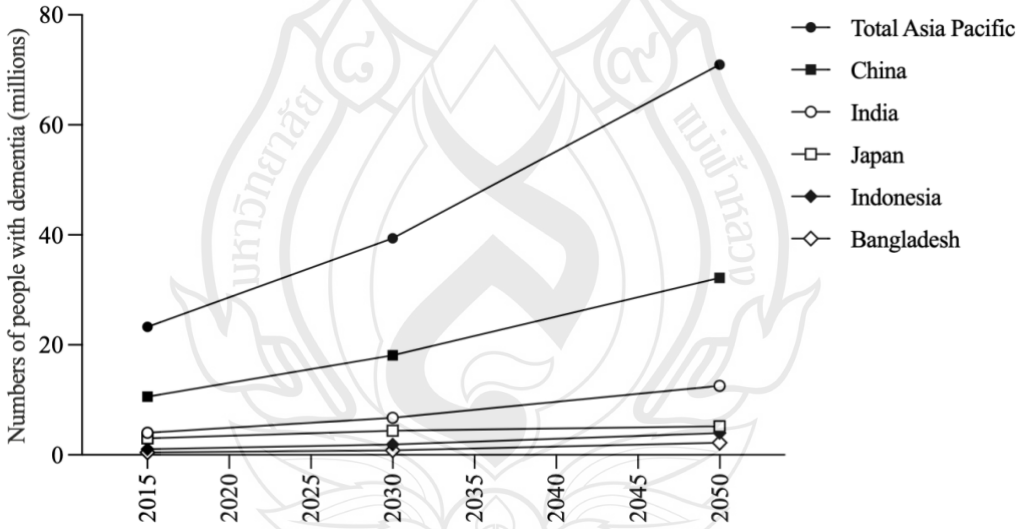
AD is the most common form of neurodegenerative dementia, affecting about 5-6% of individuals aged 65 and above and up to 30% of those over 85. Approximately 5% of cases occur before age 65, known as early-onset AD. The condition usually begins with a gradual memory decline, and the average survival time after symptom onset is 10-12 years (Gale et al., 2018; Tahami Monfared et al., 2022).

From 2020, the global population with dementia was estimated at 59 million, reaching 82 million in 2030 and 152 million in 2050. In 2050, Asia will have the highest prevalence at 82 million, followed by the Americas at 32 million, Europe at 21 million, and Africa at 17 million respectively (Alzheimer's Disease International, 2020) (Figure 2.2). The World Health Organization (WHO) reported that AD and other forms of dementia were the 7th leading cause of death in 2019 (World Health Organization, 2020). Globally, deaths from dementia in 2019 were estimated at 1.62 million, with a higher incidence observed among 1.06 million women compared to 0.56 million men (GBD 2019 Collaborators, 2021). According to the 2023 report, there are estimated total payments of $345 billion for healthcare and long-term care for individuals aged 65 and older with dementia (Alzheimer's Association, 2023) and tends to increase every year with $465 billion in 2030, $763 billion in 2040, and reaching $1 trillion by 2050 (Skaria, 2022). However, the total costs significantly associated with AD stages, as determined by the Mini-Mental State Examination (MMSE) scale, are $14,675 for the mild stage, $19,975 for the moderate stage, and $29,708 for the severe stage, as annual expenses (Marešová et al., 2020). This process aims to maintain quality of life, optimize daily functioning, improve cognition, mood, and behavior, establish a secure environment, and encourage social engagement.



Source Alzheimer's Disease International (2020)

Figure 2.2 The estimated the global number of people with dementia

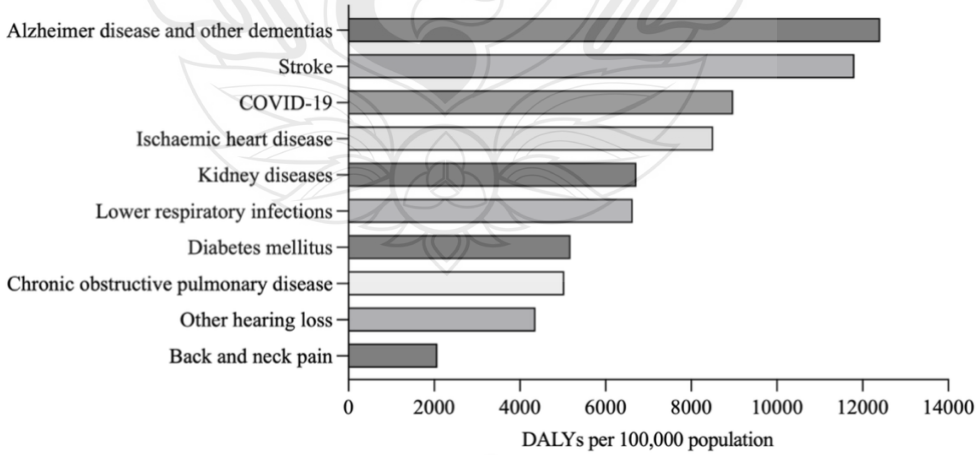


Source Alzheimer's Disease International (2014)

Figure 2.3 The estimated the number of people with dementia in the Asia-Pacific region

AD and other dementias in the Asia-Pacific region impact social and economic aspects. According to Alzheimer's Disease International (ADI) 2014 report, the estimated number of people with dementia was 23 million in 2015, reaching 39 million in 2030 and 71 million in 2050, with the highest 3 countries are China with 32 million, India with 13 million, and Japan with 5 million (Figure 2.3). The total costs associated with dementia for medical and social care in 2015 amounted to $185 billion, which is expected to continue increasing, particularly in countries with emerging economies and large populations such as China, India, Japan, and Australia, (Table 2.1) which depends on various factors including urbanization, cultural and family structures, public health infrastructure, and governmental policies (Alzheimer's Disease International, 2014).

The prevalence of dementia in Thailand was 2.35% to 3.1%, primarily attributable to AD (Dharmasaroja et al., 2021). According to the 2019 report, there were 670,047 people with dementia, and it will reach 2,391,672 in 2050 (Nichols et al., 2022). AD and other dementias were responsible for 16,278 deaths, accounting for 1.86% of total deaths (World Health Organization, 2019). Among individuals aged 85 years and older, it ranked as 1st cause of Disability-adjusted life years (DALYs), with a rate of 12,409.15 per 100,000 (World Health Organization, 2020) (Figure 2.4). The estimated cost of dementia in 2015 amounted to $1.8 million, (Table 2.1) comprising medical expenses of $89, non-medical costs of $721, and informal care costs of $854 (Alzheimer's Disease International, 2014).



Source World Health Organization (2020)

Figure 2.4 The cause of DALYs rate in Thai elderly aged 85 and above

Table 2.1 The top 10 country with the highest cost of dementia and estimated number of people in the Asia Pacific region

Country	Estimated Costs (US$)	Estimated Number of people with Dementia		
		2015	2030	2050
Japan	93,240,000	3,014,000	4,421,000	5,214,000
China	44,619,000	10,590,000	18,116,000	32,184,000
Australia	12,892,000	328,000	520,000	864,000
Korea	8,676,000	462,000	974,000	2,113,000
Taiwan	6,990,000	260,000	461,000	840,000
India	4,620,000	4,031,000	6,743,000	12,542,000
Hong Kong	3,227,000	115,000	212,000	436,000
Thailand	1,810,000	600,000	1,117,000	2,077,000
Indonesia	1,777,000	1,033,000	1,894,000	3,979,000
Singapore	1,664,000	45,000	103,000	241,000

Source Alzheimer's Disease International (2014)

2.1.3 Current Therapeutic Approaches and Challenges

Dementia remains a major public health challenge, and despite intensive research efforts, there is still no curative treatment for dementia or AD (Zhang et al., 2024). However, recent studies have led to significant advances in therapies that may modify disease progression, particularly in early-stage AD, the most common form of dementia (Wu & Fuh, 2025). Current therapeutic strategies primarily focus on symptom management and slowing disease progression rather than reversing the underlying neurodegenerative process (Pache et al., 2025). The complexity of dementia pathogenesis, which involves amyloid-β accumulation, tau hyperphosphorylation, oxidative stress, neuroinflammation, and gut–brain axis dysregulation, makes single-target therapies insufficient to fully control disease progression (Yiannopoulou & Papageorgiou, 2013).

Pharmacological treatments aim to maintain quality of life, support daily functioning, and improve cognition, mood, and behavior. These include drugs targeting both cognitive and behavioral-psychological symptoms (Dementia Australia, 2025). Medications for cognitive symptoms in AD primarily include cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine and the NMDA receptor antagonist memantine (Singh et al., 2024). Cholinesterase inhibitors work by inhibiting acetylcholinesterase, thereby increasing acetylcholine levels in the synaptic cleft to improve neurotransmission and cognitive performance (Singh & Sadiq, 2025). Donepezil, the most prescribed which shows moderate efficacy in mild to moderate AD, while rivastigmine and galantamine provide similar benefits with slight differences in tolerability (Birks & Harvey, 2018; Khoury et al., 2018). Memantine, used for moderate to severe stages, regulates glutamate signaling to prevent excitotoxic neuronal death (McShane et al., 2019). Nevertheless, these drugs offer only temporary and modest improvements, do not halt neurodegeneration, and can cause side effects such as nausea, vomiting, and bradycardia (Miculas et al., 2022). In addition, poor blood–brain barrier penetration and short systemic half-lives limit their long-term efficacy (Pardridge, 2009).

Non-pharmacological therapies play a supportive role in improving quality of life and delaying cognitive decline. These include dietary interventions (e.g., Mediterranean or DASH diets), physical exercise, meditation, computerized cognitive training, and sleep optimization, all of which have demonstrated measurable improvements in memory, attention, processing speed, and executive function (Livingston et al., 2020; Sachdeva et al., 2015).

Recently, increasing attention has focused on the gut–brain axis as a potential therapeutic target. Gut dysbiosis can disrupt intestinal and BBB integrity, elevate endotoxin (LPS) and other pro-inflammatory microbial metabolites, and activate microglia, thereby contributing to neuroinflammation and cognitive decline (Saji et al., 2022). Interventions such as probiotics, prebiotics, and polyphenol-rich foods have shown potential to restore gut microbiota balance, reduce oxidative stress, modulate

systemic inflammation, and improve cognitive function in experimental and early clinical studies (Lei et al., 2025; Naomi et al., 2021).

In this context, complementary and alternative treatments, such as herbal medicines, have increasingly emerged as viable options (May et al., 2009). These herbal medicines, rich in phytochemical compounds including flavonoids, phenolics, and terpenoids, may exert neuroprotective, antioxidant, and anti-inflammatory effects and modulate multiple pathways involved in cognitive decline. However, their clinical application faces several challenges, including limited high-quality clinical trials, variability in formulations and phytochemical content, inconsistent dosing regimens, and lack of standardization across studies (Cave et al., 2023; Liu et al., 2021). Addressing these limitations is essential for future research, which should focus on integrating conventional pharmacotherapy with safe, multitargeted natural products that modulate the gut–brain axis and counteract neuroinflammation.

2.2 *Bacopa monnieri*: An Overview

2.2.1 Introduction

BM known as brahmi, phromi, water hyssop, is a member of the family Plantaginaceae, formerly Scrophulariaceae (Ingkhaninan, 2018). It is a creeping aquatic perennial plant with small, oblong, semi-succulent leaves measuring 1-1.5 cm in length and 0.3-0.5 cm in width. It produces bell-shaped flowers that are white to purple in color, with five petals, one of which is larger than the others (National Parks, 2023). This plant is typically found in warm wetlands, subtropical, and tropical regions, including East Asia, Australia, (Aguiar & Borowski, 2013; Nemetchek et al., 2017) and the United States (Barrett & Strother, 1978). In Thailand, BM can be found in every region of the country, from the northern region in Mae Hong Son province, to the central region in Prachuap Khiri Khan province, and even in the southern region, including Phang Nga Province (Forest, 1990).

BM has been used in traditional Ayurvedic medicine for centuries (Pareek & Kumar, 2014), valued for its many potential health benefits, including memory

enhancement, anti-inflammatory, analgesic, antipyretic, sedative, and antiepileptic properties (Russo & Borrelli, 2005). However, it is best known as a nootropic booster or neural tonic, known for its ability to enhance intelligence, memory, and brain function, as well as promote longevity (Sukumaran et al., 2019). Many Ayurvedic herbal formulations containing BM can effectively target CNS and help manage anxiety, poor cognition, and lack of concentration (Aguiar & Borowski, 2013). Currently, both natural and synthetic compounds derived from BM are under study for their potential in treating AD and other forms of dementia (Agarwal et al., 2023; Dubey et al., 2009).

In a study involving healthy elderly participants, intervention with 300 mg/day of BM extract for 12 weeks significantly improved memory performance. Specifically, there were enhancements in verbal learning, memory acquisition, and delayed recall, as measured by Auditory Verbal Learning Test (AVLT), compared to the placebo group. However, some participants reported gastrointestinal side effects such as increased stool frequency, abdominal cramps, and nausea (Morgan & Stevens, 2010). Subsequently, BM was studied in a newly diagnosed with AD. Participants received an intervention of 300 mg of standardized BM extract twice/day for 6 months. The results showed a significant improvement in cognition, as indicated by higher MMSE scores. Particularly in orientation of time, place, and person, attention, and language components, including reading, writing, and comprehension, compared to the pre-drug MMSE score. Additionally, improvements in quality of life were reported, along with decreases in irritability and insomnia (Goswami et al., 2011). Furthermore, in a study by Sadhu et al. (2014) a polyherbal formula containing extracts of BM, *Hippophae rhamnoides* (sea buckthorn), and *Dioscorea bulbifera* (dioscorea) was used in the management of senile dementia in patients with AD type (SDAT). The results of the trial showed that administration of the formulation at a dose of 500 mg/day for 12 months was effective in improving cognitive functions in SDAT, as measured by Digit Symbol Substitution Test (DSST), word recall immediate and attention span. Similarly, significant improvements in cognitive measures, including MMSE, DSST, and word recall delayed, were observed in healthy elderly individuals compared to the placebo

group. Moreover, the trial results reported a reduction in inflammation and oxidative stress levels, provide a neuroprotective effect of the formulation in managing cognitive decline.

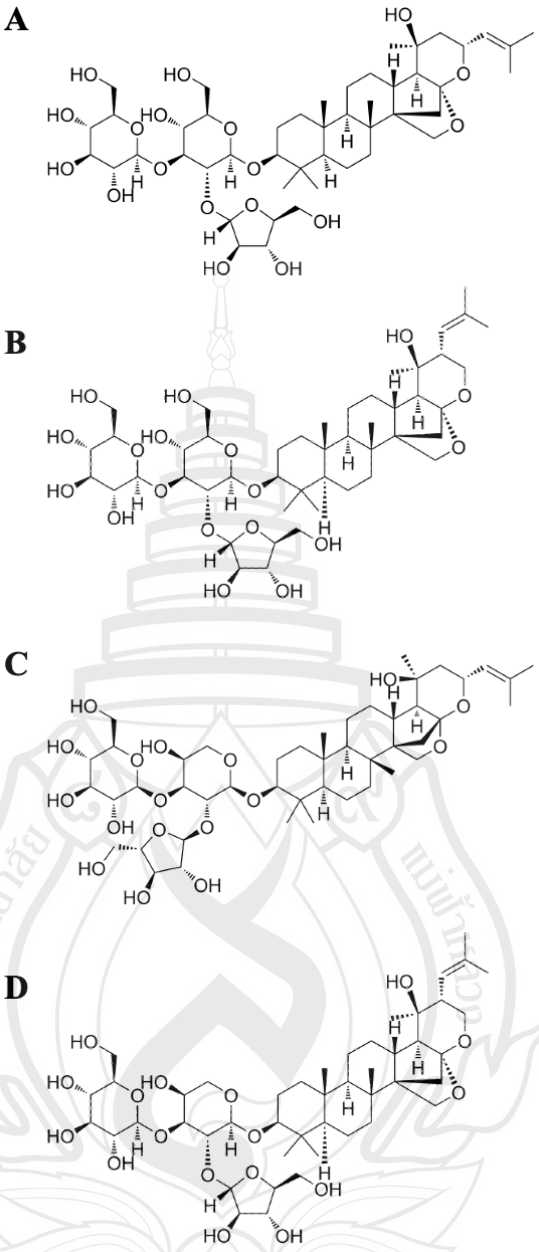
The various active constituents produced by BM include saponins, alkaloids, steroids, and flavonoids (Al-Snafi, 2013). The key constituents reported to be associated with neuroprotective and memory-enhancing effects are saponin glycosides. These compounds consist of two main parts including the glycone, a sugar molecule, and the aglycone, typically a steroid or triterpenoid structure, comprising 1-3% of the dry weight of BM (Ingkhaninan, 2018). The saponin glycosides found in BM include bacosides and bacopasides, with common variants being bacoside A and bacopaside I. These compounds have concentrations of 9.989 mg/g for bacoside A and 3.978 mg/g for bacopaside I in fresh whole-plants of BM (Srivastava et al., 2012).

From all the constituents mentioned, only bacoside A and bacoside B, which are isomers with optically active components, have showed cognitive enhancement properties (Deepak & Amit, 2004). However, bacoside A is more pharmacologically active than bacoside B (Fatima et al., 2022). Bacoside A is a mixture composed of several compounds, including bacoside A_3, bacopaside II, bacopaside X, and bacopasaponin C (Figure 2.5). Bacoside A is found in various quantities in BM such as 1g/100g in the fresh whole plant, 45g/100g in the dried whole plant, (Pal & Sarin, 1992) and 4.35-10.50% w/w in the leaves (Ahmed et al., 2015). In the mixture of bacoside A, bacoside A_3 constitutes 19.5% w/w, bacopaside II 26.9% w/w, bacopaside X 32.3% w/w, and bacopasaponin C 17.2% w/w (Malishev et al., 2017).

These compounds have been studied for their neuroprotective properties, including anti-inflammatory, antioxidant, anti-apoptotic, antiseizure, and anti-epileptic effects, as well as their ability to enhance neurogenesis (Valotto Neto et al., 2024). According to Table 2.2, bacoside A has been the most extensively studied for neuroprotective effects both *in vitro* and *in vivo* models. In contrast, other constituents of mixture have been studied much less, with some lacking any research. This

highlights that the neuroprotective effects of these compounds have not yet been investigated at the clinical level.

However, bacoside A have limited bioavailability because of their low aqueous solubility and bitter taste (Chandel, 1976). Additionally, they are sensitive to heat, showing a significant decrease in stability at 80 °C. It is also sensitive to acidic conditions, with decreased stability observed at a pH of 1.2. Moreover, it is prone to moisture absorption, all of which contribute to decreased quality and efficacy (Phrompittayarat et al., 2008).



Source MedChemExpress (2024)

Figure 2.5 The structure of bacoside A mixtures in *Bacopa monnieri*. (A) bacoside A_3, (B) bacopaside II, (C) bacopaside X, and (D) bacopasaponin C

Table 2.2 Neuroprotective effects studies of bacoside A and mixtures.

Active consituents	***In vitro***	***In vivo***	**Clinical trial**	**References**
Bacoside A	N2a cells (0.4 mg/ml)		N/A	(Bhardwaj et al., 2018)
(Fresh whole plant: 1g/100g)	SH-SY5Y cells (50 μM)			(Malishev et al., 2017)
(Dried whole-plant: 45g/100g)		C57BL/6 mice (10 and 20 mg/kg)		(Madhu et al., 2019)
(Leaf: 4.35-10.50% w/w)		Swiss mice (5 mg/kg)		(Agarwal et al., 2023)
		Swiss mice (5 mg/kg)		(Bist et al., 2021)
		Swiss mice (5 mg/kg)		(Chaudhary & Bist, 2017)
		Swiss mice, Wistar rat (32.5 mg/kg)		(Mishra et al., 2018)
		Wistar neonatal rats (50 mg/kg)		(Thomas et al., 2013)
		Wistar rats (10 mg/kg)		(Anbarasi et al., 2006)
		Wistar rats (10 mg/kg)		(Sumathi et al., 2011)
		Wistar rats (150 mg/kg)		(Mathew et al., 2010)
Bacoside A3	U87MG cells (0.5-5 uM)	N/A	N/A	(Bai & Zhao, 2022)
(Aerial parts of plant: 1.13% w/w)				
(19.5% w/w of bacoside A)				
Bacopaside Il	N/A	N/A	N/A	
(26.9% w/w of bacoside A)				
Bacopaside X	N/A	Swiss mice (20 mg/kg)	N/A	(Kraus et al., 2023)
(32.3% w/w of bacoside A)				
Bacopasaponin C	N/A	N/A	N/A	
(17.2% w/w of bacoside A)				

2.2.2 Pre-clinical Studies on *Bacopa monnieri* in Cognitive Enhancement

From the neuroprotective effect of BM in improving memory, learning, and attention, there is interest in exploring its mechanisms of action and potential therapeutic applications for cognitive enhancement through pre-clinical studies. These investigations primarily use rodent models such as mice and rats to explain the effects on cognitive function. Rodents are chosen because of their genetic similarity to humans, allowing targeted investigations into specific genes and pathways involved in cognitive processes, thereby enhancing the ability to develop interventions that could mitigate cognitive impairment (European Animal Research, 2023). Their physiological similarities to humans promote controlled experiments that reveal underlying neural mechanisms and potential therapeutic applications. Additionally, rodents have short lifespans and rapid reproductive cycles, enabling longitudinal studies important for investigating age-related cognitive decline and testing cognitive enhancement. Therefore, rodents play a crucial role in advancing for understanding of BM and other cognitive enhancement, showing valuable insights into improving cognitive function and managing cognitive decline (Bryda, 2013; Rapaka et al., 2022).

For studies on the prevention of cognitive impairment in induced cognitive and memory impairment models associated with AD, various chemicals or drugs are used to induce changes in rodent models. These changes can involve oxidative stress, neuroinflammation, neurotransmitter systems, interactions with various receptors, proteins, and enzymes, among other factors, which can also lead to behavioral changes and psychological symptoms. Table 2.3 presents various models induced for cognitive and memory impairment associated with AD. It is noted that most studies are conducted on male animals rather than females. The dosage and administration of drugs depend on the type and weight of the experimental animals. Each model aims to study different aspects of cognitive impairment mechanism or disease, and the advantages and disadvantages of each model, as shown in Table 2.4, are crucial for selecting the appropriate model for specific research objectives.

Table 2.3 Animal model induced for cognitive and memory impairment associate with Alzheimer's disease

Model	Species	Age	Gender	Biomarker	Dose	References
Streptozotocin	Sprague-Dawley rats	5 months	Female	Amyloid plaques,	1.5 mg/kg (i.c.v)	(Zeng et al., 2017)
	Wistar albino rats	3-4 months	Male	tauopathy, gliosis,	3 mg/kg (i.c.v)	(Noor et al., 2022)
	Sprague-Dawley rats	1 years	Male	atrophy of parenchyma, neurochemical alterations, oxidative stress, neuroinflammation	0.25 mg/µl (i.c.v)	(Lannert & Hoyer, 1998)
Amyloid-β	Fisher rats	18-20 weeks	Male	Amyloid plaques,	20 µg/3 days (i.c.v)	(Nakamura et al., 2001)
	Long-Evans rats	2-3 months	Male	Tau phosphorylation, cholinergic deficits, neuroinflammation, glutaminergic alterations	4 µg/µl (i.c.v)	(Hruska & Dohanich, 2007)
Scopolamine	C57BL/6J mice	7-12 weeks	Female	Tau pathology,	10 mg/kg (i.p)	(Riedel et al., 2009)
	Albino rats	4 months	Male	Cholinergic	1 mg/kg (i.p)	(Abu Almaaty et al., 2021)
	Swiss mice	10-12 weeks	Male/ female	impairment, monoamine alterations, neuroinflammation	1 mg/kg (i.p)	(Khurana et al., 2021)
Ethylcholine aziridinium ion	Wistar-Imamichi rats	7-8 weeks	Male	Cholinergic deficits,	2 nmol/2 µl (i.c.)	(Fisher & Hanin, 1986)
	Sprague-Dawley rats	5 weeks	Male	astrogliosis	6 nmol/6 µl (i.c.v)	(Yamada et al., 2010)

Table 2.3 (continued)

Model	Species	Age	Gender	Biomarker	Dose	References
Alcohol	Wistar rats	2-3 months	Male	Deficits in spatial memory, oxidative stress,	20% v/v; 4 g/kg (p.o.)	(Hashemi Nosrat Abadi et al., 2013)
	Wistar rats	2-3 months	Male	neuroinflammation,	4 g/kg (p.o.)	(Vaghef et al., 2019)
	Sprague-Dawley rats	2 months	Male	neuronal loss	20% v/v; 4 g/kg (p.o.)	(Baird et al., 1998)
	Long–Evans rats and Sprague-Dawley rats	2-3 months	Male		20% v/v; 1 or 4 g/kg (p.o.)	(Spinetta et al., 2008)
Colchicine	Wistar rats	8-10 weeks	Male	Amyloid plaques,	15 µg/5 µl (i.c.v)	(Kumar et al., 2007)
	Adult albino rat	6-8 weeks	Male	neuroinflammation,	15 µg/5 µl (i.c.v)	(Ganguly & Guha, 2008)
	Adult albino rat	6-8 weeks	Male	cognitive deficits, NMDA activation, Neurodegeneration	7.5 µg/10 µl (i.c.v)	(Sil & Ghosh, 2016)
^{192}IgG-saporin	Sprague-Dawley rats	2 months	Male	Cholinergic deficits,	100, 237.5, or 375 ng/0.3 µl (i.c.v)	(Walsh et al., 1996)
	Sprague-Dawley rats	2 months	Male	cognitive deficits, synaptic changes,	8 µg/0.3 µl (i.c.v)	(Dobryakova et al., 2018)
	Sprague-Dawley rats	2 months	Male	glial changes	4 µg/0.3 µl (i.c.v)	(Walsh et al., 1995)
Aluminium	Wistar albino rats	6-12 months	Male/ female	Amyloid plaques, tangles,	4.2 mg/kg (i.p.)	(Bitra et al., 2014)
	Sprague-Dawley rats	15 weeks	Male	neurotransmitter	100 mg/kg (p.o.)	(Rapaka et al., 2019)
	Wistar rats	6-12 months	Male	alterations,	10 mg/kg, (i.p.)	(Khan et al., 2013)
	Sprague-Dawley rats	12 weeks	Male		100 mg/kg (p.o.)	(Rapaka et al., 2021)

Table 2.3 (continued)

Model	Species	Age	Gender	Biomarker	Dose	References
Methionine	Wistar rats	3 months	Male	Cholinergic deficits, endothelial dysfunction, neuroinflammation, gliosis, cognitive deficits, oxidative stress	1.7 g/kg (p.o.)	(Sain et al., 2011)
	Wistar albino rats	3 months	Male		1.7 g/kg (p.o.)	(Khodir et al., 2022)
Okadaic acid	Sprague-Dawley rats	2 months	Male	Tau phosphorylation, amyloid-β plaques, oxidative stress, cognitive deficits	200 ng/5 µl (i.c.v)	(Song et al., 2013)
	Sprague-Dawley rats	3 months	Female		7 or 70 ng/day (i.c.v)	(Zhang & Simpkins, 2010)
Ibotenic acid	Sprague-Dawley rats	2-3 months	Male	Oxidative stress, apoptosis, cognitive deficits, excitotoxicity	2 µg/µl (i.c.v)	(He et al., 2018)
Clonidine	Wistar rats	2-3 months	Male	Cognitive deficits	0.1 mg/kg (i.p.)	(Genkova-Papazova et al., 1997)
	Wistar rats	2-3 months	Male		0.1 or 0.2 mg/kg (i.p.)	(Dyr et al., 1983)
Clozapine	Sprague-Dawley rats	2-3 months	Female	Cognitive deficits	1.25 and 2.5 mg/kg (s.c)	(Levin et al., 2009)
	Sprague-Dawley rats	2-3 months	Female		1.25 and 2.5 mg/kg (s.c)	(Pocivavsek et al., 2006)

Table 2.3 (continued)

Model	Species	Age	Gender	Biomarker	Dose	References
Lignocaine	Wistar rats	2-3 months	Male	Cognitive deficits	4% w/v; 0.5 µl (i.c.v)	(Holahan & Routtenberg, 2011)
	Wistar rats	2-3 months	Male		2% w/v; 1 µl (i.c.v)	(Pérez-Ruiz & Prado-Alcalá, 1989)
Cycloheximide	C57BL/6J mice	8-10 weeks	Male	Cholinergic deficits, cognitive disturbances, neurotransmitter alterations	3.5, 7, 15, 30, 75, 150 mg/kg (s.c.)	(Quinton & Kramarcy, 1977)
	Sprague-Dawley rats	8-10 weeks	Male		1 mg/kg (s.c.)	(Lu et al., 2007)
Phenytoin	Wistar rats	2-3 months	Male	Oxidative stress, cognitive deficits	75 mg/kg (p.o.)	(Reeta et al., 2009)
	Wistar rats	2-3 months	Male		5, 12.5, 25, 50, or 75 mg/kg (i.p.)	(Sudha et al., 1995)
D-Galactose	Swiss albino mice	6-8 weeks	Male	Amyloid-β, neuroinflammation, oxidative stress, cognitive deficits	150 mg/kg (s.c.)	(Chowdhury et al., 2018)
	C57BL/6J mice	6-8 weeks	Male		150 mg/kg (i.p.)	(Zhong et al., 2020)
	C57BL/6J mice	12 weeks	Male		100 mg/kg (s.c.)	(Wei et al., 2008)
	C57BL/6J mice	6-8 weeks	Male		150 mg/kg (s.c.)	(Zhong et al., 2019)
	C57BL/6J mice	19-20 weeks	Male		100 mg/kg (s.c.)	(Tsai et al., 2011)
	Wistar rats	2-3 months	Male		150 mg/kg (s.c.)	(Kenawy et al., 2017)
	Sprague-Dawley rats	4 months	Male		160 mg/kg (s.c.)	(Hong et al., 2016)
Dizocilpine	Wistar rats	8-10 weeks	Male	Cognitive deficits, motor disturbances	1 mg/kg (i.p)	(Sadek et al., 2016)

Table 2.3 (continued)

Model	Species	Age	Gender	Biomarker	Dose	References
Diazepam	Swiss albino mice	Young (3–4 months) and aged (12–15 months)	Male	Cognitive deficits	1 mg/kg, (i.p.)	(Mani et al., 2012)
	Swiss albino mice	3 months	Male		1 mg/kg, (i.p.)	(Dhingra et al., 2004)
Lipopolysaccharide	Sprague-Dawley rats	3 months	Male	Neuroinflammation, oxidative stress,	0.25 µg/h (i.c.v.)	(Hauss-Wegrzyniak et al., 1998)
	Long–Evans rats and Sprague-Dawley rats	35 days, 10-12 weeks	Male/ female	neurochemical alterations, amyloid plaques, tauopathy, gliosis	0.125, 0.25 and 0.50 mg/kg (i.p.)	(Pugh et al., 1998)
	Wistar rats	13-14 weeks	Male		0.5-5 µg/2 µl (i.c.v.)	(Yamada et al., 2010)
	Wistar rats	8-12 weeks	Male		100 and 250 µg/kg (i.p.)	(Shaw et al., 2001, 2005)
	Wistar rats	8-10 weeks	Male		5 mg/kg (i.p.)	(Bossù et al., 2012)
	Wistar rats	8 weeks	Male		1 mg/kg (i.p.)	(Anaeigoudari et al., 2015; Anaeigoudari et al., 2016)
	C57BL/6J mice	11–12 weeks	Male		500 and 750 µg/kg (i.p) 12 µg/3 µL (i.c.v)	(Zhao et al., 2019)
	Swiss albino mice	6-8 weeks	Male		0.25 mg/kg (i.p.)	(Thingore et al., 2021)

Table 2.4 Advantages and limitations of animal model induced for cognitive and memory impairment associate with Alzheimer's disease

Model	Advantages	Limitations
Streptozotocin	1.Reliable model for studying AD 2. Replicates amyloid plaques, neurofibrillary tangles, oxidative stress, neuroinflammation 3. Resembles human sporadic AD pathology	1. Invasive with high mortality rate 2. Requires large number of animals
Amyloid-β	1. Replicates amyloid plaques, neurofibrillary tangles, neurodegeneration 2. Allows behavioral assessments 3. Specific for AD drug screening	Potential confounding effects of invasive intracerebral injection
Scopolamine	1. Easy administration of scopolamine 2. Broad study of cholinergic deficits	Does not replicate AD pathological hallmarks
Ethylcholine aziridinium ion	Study of cognitive deficits in learning and memory	1. Invasive intracerebral injections could cause trauma 2. Does not replicate AD hallmarks
Alcohol	1. Non-invasive induction of memory deficits 2. Evaluation of memory enhancing agents and nootropic activity	1. Slow process 2. Does not replicate AD pathological hallmarks
Colchicine	1. Replicates behavioral, biochemical, and neurochemical alterations 2. Screens nootropic agents and cholinesterase inhibitors	1. High mortality rate 2. Ethical concerns due to adverse effects

Table 2.4 (continued)

Model	Advantages	Limitations
^{192}IgG-saporin	Evaluates behavioral and electrophysiological consequences of cholinergic neuron degeneration	1. Invasive administration 2. Does not express all AD hallmarks
Aluminium	1. Replicates all AD pathological hallmarks 2. Reliable and reproducible	1. Pathologically different amyloid plaques from human AD 2. Less mortality rates
Methionine	1. Study of vascular dementia 2. Safe administration for drug screening	Does not replicate AD pathological hallmarks
Okadaic acid	1. Screens dementia drugs 2. Replicates all AD pathological hallmarks	Invasive administration with high mortality risk
Ibotenic acid	Valid model for studying memory deficits in AD	1. High mortality rate 2. Invasive procedure
Clonidine	1. Evaluates monoamine influence on cognitive deficits 2. Behavioral effects study	1. Does not replicate AD hallmarks 2. Non-reliable model, depressive effects
Clozapine	1. Studies cholinergic system influence on behavior 2. Cognitive deficits evaluation	1. Does not replicate AD pathological hallmarks 2. Adverse effects require close monitoring
Lignocaine	Explores long-term and short-term memory dysfunctions	1. Does not replicate AD pathological hallmarks 2. Invasive route with mortality risks

Table 2.4 (continued)

Model	Advantages	Limitations
Cycloheximide	Study of cognitive deficits and neurotransmitter roles in memory	Better suited for cognitive deficits, not AD pathology
Phenytoin	1. Explores cognitive deficits in dementia and epilepsy 2. Screens memory enhancing agents	Non-reliable model for AD pathology
D-Galactose	1. Widely used for investigating aging brain and AD pathology 2. Replicates all AD hallmarks	Insulin resistance observed, potential for diabetes
Dizocilpine	1. Studies cognitive deficits 2. Screens drugs for cognitive dysfunction	1. Animals exhibit schizophrenia-like behavior 2. Does not replicate AD hallmarks
Diazepam	1. Studies cognitive deficits 2. Screens memory enhancing agents	1. Does not replicate AD pathological hallmarks 2. Causes CNS depression
Lipopolysaccharide	1. Induces neuroinflammation similar to AD 2. Allows study of inflammatory mechanisms	1. Variable model outcomes depending on dosage and timing 2. Invasive administration

Source Rapaka et al. (2022); Zakaria et al. (2017)

Based on the review of experimental studies on BM in animal models in Table 2.5, it was found that various models inducing cognitive impairment through different mechanisms were used. The mechanisms of action of BM involve antioxidant neuroprotection, anti-inflammatory activity, anti-apoptotic activity, and interactions with various receptors, proteins, and enzymes. Additionally, BM can modulate neurotransmitters, leading to improved learning and memory (Sukumaran et al., 2019), and it can reduce psychological symptoms such as anxiety.

According to Table 2.6 BM was administered via oral gavage in various ways, including as a pre-treatment at doses of 20-120 mg/kg, co-treatment at doses of 40-500 mg/kg, and post-treatment at doses of 50-100 mg/kg. The duration of administration ranged from 7 days to 8 months. All studies used male rats or mice. Only 3 studies included comparisons with positive control groups, specifically using donepezil, which helps improve attention, memory, and the ability to complete daily tasks in dementia, and piracetam, which is used for epilepsy, dizziness, and a learning disorder marked by difficulty reading, as well as for the treatment of memory impairment. The results as Table 2.7 showed that BM extract at doses of 10-500 mg/kg could enhance cognitive function by increasing learning and spatial memory, reducing oxidative stress, and enhancing neurotransmitter activity similarly to both drugs, but not significantly better.

For antioxidant neuroprotection, BM showed mechanisms involving various actions such as reducing ROS levels, which decrease intracellular protein oxidation, lowering lipid peroxidation (LPO) and 3,4-methylenedioxyamphetamine (MDA) levels, which are causes of cell injury, and reducing free radicals by increasing the activity of superoxide dismutase (SOD) and catalase (CAT). Additionally, it includes normalizing reduced glutathione (GSH) levels and increasing the activities of glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST). According to the study by Saini et al. (2012), the administration of BM extract at 50 mg/kg in dementia induced by colchicine reduced oxidative stress. This reduction included increased 38% GPx levels in the cortex, elevated 20% SOD levels in the hippocampus, and enhanced activity of CAT (27%, 32%), GSH (33%, 48%), GR (34%, 38%), and GST (21%, 32%) in both the cortex and hippocampus. That showed prevention of cognitive decline through free radical scavenging activity and antioxidant enzyme action.

Table 2.5 Characteristics of animals in animal studies of *Bacopa monnieri* with cognitive and memory impairment associate with Alzheimer's disease

Species	Sex	Age	Weight (g)	Animals (n)	Group (n)	Animal per group (n)	References
Wistar rats	Male	N/A	220-260	30	5	6	(Murugaiyan & Bhargavan, 2021)
Wistar rats	Male	N/A	220-260	60	5	12	(Murugaiyan & Bhargavan, 2020)
Wistar rats	Male	3 months	220-260	30	5	6	(Murugaiyan & Bhargavan, 2020)
Albino rats	Male	3 months	200±50	24	4	6	(Mahitha & John Sushma, 2017)
Wistar rats	Male	N/A	200-250	24	4	6	(Saini et al., 2019)
Wistar rats	Male	N/A	200-250	24	4	6	(Saini et al., 2012)
Wistar rats	Male	8 weeks	180-200	48	6	8	(Uabundit et al., 2010)
Swiss albino mice	Male	3-5 months	25-35	56	7	8	(Saraf et al., 2011)
Wistar rats	Male	N/A	130-150	42	7	6	(Pandareesh et al., 2016)
Sprague-Dawley rats	Male	2-3 months	250-300	36	12	3	(Deore et al., 2023)

Table 2.5 (continued)

Species	Sex	Age	Weight (g)	Animals (n)	Group (n)	Animal per group (n)	References
Swiss albino mice	Male	14 weeks	N/A	28	4	7	(Pandey & Prasad, 2017)
Wistar albino rats	Male	6-8 months	450-500	32	4	8	(Khan et al., 2015)
PSAPP mice	Male	2 months	N/A	54	6	7-11	(Holcomb et al., 2006)

Table 2.6 Characteristics of models in animal studies of *Bacopa monnieri* with cognitive and memory impairment associate with Alzheimer's disease

Induced	Negative control	Positive control	Treatment	Active compound	Timeline of the treatment	Induced	References
aluminium chloride	$AlCl_3$ (25 mg/kg; p.o.)	N/A	BM extract (100 and 200 mg/kg; p.o.)	Contain 46% of bacosides	Co-treatment	4 weeks	(Murugaiyan & Bhargavan, 2021)
aluminium chloride	$AlCl_3$ (25 mg/kg; p.o.)	N/A	BM extract (100 and 200 mg/kg; p.o.)	Contain 40% of bacosides	Co-treatment	4 weeks	(Murugaiyan & Bhargavan, 2020)
aluminium chloride	$AlCl_3$ (25 mg/kg; p.o.)	N/A	BM extract (100 and 200 mg/kg; p.o.)	Contain 40.51% of bacosides	Co-treatment	4 weeks	(Murugaiyan & Bhargavan, 2020)
aluminum maltolate	AlM (100 mg/kg; p.o.)	N/A	BM extract (40 mg/kg)	N/A	Co-treatment	5 weeks	(Mahitha & John Sushma, 2017)
colchicine	Colchicine (15 µg/5 µl; i.c.v)	N/A	BM extract (50 mg/kg; p.o.)	N/A	Post-treatment	15 days	(Saini et al., 2019)
colchicine	Colchicine (15 µg/5 µl; i.c.v)	N/A	BM extract (50 mg/kg; p.o.)	N/A	Post-treatment	15 days	(Saini et al., 2012)
ethylcholine aziridinium ion	AF64A (2 nmol/2 µl; i.c.v)	Donepezil (N/A)	BM extract (20, 40, and 80 mg/kg; p.o.)	Contain 5% (w/w) of total saponins	Pre-treatment and post-treatment	2 weeks (pre) and 1 week (post)	(Uabundit et al., 2010)

Table 2.6 (continued)

Induced	Negative control	Positive control	Treatment	Active compound	Timeline of the treatment	Induced	References
scopolamine	SCO (0.1 and 0.5 mg/kg; i.p)	N/A	BM extract (120 mg/kg; p.o.)	Contain 55.35% of bacosides	Pre-treatment	7 days	(Saraf et al., 2011)
scopolamine	SCO (2 mg/kg; i.p)	Piracetam (200 mg/kg; i.p.)	BM extract (10, 20, and 40 mg/kg; p.o.)	Contain 16% of bacoside A	Pre-treatment	8 days	(Pandareesh et al. 2016)
scopolamine	SCO (N/A)	Donepezil (N/A)	BM extract (200 and 500 mg/kg; p.o.) and BM bacoside-enriched (200 and 500 mg/kg; p.o.)	BM extract contain 13.11% of bacosides and BM bacoside-enriched contain 8.3% of bacosides	Co-treatment	2 weeks	(Deore et al., 2023)
streptozotocin	STZ (100 mg/kg; i.p.)	N/A	BM extract (100 mg/kg; p.o.)	Contain 58% of bacoside A and B	Post-treatment	2 weeks	(Pandey & Prasad, 2017)
streptozotocin	STZ (3 mg/kg; i.c.v)	N/A	BM extract (30 mg/kg; p.o.)	N/A	Pre-treatment	2 weeks	(Khan et al. 2015)
Genetic modified	-	N/A	BM extract (40 and 160 mg/kg)	N/A	Treatment	2 months and 8 months	(Holcomb et al. 2006)

Table 2.7 Cognitive enhancement effects of *Bacopa monnieri* with cognitive and memory impairment associate with Alzheimer's disease

Model	**Aluminium chloride**	**Aluminum maltolate**	**Colchicine**	**Ethylcholine aziridinium ion**	**Scopolamine**	**Streptozotocin**	**Genetic modified**
Oxidative stress markers	↑CAT ↑SOD ↑GPx ↓MDA	↑SOD ↑CAT ↑GPx ↓TBARS	↑SOD ↑CAT ↓GSH ↑GPx ↑GR ↑GST ↓MDA ↓protein levels ↓ROS ↓NO	N/A	↑SOD ↑CAT ↓GSH ↑GPx ↑GR ↓MDA ↓total protein	↑SOD ↑CAT ↓GSH ↑GPx ↑GST ↓TBARS	N/A
Neuroinflamatory markers	N/A	N/A	↓IL-6 ↓TNF-α ↓MCP-1	↑viable neurons ↑cholinergic neurons	N/A	N/A	N/A
Apoptosis markers	↑viable neurons ↓dark neurons	N/A	N/A	N/A	N/A	↑neuronal spine density	↓Aβ levels

Table 2.7 (continued)

Model	Aluminium chloride	Aluminum maltolate	Colchicine	Ethylcholine aziridinium ion	Scopolamine	Streptozotocin	Genetic modified
Receptors interaction	N/A	N/A	N/A	N/A	↑BDNF ↑MUS-1	N/A	N/A
Protein interaction	N/A	N/A	↓Aβ	N/A	N/A	↓lipofuscin accumulation	N/A
Enzyme interaction	↓AChE	N/A	↓AChE ↓COX-2 ↓iNOS ↓AChE ↑Na+K+ATPase	N/A	↓AChE ↑%inhibition AChE	↓AChE ↑Na+K+ATPase levels	N/A
Neurotransmitter	N/A	N/A	N/A	N/A	↑Ach ↑5-HT ↓glutamate ↓DA ↓DOPAC ↓NE levels	N/A	N/A
Biochemical marker	↓plasma corticosterone	N/A	N/A	N/A	N/A	N/A	N/A

Table 2.7 (continued)

Model	Aluminium chloride	Aluminum maltolate	Colchicine	Ethylcholine aziridinium ion	Scopolamine	Streptozotocin	Genetic modified
Behaviour	EPM; ↑%open arm entries, ↑rearing frequency, ↓grooming frequency, ↓defecation index MWM; ↓escape latency, ↑retention time	N/A	MWM; ↓escape latency, ↑retention time, ↓path distance EPM; ↑%retention	MWM; ↓escape latency	MWM; ↓escape latency and ↑retention time EPM; ↓transfer latency, MWM; ↓escape latency and ↑retention time NOS; ↑discrimination index Y-maze; ↑%alteration, MWM; ↓escape latency and ↑retention time	RAM; ↓working and reference error MWM; ↓escape latency and ↑retention time	Y-maze; ↓entries acquisition, Open field ↓recognition, open field; ↓locomotion and ↓behavior

The anti-inflammatory activity of BM involves regulating pro-inflammatory cytokines and chemokines that can lead to apoptosis. Saini et al. (2019) investigated its anti-inflammatory effects in colchicine-induced dementia. They showed that administration of 50 mg/kg of BM extract reduced levels of IL-6 and TNF-α, which are pro-inflammatory cytokines, to near normal levels (120 and 40 pg/mg protein, respectively), along with the chemokine monocyte chemoattractant protein-1 (MCP-1) in the cortex and hippocampus of rats (300 and 180 pg/mg protein, respectively). These results suggest potential improvements in cognitive function and may have an effect on ameliorating dementia symptoms.

In studies of apoptosis induced by aluminum chloride ($AlCl_3$), it was found that BM extract at a minimum dose of 100 mg/kg could protect neurons in the dentate gyrus of the hippocampus. This protection was evidenced by an increase in the number of viable neurons and a reduction in degenerated neurons (41.07 and 7.17 neurons/250 μm length, respectively) (Murugaiyan & Bhargavan, 2020) Similarly, a study by Uabundit et al. (2010) showed that the administration of a minimum dose of BM extract at 20 mg/kg increased neuron density in the Cornu Ammonis (CA1, CA2, CA3) and dentate gyrus (DG) subregions of the hippocampus (90, 90, 60, and 210 neurons/μm^2, respectively). The administration of 40 mg/kg mitigated the reduction of cholinergic neurons in only the CA1 and CA2 subregions (27 cells/μm^2) in rats induced with ethylcholine aziridinium ion (AF64A) in an AD model. This suggests that mitigating apoptosis can potentially maintain and enhance cognitive function.

Furthermore, BM shows a neuroprotective role through interactions with various receptors. According to a study by Pandareesh et al. (2016) on cognitive impairment induced by scopolamine, BM was found to up-regulate the expression of brain-derived neurotrophic factor (BDNF) and muscarinic M1 (MUS-1) receptors by 0.33 and 0.24-fold in the hippocampus, stimulating neurogenesis and protecting neurons from degeneration, creating a supportive environment for cognitive enhancement.

The accumulation of Aβ proteins is another factor that plays a major role in the progression of AD. According to a study by Holcomb et al. (2006), administration of BM extract at doses of 40 and 160 mg/kg in PSAPP mice showed evidence of lower Aβ1-40 and Aβ1-42 levels in the cortex (14 and 10 pg/mg protein, respectively). This

treatment also reversed cognitive impairment in behavioral tests, as seen in the Y-maze, by decreasing entries during acquisition (103±17.3(control), 50±5.4(BM); $p<0.05$) and reducing hyperlocomotion in the open field test (195±44(control), 81±8(BM); $p<0.05$), thereby enhancing short-term working memory and spatial navigation.

Additionally, BM extract can reduce the accumulation of lipofuscin, a cellular waste material indicative of aging and prevalent in neurodegenerative diseases like AD. At a dose of 100 mg/kg, BM extract decreased the integrated density value of lipofuscin accumulation in the prefrontal cortex (0.3) and hippocampus (0.4) in a type 2 diabetes mellitus model induced by streptozotocin, demonstrating its neuroprotective role in mitigating aging effects associated with diabetes mellitus type 2 (DM2) (Pandey & Prasad, 2017).

Importantly, cognitive impairment is associated with various enzymes that influence different mechanisms, all of which can potentially be reversed to normal with the administration of BM. The acetylcholinesterase (AChE) enzyme is responsible for metabolizing acetylcholine, a critical neurotransmitter in the nervous system that is essential for cognitive functions. Murugaiyan and Bhargavan (2020) studied the reduction of AChE activity in amnesic rats induced by $AlCl_3$ and found that BM extract at doses of 100 and 200 mg/kg significantly reversed the decreased AChE activity (252.46±6.00(AlCl3), 170.30±6.00(BM100), 118.17±4.50(BM200); $p<0.05$). Similarly, Deore (2023) showed an increase in percent inhibition of AChE (14.39±4.92%(SCO), 69.3±12.98%(BM) in a rat model of AD induced by scopolamine, using a minimum dose of 200 mg/kg.

Enzymes associated with inflammation, such as inducible nitric oxide synthase (iNOS) and Cyclooxygenase-2 (COX-2), can contribute to chronic neuroinflammation, leading to neuronal damage and cognitive decline. BM extract at a dose of 50 mg/kg has been shown to mitigate these effects by reducing the relative expression levels of iNOS (1.0 and 0.9, respectively) and COX-2 (0.55 and 0.35, respectively) in the cortex and hippocampus of rats induced with colchicine to simulate dementia. (Saini et al., 2019)

Similarly, the sodium-potassium adenosine triphosphatase (Na^+K^+ATPase) enzyme plays a crucial role in neuronal function, influencing cognitive processes such as learning, memory, and information processing in the brain. Increased enzyme

activity observed in STZ-induced conditions (1.2±0.2(STZ), 1.8±0.4(BM); $p<0.05$) is restored by BM extract at a minimum dose of 30 mg/kg in the cortex (2.86±0.62(COL), 3.31±0.35(BM); $p<0.05$) and hippocampus (1.89±0.38(COL), 2.64±0.45(BM); $p<0.05$), similar to colchicine-induced conditions. (Khan et al., 2015; Saini et al., 2012)

Pandareesh et al. (2016) studied neurotransmitter levels in the hippocampus of scopolamine-induced rats. BM extract at a minimum dose of 10 mg/kg significantly ($p<0.05$) increased levels of acetylcholine (ACh) (0.683±0.054(SCO), 1.130±0.067(BM)) and serotonin (5-HT) (163.24±18.9(SCO), 223.75±20.1(BM)), important for memory regulation and long-term potentiation. It also reduced levels of glutamate (Glu) (380.09±23.83(SCO), 176.43±17.48(BM)), dopamine (DA) (406.89±32.4(SCO), 356.21±21.4(BM)), dihydroxyphenylacetic acid (DOPAC) (260.58±22.5 (SCO), 207.55±27.2(BM)), and norepinephrine (NE) (362.76±31.6 (SCO), 237.65±20.1(BM)), thereby enhancing learning and memory, concentration, cognitive processes, and motor activity.

Apart from assessing cognitive function using various biological markers, it can also be assessed through behavioral experiments in animal studies, each employing different methodologies. BM extract at different doses has demonstrated cognitive enhancement in several aspects. These include improving learning and memory by reducing escape latency, increasing retention time, and using shorter path distances in the MWM test. Additionally, it enhances retention percentage in the Elevated Plus Maze (EPM) test, improves short-term working memory by reducing entry acquisition and discrimination index in the Y-maze test and Novel Object Recognition (NOR) test respectively, and enhances memory by decreasing working and reference errors in the Radial Arm Maze (RAM). Furthermore, the EPM has been utilized to assess anxiety, a psychological symptom of AD that show BM extract can reduce anxiety by increasing the percentage of open arm entries and rearing frequency, while decreasing grooming frequency and defecation index.

In conclusion, pre-clinical studies on BM highlight its potential for cognitive enhancement through various mechanisms, including reduction of oxidative stress, modulation of neurotransmitter levels, inhibition of neuroinflammation, modulation of apoptotic activity, and interactions with various receptors, proteins, and enzymes. These studies show BM ability to improve memory, learning, and cognitive functions

in experimental models. However, several limitations need consideration, such as variations in study designs, dosages, and duration of treatment across experiments, which may affect the reproducibility and generalizability of results. Additionally, translating findings from animal models to human clinical trials remains a crucial step to validate its efficacy and safety in cognitive enhancement.

2.2.3 Effectiveness of *Bacopa monnieri* in the Prevention and Treatment of Cognitive Impairment: A Aystematic Review of Randomized Clinical Trials

BM, an herb traditionally used in Ayurvedic medicine, has attention for its potential cognitive benefits. Pre-clinical studies, including *in vitro* and *in vivo* research, have clarified promising mechanisms of action and initial efficacy profiles. These studies provide practical insights into neuroprotective and cognitive-enhancing properties, suggesting potential utility in preventing and treating cognitive impairment. However, translating these findings from pre-clinical studies to clinical practice requires evaluation human trials. Human physiology and responses to interventions can clearly be different from animal models or laboratory settings which necessary clinical trials to validate and quantify effects in real-world scenarios for establishing efficacy, safety, and optimal dosage in human populations which crucial for informing healthcare decisions. A systematic review of clinical trials is necessary to synthesize and critically evaluate existing evidence, providing a comprehensive assessment effectiveness of BM in managing cognitive impairment. This review aims to consolidate findings and provide evidence-based recommendations for healthcare practitioners and researchers interested in utilizing BM for cognitive health.

A systematic review was conducted to study the effectiveness of BM in the prevention and treatment of cognitive impairment. A systematic review used the specialized framework of Population, Intervention, Comparison, and Outcome (PICO), as outlined in Table 2.8, and used keywords as shown in Table 2.9 to search databases, including PubMed, SCOPUS, and MDPI. The keywords include bacopa, bacopaside Brahmi, bacoside, neuroprotective, neurodegenerative, AD, dementia, and cognitive. The inclusion criteria were limited to randomized clinical trials involving healthy individuals or patients with AD or dementia, aged 18 years and older, investigating a BM extract. There were no restrictions on the type of intervention or combination of

Table 2.8 PICO framework for systematic review study

Component	Description
Population	Healthy people or patient with Alzheimer disease or dementia, aged 18 years and older.
Intervention	Administration of *Bacopa monnieri* extracts or isolated compounds, with no restrictions on the type of intervention or combination interventions.
Control	Evaluated the effectiveness of *Bacopa monnieri* or isolated compounds compared with placebo and/or control.
Outcome	Neuropsychological, neuropsychiatric, cognitive function, memory, moods, depression, anxiety, biochemical markers, biological markers.

Table 2.9 Keywords used for systematic review study

Database	Search (May 27, 2024)
PubMed	("Bacopa*" OR "Bacoside*" OR "Brahmi") AND ("Neuroprotective" OR "Neurodegenerative" OR "Alzheimer's" OR "Dementia" OR "Cognitive")
SCOPUS	TITLE-ABS-KEY ((‘bacopa’ OR 'bacoside' OR ‘brahmi’) AND (‘neuroprotective’ OR ‘neurodegenerative’ OR ‘alzheimer’s’ OR ‘dementia’ OR ‘cognitive’))
MDPI	("Bacopa" OR "Bacopaside" OR "Bacoside" OR "Brahmi") AND ("Neuroprotective" OR "Neurodegenerative" OR "Alzheimer's" OR "Dementia" OR "Cognitive")

interventions compared to placebo or control groups, and studies had to be published in English. The exclusion criteria included studies not in English, *in vitro, in vivo, and in silico* studies, case reports, case studies, and clinical trial protocols. From the search of the selected database, a total of 439 articles were found. Following removing duplicates and applying inclusion and exclusion criteria, 13 clinical trials met the specified conditions, as shown in Figure 2.6. Afterward, the articles that were obtained were extracted according to the PICO framework outlined in Table 2.10. The results of the studies utilized various measurement tools to assess changes from different perspectives. (Table 2.11) Most of these studies involved healthy participants, with only one research article focusing on individuals with AD or mild cognitive impairment due to AD (MCI-AD). A survey by Prabhakar et al. (2020) looked at how well BM (300 mg/daily) worked compared to donepezil (10 mg/daily), a drug used to treat AD and MCI-AD. They did this for 12 months and found that there were no big differences between the two treatments when it came to cognitive function and quality of life, as measured by the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and the Postgraduate Institute (PGI) Memory Scale. Although the results appeared promising, the study had participant dropouts, preventing conclusive claims about the comparative efficacy of BM and donepezil.

Furthermore, other studies conducted on healthy participants suggested that BM consumption could be related to cognitive function and memory improvements, which can possibly slow cognitive degeneration and enhance daily functioning and independence in AD patients. Additionally, its impact on various neuropsychiatric aspects, such as mood, depression, and anxiety, can improve the overall mental health and quality of life of individuals with AD.

In randomized, placebo-controlled, double-blind trials involving healthy adults, various doses of BM extract ranged from 300 to 640 mg/daily. Acute effects were seen within 2 hours of giving doses of 320 and 640 mg/daily. These effects included better cognitive function, as seen in the Stroop test, letter search test, and the serial 3s subtraction task of the Cognitive Demand Battery (CBD). This may help slow down cognitive decline in people with AD. (Benson et al., 2014; Downey et al., 2012) improved positive mood was observed, as measured by the State-Trait Anxiety Inventory (STAI) test, which can reduce symptoms of depression and anxiety.

Additionally, decreased salivary cortisol levels, indicating reduced stress, may help slow the progression of AD and improve the overall quality of life for patients. (Benson et al., 2014).

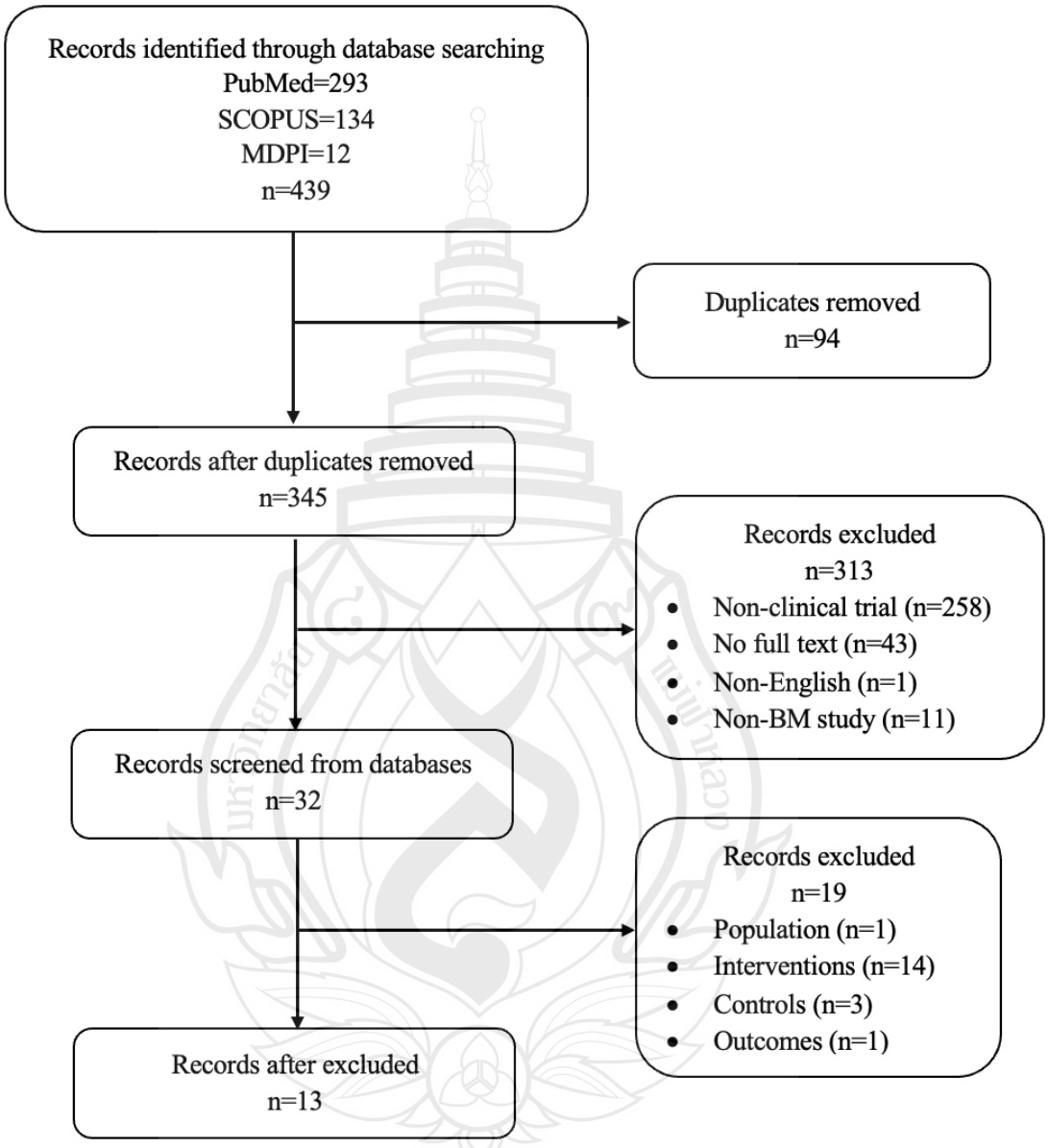


Figure 2.6 Flow diagram of systematic review study selection process

Table 2.10 Neuroprotective effects of *Bacopa monnieri* associated with cognitive and memory in clinical trials

Population	Intervention	Outcome	Result	Side effect	References
Healthy adult (18-44) n=24	BM extract (320 and 640 mg/daily) / Placebo for 2 hours	Cognitive, mood, cortisol level	↑cognitive ↑positive mood ↓cortisol level	N/A	(Benson et al., 2014)
Healthy adult (18-56) n=24	BM extract (320 and 640 mg/daily) / Placebo for 2 hours	Cognitive, stress, fatique, blood pressure	↑cognitive	gastrointestinal discomfort	(Downey et al., 2013)
Healthy adult (18-60) n=38	BM extract (300 mg/daily) / Placebo for 2 hours	Neuropsychological	No difference in cognitive	N/A	(Nathan et al., 2001)
Healthy adult (18-60) n=107	BM extract (300 mg/daily) / Placebo for 90 days	Cognitive	↑cognitive	Diarrhoea	(Stough et al., 2008)
Healthy adult (18-60) n=46	BM extract (300 mg/daily) / Placebo for 12 weeks	Neuropsychological, anxiety	↑cognitive ↑memory ↓anxiety	Nausea, dry mouth, fatigue	(Stough et al., 2001)
Healthy adult (19-22) n=60	BM extract (300 mg/daily) / Placebo for 6 weeks	Neuropsychological, biochemical	↑cognitive ↑memory ↑serum calcium level	N/A	(Kumar et al., 2016)
Healthy adult (35-60) n=72	BM extract (450 mg/daily) / Placebo for 12 weeks	Cognitive, anxiety	No difference in cognitive	N/A	(Sathyanarayanan et al., 2013)

Table 2.10 (continued)

Population	Intervention	Outcome	Result	Side effect	References
Healthy older (≥55) n=40	BM extract (250 mg/daily) / Placebo for 16 weeks	Cognitive, memory	↑cognitive ↑memory ↑mental control	Rash	(Raghav et al., 2006)
Healthy older (≥55) n=98	BM extract (300 mg/daily) / Placebo for 12 weeks	Neuropsychological	↑memory	Increase stool frequency, abdomonal cramps, nausea	(Morgan & Stevens, 2010)
Healthy older (≥55) n=28	BM extract (320 mg/daily) / Placebo for 12 weeks	Cognitive, psychological, biochemical	↑cognitive	N/A	(McPhee et al., 2021)
Healthy older (≥60) n=60	BM extract (300 and 600 mg/daily) / Placebo for 12 weeks	Cognitive, memory, biochemical	↑cognitive ↑memory ↑attention ↓AChE	N/A	(Peth-Nui et al., 2012)
Healthy older (≥65) n=54	BM extract (300 mg/daily) / Placebo for 12 weeks	Cognitive, anxiety, depression, heart rate	↑cognitive ↓anxiety ↓depression ↓heart rate	Flu-like symptoms, digestive problems	(Calabrese et al., 2008)

Table 2.10 (continued)

Population	Intervention	Outcome	Result	Side effect	References
AD and MCI-AD (≥50) n=34	BM extract (300 mg/daily) / Donepezil (10 mg/daily) for 12 months	Neuropsychological	No difference between BM and donepezil	Nausea, diarrhea, asthenia, arthralgia, headache, dizziness, anxiety, restlessness, insomnia, crying	(Prabhakar et al., 2020)

Table 2.11 Measurement tools used in clinical trials of *Bacopa monnieri* associated with cognitive and memory

Measurement tools	Purpose	Interpretation
Memory Tests		
Alzheimer's Disease Assessment Scale (Cognitive) (ADAS-Cog)	Assesses the severity of cognitive symptoms of AD	Higher scores indicate greater cognitive impairment
Alzheimer's Disease Cooperative Study (ADCS) Activities of Daily Living Inventory	Evaluates daily living activities in individuals with AD	Lower scores indicate higher impairment in daily functioning
Auditory Verbal Learning Test (AVLT)	Measures verbal learning and memory	Performance is evaluated based on the number of words recalled over several trials and delayed recall
Digit Backward	Assesses working memory and attention by having participants recall a sequence of digits in reverse order	Lower accuracy indicates potential working memory issues
Digit Forward	Evaluates attention and immediate memory span	Participants repeat a sequence of digits as presented. Shorter spans suggest attentional or memory difficulties
Digit Span (DS)	Tests both forward and backward digit recall, providing a comprehensive assessment of working memory capacity	Lower scores may indicate cognitive deficits
Logical Memory Test (Story Recall)	Measures episodic memory by having participants recall a story	Performance is based on the amount of information accurately recalled
Memory Complaint Questionnaire	Assesses subjective memory complaints, providing insight into perceived memory problems	Higher scores suggest greater perceived memory difficulties

Table 2.11 (continued)

Measurement tools	Purpose	Interpretation
Memory Span for Nonsense Syllables	Assesses short-term memory using non-meaningful syllables to minimize the influence of prior knowledge	Lower scores indicate short-term memory challenges
Paired Associate Task	Evaluates associative memory by requiring participants to remember pairs of unrelated items	Poor performance may suggest associative memory issues
Postgraduate Institute (PGI) Memory Scale	Comprehensive tool for assessing various aspects of memory, including short-term, long-term, and working memory	Lower scores indicate potential memory impairments
Prospective and Retrospective Memory Questionnaire (PRMQ)	Measures self-reported memory failures in daily life, distinguishing between prospective (future-oriented) and retrospective (past-oriented) memory	Higher scores indicate more frequent memory issues
Quality of Life-AD	Assesses the quality of life in individuals with AD, focusing on the impact of cognitive decline	Lower scores indicate a diminished quality of life
Rey-Osterrieth Complex Figure Test (CFT)	Evaluates visuospatial constructional ability and visual memory by having participants reproduce a complex figure	Poor performance indicates potential visuospatial or memory issues.
Visual Reproduction	Measures visual memory by having participants recall and reproduce visual stimuli	Lower accuracy indicates potential visual memory deficits

Table 2.11 (continued)

Measurement tools	Purpose	Interpretation
Attention and Processing Speed		
Animal Names Test (Categorical)	Assesses verbal fluency by having participants name as many animals as possible within a time limit	Lower scores may indicate cognitive or linguistic impairments
Digit Symbol Substitution Test (DSST)	Measures processing speed, attention, and executive function by requiring participants to match symbols to numbers under time constraints	Lower scores indicate slower processing speed
Divided Attention Task (DAT)	Evaluates the ability to divide attention between tasks.	Poor performance suggests difficulties in multitasking and divided attention
Inspection Time (IT)	Assesses the speed of perceptual processing by measuring how quickly participants can identify visual stimuli	Shorter times indicate faster processing speed
Letter Search	Tests visual attention and processing speed by having participants identify specific letters within a sequence	Lower accuracy or slower speed indicates attentional deficits
Rapid Visual Information Processing Task (RVIP)	Measures sustained attention and processing speed by having participants identify target sequences of numbers	Poor performance indicates attentional issues
Reaction Time (RT)	Assesses the speed of response to stimuli	Longer reaction times may indicate slower cognitive processing or attentional deficits
Serial 3s or 7s Subtraction Task	Measures attention and working memory by having participants subtract 3s or 7s from a starting number continuously	Errors or slower performance indicate attentional or memory issues

Table 2.11 (continued)

Measurement tools	Purpose	Interpretation
Speed of Comprehension Test (SCT)	Evaluates reading comprehension speed and cognitive processing by having participants read and comprehend passages under time constraints	Lower scores suggest processing speed deficits
Stroop Task	Assesses cognitive flexibility and executive function by having participants name the color of the ink rather than the word itself	Higher interference scores indicate cognitive control issues
Symbol Digit Modalities Test (SDMT)	Measures processing speed, attention, and visual-motor coordination	Lower scores indicate slower processing speed or attentional deficits
Trail Making Test (TMT)	Evaluates visual attention and task switching by requiring participants to connect a sequence of numbers/letters in order	Longer times indicate potential cognitive flexibility issues
Verbal Fluency-Controlled Oral Word Test (Phonemic)	Assesses verbal fluency by having participants generate words starting with a specific letter within a time limit	Lower scores indicate linguistic or cognitive impairments
Visual Tracking	Measures sustained attention and visual-motor coordination by having participants track moving objects	Poor performance indicates attentional or coordination deficits
Wechsler Adult Intelligence Scale (WAIS)	Comprehensive tool assessing various cognitive domains including verbal comprehension, perceptual reasoning, working memory, and processing speed	Lower scores suggest broader cognitive deficits

Table 2.11 (continued)

Measurement tools	Purpose	Interpretation
Comprehensive Cognitive Assessments		
CASP-19 Scale	Measures quality of life in older adults, focusing on control, autonomy, self-realization, and pleasure	Lower scores indicate diminished quality of life
Cognitive Drug Research (CDR) Computerized Assessment System	Provides a comprehensive assessment of cognitive function using a battery of computerized tests	Results indicate overall cognitive performance and specific domain deficits
Cognometer Tests of Working Memory	Evaluates various aspects of working memory through a series of computerized tasks	Lower scores indicate working memory deficits
CogTrack™	Computerized cognitive assessment tool measuring various cognitive functions such as memory, attention, and processing speed	Results provide a detailed cognitive profile
Mini-Mental Status Examination (MMSE)	Screens for cognitive impairment by assessing functions such as arithmetic, memory, and orientation	Lower scores indicate higher levels of cognitive impairment
Event-Related and Specialized Assessments		
Event-Related Potential Assessment	Mental Arithmetic	Mental Control
Measures brain response to specific sensory, cognitive, or motor events using EEG	Assesses numerical reasoning and working memory by having participants solve arithmetic problems mentally	Measures cognitive control and flexibility by having participants perform tasks requiring mental manipulation of information
Abnormalities indicate potential neural processing issues	Errors or slow performance indicate cognitive deficits in these areas	Poor performance suggests executive function deficits

Table 2.11 (continued)

Measurement tools	Purpose	Interpretation
Psychological Assessment		
Center for Epidemiologic Studies Depression Scale (CESD-10)	Screens for depressive symptoms in the general population	Higher scores suggest more severe depressive symptoms
Profile of Mood States (POMS)	Assesses various mood states (e.g., tension, depression, anger, vigor) in individuals	Higher scores may indicate higher levels of negative mood or lower vigor
State-Trait Anxiety Inventory (STAI)	Measures anxiety as a temporary condition (state anxiety) and as a more general trait (trait anxiety)	Differentiates between temporary anxiety symptoms (state) and more enduring personality characteristics related to anxiety (trait)
Visual Analogue Scale (VAS)	Provides a subjective assessment of various phenomena (e.g., pain, mood) using a continuous line with endpoints representing different degrees of intensity	Participants mark on a line to indicate their perception of the phenomenon being measured

Source Alzheimer's Association (2024), Chun et al. (2021), Lorente et al. (2020)

Two out of three studies show positive effects on cognitive functions at a dose of 320 mg/daily, significantly improving Stroop test scores at 1- and 2-hours post-treatment (406.47±29.47 (baseline), 46.47 (change from baseline); p=0.028 and 406.47±29.47 (baseline), 98.24 (change from baseline); p<0.001, respectively). Similarly, the letter search test scores improved at 1- and 2-hours post-treatment (5405.29±321.52 (baseline), 450.29 (change from baseline); p=0.012 and 5405.29±321.52 (baseline), 676.18 (change from baseline); p=0.003, respectively). Additionally, the serial 3s subtraction task of the Cognitive Demand Battery (CBD) also showed significant enhancement compared to placebo during the first repetition (36.30±4.05(baseline), 4.27(change from baseline); p=0.05) and the fourth repetition (36.30±4.05(baseline), 9.09(change from baseline); p=0.02), with a significant main effect for time. However, BM at a dose of 640 mg/daily only significantly improved Stroop test scores at 1- and 2-hours post-treatment (380.00±35.54(baseline), 77.06(change from baseline); p=0.001 and 380.00±35.54(baseline), 63.53(change from baseline); p=0.003).

The Digit span backward, the Logical Memory Test, the Spatial Working Memory Test, the Rapid Visual Information Processing (RVIP) Test, the Auditory Verbal Learning Test (AVLT), and the Inspection time Test (IT) all showed improved memory and cognitive function after taking BM at a dose of 300 mg/daily for at least six weeks. (Kumar et al., 2016; Stough et al., 2001; Stough et al., 2008) These enhancements suggest the potential for BM to manage AD symptoms by improving memory, increasing the learning rate (5.9±2.1(before), 5.7±2.2(after); p=0.042), and enhancing memory consolidation, which includes reducing proactive interference (-0.9±2.7(before), 0.1±2.0(after); p=0.042) and decreasing the forgetting rate (1.2±1.2(before), 1.0±1.9(after); p=0.03). Additionally, BM improves attention and processing speed by enhancing inspection time (94.4±47.9(before), 64.5±16.7(after); p=0.018) and reducing false records in rapid visual information processing (10.00±10.40(before), 6.52±5.84(after); p=0.029). It also improves working memory and spatial awareness by increasing working memory capacity (185.11±13.16 (before), 190.64±9.39 (after); p=0.035).

Furthermore, the study by Kumar et al. (2016) showed that an increase in serum calcium levels supports neuronal health and function, potentially improving cognition, which typically declines with the progression of the disease and results in cognitive decline. (Ma et al., 2021) Additionally, the STAI test results demonstrated decreased anxiety with prolonged administration of at least 12 weeks. (Stough et al., 2001)

The trials involving healthy elders have similar results. The AVLT, Stroop test, Digit span forward, Logical memory, Spatial Recognition Task (SRT), Spatial Working Memory Task (SWM-O RT), Event-Related Potential (ERP), Paired Associate Learning, and Quality and Speed of Memory all show that giving BM extract at a dose of at least 250 mg per day improves cognitive function and memory over time. (Calabrese et al., 2008; McPhee et al., 2021; Morgan & Stevens, 2010; Peth-Nui et al., 2012; Raghav et al., 2006) Additionally, it has been shown to modulate various moods, reducing anxiety and depression, as measured by STAI and the Center for Epidemiologic Studies Depression Scale (CESD-10), respectively. (Calabrese et al., 2008) While also enhancing overall mental control of the Wechsler Memory Scale (WMS) (Raghav et al., 2006) and increasing participant attention, as measured by the continuity and power of attention test. Furthermore, these trials showed a reduction in AChE, another significant factor in AD. (Peth-Nui et al., 2012) These effects could improve the quality of life for AD patients and possibly slow the disease progression.
Although the use of BM has shown effectiveness in various treatment aspects, there are also reports of adverse events and side effects in participants who received BM. These side effects include diarrhea, abdominal cramps, nausea, digestive problems, dry mouth, fatigue, headaches, and dizziness. (Table 2.10) Therefore, it is essential to conduct further clinical trials to study the safety and side effects and provide evidence to support the pharmacological efficacy and safety profile of BM.

This systematic review study consolidates compelling scientific evidence that underscores BM potential in preventing cognitive decline among healthy adults and the elderly, accompanied by potential improvements in mood and related psychological aspects, as documented in Table 2.12 and Table 2.13. The review highlights the diverse doses of BM studied, each showing promising effects across cognitive and mood states. However, the efficacy of BM as a treatment for cognitive impairment-related diseases such as AD or dementia remains inconclusive, primarily due to the scarcity and

limitations of clinical studies in this specific patient population. Therefore, further research is imperative to establish definitive conclusions regarding effectiveness and safety in clinical settings, particularly for individuals suffering from cognitive impairments.

Table 2.12 Characteristics of the participants in clinical trials of *Bacopa monnieri* associated with cognitive and memory

Study	Population	Age	Number (Treatment/Control)	Gender (M/FM)	References
RD, DB, CO	Healthy	18-44 (25.23±5.97)	17	4/13	(Benson et al. 2014)
RD, DB, CO	Healthy	18-56 (25.25±9.30)	24	4/20	(Downey et al. 2012)
RD, DB	Healthy	18-60 (37.4±11.3)	18/20	11/27	(Nathan et al. 2001)
RD, DB	Healthy	18-16 (BM 41.6±13.4) (P 44.3±11.3)	33/29	21/41	(Stough et al. 2008)
RD, DB	Healthy	18-61 (39.4±11.4)	23/23	11/35	(Stough et al. 2001)
RD, DB	Healthy	19-22 (N/A)	60	N/A	(Kumar et al. 2016)
RD, DB	Healthy	35-60 (42±6.9)	33/33	21/45	(Sathyanarayanan et al. 2013)
RD, DB	Healthy	55-70 (N/A)	20/20	37/3	(Raghav et al. 2006)
RD, DB	Healthy	55-86 (65±7.53)	49/49	46/52	(Morgan & Stevens, 2010)
RD, DB	Healthy	57-78 (BM 68.87±5.59) (P 66.85±5.93)	15/13	12/16	(McPhee et al. 2021)
RD, DB	Healthy	≥60 (62.62±6.46)	20 (BM 300 mg) 20 (BM 600 mg) 20 (P)	23/37	(Peth-Nui et al. 2012)

Table 2.12 (continued)

Study	Population	Age	Number (Treatment/Control)	Gender (M/FM)	References
RD, DB	Healthy	≥65 (73.5)	N/A	22/32	(Calabrese et al., 2008)
RD, DB	AD and MCI-AD (HIS < 5 points MMSE > 10 with brain MRI, brain FDG PET, and CSF of Aβ and total tau)	≥50 (BM 70.18±6.73) (D 68.71±9.54)	17/17	22/12	(Prabhakar et al., 2020)

Table 2.13 Characteristics of the drugs in clinical trials of *Bacopa monnieri* associated with cognitive and memory

Treatment		**Control**		**References**
Detail	**Dose/day**	**Detail**	**Dose/day**	
KeenMind® (CDRI 08) 160 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 or 4 capsule (320 or 640 mg)	Capsule of inert plant-based materials 160 mg of plant-based materials per capsule Same shape, smell, taste, and weight as BM	1 capsule	(Benson et al., 2014)
KeenMind® (CDRI 08) 160 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 or 4 capsule (320 or 640 mg)	Capsule of inert plant-based materials 160 mg of plant-based materials per capsule Same shape, smell, taste, and weight as BM	1 capsule	(Downey et al., 2012)
KeenMind® 150 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 capsules (300 mg)	Same shape, smell, taste, and weight as BM	N/A	(Nathan et al., 2001)

Table 2.13 (continued)

Treatment		Control		References
Detail	**Dose/day**	**Detail**	**Dose/day**	
KeenMind® 150 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 capsules (300 mg)	Same shape, smell, taste, and weight as BM	N/A	(Stough et al., 2008)
KeenMind® 150 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	3 capsules (300 mg)	Same shape, smell, taste, and weight as BM	N/A	(Stough et al., 2001)
150 mg of BM extract per tablet Standardized (%w/w) 4.57 Bacoside A3, 2.13 Bacopaside II, 1.70 Bacopaside X, 2.98 Bacopasaponin C	2 tablets (300 mg)	Same shape, smell, taste, and weight as BM	2 tablets	(Kumar et al., 2016)
Bacomind™ 225 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 capsules (450 mg)	Capsule of starch Same shape and size as BM	2 capsules	(Sathyanarayanan et al., 2013)

Table 2.13 (continued)

Treatment		Control		References
Detail	**Dose/day**	**Detail**	**Dose/day**	
Standardized BM extract 125 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 capsules (250 mg)	N/A	2 capsules	(Raghav et al., 2006)
Bacomind™ 225 mg of BM extract per tablet Standardized for 40%-50% of total bacosides	1 tablet (300 mg)	Same size, color, and shape as BM	1 tablet	(Morgan & Stevens, 2010)
KeenMind® (CDRI 08) 160 mg of BM extract per capsule Standardized for no less than 55% of total bacosides	2 capsules (320 mg)	Same size and color as BM	1 capsule	(McPhee et al., 2021)
Standardized BM extract 300 and 600 mg of BM extract per tablet Standardized (BM 2 mg/mL) contain 5% (w/w) of total saponins	1 tablet (300 or 600 mg)	Same appearance, odor, and texture of BM	1 tablet	(Peth-Nui et al., 2012)

Table 2.13 (continued)

Treatment		Control		References
Detail	**Dose/day**	**Detail**	**Dose/day**	
Standardized BM extract 300 mg of BM extract per tablet Standardized for 150 mg of bacosides A and B	1 tablet (300 mg)	Same appearance, odor, and texture as BM	2 tablets	(Calabrese et al., 2008)
Standardized BM extract 300 mg of BM extract per capsule Standardized contain of bacoside-A and bacoside-B	1 capsule (300 mg)	10 mg donepezil per capsule	1 capsule (10 mg)	(Prabhakar et al., 2020)

2.3 Microencapsulation: The Innovations in Delivery

2.3.1 Overview of Microencapsulation Technology

Microencapsulation is a technology in foodsnand the therapeutic delivery of drugs employing microparticles, microspheres, or microcapsules within the size range of 1-1000 μm. (Lengyel et al., 2019) The first study of microencapsulation was in 1931 by Barrett K. Green to prepare gelatin spheres using the coacervation technique. Subsequently, the pharmaceutical industry researched and developed various dosage forms and coating materials in microencapsulated (Wen et al., 2014). Microcapsules consist of the core or base layer and the shell or outer covering (Chaturvedi & Sharma, 2024). The core material can be solid, liquid, or gaseous and may comprise various substances, including drugs, pesticides, enzymes, vitamins, flavors, aromas, and dyes (Dubey et al., 2009; Mehta et al., 2022). The shell material can be a film of a natural, semi-synthetic, or synthetic polymer such as gum Arabic, starch, gelatin, alginate, and protein (Saberi-Riseh et al., 2021). Selecting material by considering product requirements such as stabilization, reduced volatility, release characteristics, and environmental conditions (Roy et al., 2012).

The benefits of microencapsulation can enhance the properties of various biologically active compounds, including enhancing stability, improving shelf life, reducing degradation, enhancing physical performance, masking unpleasant tastes and odors, targeted delivery, and reducing toxicity (Mudrić et al., 2018). As a result, it protects the active material from humidity, oxygen, light, and other environmental factors (Calderón-Oliver & Ponce-Alquicira, 2022). In the case of herbal drugs and extracts, their absorption within the gastrointestinal tract is poor, resulting in low bioavailability (Kesarwani & Gupta, 2013). Microencapsulation can improve solubility, stability, and absorption to enhance the bioavailability of bioactive compounds. Additionally, it can target delivery and controlled release to specific parts of the gastrointestinal tract (Grgić et al., 2020; Pudziuvelyte et al., 2019).

Microencapsulation can be performed with various techniques, categorized into two main groups: chemical and physical processes (Hussain et al., 2018), with a sub-group known as physicochemical processes (Jawarkar et al., 2023). These techniques

include spray drying, cooling, fluidized bed coating, coacervation, coextrusion, emulsification, and cyclodextrin inclusion (Choudhury et al., 2021). Each method is based on different principles, with various advantages and disadvantages depending on its appropriate application (Table 2.14).

2.3.2 Benefits of Microencapsulation for the Utilization of Medicinal Plants

Currently, there are many studies of microencapsulation in medicinal plants. Medicinal plants with neuroprotective effects have been reported to increase potential and efficiency in both *in vitro* and *in vivo* pathways. Results indicated antioxidant activity through reduced oxidative stress biomarkers, anti-inflammatory potential by decreasing neuroinflammatory cytokines, and increased neurotransmitters.

In the study by Romero-Román et al. (2021), *in vitro* on PC12 cells showed that microencapsulated *Berberis microphylla* G. Forst (Calafate berries) have antioxidant activity and neuroprotective properties by the inhibition of AChE and the prevention of the cytotoxic effects of Aβ peptide. The microcapsules were prepared using the spray drying technique and operated at air temperatures of 100, 120, and 140±3 °C, with maltodextrin as the wall material, resulting in an encapsulation efficiency approaching 50%.

In another *in vitro* study by Souza et al. (2020), *Camellia sinensis* L. (Green tea) and *Malpighia emarginata* D.C. (Acerola) showed antioxidant and anti-inflammatory properties on RAW 264.7 cells through microencapsulation. Spray drying technique was used to prepare microcapsules with maltodextrin. The gas inlet temperature was set at 110°C, while the gas outlet temperature was maintained at a minimum of 10 °C. The result of co-treatment microencapsulation showed blocking the alterations in protein thiol levels. Additionally, there was a modification in the activity of antioxidant enzymes, with an increase in SOD activity and a decrease in CAT activity. Furthermore, a reduction in inflammatory cytokines IL-1β, IL-6, IL-10, and TNF-α was observed, leading to a neuroprotective effect.

Table 2.14 Principles, advantages, and disadvantages of microencapsulation techniques.

Techniques	Principles	Advantages	Disadvantages
Spray drying	Creating an emulsion or dispersion and atomizing the mixture into a drying chamber.	1. Low operating cost 2. Simple process 3. Short time process 4. Reproducibility 5. High process efficiency 6. Encapsulated product stability	1. Highly sensitive at high temperature 2. Loss of particle in drying chamber 3. Nonuniform particles 4. Forming aggregates
Spray cooling	Dispersing the core into a liquefied shell material and atomized into a cold chamber.	1. Low operating cost 2. Short time process 3. Reproducibility 4. No use water or organic solvents 5. Suitable for heat-sensitive substances	1. Specific for hydrophobic substances 2. Rapid release of substances 3. Nonuniform particles 4. Variable encapsulation efficiency
Fluidized bed coating	Suspending solid or powdered core material particles in an air stream and coating with molten polymer.	1. Simple process 2. High process efficiency 3. Uniform coating distribution	1. Specific for hydrophilic substances 2. Formation of large-size particle 3. Forming aggregates
Coacervation	Forming three immiscible chemical layers, depositing the coating, and rigidifying.	1. High loading capacity 2. Low temperature 3. Reduced thermal degradation 4. Encapsulated product stability	1. High particle isolation cost 2. Variable encapsulation efficiency 3. Use of organic solvents 4. Toxicity of the solvents used

Table 2.14 Principles, advantages, and disadvantages of microencapsulation techniques.

Techniques	Principles	Advantages	Disadvantages
Extrusion	Extruding an emulsion of core and wall material through a high-pressure then immersing in a cold solvent bath to form pellets.	1. Low process cost 2. Short time process 3. Very long shelf life 4. No use organic solvents or pH condition	1. Formation of large-size particle 2. Difficulties with viscous solutions 3. Core material stability affected from high shear force
Emulsification	Stabilizing an emulsion of the core and wall material by adding an emulsion stabilizer.	1. Use hydrophilic and hydrophobic substances 2. High process efficiency	1. Instability of the emulsion 2. Formation of large-size particle 3. Difficulties in uniform coating distribution
Cyclodextrin inclusion	Utilizing hydrophobic interaction between the cyclodextrin surface and the guest compounds.	1. Controlled release 2. Solubility and stability of hydrophobic substances 3. Reduce compounds loss 4. Reduce volatility	1. High material cost 2. Specific for low-molecular-weight compounds 3. Forming aggregates 4. Limited capacity for larger molecules

Source Calderón-Oliver and Ponce-Alquicira (2022), Chaabane et al. (2022), Choudhury et al. (2021), Favaro-Trindade et al. (2021), García-Carrasco et al. (2023), Kłosowska et al. (2023)

In addition to the spray-drying technique, medicinal plant studies have used the freeze-drying technique to produce microcapsules. Kawvised et al. (2017) studied the neuroprotective and cognitive enhancing effects in animal models using microencapsulated *Morus alba* L. (Mulberry). Maltodextrin was used as the wall material mixed with mulberry extract in a ratio of 9:1 (w/w), freeze-drying at a temperature of -86 °C for 48 hours. The microencapsulated formulation showed *in vivo* results indicating decreased AChE activity and modification of oxidative stress markers, including increased levels of SOD, CAT, and GSH-Px, and reduced levels of MDA. Additionally, it showed enhanced Erk phosphorylation, suggesting potential neuroprotective and cognitive-enhancing effects.

The neuroprotective effects of *Acrocomia aculeata* microcapsules were studied, prepared using the coacervation technique, and coated with gelatin and gum Arabic at temperatures of 40 °C, 60 °C, and 50 °C, followed by cooling to approximately 10 °C. The in vivo results showed positive antioxidant responses, including increased levels of SOD, CAT, and GSH, along with decreased levels of MDA and protein carbonylation, potentially protecting against neurodegenerative diseases (Jacobowski et al., 2021).

Additionally, the coacervation technique has been used to study *Spinach oleracea* (Spinach), *Beta vulgaris* (Red beet), and *Brassica oleracea var. italica* (Broccoli) in animal experiments. The microcapsules were prepared by dissolving the extract in whey protein concentrate solution at a ratio of 1:10, then mixed with gum arabic. The process was conducted at 25 °C and then cooled to 5 °C before freeze-drying at –52 °C for 48 hours. The highest encapsulation efficiency was in red beet at 81.3%, followed by broccoli and spinach, with an efficiency of over 60%. The results from each sample showed an impact on acetylcholinesterase activity by inhibiting AChE and butyrylcholinesterase (BChE) activities, increasing neurotransmitters 5-hydroxytryptamine (5-HT) and dopamine (DA), BDNF levels, and reducing glial fibrillary acidic protein (GFAP) levels. Furthermore, there was a reduction in oxidative stress, with increased levels of SOD, GSH, and GSH-Px, and decreased levels of CAT, MDA, and NO, which are oxidative and inflammatory markers. These results demonstrate that plants have antioxidant, anti-inflammatory, and neuroprotective properties (Soliman et al., 2022) (Table 2.15).

At present, research on encapsulation techniques in BM is limited. Studies used polymersomes of polyethylene glycol-S-S-polylactic acid-polycaprolactone (PEG-S-S-PLA-PCL-OH) to encapsulate bacosides using emulsification. The ratio of triblock copolymer to bacosides was 1:1, 1:2, and 1:5, and the process was conducted at 4 °C, resulting in particles with sizes ranging from 110-120 nm and an encapsulation efficiency of 60%. *In vivo,* studies showed a reversal of memory loss in chemically induced amnesic mice and the epigenetic changes in their brains, supported by significant changes in the gene expression profiles of Activity-regulated cytoskeleton-associated protein (Arc), BDNF, and cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) (Goyal et al., 2018; Goyal et al., 2020).

Additionally, encapsulation techniques are used to develop innovations. Solid lipid nanoparticles (SLNs) of BM were produced and loaded into microneedles to manage Parkinson's disease (PD). This study used the emulsification method to develop various formulations using glyceryl monostearate (GMS) as the lipid shell material. Among the tested formulations, the optimal ratio of 1:1 with Tween 80 in distilled water at a temperature of 75 °C resulted in particles with a size of 128 nm and a drug release of 79.21%. The BM-SLNs were loaded into transdermal microneedle patches to investigate their neuroprotective effects in PD-induced rats. *In vivo* results showed that the microneedle patch containing BM-SLNs had better neuroprotective activity than the pure drug and enhanced drug bioavailability through transdermal absorption, as GSH, CAT and SOD. Furthermore, it improves rats' motor coordination and balancing abilities (Joy et al., 2022) (Table 2.16).

Table 2.15 Microencapsulation techniques used in neuroprotective medicinal plants.

Plant name	Techniques	Coating materials	Model	Neuroprotective effects	References
***In vitro* studies**					
Berberis microphylla G. Forst (Calafate berries)	Spray drying	Maltodextrin	PC12 cells	↑antioxidant activity ↓AChE ↓Aβ peptide cytotoxicity	(Romero-Román et al., 2021)
Camellia sinensis L. (Green tea)	Spray drying	Maltodextrin	RAW 264.7 cells	↑SOD ↑protein thiol levels ↓CAT ↓IL-1β, IL-6, IL-10, TNF-α	(Souza et al., 2020)
Malpighia emarginata D.C. (Acerola)	Spray drying	Maltodextrin	RAW 264.7 cells	↑SOD ↑protein thiol levels ↓CAT ↓IL-1β, IL-6, IL-10, TNF-α	(Souza et al., 2020)
***In vivo* studies**					
Acrocomia aculeata	Coacervation	Gelatin and gum arabic	Rats	↑SOD, CAT, GSH ↓MDA ↓ protein carbonyls	(Jacobowski et al., 2021)
Beta vulgaris (Red beet)	Coacervation	Gum arabic and whey protein	Rats	↑Dopamine, 5-hydroxytryptamine ↑BDNF ↑SOD, GSH, GSH-Px ↓AChE, BChE ↓GFAP ↓CAT, MDA, NO	(Soliman et al., 2022)
Brassica oleracea var. italica (Broccoli)	Coacervation	Gum arabic and whey protein	Rats	↑Dopamine, 5-hydroxytryptamine ↑BDNF ↑SOD, GSH, GSH-Px ↓AChE, BChE ↓GFAP ↓CAT, MDA, NO	(Soliman et al., 2022)

Table 2.15 (continued)

Plant name	Techniques	Coating materials	Model	Neuroprotective effects	References
Morus alba L. (Mulberry)	Freeze drying	Maltodextrin	Rats	↑SOD, CAT, GSH-Px ↑ACh ↑phosphorylation of Erk ↓oxidative stress ↓MDA	(Kawvised et al., 2017)
Spinach oleracea (Spinach)	Coacervation	Gum arabic and whey protein	Rats	↑Dopamine, 5-hydroxytryptamine ↑BDNF ↑SOD, GSH, GSH-Px ↓AChE, BChE ↓GFAP ↓CAT, MDA, NO	(Soliman et al., 2022)
Beta vulgaris (Red beet)	Coacervation	Gum arabic and whey protein	Rats	↑Dopamine, 5-hydroxytryptamine ↑BDNF ↑SOD, GSH, GSH-Px ↓AChE, BChE ↓GFAP ↓CAT, MDA, NO	(Soliman et al., 2022)

Table 2.16 Encapsulation techniques used in *Bacopa monnieri.*

Techniques	Coating materials	Ratio	Temperature	Encapsulation efficiency	Size	References
Emulsification	PEG-S-S-PLA-PCL-OH	1:1, 1:2, 1:5	4 °C	60%	110 nm	(Goyal et al., 2018)
Emulsification	PEG-S-S-PLA-PCL-OH	1:1, 1:2, 1:5	4 °C	N/A	120 nm	(Goyal et al., 2020)
Emulsification	GMS	1:1	75 °C	79.21%	128 nm	(Joy et al., 2022)

2.3.3 Microcapsules: Enhanced Bioavailability and Targeted Delivery

The use of microencapsulation techniques is highly beneficial in overcoming various biological limitations. Several studies have shown the efficacy of enhancing bioavailability through improved solubility, leading to enhanced drug dissolution, absorption, and pharmacokinetics. Furthermore, increased drug release promotes a more significant portion of the administered dose being available for absorption and bioaccessibility. Additionally, enhancing stability to heat and light helps preserve the integrity and efficacy of the drug molecules from formulation to absorption. In the study by Ahmadian et al. (2019), microencapsulation of *Crocus Sativus* L. (saffron) was conducted using spray drying and freeze-drying techniques, with maltodextrin and pectin employed as wall materials. The results showed that microencapsulation improved solubility in water, delayed thermal degradation by reducing the percentage of weight loss at higher temperatures, and enhanced the release of polyphenols under simulated conditions of gastrointestinal digestion environments.

Additionally, increasing drug release can also promote targeted delivery through controlled release under specific conditions. Microencapsulation techniques are used to enhance release and prevent degradation during digestion in various experiments simulating gastrointestinal digestion, including oral, gastric, intestinal, and colonic phases. This controlled release allows for specific targeting of organs, thereby increasing the bioavailability and efficacy of drugs or substances. In the study by González et al. (2019), microencapsulation of *Olea europaea* (olive) leaf oil was conducted using spray drying techniques with sodium alginate as wall materials. The results showed that microencapsulation can protect oleuropein, a phenolic compound, under gastric conditions and gradual release under intestinal conditions, leading to higher bioaccessibility and potential bioavailability. However, the substance and the wall material eventually degrade under colonic conditions (Table 2.17).

Table 2.17 Microencapsulation techniques used to enhance bioavailability and target delivery.

Name	Techniques	Coating materials	Ratio	Encapsulation efficiency	Effect	References
Aronia melanocarpa (Chokeberry)	Spray drying	Maltodextrin and skimmed milk	NA	27.31-97.30%	↑drug release ↑digestion stability	(Ćujić-Nikolić et al., 2019)
Crocus Sativus L. (Saffron)	Spray drying and freeze drying	Maltodextrin and pectin	1:3	64.78-86.77%	↑solubility ↑drug release ↑controlled drug release ↑heat stability	(Ahmadian et al., 2019)
Curcuma longa (Turmeric)	Coacervation	Gelatin and chitosan	30:1%	82.69%	↑drug release ↑heat stability ↑light stability	(Ang et al., 2019)
	Spray drying	HI-CAP100 and whey protein or maltodextrin and whey protein	1:1	68.01-84.99%	↑drug release ↑controlled drug release ↑heat stability	(Patel et al., 2022)

Table 2.17 (continued)

Name	Techniques	Coating materials	Ratio	Encapsulation efficiency	Effect	References
Curcuma longa (Turmeric)	Emulsification	Maltodextrin and gelatin	1:10	N/A	↑absorption ↑digestion stability ↑controlled drug release	(Ashraf et al., 2022)
	Coacervation	*Cicer arietinum* (Chickpea) protein	1:10	78.6%	↑drug release ↑heat stability ↑light stability	(Ariyarathna & Karunaratne, 2016)
Elsholtzia ciliata (Vietnamese balm)	Spray drying	Sodium caseinate, skim milk, maltodextrin, resistant-maltodextrin, gum arabic, and beta-cyclodextrin	NA	42.5-99.9%	↑solubility	(Pudziuvelyte et al., 2019)
Flourensia spp. *(F. cernua, F. microphylla, and F. retinophylla)*	Gelation	Sodium alginate	1:6	30.1-63.5%	↑drug release ↑controlled drug release	(Rodríguez et al., 2019)

Table 2.17 (continued)

Name	Techniques	Coating materials	Ratio	Encapsulation efficiency	Effect	References
Hylocereus polyrhizus (Red pitaya)	Spray drying	Maltodextrin	NA	94.94%	↑solubulity	(Vieira et al., 2024)
Olea europaea (Olive)	Spray drying	Sodium alginate	1:1.6	60.8%	↑drug release ↑controlled drug release ↑bioavailability	(González et al., 2019)
Plukenetia volubilis L. (Sacha Inchi)	Spray drying	Maltodextrin and sodium caseinate	1:10	68.94-70.28%	↑bioaccessibility ↑drug release ↑solubility ↑heat stability	(Rodríguez-Cortina & Hernández-Carrión, 2023)

CHAPTER 3

METHODOLOGY

3.1 Plant Material

The standardized alcoholic extract of BM containing 21.24% bacosides (BCP-2209147), was purchased from AP Operations Co., Ltd., Thailand. The extract was light brown to greenish brown in color.

3.2 Determination of Total Phenolic and Total Flavonoid Contents in *Bacopa monnieri* Extract

3.2.1 Total Phenolic Content

The TPC of the extract was determined using the Folin-Ciocalteu method with some modifications. Briefly, 10 µL of the 5 mg/mL sample and 100 µL of Folin-Ciocalteu reagent were added to a 96-well plate, followed by the addition of 80 µL of 20% Na_2CO_3 solution (w/v). The mixtures were thoroughly mixed and incubated in the dark at room temperature for 45 minutes. Absorbance was then measured at 630 nm. The TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

3.2.2 Total Flavonoid Content

The TFC of the extract was determined using the aluminum chloride method with some modifications. Briefly, 20 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 80 µL of distilled water and 6 µL of 5% $NaNO_2$ (w/v) and incubation in the dark for 6 minutes at room temperature. Then add 6 µL of 10% $AlCl_3$ (w/v) and incubate the mixtures in the dark for another 6 minutes. Finally, 40 µL of 1 M NaOH was added and the absorbance was measured at 510 nm. The TFC was expressed as milligrams of catechin equivalents per gram of extract (mg CAE/g extract).

3.3 Determination of Antioxidant Capacity of *Bacopa monnieri* Extract

3.3.1 Metal Chelating Assay

The metal chelating activity (MCA) was used to measure the presence of excess transition metals, using ethylenediaminetetraacetic acid (EDTA) as the standard and some method modification. A volume of 40 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 80 µL of 0.25 mM $FeSO_4$ and the mixtures were incubated in the dark at room temperature for 10 minutes. The reaction was then initiated by adding 80 µL of 0.5 mM ferrozine and incubating for another 10 minutes at room temperature. Following incubation, the absorbance was measured at 562 nm. The percentage of chelating activity was calculated using the equation: MCA %inhibition = [(OD of sample – OD of blank)/OD of control] ×100.

3.3.2 DPPH Free Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay using a colorimetric method, using trolox as the standard and some method modifications. A volume of 20 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 180 µL of 80 µM DPPH ethanol solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 5 minutes. Following incubation, the absorbance was measured at 490 nm. The DPPH free radical scavenging activity was calculated using the equation: DPPH %inhibition = [(OD of sample – OD of blank)/OD of control] ×100.

3.3.3 ABTS Free Radical Scavenging Assay

The ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay using a colorimetric method, using trolox as the standard and some method modifications. The ABTS solution was prepared by mixing 2 mM ABTS and 2.45 mM $K_2S_2O_8$ solution in a ratio 1:1, then kept the mixture in the dark at room temperature for 16 hours. Then the ABTS solution was diluted with 50 mM phosphate buffer (pH 7.4) to achieve an absorbance of 0.70±0.02 at 734 nm. A volume of 20 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 200 µL of ABTS solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 6 minutes. Following incubation, the absorbance was measured at 743 nm. The ABTS

free radical scavenging activity was calculated using the equation: ABTS %inhibition = [(OD of sample – OD of blank)/OD of control] ×100.

3.3.4 Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) assay was used to measure the reduction of ferric ions to ferrous ions, using $FeSO_4 \cdot 7H_2O$ as the standard and some method modification. The FRAP solution was prepared by mixing 10 mL of 300 mM acetate buffer, 1 mL of 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), and 1 mL of 20 mM $FeCl_3$. A volume of 20 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 180 µL of FRAP solution. The mixtures were thoroughly mixed and incubated in the dark at room temperature for 30 minutes. Following incubation, the absorbance was measured at 593 nm. The FRAP value was expressed as micromoles of $FeSO_4$ per milligram of extract (µM $FeSO_4$/mg extract).

3.3.5 Nitroblue Tetrazolium Assay

The nitroblue tetrazolium (NBT) assay was used to measure the scavenging activity of superoxide radicals, using trolox as the standard and some method modification. The reagent was prepared by mixing 1 mL of 1 mg/mL of NBT, 1 mL of 10 mg/mL riboflavin, 1 mL of 10 mM methionine, and 2 mL of 150 mM phosphate buffer (pH 7.4). A volume of 100 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 100 µL of NBT solution. The mixtures were thoroughly mixed and incubated under illumination with fluorescent lamps (20 W) at room temperature for 10 minutes. Following incubation, the absorbance was measured at 560 nm. The ability to superoxide radical was calculated using the equation: NBT %inhibition = [(OD of sample – OD of blank)/OD of control] ×100.

3.3.6 Oxygen Radical Antioxidant Capacity Assay

The oxygen radical antioxidant capacity (ORAC) assay was used to measure the scavenging of free radical through hydrogen atom transfer-based mechanism, using trolox as the standard some method modification. The fluorescein solution was prepared by mixing 40 nM fluorescein with 7.5 mM phosphate buffer (pH 7.4) in a ratio 1:9. A volume of 25 µL of the 5 mg/mL sample was added to a 96-well plate, followed by 150 µL of fluorescein solution and 25 µL of 153 mM 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH). The mixtures were thoroughly mixed and incubated in the

dark at 37 °C for 15 minutes. Following incubation, the absorbance was at an emission wavelength of 523 nm and an excitation wavelength of 485 nm with readings taken every 5 minutes for 3 hours. The ability to scavenge of free radical was expressed as micromole of trolox equivalents per milligram of extract (mg TE/mg extract).

3.4 Identification of Phytochemical Compounds in *Bacopa monnieri* Extract

The high-performance liquid chromatography (HPLC) analysis was performed using a Waters/Acquity Arc system with a PDA detector (HPLC 2998, 800 nm) from Waters Corporation, USA. Separation was achieved on a Kinetex EVO C18 reverse-phase column (150 mm × 2.1 mm, 5 µm) from Phenomenex, USA using a binary gradient mode with acetonitrile as solvent A and orthophosphoric acid as solvent B. The gradient program was as follows: 0-25 min, 30%-40% A; 25-30 min, 40%-60% A; 30-32 min, 60% A; 32–35 min, 60%–30% A; and 35–40 min, 30% A. The column temperature was maintained at 30 °C. The flow rate was set to 0.2 mL/min, and the injection volume for both sample and standard solutions was 5 µL. Chromatograms were acquired at a detector wavelength of 205 nm.

3.5 Evaluation of Toxicity in *Bacopa monnieri* Extract

3.5.1 Food Clearance Assay

The 10 µL of *Caenorhabditis elegans* (*C. elegans)* L1 stage from the synchronized S-medium buffer was mixed into a 96-well plate with 80 µL of *E. coli* OP50 (OD570 nm = 0.6) and 10 µL of the extracts at concentrations of 0.1, 0.25, 0.5, and 1.0 mg/mL. Then incubated at 20°C and measure the absorbance at 570 nm for 7 days.

3.5.2 Pharyngeal Pumping Assay

The 10 µL of *C. elegans* L4 stage from the synchronized S-medium buffer was mixed into a 96-well plate with 80 µL of *Escherichia coli (E. coli)* OP50 (OD570 nm = 0.6) and 10 µL of the extracts at concentrations of 0.1, 0.25, 0.5, and 1.0 mg/mL.

Then after incubated at 20°C for 24 hours, *C. elegans* were randomly counted by observing the contraction and relaxation of their pharyngeal muscles under a microscope for 1 minute, with each measurement repeated three times. The results were reported as the feeding rate of *C. elegans* per minute.

3.6 Cognitive Enhancement of *Bacopa monnieri* Extract

3.6.1 Animals

Male Sprague-Dawley rats (SPF grade), weighing 280-300 g were were used in this study to eliminate the influence of female hormones on the results. The animals were obtained from Beijing Huafukang Biotechnology Co., Ltd., China. The rats were housed in opaque polypropylene cages (28 × 21 × 14 cm) in groups of 5 per cage with provided with ad libitum feed under standard laboratory conditions as a temperature of 25±2 °C and a 12-hour natural light/dark cycle. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Chengde Medical College, China (Approval No. CDMULAC-20240715-033). The rats were acclimatized for 7 days to the start of the experiment and were treatment naive.

3.6.2 Drug Treatments and Experimental Procedure

Animals were randomized into 4 groups with 10 rats in each group. All drugs were dissolved in 0.9% saline and administered to the animals. The extract and drugs were administered orally or intraperitoneally injection for 28 days, while LPS was injected intraperitoneally for 7 days (days 15-21). The animal groups included: (1) control animals receiving oral administration of normal saline (0.2 mL/kg), (2) animals receiving an injection of LPS (0.175 mg/kg), (3) animals receiving an injection of LPS (0.175 mg/kg) and rivastigmine (0.175 mg/kg), and (4) animals receiving an injection of LPS (0.175 mg/kg) and oral administration of *Bacopa monnieri* extract (70 mg/kg). Behavioral assessments were conducted, including the MWM test on days 22-26 and the Y-maze test on day 27. The animals were then sacrificed on day 28.

3.6.3 Morris Water Maze Test

The MWM test measures learning and spatial memory, the procedure was used in previous published study with some modification. The water maze consisted of a circular pool with a diameter of 150 cm and a height of 50 cm, partitioned into 4 equal quadrants. A submerged platform (10 × 10 cm), painted white, was placed in 1 quadrant so that its height was 1 cm below the water surface. The pool was filled with water at 28-30°C to a depth of 30 cm and made opaque with milk powder. Several brightly colored cues were positioned around the pool for spatial orientation, and their positions remained consistent throughout the analysis.

The animals were training on day 1-4 with 4 consecutive trials with two sessions in the morning and two sessions in the afternoon. For each trial, the animals were individually placed in the pool and allowed 60 seconds to find the platform. If they failed to locate the platform within 60 seconds, they were gently guided to it and allowed to stay on it for 30 seconds. The animals were placed randomly in different quadrants each trial. The relative location of the maze and surroundings remained consistent throughout the study. On day 5, the platform was removed from the target quadrant and the total distance, total time, and number of times the animal entered the target quadrant were recorded using a computer-connected camera and calculated.

3.6.4 Y-maze Test

The Y-maze test measures working memory base on the natural exploratory of animals. The maze consisted of 3-armed design resembling the letter Y. Each arm measured 8 cm in width, 30 cm in length, and 15 cm in height, positioned 120° apart from each other. During the training trial, insert a partition in the novel arm (C) to block the rats from entering. The animals were gently placed in arm A and allowed to explore the maze for an adaptation period of 10 minutes. After a 30-minute interval, remove the partition in the novel arm (C) and record the total distance, total time, and the number of entries the rats make into the novel arm (C) for a duration of 5 minutes.

Results were evaluated based on spontaneous alteration behaviors and the number of entries into the novel arm. spontaneous alteration behaviors was defined as the sequential entry pattern ABC, CAB, and BCA. An arm entry was recorded when all four paws of the mouse were within the novel arm. The percentage of alteration was calculated using the equation: [(number of alterations) / (total arm entries - 2)] × 100.

3.7 Microencapsulation of *Bacopa monnieri* Procedures

Preparation of the procedures began with solution preparation: (A) BM extract (AP Operations Co., Ltd., Thailand) was dissolved in distilled water to obtain a 5% (w/v) solution (1,000 mL). The solution was homogenized at 10,000 rpm for 15 minutes with 1-minute pauses every 5 minutes, followed by continuous stirring for 2 hours at room temperature under a fume hood and (B) unripe banana flour (Lot No.03/2025, Musarium Co., Ltd., Thailand) was dissolved in distilled water to obtain a 10% (w/v) solution (1,000 mL). The mixture was heated to 70°C with continuous stirring for 1 hour and then allowed to cool to room temperature. Solutions (A) and (B) were then combined to a final volume of 20,000 mL and maltodextrin with a dextrose equivalent (D.E.) of 10-12 (Vicchi Enterprise Co., Ltd.) was added to achieve a final concentration of 20% (w/v). The combined mixture was homogenized again at 10,000 rpm for 15 minutes with 1-minute pauses every 5 minutes, followed by continuous stirring for another 2 hours at room temperature resulting in the final solution (C). Solution (C) was then spray-dried using a laboratory-scale spray dryer equipped with a two-fluid nozzle (Minor-mode E, Niro A/S, China) at a feed rate of 10 mL/min and a compressor pressure of 1-3 bar. The encapsulation yield and physicochemical properties of the resulting microcapsules were recorded and calculated (Osamede Airouyuwa & Kaewmanee, 2019; Vonghirundecha et al., 2022).

3.8 Physicochemical Characterization of Microcapsule

The physicochemical parameters of BM microencapsulated with maltodextrin and unripe banana flour were characterized included color parameters, particle morphology, size distribution, bulk density, tapped density, flowability, cohesiveness, moisture content, water activity, hygroscopicity, and water solubility index according to published procedures. (Vonghirundecha et al., 2022)

3.8.1 Particle Size

The average particle size and particle size distribution of the microcapsules were measured using a Malvern Scirocco 2000 particle size analyzer (Malvern Instruments, Worcestershire, U.K.). During the experiments, the sample was suspended in isopropanol and constantly stirred.

3.8.2 Particle Morphology

The morphology of the microcapsule powder was evaluated using scanning electron microscopy (SEM; SEM JSM5800LV model, Japan) at magnifications of 1500×.

3.8.3 Color

The microcapsule color measurements were performed using a color analyzer (MiniScan X.E., Model 45/0S, Hunter Associates Laboratory Inc., USA) equipped with a standard illuminant D65, an area view of 19.812 mm, and an observer angle of 10°. The measurements were made using the L^* (lightness), a^* (+; redness to -; greenness), and b^* (+; yellowness to -; blueness) instrumental scale

3.8.4 Bulk and Tapped Density

The bulk density (ρ_{bulk}) was determined as the ratio of the mass of the samples to the volume they occupy. For tapped density (ρ_{tapped}), 5 g of samples were measured into a 25 mL graduated cylinder and manually tapped repeatedly from a vertical distance of 10 cm until the volume change was minimal. The tapped density was then calculated as the mass per volume (g/mL), using the apparent volume (V) and mass (M) of the powder.

3.8.5 Flowability and Cohesiveness

The flowability and cohesiveness of the microcapsule samples were calculated based on their bulk and tapped densities and expressed as Carr's index (CI) and Hausner ratio (HR), respectively.

3.8.6 Hygroscopicity

The hygroscopicity of the sample was determined by placing 300 mg of the powder samples in a desiccator filled with a saturated NaCl solution for 1 week at 25°C (75.29% relative humidity; aw = 0.75), followed by recording the weight of the samples after storage. The hygroscopicity was presented as grams of adsorbed moisture (g/100 g) per 100 grams of the original sample.

3.8.7 Moisture Content, Water Activity, and Water Solubility Index

The moisture content of the microcapsule powder (1 g) was reported as the percentage of weight loss (%) following 4 hours of oven drying at 105 °C until a stable weight was achieved. The water activities were measured using water activity meters (Aqua Lab, Series 3 C.E., USA). The solubility of the resultant microcapsules was examined by dissolving 1 g of the powder in 12 mL of distilled water in a laboratory water bath at 30°C for 30 minutes, followed by centrifugation at 1000×g for 15 minutes. After collecting the supernatant, it was oven-dried at 105°C for 12 hours. The water solubility index (WSI) was expressed as a percentage.

3.9 Determination of Total Phenolic and Total Flavonoid Contents in Microcapsule

The microencapsulated standardized BM extract was reanalyzed for total phenolic and total flavonoid contents to determine which formulation contained the highest levels of phytochemical compounds using TPC and TFC assay.

3.10 Determination of Antioxidant Capacity of Microcapsule

The microencapsulated standardized BM extract was reanalyzed for antioxidant capacity to determine which formulation showed the strongest activity using MCA, DPPH, ABTS, FRAP, NBT, and ORAC assays.

3.11 Identification of Phytochemical Compounds in Microcapsule

3.11.1 Metabolite Profiling Analysis

The liquid chromatography-mass spectrometry (LC-MS) analysis was performed using a An Agilent LC-QTOF 6545XT 6495 system (Agilent Technologies Inc., USA). Using a binary gradient system with 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B, liquid chromatographic separation

was accomplished on a Poroshell 120 EC-C18 (100 mm × 2.1 mm, 2.7 μm) from Agilent Technologies Inc., USA. 0-0.5 min, 100% A; 0.5-10.5 min, 100% to 45% A; 10.5-12.5 min, 45% to 25% A; 12.5-14 min, 25% to 0% A; 14-17 min, 0% A; 17-17.5 min, 0% to 100% A; and 17.5-20 min, 100% A comprised the gradient program. The temperature of the column was kept at 50°C. The injection volume was 10 μL, and the flow rate was set at 0.4 mL/min. The mass spectrometer operated at high resolution in both positive and negative ionization modes: capillary voltage at 4000 V (positive) and 3000 V (negative), 325°C gas temperature, 13 L/h gas flow, 45 psi nebuliser pressure, 12 L/h sheath gas flow, and 275°C sheath gas temperature. The mass range was set at 40–1700 m/z for MS1 and 25–1000 m/z for MS2. Collision energy was 20 eV for positive mode and 10 eV for negative mode. The acquisition rate was 3.35 spectra/s, with a maximum of 10 precursors per cycle, a precursor threshold of 5000 counts, and a retention time threshold of 0.001%. The reference masses were 121.0509 m/z and 922.0098 m/z for positive mode, and 112.9856 m/z and 1033.9881 m/z for negative mode.

3.11.2 Quantification Analysis

The liquid chromatography-mass spectrometry (LC-MS) analysis was performed using a An Agilent LC-QTOF 6545XT 6495 system (Agilent Technologies Inc., USA). with an electrospray ionisation (ESI) source running in positive ionisation mode was used to perform the liquid chromatography-mass spectrometry (LC-MS) study. Using a binary gradient system with 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B, liquid chromatographic separation was accomplished on a Zorbax Eclipse Plus C18 column (50 mm × 2.1 mm, 1.8 μm) from Agilent Technologies Inc., USA. 0-0.5 min, 90% A; 0.5-5.5 min, 90% to 0% A; 5.5-6.5 min, 0% A; 6.5-6.6 min, 0% to 90% A; and 6.6-8 min, 90% A comprised the gradient program. The temperature of the column was kept at 40°C. For both BM110-180 microencapsulated formulation and bacoside A standard, the injection volume was 5 μL, and the flow rate was set at 0.4 mL/min. With the following settings, the mass spectrometer operated in multiple reaction monitoring (MRM) mode: capillary voltage at 3000 V, 150°C nitrogen gas temperature, 14 L/min gas flow, 20 psi nebuliser pressure, 11 L/min sheath gas flow, and 250°C sheath gas temperature.

3.12 Cognitive Function Improvement of Microcapsule

3.12.1 Animals

Male Sprague Dawley (weighing 250±20 g) were used in this study to eliminate the influence of female hormones on the results. The animals were obtained from the Comparative Medicine and Technology Unit, Institute of Bioscience, Universiti Putra Malaysia, Malaysia. The rats were housed in opaque polypropylene cages (28 × 21 × 14 cm) in groups of 2-3 per cage with provided with 60-80 g of feed and 200-300 ml per day under standard laboratory conditions as a temperature of 25±2 °C, 60-70% relative humidity, and a 12-hour natural light/dark cycle. All procedures were conducted in accordance with the guidelines of the IACUC of Universiti Putra Malaysia, Malaysia (Approval No. UPM/IACUC/AUP-R057/2024). The rats were acclimatized for 7 days to the start of the experiment and were treatment naive.

3.12.2 Drug Treatments and Experimental Procedure

Animals were randomized into 3 groups with 5-7 rats in each group. All drugs were dissolved in 0.9% saline and administered to the animals. The extract and drugs were administered orally or intraperitoneal injection 3 times per week for 35 days, while LPS was single intraperitoneal injection on day 17. The animal groups included: (1) control animals receiving oral administration of normal saline (0.2 mL/kg), (2) animals receiving an injection of LPS (1 mg/kg), and (3) animals receiving an injection of LPS (1 mg/kg) and oral administration of microencapsulated standardized BM extract (200 mg/kg). Behavioral assessments were conducted, including the MWM test on days 24-29, OFT on day 31, and NOR on day 32. The animals were then sacrificed on day 35.

3.12.3 Morris Water Maze Test

The MWM test measures learning and spatial memory, the procedure was used in previous published study with some modification. The water maze consisted of a circular pool with a diameter of 150 cm and a height of 60 cm, partitioned into 4 equal quadrants. A submerged platform (10 × 10 cm), painted white, was placed in 1 quadrant so that its height was 1 cm below the water surface. The pool was filled with water at 28-30°C to a depth of 30 cm and made opaque with milk powder. Several brightly

colored cues were positioned around the pool for spatial orientation, and their positions remained consistent throughout the analysis.

The animals were training on day 1-5 with 3 consecutive trials with a 120 second break between trials. For each trial, the animals were individually placed in the pool and allowed 120 seconds to find the platform. If they failed to locate the platform within 120 seconds, they were gently guided to it and allowed to stay on it for 30 seconds. The animals were placed randomly in different quadrants each trial. The relative location of the maze and surroundings remained consistent throughout the study. On day 6, the platform was removed from the target quadrant and allowed 30 seconds to find the platform. The total distance, total time, and number of times the animal entered the target quadrant were recorded using a computer-connected camera and calculated.

3.12.4 Open Field Test

The OFT measures locomotor and anxiety-like behaviors base on the natural exploratory of animals, the procedure was used in previous published study with some modification. The test consisted of white acrylic box (75 × 75 × 75 cm) with a designated central zone (15 × 15 cm) marked in the middle. (15 × 15 cm). During the trial, the animals were gently placed in the box and allowed to explore for 5 minutes. The parameters recorded over this 5-minute period included total distance traveled, mean speed, time spent in the central area, and distance traveled within the central area using a computer-connected camera and calculated.

3.12.5 Novel Object Recognition Test

The NOR measure recognition memory, the procedure was used in previous published study with some modification. The test consisted of white acrylic box (75 × 75 × 75 cm). For training trial, two identical objects were placed in the box and the animals were gently placed inside and allowed to explore for 5 minutes. After a 6 hours retention interval to assess short-term memory, one of the familiar objects was replaced with a novel object, and the animals were again allowed to explore for 5 minutes. The time spent exploring each object was recorded and the discrimination index (DI) was calculated using the following equation: (Time spent exploring the novel object – familiar object) / (Time spent exploring the novel object + familiar object).

3.13 Data Analysis

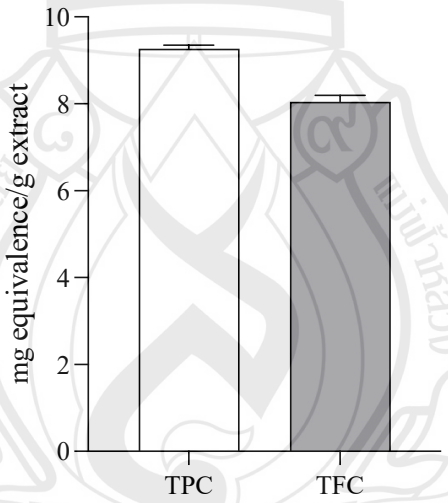
All experiments were performed in triplicate and results are presented as mean±standard deviation (SD), except for animal experiment data, which are presented as mean±standard error of the mean (SEM). Statistical analysis was conducted using a One-way ANOVA with Duncan poc-hoc in SPSS software version 25 (IBM Corp., USA). A p-value of <0.05 was considered statistically significant.

CHAPTER 4

RESULTS

4.1 Determination of Total Phenolic and Total Flavonoid Contents in *Bacopa monnieri* Extract

The TPC was determined using the Folin-Ciocalteu method. The result showed that BM extract contained 9.27±0.08 mg GAE/g extract (Figure 4.1). The TFC was determined using the aluminum chloride method. The result showed that BM extract contained 8.04±0.15 mg CAE/g extract (Figure 4.1).



Note Total phenolic content (TPC) expressed as mg gallic acid equivalence (GAE)/g extract, Total flavonoid content (TFC) expressed as mg of catechin equivalence (CAE)/g extract.

Figure 4.1 Total phenolic and total flavonoid contents in *Bacopa monnieri* extract

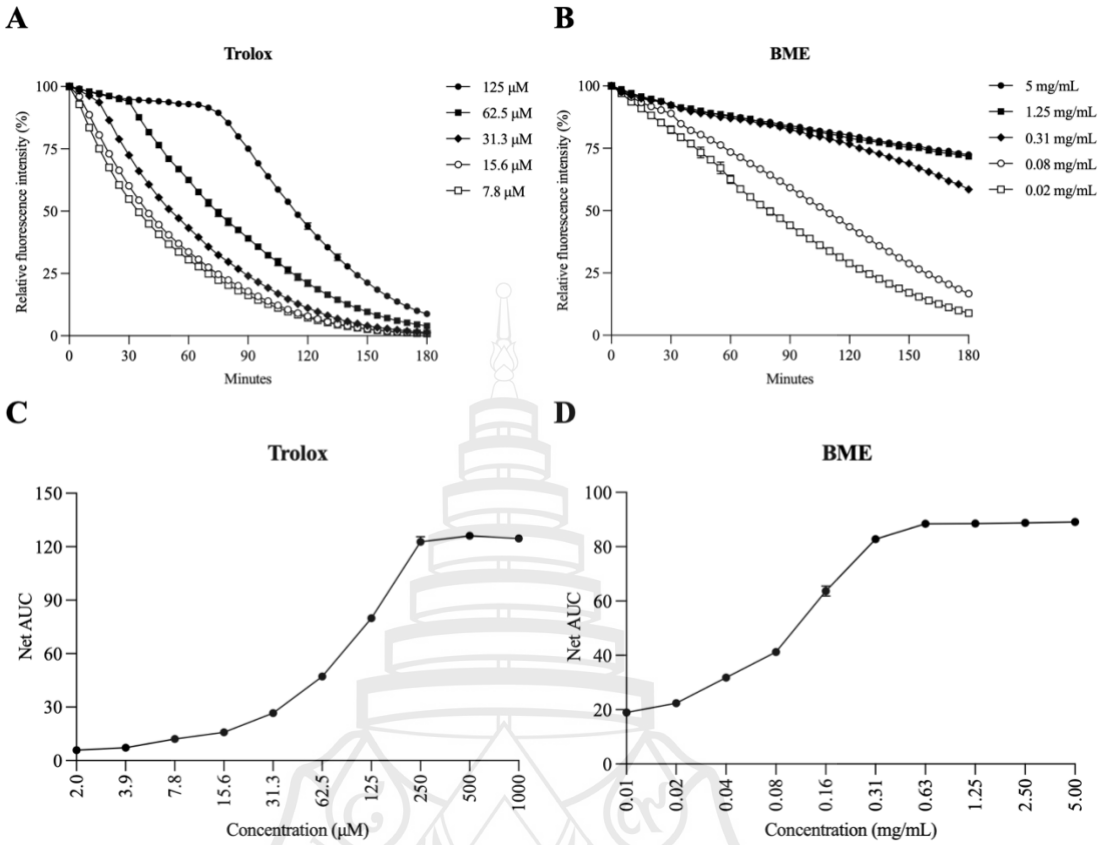
4.2 Determination of Antioxidant Capacity of *Bacopa monnieri* Extract

Antioxidant capacity of BM extract at the concentration was determined using various assays as presented in Table 4.2. The free radical scavenging activities was determined using DPPH and ABTS scavenging assay with IC_{50} value of 3.05±0.07 mg/mL and 1.95±0.04 mg/mL, respectively. FRAP assay was used to measure the reduction of ferric ions to ferrous ions showed a reducing capacity of 7.83±0.01 µM $FeSO_4$ per mg of extract. NBT assay was used to measure the scavenging activity of superoxide radicals. The result was expressed as IC_{50} value of 0.59±0.01 mg/mL. Additionally, ORAC assay was used to measure the scavenging of free radical through hydrogen atom transfer-based mechanism resulted in 1.17±0.01 µM TE/mg extract. Furthermore, Figure 4.2 presents the kinetic fluorescence decay curves of fluorescein along with the net area under the curve (AUC) values, showing additional insights into the antioxidant potential of the extract. Meanwhile, the MCA assay used to measure the presence of excess transition metals was not available for this extract

Table 4.1 Antioxidant capacity of *Bacopa monnieri* extract

Methods	**Antioxidant capacity**
MCA	N/A
DPPH	3.05±0.07 (IC_{50}, mg/mL)
ABTS	1.95±0.04 (IC_{50}, mg/mL)
FRAP	7.83±0.01 µM $FeSO_4$/mg extract
NBT	0.59±0.01 (IC_{50}, mg/mL)
ORAC	1.17±0.01 µM TE/mg extract

Note Metal chelating activity (MCA), Free radical scavenging activities of DPPH and ABTS, Ferric reducing antioxidant power (FRAP), Nitroblue tetrazolium (NBT), Oxygen radical antioxidant capacity (ORAC). Not available (N/A).



Note *Bacopa monnieri* extract (BME).

Figure 4.2 Oxygen radical antioxidant capacity (ORAC) of *Bacopa monnieri* extract. Kinetic fluorescence decay curves of fluorescein for (A) Trolox and (B) the extract, net AUC values for (C) Trolox and (D) the extract.

4.3 Identification of Phytochemical Compounds in *Bacopa monnieri* Extract

The active compound in BM extract, identified as a bacoside A mixture was analyzed using HPLC. The resulting HPLC chromatograms are present in Figure 4.3. The analysis showed data on the retention time, peak area, and concentration of the active compounds in the extract as presented in Table 4.2. Among the detected components of the bacoside A mixture, bacopasaponin C was found in the highest content (52.71±0.06 mg/kg), followed by bacopaside II (43.05±0.28 mg/kg), bacoside A_3 (33.93±0.17 mg/kg), and bacopaside X (17.44±0.33 mg/kg), respectively.

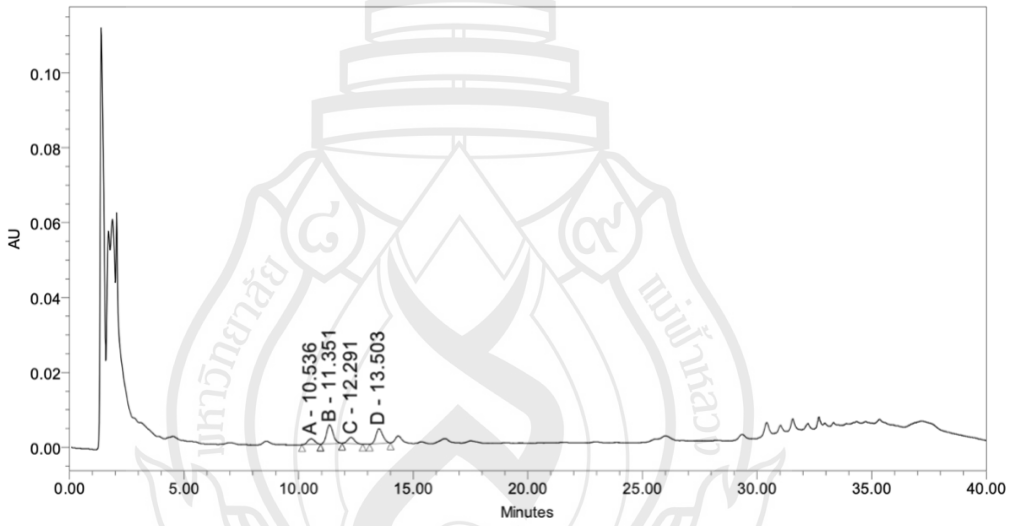


Figure 4.3 High-performance liquid chromatography (HPLC) chromatogram of bacoside A in *Bacopa monnieri* extract. The chromatogram showed the compounds in the bacoside A mixture including (A) bacoside A_3, (B) bacopaside II, (C) bacopaside X, and (D) bacopasaponin C

Table 4.2 Phytochemical compounds identified in *Bacopa monnieri* extract

The high-performance liquid chromatography (HPLC) analysis					
Compounds	**Equation**	**R^2**	**Retention time (min)**	**Peak area**	**Content (mg/kg extract)**
Bacoside A_3	y = 794.21x + 5729.1	0.99	10.56±0.03	32675.33±136.95	33.93±0.17
Bacopaside II	y = 2133.7x + 7204.9	0.99	11.37±0.03	99054.00±603.03	43.05±0.28
Bacopaside X	y = 1456.3x + 8350.6	0.99	12.31±0.03	33749.00±479.70	17.44±0.33
Bacopasaponin C	y = 1645.1x - 4583.3	0.99	13.52±0.04	82136.00±106.04	52.71±0.06

4.4 Evaluation of Toxicity in *Bacopa monnieri* Extract

4.4.1 Pharyngeal Pumping Assay

The feeding rate of *C. elegans* was assessed by counting the number of pharyngeal muscle contractions and relaxations per minute at various concentrations of BM extract. The results showed that BM extract at 0.1 mg/mL had no significant effect on the pharyngeal pumping rate of *C. elegans*, with a rate of 84.00±0.00 pumps/min compared with 88.00±2.00 pumps/min in the control group (p<0.05; Figure 4.4A). However, at concentrations of 0.25, 0.5, and 1.0 mg/mL, the pharyngeal pumping rate of *C. elegans* was significantly decreased compared with the control group (p<0.05; Figure 4.4A), with rates of 77.33±4.16, 68.67±1.15, and 57.33±5.03 pumps/min, respectively.

4.4.2 Food Clearance Assay

The toxicity testing of *C. elegans* was performed by measuring the absorbance associated with decreased turbidity of *E. coli* OP50 in the liquid medium used to culture *C. elegans* for 7 days at high-dose concentrations. The result showed that the BM extract significantly affected on feeding behavior of *C. elegans* at concentrations of 0.5 and 1.0 mg/mL compared to the control group (p<0.05; Figure 4.4B). At both concentrations, absorbance values were significantly reduced relative to the control on day 1, increased to significantly higher levels on days 2-4, and subsequently declined to significantly lower levels again on days 5-7 (p<0.05; Figure 4.4B).

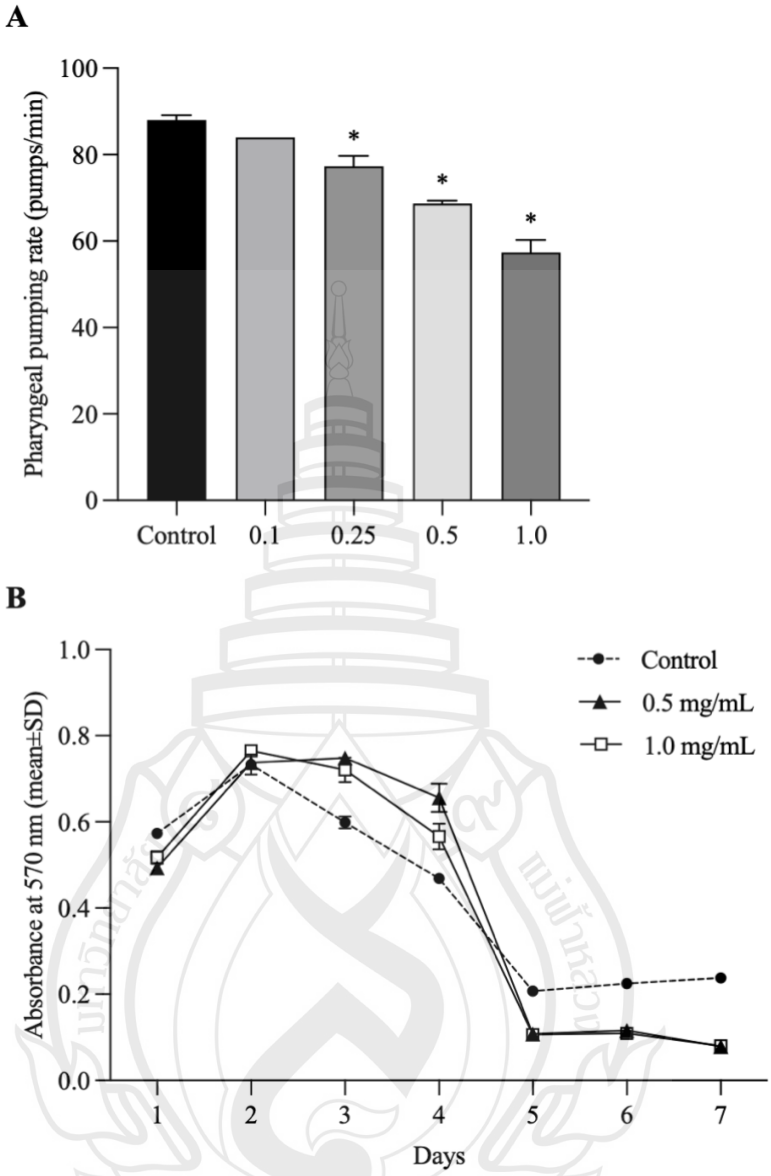


Figure 4.4 Effects of *Bacopa monnieri* extract on food-intake behavior of *C. elegans* for toxicity evaluation. (A) Bacteria clearance assay (B) Pharyngeal pumping rate. The statistically differences with control group $^{*}p<0.05$

4.5 Cognitive Function Improvement of *Bacopa monnieri* Extract

4.5.1 Effects of *Bacopa monnieri* Extract on Memory Acquisition in LPS-Induced Rats

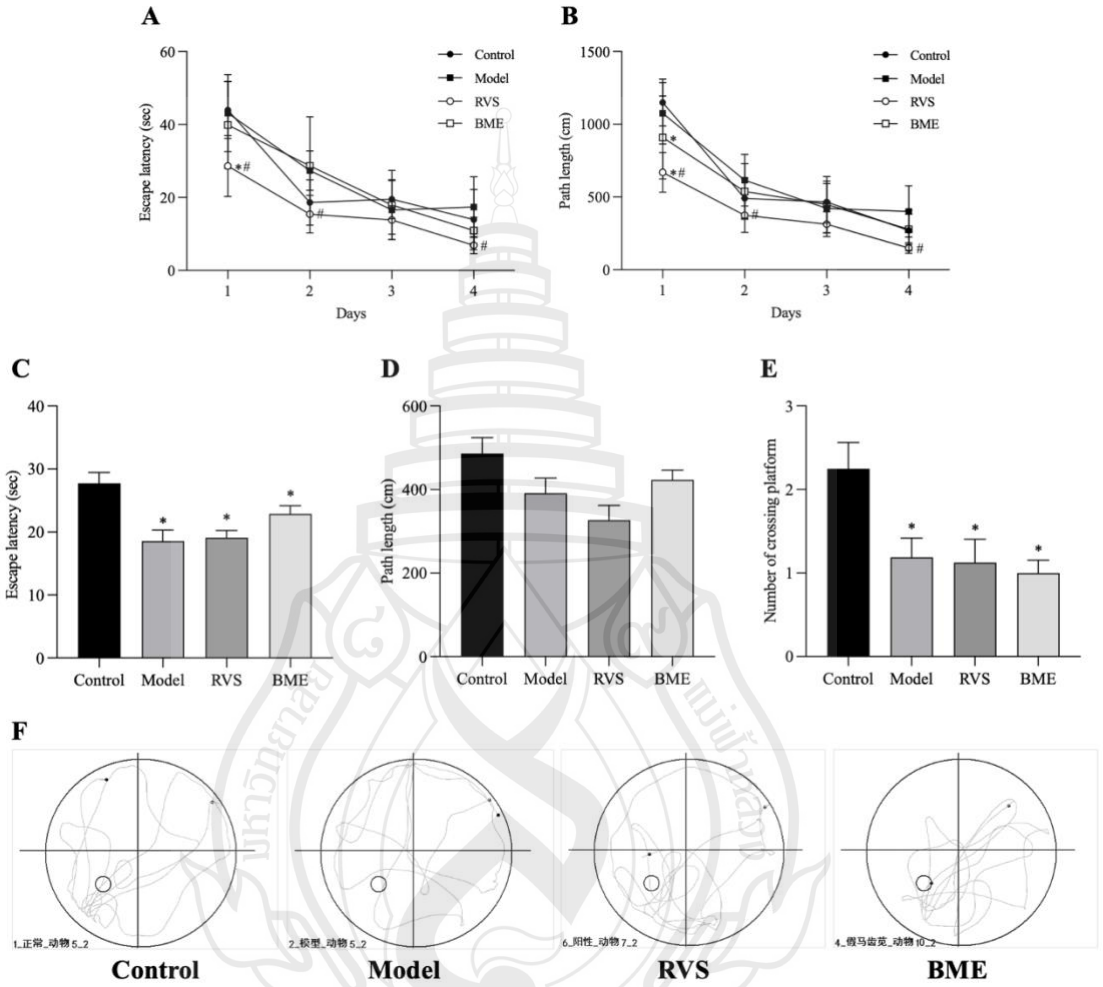
The MWM was used to assess spatial learning and memory performance. All groups showed decreases in escape latency and path length to find the platform during training trial from days 1 to 4 (Figure 4.5). The BME group showed a non-significant decrease in escape latency to find the platform from day 1 to day 4 (39.87±3.99, 28.66±4.49, 17.94±3.60, and 10.93±1.38 sec) compared with the model group (43.14±3.34, 27.40±2.05, 16.58±2.85, and 17.35±2.94 sec, $p>0.05$; Figure 4.5A). Similarly, for path length to find the platform, the BME group showed a non-significant decrease from day 1 to day 4 (909.53±100.74, 537.63±63.95, 448.54±72.91, and 277.57±39.35 cm) compared with the model group (1075.05±74.70, 615.14±62.94, 422.08±60.21, and 400.21±62.42 cm, $p>0.05$; Figure 4.5B).

On the testing trial, the BME group showed a 23.30% increase in escape latency in the target quadrant (22.86±1.32 sec) compared with the model group (18.54±1.77 sec), though the difference was not statistically significant ($p>0.05$; Figure 4.5C). Similarly, the path length in the target quadrant increased by 8.21% in the BME group (423.37±22.93 cm) compared with the model group (391.24±36.39 cm, $p>0.05$; Figure 4.5D). In contrast, the frequency of platform crossings decreased by 15.97% in the BME group (1.00±0.15 times) compared with the model group (1.19±0.23 times), also without statistical significance $p>0.05$; Figure 4.5E). These behavioral patterns are shown in the representative trajectories from the tracking system (Figure 4.5F).

4.5.2 Effects of *Bacopa monnieri* Extract on Memory Retention in LPS-Induced Rats

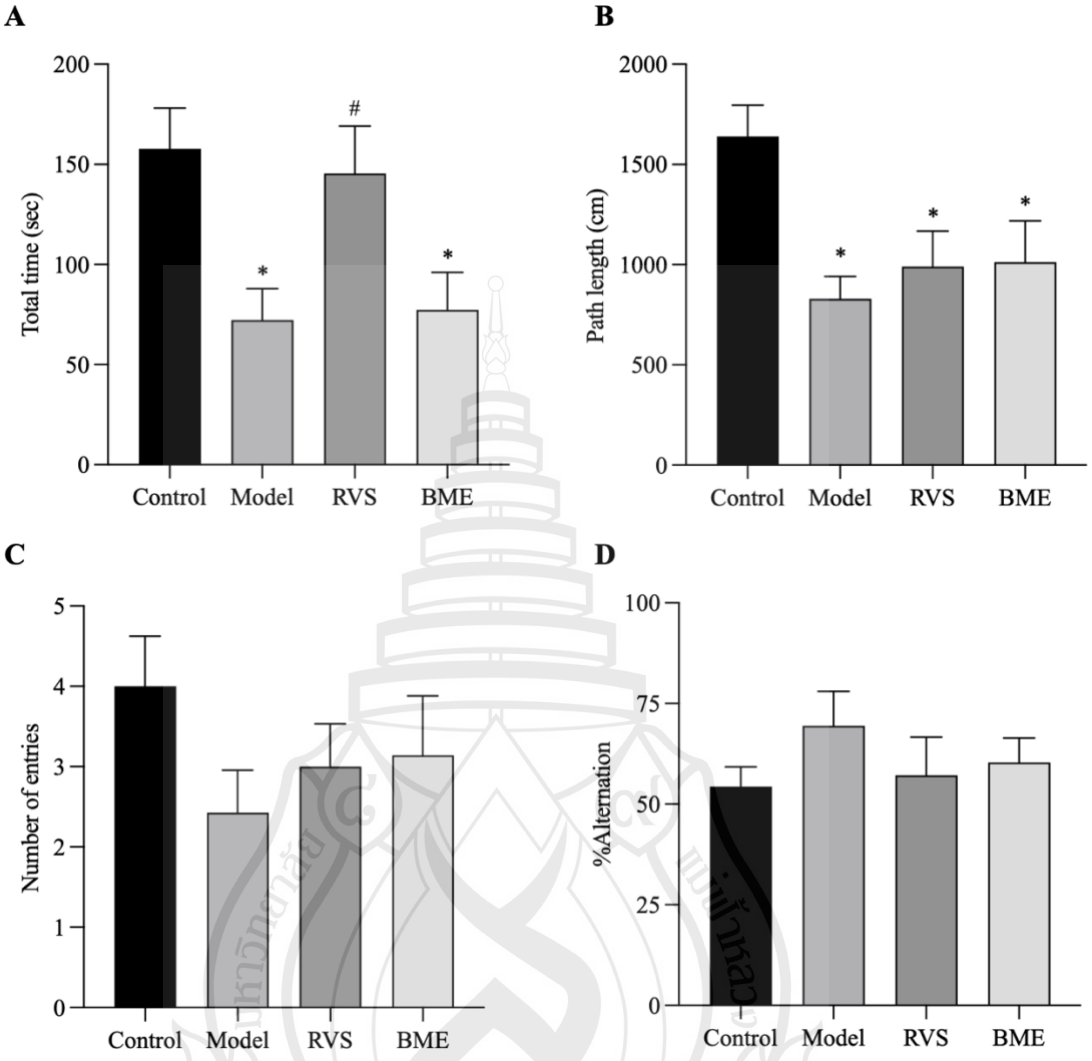
The Y-maze was used to assess working memory and short-term memory performance. The BME group showed a 7.07% increase in total time spent (77.40±18.75 sec), a 21.96% increase in path length (1011.61±206.49 cm), and a 29.22% increase in novel arm entries (3.14±0.74 times) compared with the model group (72.29±15.63 sec, 829.42±112.22 cm, and 2.43±0.53 times), though the difference was not statistically significant ($p>0.05$; Figure 4.6A-C). In contrast, alternation behavior in

the BME group decreased by 0.87% (60.38±6.05%) compared with the model group (69.46±8.53%, p>0.05; Figure 4.5E).



Note Rivastigmine group (RVS), *Bacopa monnieri* extract group (BME).

Figure 4.5 Effects of *Bacopa monnieri* extract on memory acquisition in morris water maze test of LPS-induced rats (A) Escape latency to find the platform on training trial (B) Path length to find the platform on training trial (C) Escape latency in the target quadrant on testing trial (D) Path length in the target quadrant on testing trial (E) Number of platform crossings on testing trial (F) Representative trajectory from the tracking system on testing trial. The statistically differences compared with control group $^{*}p$<0.05; compared with model group $^{\#}p$<0.05



Note Rivastigmine group (RVS), *Bacopa monnieri* extract group (BME).

Figure 4.6 Effects of *Bacopa monnieri* extract on memory retention in Y-maze test of LPS-induced rats. (A) Total time spent in the novel arm (B) Path length in the novel arm (C) Number of entries into the novel arm (D) Spontaneous alternation behavior. The statistically differences compared with control group $^{*}p<0.05$; compared with model group $^{\#}p<0.05$

4.6 Microencapsulation of *Bacopa monnieri* Procedures

The BM microencapsulation was developed using the formulation in Table 4.3 using spray-dried technique. Each formulation varied in the ratio of core to wall material with ratios of 1:10 and 1:20 w/w. The core material consisted of BM extract in 95% ethanol, while the wall material comprised 100 g of banana flour and 400 g of maltodextrin. Additionally, the inlet and outlet temperatures varied. For the formulation with an inlet temperature of 120 °C, the outlet temperature was 75 °C, while for the formulation with an inlet temperature of 180 °C, the outlet temperature was 105 °C. After producing the microcapsules, the encapsulation yield was calculated and showing values ranging from 37.14% to 40.62%. The best formulation was BM110-120, followed by BM120-180, BM110-180, and BM120-120, respectively (Table 4.3).

Table 4.3 Ratios of wall to core materials and encapsulation yields of spray-dried *Bacopa monnieri* microcapsules prepared at various spray-drying air temperatures

Treatment code	**Ratio of core: wall material**	**Temperature (°C)**		**Encapsulation yield (%)**
		Inlet	**Outlet**	
BM110-120	1:10	120	75	40.62
BM110-180	1:10	180	105	39.08
BM120-120	1:20	120	75	37.14
BM120-180	1:20	180	105	39.14

4.7 Physicochemical Characterization of Microcapsules

4.7.1 Particle Size

The particle size distribution of the microcapsules showed result that the BM110-180 formulation had the smallest mean particle size (45.57±25.10 μm), followed by BM110-120 (47.49±29.14 μm), BM120-180 (47.73±29.84 μm), and BM120-120 (48.32±27.51 μm). Additionally, the particle size distribution was analyzed using the D_{10}, D_{50}, and D_{90} values, representing the particle diameters at which 10%, 50%, and 90% of the particles are smaller. The results showed the following by BM110-180 (15.45, 43.58, 74.53 μm), BM110-120 (16.54, 44.29, 76.94 μm), BM120-180 (16.79, 43.94, 77.32 μm), and BM120-120 (17.83, 44.87, 80.12 μm) in order of smallest to largest size (Table 4.4; Figure 4.7).

4.7.2 Particle Morphology

The particle morphology of the microcapsules showed result that most particles were spherical with a rough and dimpled surface, while some particles showed a sickle-like shape with varying sizes in the powder as observed in the scanning electron micrographs. (Figure 4.7).

4.7.3 Color

The color of the microencapsulates was characterized by L^* values (lightness), a^* values (redness to greenness), and b^* values (yellowness to blueness). The results showed value of BM110-120 (82.03±0.04, 1.45±0.02b, 13.96±0.05), BM110-180 (82.16±0.17, 1.43±0.04, 13.55±0.07), BM120-120 (79.67±0.08, 1.67±0.02, 15.64±0.14), and BM120-180 (79.70±0.01, 1.68±0.00, 15.73±0.02) with statistically significant differences at $p<0.05$ (Table 4.4) All formulations resulting in a light yellowish appearance as presented in Figure 4.7.

Table 4.4 Color parameters and particle size characteristics of spray-dried *Bacopa monnieri* microcapsules

Treatment code	Particle size (μm)						Color		
	Range	Mode	Mean±SD	D_{10}	D_{50}	D_{90}	*L**	*a**	*b**
BM110-120	0.791-373.1	50.22	47.49±29.14	16.54	44.29	76.94	82.03±0.04^{a}	1.45±0.02^{b}	13.96±0.05^{b}
BM110-180	0.868-309.6	50.22	45.57±25.10	15.45	43.58	74.53	82.16±0.17^{a}	1.43±0.04^{b}	13.55±0.07^{c}
BM120-120	0.721-309.6	50.22	48.32±27.51	17.83	44.87	80.12	79.67±0.08^{b}	1.67±0.02^{a}	15.64±0.14^{a}
BM120-180	0.791-339.9	50.22	47.73±29.84	16.79	43.94	77.32	79.70±0.01^{b}	1.68±0.00^{a}	15.73±0.02^{a}

Note Different superscript letters indicate statistically significant differences at $p<0.05$. *L**: Lightness, *a**: Redness to greenness, *b**: Yellowness to blueness. D_{10}: The particle diameter at which 10% of the particles are smaller, D_{50}: The particle diameter at which 50% of the particles are smaller, D_{90}: The particle diameter at which 90% of the particles are smaller.

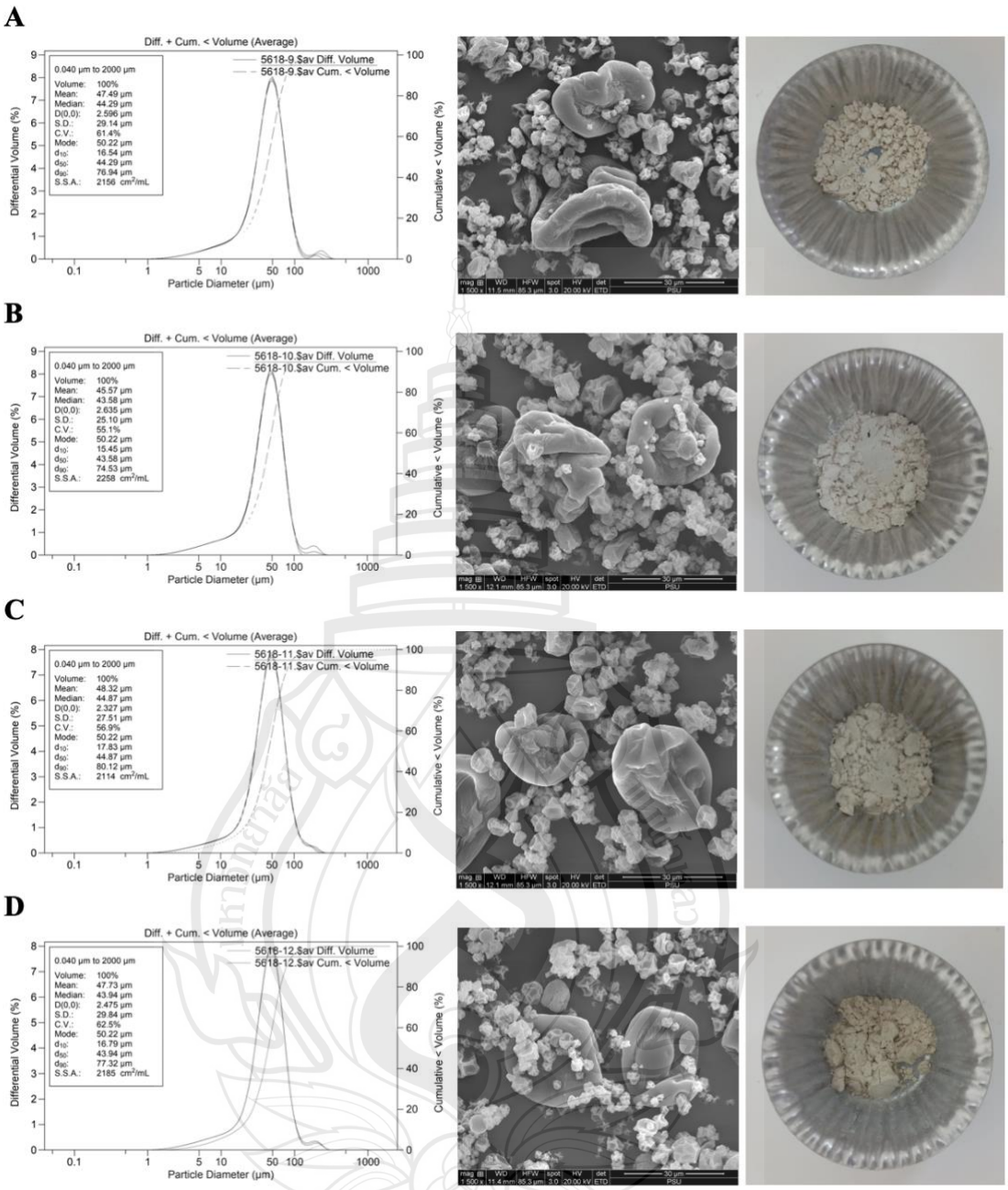


Figure 4.7 Particle size distribution (left), scanning electron micrographs (middle), and physical appearance (right) of spray-dried *Bacopa monnieri* microcapsules. (A) BM110-120 (B) BM110-180 (C) BM120-120 (D) BM120-180

4.7.4 Bulk Density

The bulk density of the microencapsulates showed result that the BM110-180 formulation had the highest bulk density (0.33±0.01 g/mL), followed by BM110-120 (0.31±0.01 g/mL), BM120-180 (0.29±0.00 g/mL), and BM120-120 (0.28±0.01 g/mL) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.5 Tapped Density

The tapped density of the microencapsulates showed result that the BM120-180 formulation had the highest tapped density (0.52±0.02 g/mL), followed by BM120-120 (0.51±0.02 g/mL), BM110-120 (0.50±0.01 g/mL), and BM110-180 (0.48±0.02 g/mL) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.6 Flowability

The flowability of the microencapsulates was expressed as Carr's index range showed result that the BM120-180 formulation had the highest flowability (0.23±0.02%), followed by BM120-120 (0.22±0.02%), BM110-120 (0.19±0.01%), and BM110-180 (0.16±0.01%) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.7 Cohesiveness

The cohesiveness of the microencapsulates was expressed as Hausner's ratio showed result that the BM120-180 formulation had the highest cohesiveness (1.79±0.08), followed by BM120-120 (1.78±0.05), BM110-120 (1.62±0.03), and BM110-180 (1.47±0.04) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.8 Hygroscopicity

The hygroscopicity of the microencapsulates showed result that the BM110-180 had the highest hygroscopicity (15.61±6.06%), followed by BM120-180 (13.87±0.28%), BM120-120 (11.95±0.13%), and BM110-120 (11.10±0.45%) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.9 Moisture Content

The moisture content of the microencapsulates showed result that the BM110-120 formulation had the highest moisture content (4.70±0.05%), followed by BM120-120 (4.23±0.00%), BM110-180 (3.33±0.22%), and BM120-180 (2.59±0.05%) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.10 Water Activity

The water activity of the microencapsulates showed result that the BM110-120 formulation had the highest water activity (0.27±0.00%), followed by BM120-120 (0.26±0.00%), BM110-180 (0.20±0.01%), and BM120-180 (0.18±0.00%) with statistically significant differences at $p<0.05$ (Table 4.5).

4.7.11 Water Solubility Index

The water solubility index of the microencapsulates showed result that the BM120-120 formulation had the highest water solubility index (61.79±0.75%), followed by BM120-180 (61.05±0.03%), BM110-180 (57.58±0.50%), and BM110-120 (57.19±0.65%) with statistically significant differences at $p<0.05$ (Table 4.5).

Table 4.5 Physicochemical properties of spray-dried *Bacopa monnieri* microcapsules

Parameters	**Treatment code**			
	BM110-120	**BM110-180**	**BM120-120**	**BM120-180**
Bulk density (g/mL)	0.31±0.01^{b}	0.33±0.01^{a}	0.28±0.01^{c}	0.29±0.00^{c}
Tap density (g/mL)	0.50±0.01^{ab}	0.48±0.02^{b}	0.51±0.02^{ab}	0.52±0.02^{a}
Flowability (CI, %)	0.19±0.01^{b}	0.16±0.01^{c}	0.22±0.02^{a}	0.23±0.02^{a}
Cohesiveness (HR)	1.62±0.03^{b}	1.47±0.04^{c}	1.78±0.05^{a}	1.79±0.08^{a}
Hygroscopicity (%)	11.10±0.45^{a}	15.61±6.06^{a}	11.95±0.13^{a}	13.87±0.28^{a}
Moisture content (%)	4.70±0.05^{a}	3.33±0.22^{c}	4.23±0.00^{b}	2.59±0.05^{d}
Water activity (%)	0.27±0.00^{a}	0.20±0.01^{c}	0.26±0.00^{b}	0.18±0.00^{d}
Water solubility index (%)	57.19±0.65^{b}	57.58±0.50^{b}	61.79±0.75^{a}	61.05±0.03^{a}

Note Different superscript letters indicate statistically significant differences at $p<0.05$.
Carr index (CI), Hausner ratio (HR).

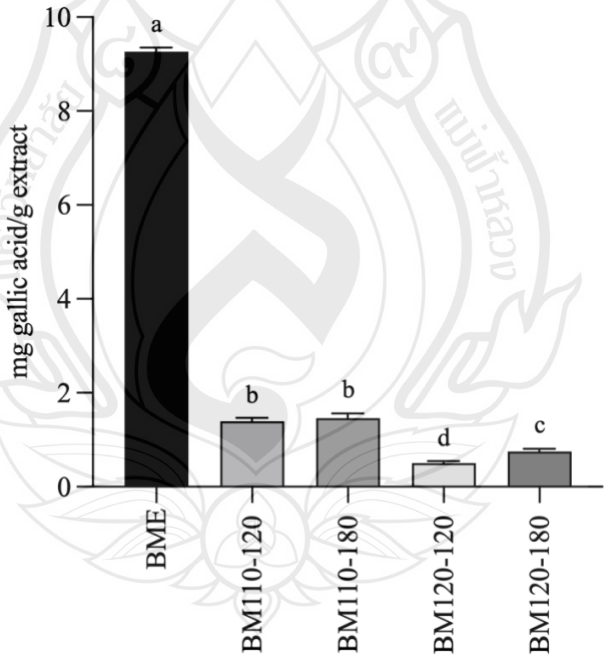
4.8 Determination of Total Phenolic and Total Flavonoid Contents in Microcapsules

4.8.1 Total Phenolic Content

The TPC was determined using the Folin-Ciocalteu method. The result was expressed as mg of GAE per g of extract. The BM110-180 formulation had the highest TPC (1.46±0.10 mg GAE/g extract), followed by BM110-120 (1.40±0.07 mg GAE/g extract), BM120-180 (0.75±0.06 mg GAE/g extract), and BM120-120 (0.50±0.05 mg GAE/g extract) with statistically significant differences at $p<0.05$ (Figure 4.8).

4.8.2 Total Flavonoid Content

The TFC was determined using the aluminum chloride method. The result showed that it was not detectable in any formulation.



Note *Bacopa monnieri* extract (BME), Microencapsulated formulations (BM)

Figure 4.8 Total phenolic content of *Bacopa monnieri* extract and spray-dried microcapsules. Different superscript letters indicate statistically significant differences at $p<0.05$

4.9 Determination of Antioxidant Capacity of Microcapsules

4.9.1 The Metal Chelating Activity Assay

The MCA assay was used to measure the presence of excess transition metals. The result showed that is was not detectable in any formulation.

4.9.2 DPPH Free Radical Scavenging Assay

The DPPH assay was used to measure the ability to neutralize free radicals through donating an electron or hydrogen atom. The result was expressed as IC_{50} in mg per mL. At a concentration of 5 mg/mL, the BM110-120 formulation had the highest IC_{50} value (12.56±0.33 mg/mL), followed by BM110-180 (13.07±0.24 mg/mL), BM120-180 (20.29±0.24 mg/mL), and BM120-120 (20.54±0.30 mg/mL) with statistically significant differences at $p<0.05$ (Figure 4.9A).

4.9.3 ABTS Free Radical Scavenging Assay

The ABTS assay was used to measure the ability to neutralize free radicals through donating electrons. The result was expressed as IC_{50} in mg per mL. At a concentration of 5 mg/mL, the BM110-180 formulation had the highest IC_{50} value (5.87±0.22 mg/mL), followed by BM110-120 (6.31±0.02 mg/mL), BM120-180 (11.81±0.38 mg/mL), and BM120-120 (12.70±0.30 mg/mL) with statistically significant differences at $p<0.05$ (Figure 4.9B).

4.9.4 Ferric Reducing Antioxidant Power Assay

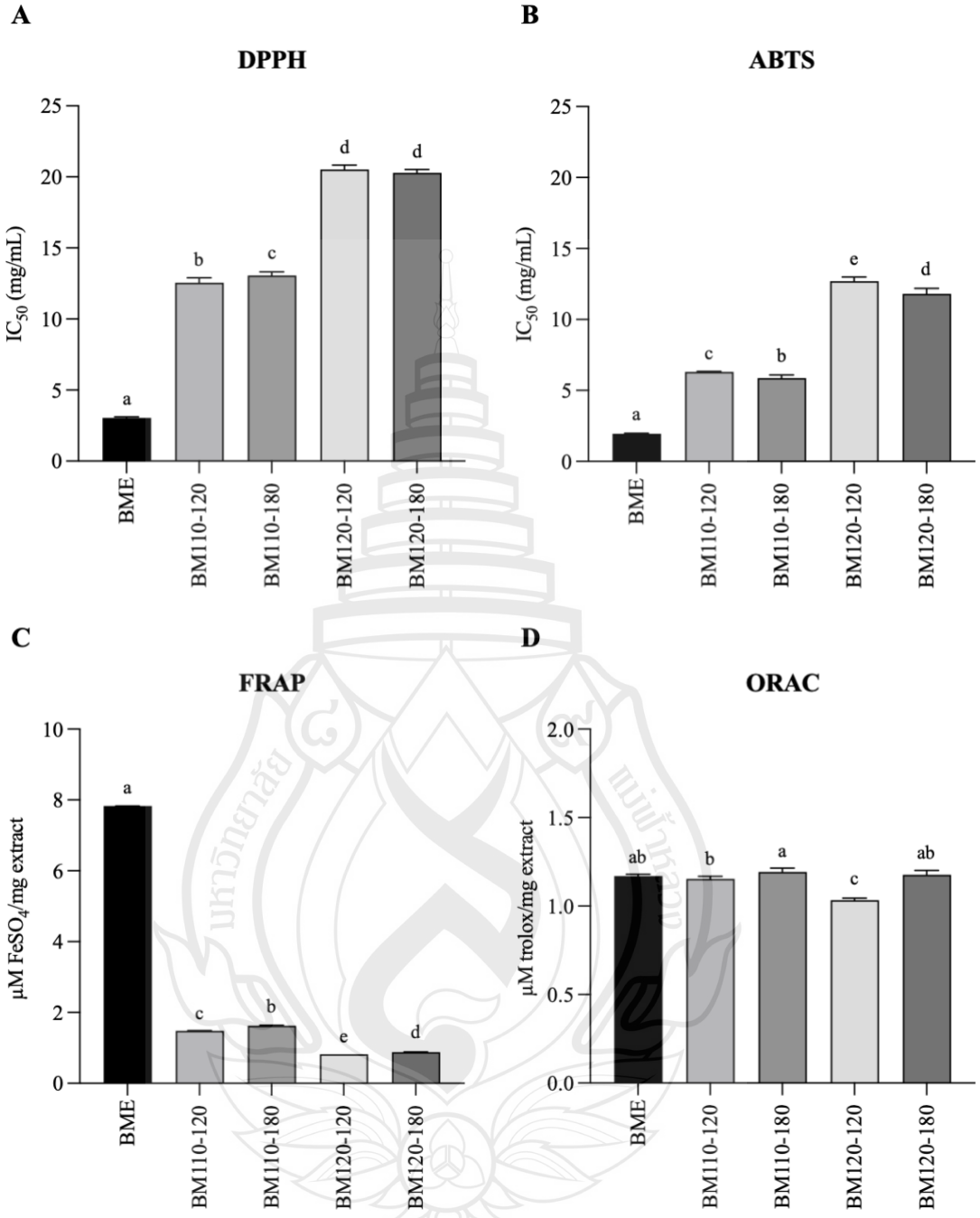
The FRAP assay was used to measure the reduction of ferric ions to ferrous ions. The result was expressed as µM of ferrous sulfate ($FeSO_4$) per mg of extract. At a concentration of 5 mg/mL, the BM110-180 formulation had the highest ability (1.63±0.01 µM $FeSO_4$/mg extract), followed by BM110-120 (1.48±0.01 µM $FeSO_4$/mg extract), BM120-180 (0.88±0.01 µM $FeSO_4$/mg extract), and BM120-120 (0.82±0.00 µM $FeSO_4$/mg extract) with statistically significant differences at $p<0.05$ (Figure 4.9C).

4.9.5 Nitroblue Tetrazolium Assay

The NBT assay was used to measure the scavenging activity of superoxide radicals. The result showed that is was not detectable in any formulation.

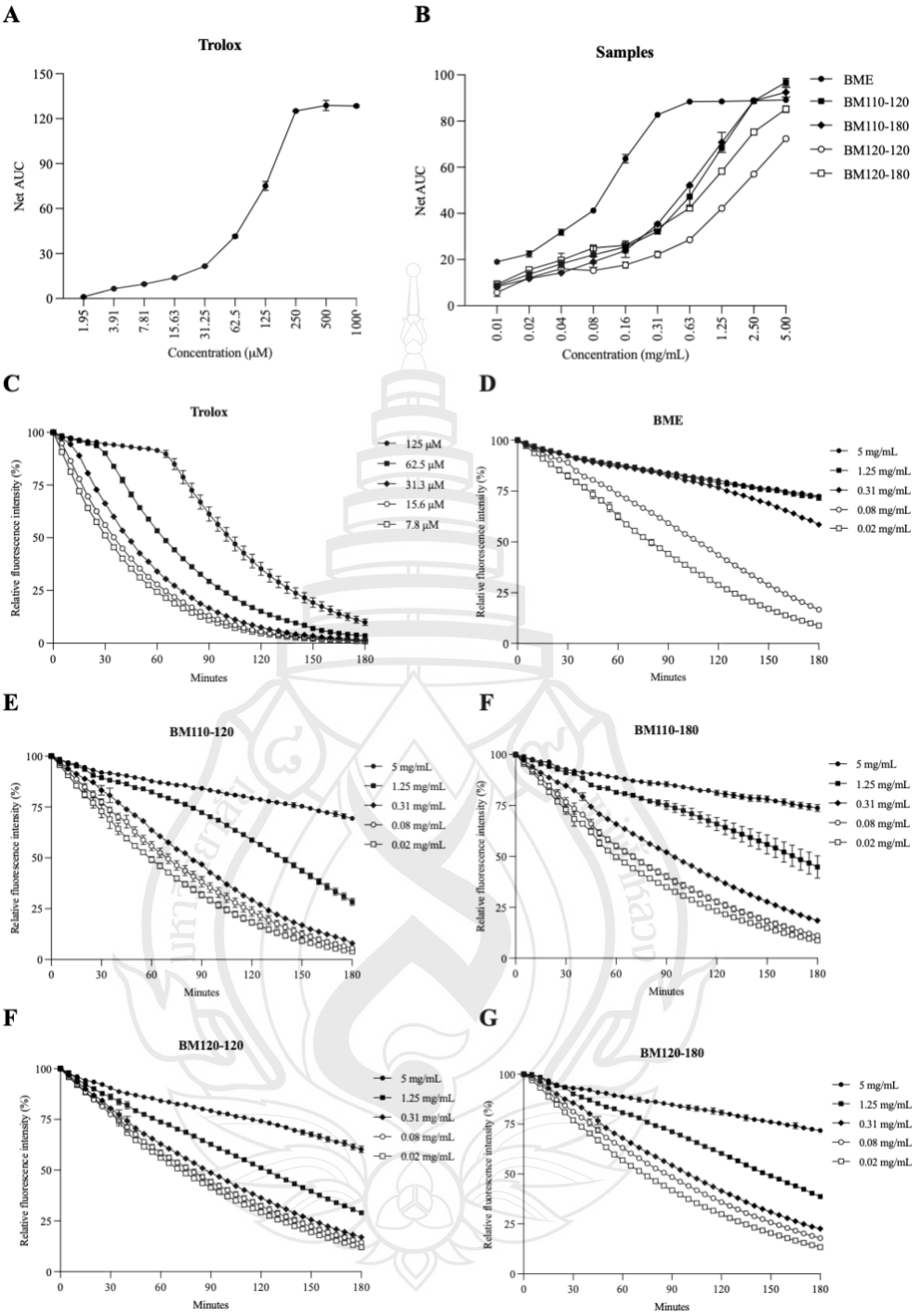
4.9.6 Oxygen Radical Antioxidant Capacity Assay

The ORAC assay was used to measure the ability of antioxidants to protect a fluorescent probe from degradation by peroxyl radicals through electron donation or hydrogen atom transfer mechanisms. The result was expressed as μM of TE per mg of extract. At a concentration of 5 mg/mL, the BM110-180 formulation had the highest ability (1.19±0.02 μM TE/mg extract), followed by BM120-180 (1.18±0.03 μM TE/mg extract), BM110-120 (1.15±0.02 μM TE/mg extract), and BM120-120 (1.03±0.01 μM TE/mg extract) with statistically significant differences at $p<0.05$ (Figure 4.9D). Furthermore, Figure 4.10 presents the kinetic fluorescence decay curves of fluorescein along with the net AUC values, offering additional insights into the antioxidant capacity of the microcapsules.



Note *Bacopa monnieri* extract (BME), Microencapsulated formulations (BM)

Figure 4.9 Antioxidant capacity of *Bacopa monnieri* extract and spray-dried microcapsules. (A) DPPH free radical scavenging assay. (B) ABTS free radical scavenging assay. (C) Ferric reducing antioxidant power assay (FRAP). (D) Oxygen radical antioxidant capacity assay (ORAC). Different superscript letters indicate statistically significant differences at $p<0.05$



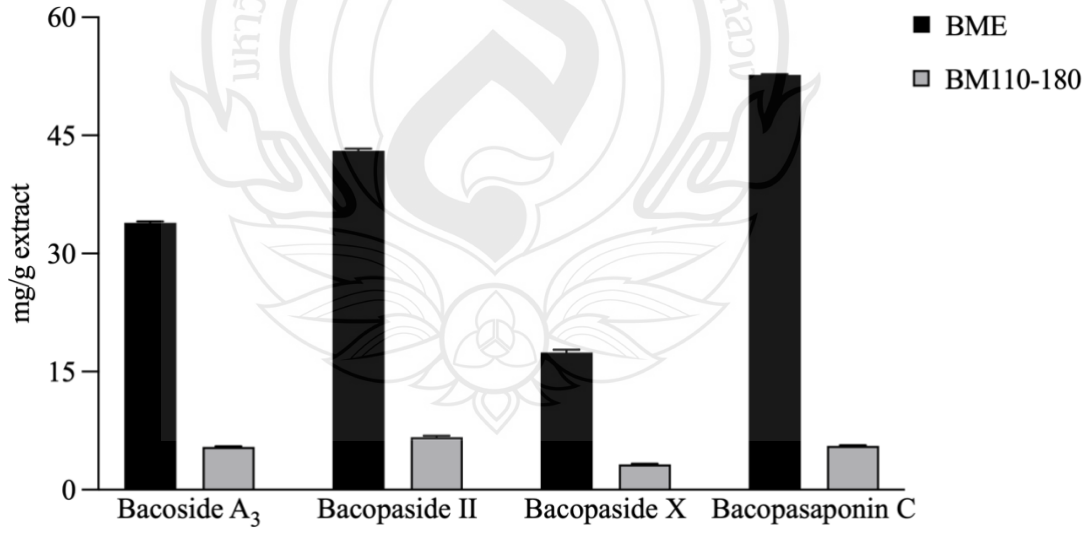
Note *Bacopa monnieri* extract (BME), Microencapsulated formulations (BM)

Figure 4.10 Oxygen radical antioxidant capacity of *Bacopa monnieri* extract and spray-dried microcapsules. Net area under curve values for of fluorescein for (A) Trolox and (B) samples, kinetic fluorescence decay curves (C) Trolox and (D) *Bacopa monnieri* extract, (E) BM110-120, (F) BM110-180, (G) BM120-120, (H) BM120-180

4.10 Identification of Phytochemical Compounds in Microcapsule

The antioxidant capacity of the microencapsulates showed that BM110-180 had the best overall antioxidant performance, then was selected for the determination of metabolite profiling using LC-MS analysis. The result showed that bacoside A_3, a key compound expected in both BM extract and microcapsule was identified (Table 4.6). Additionally, bacopaside X was detected in low intensity but was not prominent enough to be included in the table. Other identified compounds included triterpene saponins, flavonols, flavones, alkaloids, coumaric acids and their derivatives. However, the microencapsulate showed lower intensity compared to the extract.

To confirm the metabolite profile, the quantity of bacoside A was determined based on the retention time, peak area, and concentration of the active compounds in the extract. Among the detected compounds, bacopaside II was found in the highest concentration (6.70±0.17 mg/g), followed by bacopasaponin C (5.58±0.09 mg/g), bacoside A_3 (5.42±0.11 mg/g), and bacopaside X (3.22±0.07 mg/g), respectively (Figure 4.11). The concentrations of the active compounds were found in BM110-180 to be approximately 5-9 times lower than the BM extract.



Note *Bacopa monnieri* extract (BME), Microencapsulated formulations (BM)

Figure 4.11 Amounts of compounds identified of *Bacopa monnieri* extract and BM110-180 microencapsulated formulation.

Table 4.6 Metabolite profile of *Bacopa monnieri* extract and spray-dried microcapsules

Ontology	Metabolite name	Average RT (min)	Average m/z	Total score	Intensity	
					BME	BM110-180
1-acyl-sn-glycero	PC(16:0/0:0)	12.45	496.34	1.17	+	#
-3-phosphocholines	LysoPC(0:0/18:0)	13.71	524.37	1.13	+	#
Alkaloids and derivatives	Piperine	10.143	286.14	1.57	#	+
Alpha amino acids	Betaine	0.82	140.07	1.00	+	#
Benzoyl derivatives	Benzaldehyde	3.80	107.05	1.14	+	#
Cholines	Choline; CE10; OEYIOHPDSNJKLS-UHFFFAOYSA-N	0.66	104.11	1.32	+	+
Coumaric acids and derivatives	Rosmarinic acid	5.96	163.04	1.20	+	#
Flavones	Luteolin	6.68	287.06	1.38	+	#
Flavonols	Kaempferol	6.28	287.06	1.09	+	#
Hydroxyanthraquinones	1,3,8-trihydroxy-6-methylanthra-9,10-quinone	7.41	271.06	1.54	+	#
Macrolides and analogues	Pesticide4_Doramectin _C50H74O14_Dectomax	9.179	921.51	1.20	#	+
Phenylalanine and derivatives	Aurantiamide acetate	10.69	445.21	1.20	+	#
Phosphatidylcholines	PC(16:0/18:1(9Z))	14.15	760.59	1.21	+	-
Triterpene saponins	Saikosaponin D	12.13	781.47	1.23	+	#
	Soyasapogenol B base + O-HexA-dHex-Pen	11.57	911.51	1.03	+	#
Triterpenoids	Bacoside A_3	9.62	929.51	1.09	+	#
	Bayogenin	10.53	511.34	1.33	+	#
	Glycyrrhetic acid	13.81	471.35	1.35	+	#
	Glycyrrhetinic Acid	12.94	471.35	1.17	+	#
	Saikogenin D	12.35	495.34	1.43	+	+
	Scabioside C	9.59	789.44	1.34	+	+
	Soyasapogenol A	12.83	497.36	1.00	+	#
	Ziyuglycoside II	10.39	627.39	1.23	+	+
Tyrosols and derivatives	Betaxolol	7.24	308.22	1.24	+	#

Note +: Detected intensity more than 10^6, #: Detected intensity less than 10^6, -: Not detected, RT: Retention time.

4.11 Cognitive Function Improvement by *Bacopa monnieri* Microcapsule

4.11.1 Effects of *Bacopa monnieri* Microcapsule on Memory Acquisition in LPS-induced Rats

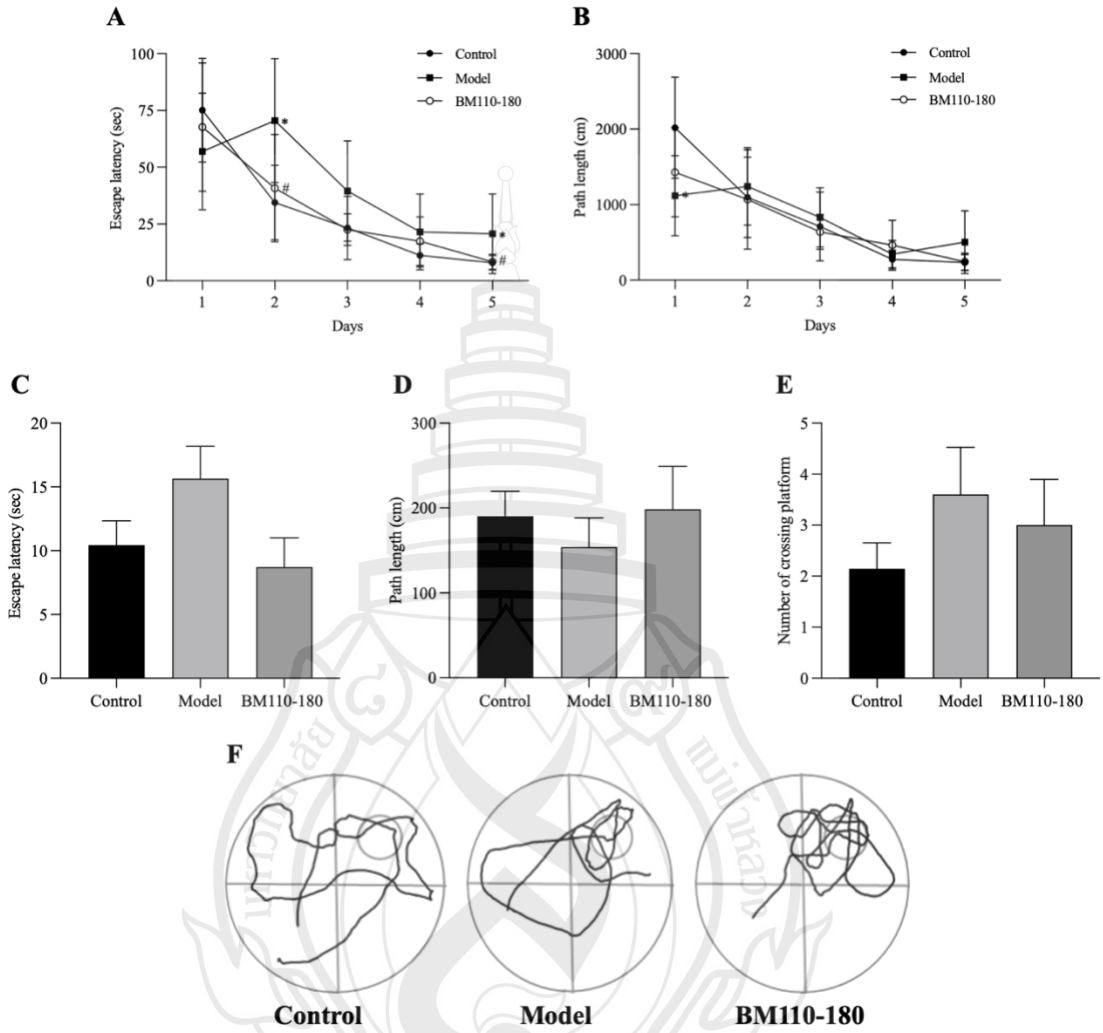
The MWM was used to assess spatial learning and memory performance. All groups showed decreases in escape latency and path length to find the platform during training trial from days 1 to 5 (Figure 4.12). The BM110-180 group showed a significant decrease in escape latency to find the platform on days 2 and 5 (40.75±10.58 and 8.37±1.51 sec) compared with the model group (70.55±12.18 and 20.69±7.84 sec, $p>0.05$; Figure 4.12A). However, the BM110-180 group showed a non-significant decrease in path length to find the platform compared with the model group from day 1 to day 5 (Figure 4.12B).

On the testing trial, the BM110-180 group showed a 28.83% increase in path length in the target quadrant (198.46±50.55 cm) compared with the model group (154.04±34.36 cm), though the difference was not statistically significant ($p>0.05$; Figure 4.12C). In contrast, escape latency decreased by 16.48% (8.72±2.28 sec vs. 10.44±1.90 sec), and the frequency of platform crossings decreased by 16.67% (3.00±0.89 times vs. 3.60±0.93 times) in the BM110-180 group compared with the model group, also without statistical significance ($p>0.05$; Figure 4.12C, E). Representative trajectories from the tracking system are shown in Figure 4.12F.

4.11.2 Effects of *Bacopa monnieri* Microcapsule on Locomotor Activity and Anxiety-like Behavior in LPS-induced Rats

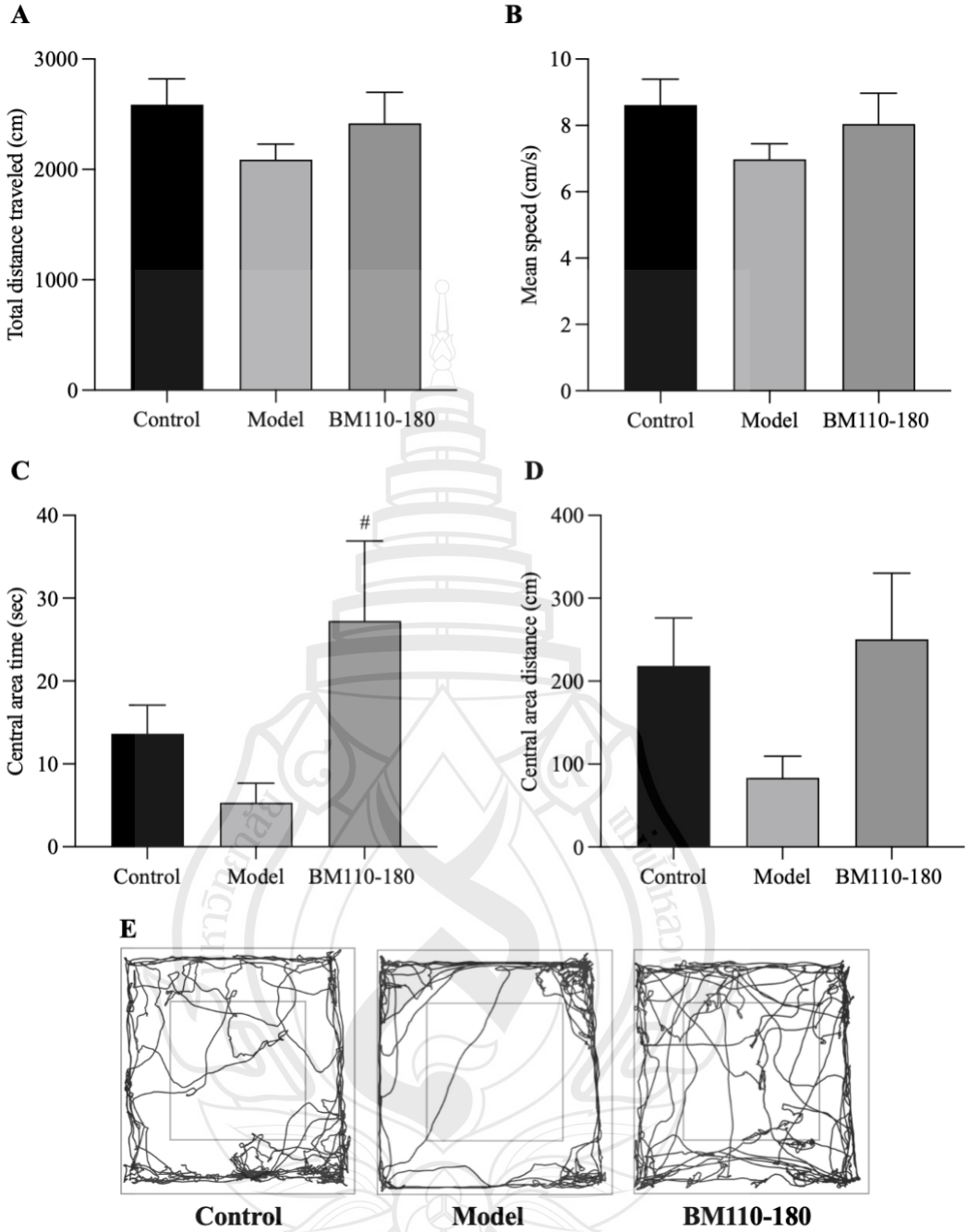
The OFT was used to assess locomotor activity and anxiety-like behavior. The BM110-180 group showed a 15.84% increase total distance traveled (2418.54±280.03 cm) and 15.18% increase mean speed (8.04±0.93 cm/s) compared with the model group (2087.94±141.67 cm and 6.98±0.47 cm/s), though the difference was not statistically significant ($p>0.05$; Figure 4.13A, B). Regarding anxiety-like behavior, the BM110-180 group spent significantly more time in the central area, approximately 4-fold higher (27.26±9.65 sec) than the model group (5.28±2.35 sec, $p<0.05$; Figure 4.13C), and showed a 2-fold higher distance traveled in the central area (250.64±79.49 cm) compared with the model group (83.32±26.04 cm), though the difference was not

statistically significant (p>0.05; Figure 4.13D). These behavioral patterns are shown in the representative trajectories from the tracking system (Figure 4.13F).



Note Rivastigmine group (RVS), *Bacopa monnieri* spray-dried microcapsule group (BM110-180)

Figure 4.12 Effects of *Bacopa monnieri* microcapsule (BM110-180) on memory acquisition in morris water maze test of LPS-induced rats. (A) Escape latency to find the platform on training trial (B) Path length to find the platform across on training trial (C) Escape latency in the target quadrant on testing trial (D) Path length in the target quadrant on testing trial (E) Number of platform crossings on testing trial (F) Representative trajectory from the tracking system on testing trial. The statistically differences compared with control group $^{*}p$<0.05; compared with model group $^{\#}p$<0.05

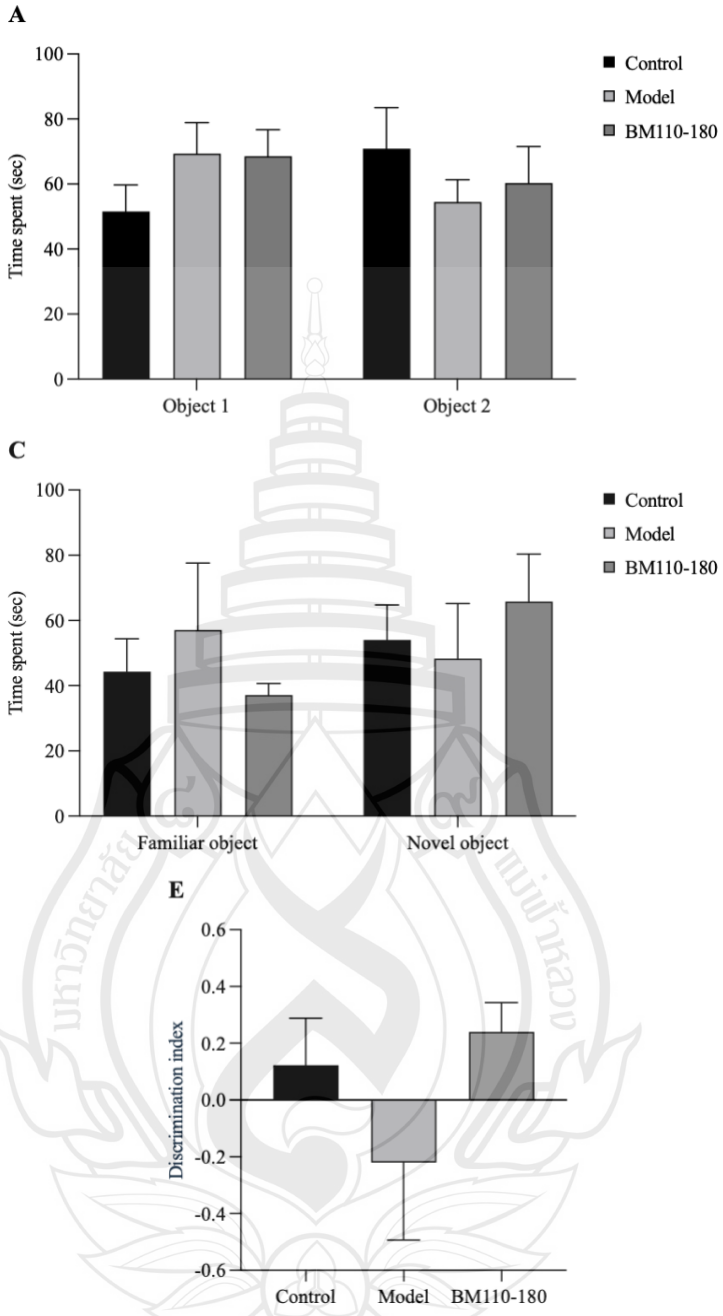


Note Rivastigmine group (RVS), *Bacopa monnieri* spray-dried microcapsule group (BM110-180)

Figure 4.13 Effects of *Bacopa monnieri* microcapsule (BM110-180) on locomotor activity and anxiety-like behavior in open field test of LPS-induced rats. (A) Total distance traveled (B) Mean speed (C) Time spent in the central area (D) Distance traveled in the central area (E) Representative trajectory from the tracking system. The statistically differences compared with control group $^{*}p<0.05$; compared with model group $^{\#}p<0.05$.

4.11.3 Effects of *Bacopa monnieri* Microcapsule on Recognition Memory in LPS-induced Rats

The NOR test was used to assess recognition memory performance. The BM110-180 group showed no significant difference in the time spent exploring objects 1 and 2 (68.54±8.20 and 60.32±11.20 sec) compared with the model group (69.38±9.52 and 54.48±6.87 sec, $p>0.05$) during the training trial (Figure 4.14A). In the testing trial, the BM110-180 group showed a 34.85% decrease in exploration time of the familiar object (37.14±3.53 sec) and a 36.24% increase in exploration time of the novel object (65.80±14.60 sec) compared with the model group (57.10±20.52 sec and 48.30±16.89 sec), although these differences were not statistically significant ($p>0.05$; Figure 4.14B). Similarly, the discrimination index of the BM110-180 group increased to 0.22±0.11 compared with -0.23±0.27 in the model group, also without statistical significance ($p>0.05$; Figure 4.14C).



Note Rivastigmine group (RVS), *Bacopa monnieri* spray-dried microcapsule group (BM110-180)

Figure 4.14 Effects of *Bacopa monnieri* microcapsule on recognition memory in novel object recognition test of LPS-induced rats. (A) Time spent exploring object 1 and object 2 during the training trial (B) Time spent exploring the familiar and novel objects during the testing trial (C) Discrimination index. No statistically significant differences were observed among groups (p>0.05).

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigated the effects of BM extract and its microencapsulated formulations on cognitive functions in LPS-induced dementia *in vivo* model. BM is a well-known medicinal plant with neuroprotective and antioxidant properties, which may help counteract neuroinflammation and oxidative stress associated with cognitive decline. Microencapsulation was applied to enhance the stability, bioavailability, and efficacy of the extract, aiming to improve its therapeutic potential. Overall, the findings of this study provide insight into the ability of both BM extract and its microcapsules to support cognitive function and mitigate neuroinflammation, offering potential strategies for functional interventions in dementia.

The variety of phytochemicals has been reported in BM including triterpenoid saponins, flavonoids, alkaloids, phenols, and tannins (Parveen et al., 2016). In this study, phenolic and flavonoid compounds were detected in the ethanolic extract. These two groups are major classes of bioactive compounds commonly found in plants. TPC is generally higher than TFC because flavonoids represent only one subclass within the broader category of phenolic compounds, which also includes phenolic acids, tannins, lignans, and other related compounds. (Hassanpour & Doroudi, 2023; Sun & Shahrajabian, 2023). Compared with previous studies on ethanolic extracts of BM, Shanmugam et al. (2025) reported a TPC about 16 times higher, while Otari and Ghane (2024) reported a TFC about 3 times higher than those observed in the present work. The observed variations may be attributed to differences in sample collection and preparation, which influence the measured levels of these compounds. Both studies also highlighted the strong effect of solvent type on TPC and TFC values as for example, acetone typically yields higher TPC and TFC than ethanol because its intermediate polarity dissolves a broader range of phenolic compounds and penetrates plant tissues more effectively (Abubakar & Haque, 2020). Given that phenolic and flavonoid

compounds are widely recognized as major contributors to antioxidant activity due to their ability to scavenge free radicals, donate hydrogen or electrons, and chelate pro-oxidant metals, the measured TPC and TFC values in the present extract provide a strong basis for its antioxidant potential (Asfaw, 2023).

The antioxidant activity of the BM extract in this study reflects its rich phytochemical composition, particularly its phenolic and flavonoid content. Evaluation using DPPH and ABTS radical scavenging assays indicated that the extract functions through both hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, consistent with the behavior of phenolic compounds (Johari & Khan, 2022). These assays are simple, cost-effective colorimetric methods that employ stable, colored radical species which react with antioxidants, enabling rapid assessment of radical-scavenging capacity (Sadeer et al., 2020). Notably, the results of this study showed that the IC_{50} value for DPPH was higher than that for ABTS. This difference can be attributed to solvent compatibility, as DPPH is soluble only in organic solvents while ABTS dissolves in both aqueous and organic solvents, allowing it to assess both lipophilic and hydrophilic compounds and making it more suitable for diverse sample types. Furthermore, ABTS reacts more rapidly with free radicals than DPPH, which likely accounts for its superior radical-scavenging efficiency (Floegel et al., 2011; Lee et al., 2015).

After screening for antioxidant activity using DPPH and ABTS assays, the study further investigated specific antioxidant mechanisms. SET activity of BM extract was showed in the FRAP assay, where antioxidant molecules donate electrons to reduce ferric ions (Fe^{3+}) to the more stable ferrous form (Fe^{2+}), forming a colored Fe^{2+}–ligand complex that can be measured spectrophotometrically (Munteanu & Apetrei, 2021). This electron-donating activity stabilizes radicals and terminates ROS chain reactions, thereby reducing oxidative stress and preventing the oxidation of cellular molecules such as proteins, carbohydrates, lipids, and DNA, ultimately protecting cells from free radical-induced damage (Anu et al., 2025). Additionally, superoxide anion scavenging activity was observed using the NBT assay, in which superoxide radicals ($O_2^{\bullet -}$) reduce NBT to formazan, while antioxidants inhibit this reduction by donating electrons to neutralize the radicals (Xu et al., 2013). This indicates the inhibition of superoxide radicals is particularly important, as it contributes significantly to oxidative stress and

is implicated in the development of diseases such as cancer, cardiovascular disorders, diabetes, and neurological disorders including AD (Jomova et al., 2023).

Furthermore, peroxyl radical scavenging capacity, which operates via HAT mechanisms, was confirmed using the OARC assay. In this assay, antioxidants react with peroxyl radicals (ROO•) generated from AAPH decomposition, terminating radical chain reactions and protecting the fluorescent probe. (Prior, 2015). This activity neutralizes peroxyl radicals formed during lipid peroxidation, when molecular oxygen reacts with lipid radicals (L•) in cell membranes or lipoproteins. By inhibiting these radicals, cellular oxidative stress can be reduced, helping to protect against diseases associated such as colon cancer and neurodegenerative diseases including AD and PD with oxidative damage (Kurutas, 2016; Skrzydlewska et al., 2005).

In contrast, MCA, which reflects preventive antioxidant potential, was not detectable in this study. Normally, ferrous ions (Fe^{2+}) react with ferrozine to form a blue-colored complex, but this complex formation can be disrupted if Fe^{2+} binds to hydroxyl groups (–OH) on phenolic compounds, including flavonoids, phenolic acids, and other polyphenols (Gulcin & Alwasel, 2022; Santos et al., 2017). The absance of detectable MCA despite measurable total phenolic and flavonoid content in the BM extract may be due to the hydroxyl groups already forming complexes or hydrogen bonds with themselves or with other molecules such as proteins, amino acids, or other plant constituents, or undergoing reactions that render them unavailable for chelation with Fe^{2+} in the ferrozine assay. (Chen et al., 2024) Similar findings have been reported by Lee et al., (2016) in their study on *Perilla frutescens* var. *acuta* Kudo juice concentrate and water extracts, which showed high TPC but showed no detectable MCA.

Overall, BM extract showed antioxidant activity primarily through electron transfer and hydrogen atom transfer mechanisms, indicating that its effects are mainly mediated via radical scavenging and reducing pathways rather than metal ion chelation. Similarly, the study by Shanmugam et al. (2025) reported that the leaf extract of BM showed strong antioxidant activity through both SET and HAT mechanisms, as evaluated by DPPH, ABTS, and FRAP assays. The observed activity depended on the extraction solvent, with the highest activity detected using aqueous solvent for DPPH and FRAP, and acetone for ABTS, highlighting how solvent polarity can influence the

extraction of phenolic and flavonoid compounds and thereby affect the measured antioxidant potential.

Additionally, phytochemical analysis in this study identified bacoside A as the primary bioactive compound in BM extract, which is known for its neuroprotective effects (Sekhar et al., 2019). This study successfully detected key constituents of the bacoside A mixture, including bacoside A_3, bacopaside II, bacopaside X, and bacopasaponin C. In comparison, Otari and Ghane (2024), who used methanol to extract the whole plant, reported bacoside A levels approximately 25-100 times higher. Similarly, Shahid et al. (2016), who performed methanol extraction of the aerial parts of BM, reported bacoside A levels about 36-1,100 times higher than those observed in the present study. These differences in compound levels are likely due to several factors. First, the choice of solvent affects extraction efficiency such as methanol is more polar than ethanol, allowing it to dissolve polar compounds such as bacosides more effectively and penetrate plant cells more efficiently (Widyawati et al., 2014). Second, different studies used different plant parts which naturally contain varying levels of bioactive compounds with the stolon and leaves showing the highest concentrations and flowers the lowest (Naik et al., 2012). Additionally, other factors such as cultivation practices, harvesting time, and environmental conditions can also influence the concentration of bacoside A in the plant material (Bansal et al., 2016; Phrompittayarat et al., 2011).

To assess the potential toxicity of BM extract, pharyngeal pumping and food clearance assays were performed in *Caenorhabditis elegans*. The pharyngeal pumping assay directly measures the rate of pharyngeal contractions, provides information about the effects on neuronal and muscular function, specifically related to feeding behavior (Calahorro et al., 2021). This study showed no acute toxicity of pharyngeal pumping was unaffected at lower concentrations that suggesting no acute neurotoxicity at low concentrations of 0.1 mg/mL. However, at higher concentrations (0.25, 0.5, and 1.0 mg/mL), pharyngeal contractions were significantly reduced reflecting a concentration-dependent decrease in pharyngeal movements that suggests the extract may have neurotoxic effects leading to reduced food intake behavior. According to Brimson et al. (2022) reported that the hexane extract of BM did not show toxicity at dose of 5 and 10 µg/mL when compared to the control group fed with laboratory food source.

Furthermore, Phulara et al. (2015) observed no toxic effects at any of the tested concentrations (0.001, 0.01, and 0.1 mg/mL) of aqueous extract of BM during the first 3 days of exposure. However, a significant decline in pharyngeal pumping rate was observed at later days, suggesting that toxic effects may manifest upon prolonged exposure.

To further investigate potential toxicity at higher doses and during prolonged exposure, the food clearance assay was employed. This assay measures the reduction in optical density of *Escherichia coli* in the medium, reflecting the feeding activity and overall health of the nematodes (Mudd & Liceaga, 2022). Contrary to expectations, BM extract at higher concentrations (0.5 and 1.0 mg/mL) significantly reduced feeding behavior during the first 4 days compared to the control group indicating a mild toxic effect during this period, followed by a significant recovery in feeding behavior during days 5-7 suggesting that the toxic effects were transient. This pattern suggesting no overt toxicity and also highlight the complex and biphasic nature of BM effects on *C. elegans* feeding.

After investigating toxic effects, the extract's ability to improve cognitive function was examined using behavioral assays in animal models. Normal rats present consistent behaviors that reflect their physiological and psychological health. They typically consume about 10 g of food and 10-15 mL of water per 100 g body weight daily, often in multiple short bouts. Rats regularly groom to maintain clean, glossy fur and engage in locomotor activities such as exploration, walking, climbing, and occasional wheel use, with peak activity during the dark cycle. Social behaviors, including sniffing, rearing, and affiliative grooming, are also commonly observed in group-housed animals (Koolhaas, 2010; Whishaw & Kolb, 2020). These experiments targeted cognitive dysfunction, particularly memory and learning impairments, which are hallmark features of neurodegenerative diseases such as AD. Rats with experimentally induced cognitive deficits showed decreased performance in learning and memory tasks, reduced exploratory activity, lower rearing frequency, and increased thigmotaxis, reflecting heightened anxiety. Feeding and drinking behavior, grooming and self-care, and social interactions were also frequently diminished (Ghafarimoghadam et al., 2022; Puzzo et al., 2014; Kirsten et al., 2015; Zhu et al., 2014).

In this study, a low dose of BM extract was administered daily for 28 days prior to LPS induction to evaluate its protective effects against cognitive impairment. BM extract at a dose of 70 mg/kg showed a trend toward improving cognitive performance by attenuating LPS induced deficits associated with neuroinflammation. BM showed neuroprotective effects against LPS, which reliably induces systemic and CNS inflammation through Toll-like receptor 4 (TLR4) activation and mirrors key pathological processes seen in neurodegenerative disorders such as AD including microglial activation elevated pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6), oxidative stress, and impaired synaptic plasticity, thereby producing reproducible learning and memory impairments (Engler-Chiurazzi et al., 2023; Zhao et al., 2019). Furthermore, no abnormal behaviors were observed in the experimental rats, except in the LPS-induced group, which showed impaired performance in learning and memory tasks, while other normal behaviors remained unaffected.

The MWM results show that the extract improved escape latency and path length during both the training and testing trials, indicating enhanced spatial learning and memory compared with the LPS group, which presented cognitive dysfunction. However, the number of platform crossings was slightly lower. These effects may involve the CA subregions of the hippocampus, including CA1 and CA3, which are essential for memory but have distinct functions. CA1 primarily supports rapid encoding and retrieval of episodic memories, whereas CA3 is critical for pattern completion and one-step learning. Together, they facilitate spatial learning and memory formation (Dong et al., 2021; Othman et al., 2022). Similarly, Rani and Prasad (2015) reported that pre-treatment with BM extract (200 mg/kg) restored memory in cobalt chloride ($CoCl_2$) induced hypoxic mice, improving escape latency, path length, platform crossings, and time spent in the target quadrant compared with untreated hypoxic controls. In addition, Kunte and Kuna (2013) showed that pre-treatment with BM extract (100 mg/kg) for approximately 180 days in a mouse model of AD induced by D-galactose and sodium nitrite ($NaNO_2$) initially resulted in impaired performance but progressively improved, with escape latency approaching normal levels by day 165. This indicates that early BM administration mitigated the development of cognitive deficits rather than reversing established impairments. Taken together, these findings suggest that BM possesses neuroprotective potential, particularly when administered

before a pathological insult, even if some measures of spatial memory are not completely restored.

Furthermore, BM extract showed a trend toward a protective effect on working and short-term memory, as demonstrated by the Y-maze test. The extract resulted in slight improvements in time spent and path length in the novel arm, as well as the number of entries, compared with the LPS group, which showed memory deficits. These findings suggest that the extract may support spatial working and reference memory, which involve the CA1 and CA3 subregions of the hippocampus and the prefrontal cortex, a region critical for decision-making, attention, and the temporal organization of behavior, as reflected in spontaneous alternation performance. (Funahashi, 2017; Hauser et al., 2020; Kraeuter et al., 2019). In contrast, the reduction in spontaneous alternation in this study suggests that the extract may have limitations or inconsistencies in supporting certain aspects of spatial memory related to executive function. This decline reflects a reduced ability of the animals to remember previously visited arms and to make choices to explore new ones, which is associated with the prefrontal cortex (De Saint Blanquat et al., 2010). Consistent with the pre-treatment study by Pham et al. (2019), in which BM extract at 50 mg/kg ameliorated cognitive deficits in trimethyltin-induced mice by significantly increasing time spent in the novel arm, this study results suggest a similar trend, although the effect did not reach statistical significance.

Pre-treatment with BM extract in LPS-induced rats showed a trend toward enhancing spatial working and reference memory, as reflected by modest improvements in escape latency and path length in the target quadrant of the MWM, as well as increased time spent in the novel arm in the Y-maze, although some measures did not reach statistical significance. These trends are consistent with observations from other studies in which pre-treatment with plant extracts mitigated LPS-induced cognitive impairments. For instance, pre-treatment with Pu'er tea water extract at doses of 150 and 300 mg/kg, administered orally once daily for 4 weeks before and during the final week of LPS injection, improved spatial learning and memory in MWM (Jeong et al., 2020). Similarly, oral pre-treatment with *Tacca chantrieri* Andre extract at doses of 100 and 200 mg/kg for 7 days prior to LPS administration enhanced performance in the Y-maze and novel object recognition tests (Kamsrijai et al., 2023). Collectively,

these findings indicate that early intervention with neuroprotective phytochemicals, including BM, can prevent or attenuate LPS-induced cognitive impairments and reduce neuroinflammatory markers, highlighting its potential as a preventive agent against inflammation-associated cognitive decline.

In this study, microencapsulation using the spray-drying technique was employed due to its cost-effectiveness, simplicity, and ability to produce free-flowing powders that protect bioactive compounds in natural products (Mardani et al., 2024). This approach was used to overcome the limitations of the main active compound, which exhibits poor bioavailability due to low water solubility and is prone to rapid degradation under heat, moisture, and acidic conditions. Moreover, the compound may not be efficiently absorbed in its natural form, leading to further absorption challenges (Devaraj Reddy et al., 2024; Phrompittayarat et al., 2008). Maltodextrin and unripe banana flour were selected as coating agents because they are widely used coating materials, known for their good solubility, low viscosity, film-forming ability, and affordability (Nur Hanani & Abdullah, 2016; Xiao et al., 2022). Additionally, banana flour is a nutritionally valuable and highly functional food ingredient, rich in resistant starch and dietary fiber, making it suitable for future development into functional food products (Munir et al., 2024). The results showed that lower inlet and outlet drying temperatures led to higher encapsulation yields, as high temperatures can cause thermal degradation of bioactive compounds and increase stickiness, reducing the amount of recoverable powder (Fitzpatrick et al., 2007). Additionally, a higher core-to-wall material ratio further improved encapsulation yield. A greater proportion of wall material provides more coverage around the core, effectively encapsulating it and minimizing leakage or exposure to external conditions (Delaporte et al., 2024). Therefore, selecting appropriate core-to-wall ratios and drying temperatures is crucial for optimizing encapsulation yield. These results align with the findings of Duangchuen et al. (2020), who investigated the effect of spray-drying inlet air temperature on skimmed coconut milk powder. They reported that higher inlet temperatures generally led to lower yield percentages, likely due to thermal degradation of the product, which reduced the amount of powder successfully collected. Similarly, Grassia et al. (2021) encapsulated phenolic extracts from cocoa shells using maltodextrin via spray drying with different core-to-wall ratios and temperatures. Their results also demonstrated that

encapsulation yield was strongly influenced by both the core-to-wall ratio and the drying temperature.

After microencapsulation development, the resulting microencapsulated standardized BM showed differences in particle size distribution. The formulations with higher core-to-wall ratios (1:10) had smaller particle sizes than the formulations with lower core-to-wall ratios (1:20). This is due to the fact that increasing the wall material ratio can thicken the mixture, which affects how the liquid breaks into droplets during spray drying. Thicker mixtures tend to form larger droplets, resulting in larger microcapsule particle size (Rajam & Anandharamakrishnan, 2015). Furthermore, at the same core-to-wall ratios, the formulations processed at higher temperatures also resulted in smaller particle sizes. Similarly, Santiago-Adame et al. (2015) reported that spray drying of cinnamon infusions with maltodextrin at various inlet temperatures resulted in smaller particle sizes at higher temperatures. This is due to faster moisture evaporation and more rapid solidification of droplets at higher temperatures, leading to greater shrinkage and ultimately smaller particles (Sun et al., 2020).

The physical properties of the microcapsule powders, including bulk density, tap density, flowability, and cohesiveness provide important insights into their handling and suitability for further processing. These properties are critical as they influence the powder's flow behavior, packing efficiency, stability, and ease of handling during manufacturing and storage. Higher bulk and tapped densities are generally favorable because they indicate better packing and more efficient storage and transportation. Good flowability, indicated by lower CI and HR, is essential for consistent dosing and smooth processing. Moderate cohesiveness is desirable to maintain powder integrity, but excessive cohesiveness can lead to poor flow and processing difficulties (Koca et al., 2015; Tatasciore et al., 2024). The result found that higher concentrations of wall materials generally led to increased bulk and tapped densities, as well as enhanced flowability and cohesiveness. However, increasing the inlet temperature had the opposite effect, reducing these physical properties. These findings are consistent with the studies by Vonghirundecha et al. (2022), who investigated the microencapsulation of *Moringa oleifera* leaf polyphenol-rich extract using maltodextrin as the wall material, and Osamede Airouyuwa and Kaewmanee (2019), who used vegetable protein as the wall material. Both studies showed that the wall material ratio and inlet

temperature significantly influenced the overall physical properties of the microcapsules.

This study also assessed the chemical properties of the microencapsulated powders. The results showed that temperature is a critical factor influencing hygroscopicity, moisture content, and water solubility. Higher temperatures led to decreased moisture content due to enhanced evaporation during spray drying. As a result, the powders became drier and showed increased hygroscopicity, as drier powders tend to absorb more moisture from the environment. These findings are consistent with the study by Wijayanti et al. (2023), who reported that increasing the spray drying inlet temperature for black garlic extract powder resulted in reduced moisture content and higher hygroscopicity. The water activity also decreased, indicating lower available energy of water in the microencapsulated powders, which contributes to longer shelf life and reduced degradation. Similar findings were reported by Tao et al. (2024), studied the physicochemical properties of grapeseed oil microcapsules using maltodextrin as the wall material and found that higher inlet temperatures reduced both moisture content and water activity. Furthermore, the water solubility index of the microcapsules increased after the spray-drying process compared to the original BM extract, which had a water solubility of 15.31% (Mishra et al., 2015). This improvement suggests that microencapsulation can help overcome the limitation of BM's naturally low water solubility.

However, the microencapsulation process significantly reduced the TPC and TFC. Formulations with a higher core-to-wall ratio (1:10) retained more of these compounds. This reduction is likely due to the high temperatures used during microencapsulation, which can cause thermal degradation of phenolic and flavonoid compounds in the BM extract (Antony & Farid, 2022). High temperatures degrade these compounds by breaking glycosidic bonds, oxidizing hydroxyl (–OH) and carbonyl (C=O) groups, and disrupting ring structures. These alterations decrease the number of reactive groups available for the Folin–Ciocalteu and $AlCl_3$ assays, resulting in lower measured TPC and TFC values (ElGamal et al., 2023).

This trend of thermal degradation was also reflected in the antioxidant activity, with microencapsulation significantly reducing the antioxidant effects compared to the original BM extract after processing. The results showed that formulations with a higher

core-to-wall ratio showed better antioxidant activity in DPPH, ABTS, FRAP, and ORAC assays, likely due to greater retention of antioxidant compounds within the microcapsules, reflecting both SET and HAT mechanisms. Among them, BM110-180 demonstrated the highest overall antioxidant activity. However, no antioxidant capacity was detectable in any formulation in the NBT assay, likely due to the degradation of phenolic and flavonoid cpmpound, which can no longer donate electrons to neutralize the superoxide anion and thereby fail to inhibit the reduction of NBT to blue-colored diformazan (Sadeer et al., 2020). These findings are consistent with the study by Polanco et al. (2024), who encapsulated boldo leaf extract using maltodextrin via spray-drying and reported that variations in core-to-wall ratio, temperature, and drying time influenced antioxidant capacity. Their microcapsules showed high radical scavenging activity in DPPH and ABTS assays and tended to show higher in FRAP, reflecting a consistent trend across different antioxidant assessment methods. Similarly, Moreno et al. (2016) encapsulated grape marc extract using maltodextrin, whey protein, or pea protein through spray-drying under varying inlet and outlet temperatures, and all production conditions preserved the peroxyl radical scavenging capacity of the extract. These studies support the trends observed in the present work, where formulations with higher core-to-wall ratios retained greater antioxidant capacity across multiple mechanisms.

The metabolite profiling of BM110-180 showed that it contained the same 2 key metabolites as the original BM extract, namely bacoside A3 and bacopaside X along with other compounds such as triterpene saponins, flavonols, flavones, alkaloids, coumaric acids, and their derivatives. However, detection of some compounds was initially limited due to similar molecular weights (MW), such as bacopaside II (MW 929.10, PubChem) corresponding to bacoside A3 and bacopasaponin C (MW 899.07, PubChem) corresponding to bacopaside X (Fang et al., 2015). To confirm whether BM110-180 still retained these key bioactive compounds, quantitative analysis was performed for all 4 metabolites. The results confirmed the presence of all compounds, although their concentrations were approximately 5-9 times lower than in the original extract, likely due to the high core-to-wall ratio used in the formulation (1:10), which exceeded expectations and led to a reduction of active compounds by roughly 10-fold relative to the core material. Similarly, Vonghirundecha et al. (2022) reported that

microencapsulation of *Moringa oleifera* leaf extract and its phenolic-rich fraction using higher wall-to-core ratios with maltodextrin resulted in a marked decrease in active constituents, in some cases rendering compounds undetectable.

The BM110-180 was administered three times per week for 35 days prior to LPS induction to evaluate its potential protective effects against cognitive impairment. At a dose of 200 mg/kg, BM110-180 showed a tend to improve cognitive performance by mitigating LPS-induced deficits associated with neuroinflammation. In the LPS group, abnormal behaviors were observed, including reduced locomotor activity, difficulty walking, minimal exploratory behavior, and decreased grooming, resulting in unkempt fur. Some animals also showed bloody stools, pallor, and diminished social interactions. Notably, these symptoms improved within three days, and the animals' behaviors gradually returned to normal. In contrast, rats in the BM110-180 group showed milder behavioral alterations, suggesting a protective effect of BM110-180 against LPS-induced behavioral disturbances. The observed behavioral alterations in this study may have resulted from the relatively LPS dose used. A single high-dose injection likely induced strong physiological stress, immune activation, and neuroinflammation, which interfered with normal behaviors. In contrast, using a lower dose (e.g., 0.25 mg/kg to 0.75 mg/kg) administered repeatedly over 5-7 days would be expected to induce cognitive deficits while minimizing overt behavioral disturbances, allowing clearer observation of subtle behavioral changes (da Silva et al., 2024; Skrzypczak-Wiercioch & Sałat, 2022).

In the MWM, pre-treatment of BM110-180 improved both the escape latency and path length during the training trials, as well as the path length in the target quadrant during the testing trial, indicating enhanced spatial learning and memory. Although escape latency and the number of platform crossings showed only slight improvements, the overall trend suggests a protective effect on cognitive function. These results indicate that BM110-180 may help mitigate LPS-induced impairments in hippocampal-dependent learning and memory, particularly within the CA1 and CA3 subregions. Similar to the findings of Martin et al. (2023), a low-dose pre-treatment with astaxanthin microencapsulated within spirulina at dose of 80 mg/kg in female rats prevented LPS-induced cognitive deficits and improved long-term memory in the MWM, as evidenced by reduced escape latency. These findings collectively

demonstrate that microencapsulation can preserve and enhance the neuroprotective efficacy of bioactive compounds.

Moreover, results from the NOR test further support the cognitive-enhancing effects of BM110-180. The treated group spent more time exploring the novel object, less time with the familiar object, and showed a higher discrimination index compared with the LPS group, indicating improved recognition memory. These findings are consistent with the known roles of key brain regions in object recognition memory, particularly the perirhinal cortex and hippocampal CA1. The perirhinal cortex is critical for evaluating whether an object is familiar or novel, particularly over short retention intervals, and contributes to representing basic object familiarity. The hippocampus, especially the CA1 region, plays a significant role in forming strong object memories, and its inactivation can impair these memories. It also supports long-term object recognition and encodes contextual information, complementing the perirhinal cortex's role in short-term processing (Antunes & Biala, 2012; Barker & Warburton, 2011; Cinalli et al., 2020). Similarly, consistent with Kwon et al. (2018), administration of BM at 200 mg/kg significantly improved novel object recognition in healthy mice, as evidenced by increased exploration time of the novel object compared with the familiar object and a higher discrimination index. Likewise, Preethi et al. (2016) reported similar results, showing that administration of 80 mg/kg of standardized BM extract (CDRI-08) enhanced novel object recognition in healthy rats, with increased exploration of the novel object and an elevated discrimination index. Collectively, these results indicate that BM110-180 preserves the neuroprotective and cognitive-enhancing efficacy of the original extract.

Taken together, the MWM and NOR results indicate a trend toward cognitive-enhancing effects of BM110-180 after the microencapsulation process. The lack of statistically significant improvement may be attributed to the relatively low dose of BM110-180 (200 mg/kg) and the non-daily LPS injections used to induce neuroinflammation (3 days per week) instead of daily administration. Nevertheless, these findings suggest that spray-dried microencapsulation preserves the extract's neuroprotective potential and cognitive-enhancing effects.

Another noteworthy finding is that BM110-180 appeared to reduce anxiety-like behavior in the OFT which induced-LPS neuroinflammation, as demonstrated by a

significant increase in time spent in the central area and a non-significant increase in distance traveled within the central area, without any differences in overall locomotor activity. This suggests that BM110-180 may help counteract neuroinflammation-induced anxiety-like behaviors, which in rodent models arise mainly through activation of microglia and astrocytes, release of pro-inflammatory cytokines and other mediators, and subsequent disruption of neuronal function and synaptic plasticity which particularly in the hippocampus and amygdala (Guo et al., 2023; Wang et al., 2018). This finding is supported by Rathore et al. (2019), who studied BacoMind™, a standardized phytochemical composition derived from BM and reported that oral administration at doses of 30 mg/kg and 60 mg/kg significantly reduced anxiety while increasing the number of entries into the central area as well as both rearing and assisted rearing in the OFT

5.2 Conclusion

The BM extract contained high levels of key phytochemical compounds, including bacoside A, phenolics, and flavonoids, and showed strong antioxidant activity through both SET and HAT mechanisms. It showed no toxicity that did not affect feeding behavior in *C. elegans*, and showed a trend toward improving cognitive function, including spatial learning, memory, and recognition memory in LPS-induced dementia rats, indicating neuroprotective effects. The microencapsulated BM extract, prepared with maltodextrin and unripe banana flour at different core-to-wall ratios and drying temperatures, effectively preserved these phytochemical compounds and antioxidant activity, although some reduction presented compared with the original extract, likely due to heat exposure during spray-drying. Among the formulations, BM110-180 showed the highest antioxidant activity and a clear trend toward enhancing cognitive function, along with a reduction in anxiety-like behavior. These findings suggest that microencapsulation preserves the bioactivity of BM extract while providing enhanced stability, making BM110-180 a promising candidate for functional interventions aimed at mitigating cognitive deficits associated with dementia and neuroinflammation.

5.3 Limitation

5.3.1 Bioactive Compound Degradation

Microencapsulation and spray-drying may partially degrade key bioactive compounds, potentially reducing therapeutic efficacy.

5.3.2 Model Limitations

The LPS-induced neuroinflammation model may not fully represent the complex pathology of neurodegenerative diseases, and the short experimental duration limits generalizability.

5.3.3 Behavioral-only Assessment

Only behavioral tests were conducted, without biochemical or histological analyses, limiting understanding of the underlying neuroprotective mechanisms.

5.4 Suggestion

5.4.1 *In vitro* Investigations

Detailed investigations of drug release profiles and shelf-life stability are needed to optimize the formulation and maintain bioavailability of the extract and microcapsules.

5.4.2 *In vivo* Studies

Further studies should assess biochemical and histological changes, including neuroinflammatory markers and blood-brain barrier integrity, and include both sexes to examine hormonal influences. Toxicity assessments such as liver function and hematological tests are also recommended.

5.4.3 Clinical Trials

Well-designed clinical studies in patients with dementia or related neurodegenerative disorders are needed to evaluate the long-term safety and efficacy of the extract and its microencapsulated form.

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APPENDIX A

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CALIBRATION CURVE OF BACOSIDE A STANDARD

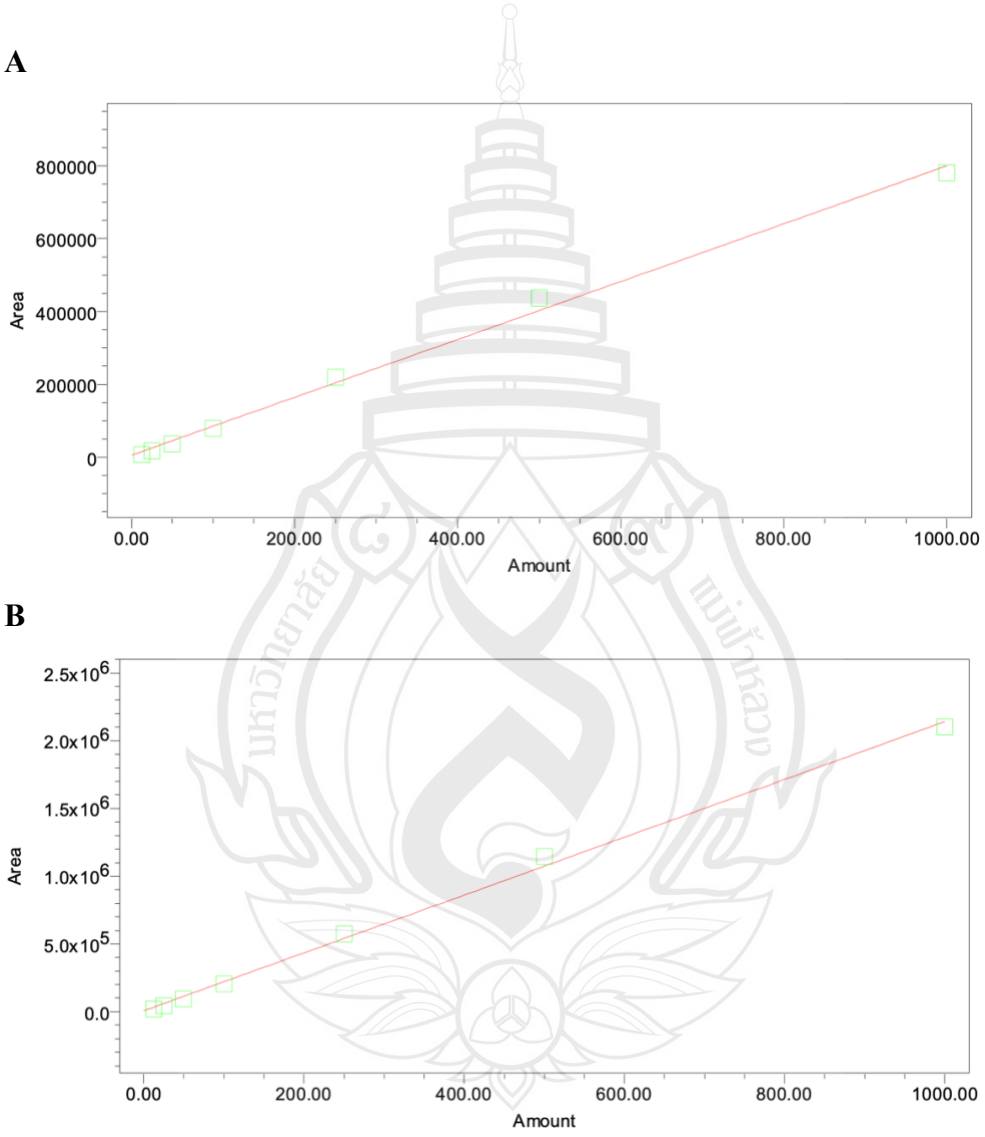
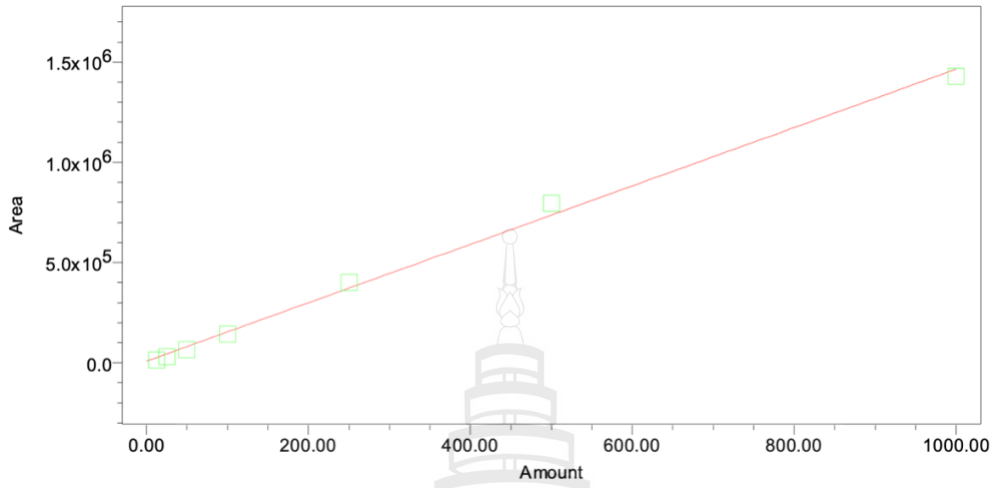


Figure A1 High-performance liquid chromatography (HPLC) calibration curve of bacoside A standard. (A) bacoside A_3, (B) bacopaside II, (C) bacopaside, and (D) bacopasaponin C

C



D

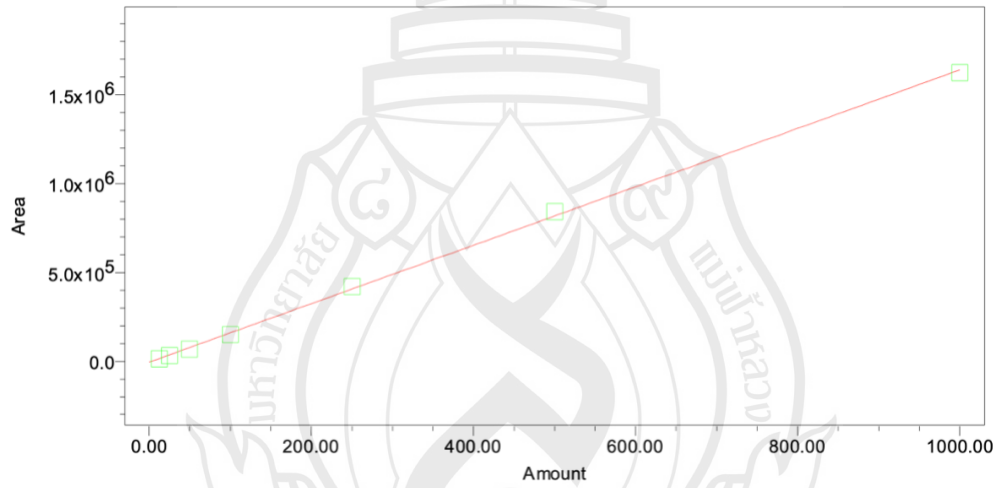


Figure A1 (continued)

APPENDIX B

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY CALIBRATION CURVE OF BACOSIDE A STANDARD

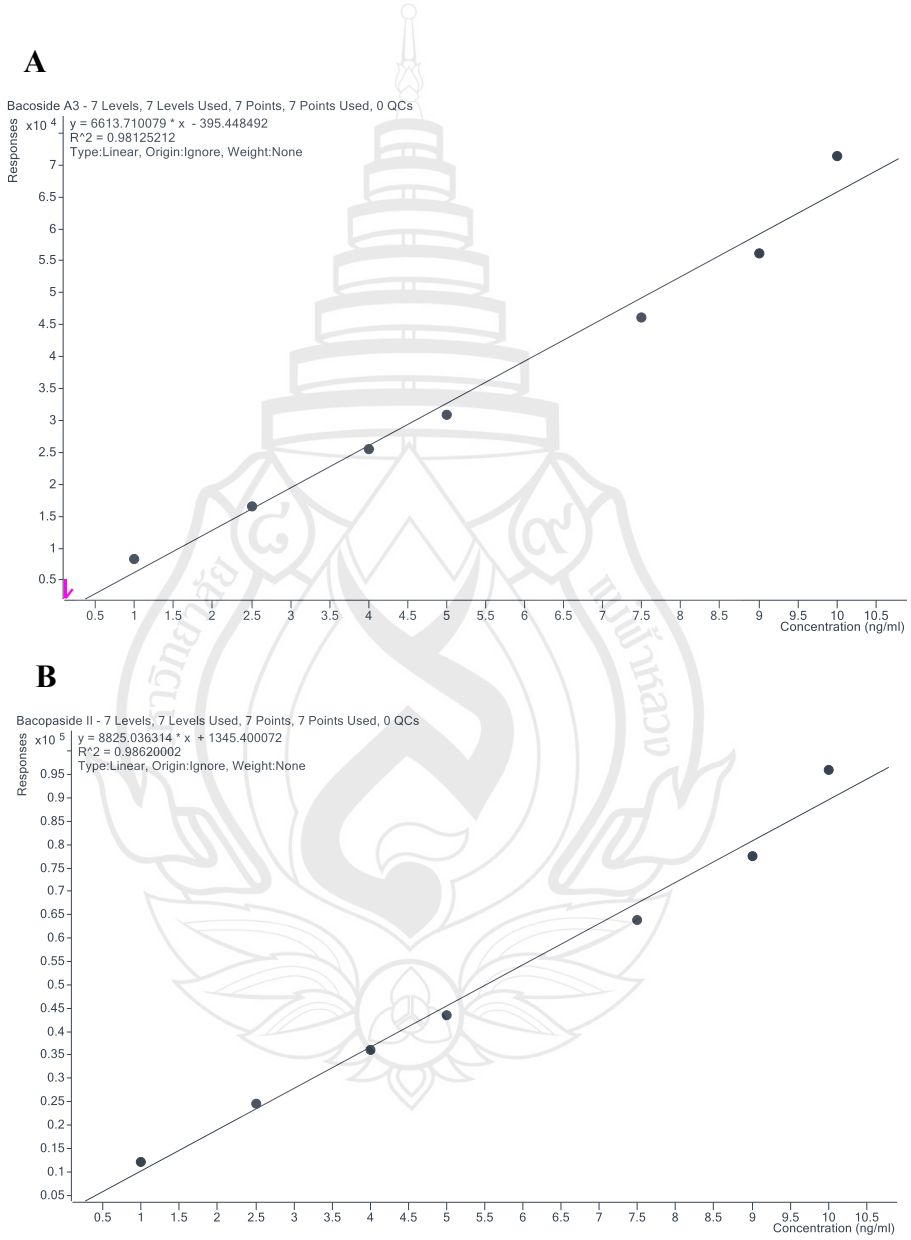
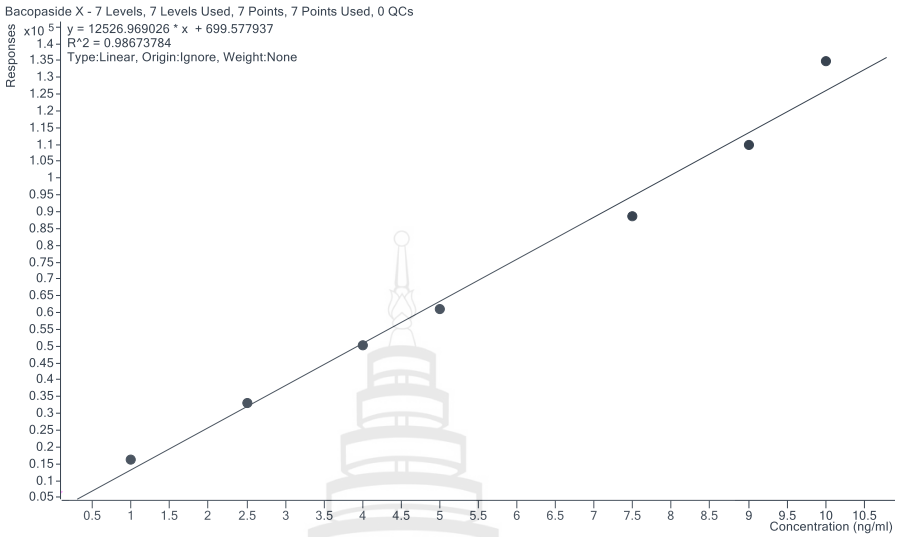


Figure A2 Liquid Chromatography-Mass Spectrometry (LC-MS) calibration curve of bacoside A standard. (A) bacoside A_3, (B) bacopaside II, (C) bacopaside, and (D) bacopasaponin C

C



D

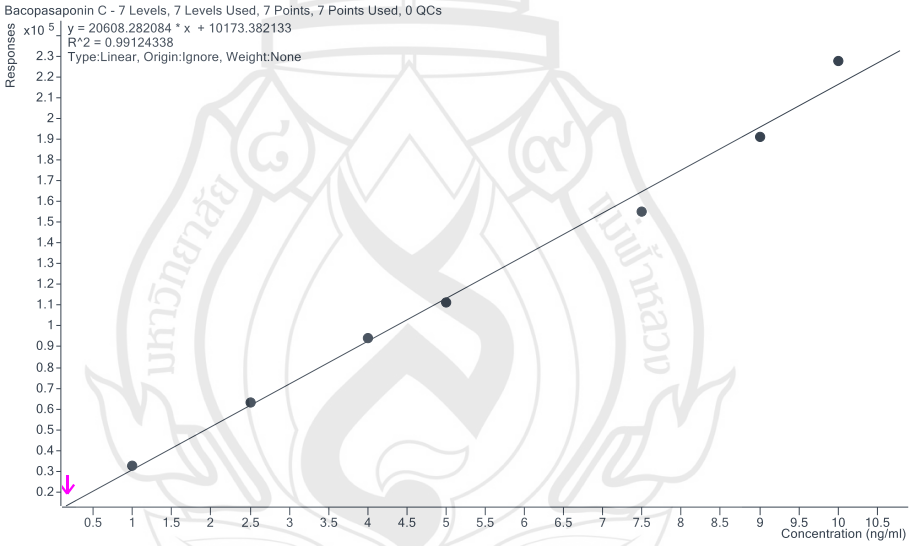


Figure A2 (continued)

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