THE EFFECTS OF YLANG-YLANG (*CANANGA ODORATA*) ESSENTIAL OIL ON BEHAVIOURAL SYMPTOMS AND PLASMA BIOMARKERS IN LONG-TERM CARE RESIDENTS WITH DEMENTIA

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by

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LIST OF ABBREVIATIONS

AChEI	Acetylcholinesterase Inhibitors
ACTH	Adrenocorticotropic Hormone
AD	Alzheimer’s disease
APP	Amyloid Beta precursor protein
Avidin-HRP	Avidin-Horseradish Peroxidase
Aβ	Amyloid Beta
BD	Becton Dickinson
BD Ab	biotinylated detection antibody
BDNF	Brain-derived neurotrophic factor
BPSD	behavioural and psychological symptoms of dementia
CCMAI	Chinese versions of Cohen-Mansfield Agitation Inventory
CDR	Clinical Dementia Rating
CFS	Clinical Frailty Scale
CI	confidence interval
CMAI	Cohens Mansfield Agitation Inventory
CNPI	Chinese version of Neuropsychiatric Inventory
CNS	central nervous system
CSDD	Cornell Scale for Depression in Dementia
CT	computerized tomography
DBD	Dementia Behavior Disturbance scale
DNA	Deoxyribonucleic acid
DNTB	Dinitrobenzoic acid
DOSM	department of statistic Malaysia
DPPH	1,1-diphenyl-2-picrylhydrazyl
DTT	Dithiothreitol
e.g.	example given
EIA	enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EP tube	Eppendorf tube
FAST	Functional Assessment Staging of Alzheimer's disease

FBP	Folate Binding Protein
FDA	Food and Drug Administration
FHS	Framingham Heart Study
GABA	gamma-aminobutyric acid
GBSS	Gottfries, Brane, Steen scale
GBSS-J	Japanese version of Gottfries, Brane, Steen scale
GRAS	generally recognized as safe
GSH	Glutathione
HDS-R	Revised version of Hasegawa's Dementia Scale (HDS-R)
HPA	hypothalamic-pituitary-adrenal
HPLC	High Performance Liquid Chromatography
hrs	Hours
ICR	Institute of Cancer Research
IDATE	Anxiety Inventory
IL-2	Interleukin-2
IL-6	Interleukin-6
KOH	potassium hydroxide
MCI	mild cognitive impairment
MDA	Malondialdehyde
MMSE	Mini-Mental State Examination
MRI	Magnetic resonance imaging
MSDS	material safety data sheet
NKEA	National Key Economic Areas
NMDA-receptor	N-methyl-D-aspartate receptor
NPI	Neuropsychiatric Inventory
NPI-Q	Neuropsychiatric Inventory- Brief Questionnaire
OD	Optical Density
OR	odds ratios
P	p-value
PAS	Pittsburgh Agitation Scale
PET	Positron emission tomography
PMIS	Picture-based Memory Impairment Screen
REC	Research Ethics Committee

ROS	reactive oxygen species
SD	standard deviation
Sdn Bhd	Sendirian Berhad
SFR	salivary flow rate
SPSS	Statistical Package for the Social Sciences
TAOC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substance
TDAS	Touch Panel-type Dementia Assessment Scale
TMB	3,3′,5,5′-Tetramethylbenzidine
TNF	Tumor necrosis factor
TSST	Trier Social Stress Test
UK	United Kingdom
US$	United States Dollar
vs	Versus
WHO	World Health Organization

LIST OF SYMBOLS

%	Percentage
<	less than, more than
>	More than
≤	Less than or equal to
≥	More than or equal
®	Registered trademark
µ	Micro
µL	Micro liter
µmol/L	micromoles per litre
E	Effect size
mL	Milliliter
mL/min	milliliters per minute
mmHg	millimeters of mercury.
N	sample size
n	number of samples
ng/mL	Nanograms per milliliter
nm	Nanometer
nmol/L	Nano mol per liter
ºC	Celsius
pg/L	picograms per liter
pg/mL	picograms per milliliter
pmol/g-tissue	Pico mol per gram of tissue
S^Δ	Standard Deviation of the change in the outcome
™	Trademark
Z	Standard normal deviate
α	Alpha
β	Beta
λ	Gamma
µg/dL	micrograms per deciliter
ρ	spearman Rho correlation

KESAN MINYAK PATI YLANG-YLANG (*CANANGA ODORATA*) TERHADAP GEJALA TINGKAH LAKU DAN BIOPENANDA PLASMA PADA PENGHUNI DENGAN DIMENSIA DI PUSAT JAGAAN JANGKA PANJANG

ABSTRAK

Gejala tingkah laku dan psikologi demensia (BPSD) merangkumi kegelisahan, psikosis, kemurungan, dan sikap tidak peduli. Penggunaan antipsikoptik dalam mengurangi BPSD telah dihadkan kerana berisiko menambahkan kesan sampingan yang tidak diingini. Terkini, penggunaan aromaterapi telah mendapat sambutan sebagai pendekatan bukan farmakologi dalam mengawal BPSD kerana kesan sampingannya yang tidak merbahaya.. Ylang-ylang (*Cananga odorata*) ialah pokok tropika yang kebanyakkan ditemui di Asia Tenggara dan bunga ylang-ylang mengandungi monoterpena seperti linalool yang berpotensi membantu pengawalan BPSD. Walau bagaimanapun, belum ada kajian mengenai kebaikan minyak pati ylang-ylang terhadap kalangan individu yang mengidap demensia. Tambahan pula, kajian keberkesanan aromaterapi melalui analisis sampel darah juga agak terhad. Oleh hal yang demikian, kajian ini mengkaji keberkesanan penggunaan minyak pati ylang-ylang berdasarkan soal selidik penilaian tingkah laku berkaitan demensia dan analisis biokimia dengan menggunakan sampel darah pengidap BPSD. Kajian kuasi-eksperimen pra-pasca telah dijalankan selama dua bulan di tiga rumah penjagaan pesakit demensia (n=28). Air dan minyak pati ylang-ylang telah disebarkan melalui penyebar ultrasonik selama 20 minit sebanyak dua kali sehari pada fasa sebelum dan semasa intervensi. Gejala tingkah laku diperhatikan dan didokumentasikan dengan menggunakan *Cohen-Mansfield Agitation Inventory* (CMAI), Skala *Cornell Scale for*

Depression in Dementia (CSDD) dan *Neuropsychiatric Inventory- Questionnaire* (NPI-Q) sebelum dan selepas intervensi aromaterapi. Di samping itu, sampel darah diambil untuk analisis biokimia. 14 penanda biologi yang dikategorikan sebagai, nuerodegeneratif, neuroninflamasi, stres hormon, stres oksidatif dan antioksida telah diuji. Skor median bagi CMAI ($p=0.002$) dan skor median bagi CSDD ($p<0.001$) menurun dengan signifikan berikutan dengan aromaterapi ylang-ylang. Tiada penemuan penting diperhatikan pada skor NPI-Q. Berkenaan dengan analisis biokimia, pengurangan signifikan dilihat dalam kepekatan homocysteine; $p=0.001$, beta amiloid (Aβ1-42); $p=0.02$, interleukin-6; $p=0.023$ dan interleukin-2; $p=0.05$ dengan inhalasi minyak pati ylang-ylang. Nisbah Aβ42/40 meningkat dengan signifikan ($p=0.013$) selepas inhalasi ylang-ylang. Keputusan lain yang tidak signifikan merangkumi stres hormon, stres oksidatif dan antioksida. Secara keseluruhannya, aromaterapi ylang-ylang telah merendahkan kegelisahan dan kemurungan bagi pesakit yang mempunyai BPSD. Aromaterapi ylang-ylang dikaitkan dengan pengurangan signifikan dalam penanda neurodegeneratif dan neuroinflamasi.

THE EFFECTS OF YLANG-YLANG (*CANANGA ODORATA*) ESSENTIAL OIL ON BEHAVIOURAL SYMPTOMS AND PLASMA BIOMARKERS IN LONG-TERM CARE RESIDENTS WITH DEMENTIA

ABSTRACT

Behavioural and psychological symptoms of dementia (BPSD) include agitation, psychosis, depression, and apathy. The use of antipsychotics in reducing BPSD is limited due to the risk of developing adverse effects. Recently, aromatherapy has gained momentum as a complementary non-pharmacological approach to controlling the symptoms of BPSD due to its negligible side effects. Ylang-ylang (*Cananga odorata*) is a tropical tree found commonly in Southeast Asia and the ylang-ylang flower contains monoterpenes such as linalool that may potentially help in the management of BPSD. However, no studies have evaluated the benefits of ylang-ylang essential oil among persons with dementia. Moreover, blood-based study on the effectiveness of aromatherapy intervention is scarce. Thus, this study examines the effectiveness of diffused ylang-ylang essential oil based on dementia-related behavioural assessment tools and biochemical analysis using blood samples of persons with BPSD. A pre-post quasi-experimental study was conducted over two months in three nursing homes with persons with dementia (n=28). Water and ylang-ylang essential oil were diffused through an ultrasonic diffuser for 20 minutes twice a day during both pre and post intervention phases. Behavioral symptoms were observed and documented by using the Cohen-Mansfield Agitation Inventory (CMAI), the Cornell Scale for Depression in Dementia (CSDD) and the Neuropsychiatric Inventory- Brief Questionnaire Form (NPI-Q) at pre and post aromatherapy. In addition, blood was taken for biochemical analysis on 14 blood biomarkers categorized as,

neurodegenerative, neuroinflammatory, stress, oxidative stress and antioxidative biomarkers. The median CMAI score ($p=0.002$) and CSDD score ($p<0.001$) decreased significantly following ylang-ylang aromatherapy. No significant finding was observed on the NPI-Q score. With regards to biochemical analysis, a significant reduction ($p<0.05$) was seen in the levels of homocysteine, amyloid beta (Aβ1-42); p, interleukin-6 and interleukin-2 with inhalation of ylang-ylang essential oil. The ratio of Aβ42/40 increased significantly ($p<0.05$) upon ylang-ylang inhalation. No changes were obtained with regard to other biomarkers of stress, oxidative stress and antioxidation. Overall, ylang-ylang aromatherapy lowered the agitation and depression of persons with BPSD and was associated with reduction in neurodegenerative and neuroinflammatory markers.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Dementia is a pathologic condition in the brain, which disturbs the higher cortical functions and affects normal brain activities that are involved in learning, thinking, memory, orientation, judgement, emotion and cognitive functions. Alzheimer's disease (AD) is the most dominant form of dementia that causes gradual memory loss and makes up 60 to 80 percent of dementia (Deture & Dickson, 2019).

To date, prevalence studies of dementia in Malaysia remain limited. AD International reported that Malaysia had approximately 123,000 people with dementia in 2015. The number of cases is expected to reach 261,000 by 2030 and rise to 590,000 in 2050 in the absence of effective intervention (Chan et al., 2019). The key challenge in taking care of and assisting persons with dementia is the management of behavioral and psychological symptoms of dementia (BPSD). BPSD is a group of symptoms often seen in persons with dementia at any time after its onset. The symptoms are classified into three categories which are symptoms related to cognitive impairments (e.g. aggression and agitation), psychological disturbances (e.g. phobias and anxiety) and a combination of both categories involving symptoms like hallucinations, paranoia and delusions (Thies & Bleiler, 2013).

Dementia is generally managed through pharmacological and non-pharmacological interventions. However, most pharmacological treatments help to manage the symptoms of dementia, and there is a limited disease-modifying drug available clinically. There are several medications such as cholinesterase inhibitors, memantine and Aducanumab which have been approved by the US Food and Drug

Administration (FDA) to treat dementia, but they are associated with adverse side effects. Besides that, antipsychotics, antidepressants, anticonvulsants and mood stabilizers have been used off-label to treat BPSD (Yaffe et al., 2002).

In the absence of a treatable condition, non-pharmacological interventions are preferred to manage and care for BPSD. Recently, aromatherapy has gained momentum as a complementary non-pharmacological approach against BPSD. This is mainly due to its negligible side effects (Scales et al., 2018). According to Ballard et. al (2009), the combination of aromatherapy and psychological treatment was proven to be effective in controlling agitation in patients with dementia (Ballard et al., 2009). Previous clinical studies have reported that lavender, lemon balm, geranium, rosemary, orange, lemon, marjoram, patchouli, chamomile, tea tree and thyme were among the essential oils that were studied in persons with dementia (Dong & Jacob, 2016; Fu et al., 2013; Gray & Clair, 2002; Jimbo et al., 2009).

Ylang-ylang (*Cananga Odorata*) is postulated to have a calming effect, but it is still unknown whether similar effects are seen in persons with dementia. Several studies conducted on healthy volunteers showed its beneficial effects on mood, reduced anxiety and boosted self-esteem (Gnatta et al., 2014; Hongratanaworakit & Buchbauer, 2006; Hwang, 2006; Moss et al., 2009; Nan et al., 2016). Thus, considering these findings and safety measures, evaluating the use of ylang-ylang and its effects on BPSD is imperative to possibly lead to reduced use of sedative medication and help to reduce side effects of pharmacotherapy.

1.2 Problem Statement

The increasing prevalence of older people with dementia worldwide corresponds with a significant burden to caregivers, especially in managing BPSD. BPSD can be reduced by providing comfort to patients using patient-centered and caregiver-centered approaches accompanying interventions (Sakka et al., 2019). As the current treatment of using antipsychotics leads to undesired side effects in persons with dementia, there is a need to explore alternatives to antipsychotics with fewer side effects and cost-effectiveness. In Malaysia, the prevalence of dementia is rising, yet not many studies on its potential treatment have been done in the local setting. The annual direct and indirect cost of dementia care for one individual ranges between RM12,613 to RM18,730 (Koris, 2018). Yet, the exploration of non-pharmacological treatments is still lacking. For example, the effectiveness of aromatherapy as a complementary non-pharmacological approach against BPSD can be explored.

In previous studies, the effect of aromatherapy intervention has been assessed using behavioral parameters by interviewing caregivers. The accuracy and biases of this method are still subjective to patients' and caregivers' demographic status. In addition, several studies have used saliva to test the effectiveness of aromatherapy in persons with dementia (Kikukawa et al., 2021; Schaub et al., 2018) and to look at saliva as a potential biomarker. There is still a lack of human trials using blood analysis to detect the benefits of aromatherapy. Hence, it is imperative to explore the blood biomarkers associated with dementia to provide evidence of the potential benefits of aromatherapy.

To date, many studies have reported the benefits of lavender and lemon balm essential oils in persons with dementia (Watson et al., 2019). Ylang-ylang essential

oils which are extracted from the local flower have not been assessed for their effectiveness in persons with BPSD. Ylang-ylang essential oil is postulated to have calming effects, but less is known about whether similar effects are seen in persons with dementia. The present study is undertaken to evaluate the effect of ylang-ylang among persons with dementia using behavioral tools and biochemical analysis. This study aimed to elucidate the mechanisms of ylang-ylang on blood biomarkers related to neurodegeneration, neuroinflammatory, hormonal and antioxidant status among people with dementia in Malaysia.

1.3 Research Questions

Based on the research gaps identified from the literature, the proposed study postulated these research questions:

1. What are the effects of ylang-ylang essential oil on dementia-related behavior in persons with BPSD?

2. What are the effects of ylang-ylang essential oil on blood biomarkers related to neurodegeneration, neuroinflammation, stress hormonal and antioxidant status in persons with BPSD?

1.4 Research Hypotheses

It is hypothesized that diffused ylang-ylang essential oil will reduce BPSD in terms of behavioral symptoms and blood biomarkers. If ylang-ylang essential oil is effective in reducing BPSD, it can be promising non-pharmacological management and may benefit in reducing caregivers' burden.

1.5 Research Objectives

The main objective of the study is to identify the effects of ylang–ylang essential oil in the management of persons with BPSD.

The specific objectives of the study are as follows:

1. To examine dementia-related behavioral changes using CMAI and other established behavioral assessment tools upon ylang-ylang aromatherapy.
2. To determine the effects of ylang-ylang aromatherapy on blood biomarkers related to neurodegeneration, neuroinflammation, stress hormones, oxidative stress and antioxidant status in persons with BPSD.

1.6 Significance of Study

This study is essentially in line with the National Key Economic Areas (NKEA) under healthcare in expanding the opportunity of improving aged-care services, especially for people with dementia in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Dementia

Dementia results from diseases that primarily affect the brain. Types of dementia include AD, frontotemporal dementia, Lewy body dementia, vascular dementia and mixed dementia (Livingston et al., 2020). Among them, AD is the most common type of dementia worldwide that causes the irreversible loss of memory and cognitive function (Cacabelos, 2020; Cacabelos et al., 2016). Dementia causes brain nerves, which involve thinking, learning and memory to impair (Livingston et al., 2020). Loss of connection between the neurons in the brain nerve cells causes the brain to shrink, the cerebral cortex to shrivel, and the ventricles to expand (Blinkouskaya & Weickenmeier, 2021). Neuritic senile plaques and intracellular neurofibrillary tangles, which are driven on by the accumulation of amyloid-beta (Aβ) peptide and insoluble aggregates of tau proteins are the predominant pathogenic hallmarks of AD (Martínez-Iglesias et al., 2021; Rajmohan & Reddy, 2017).

2.1.1 Prevalence of Dementia

In 2015, 50 million people had dementia globally, and the prevalence of dementia is progressively increasing every year. Consistently, there are almost 10 million new dementia cases every year. The total number of people living with dementia is expected to rise to 82 million in 2030 and 152 million in 2050 (Minghui, 2019). In Malaysia, World Health Organization (WHO) reports that in 2015, 123,000 persons were diagnosed with dementia. By the year 2030, approximately 261,000 people are projected to live with dementia and this number is expected to increase to 590,000 people by 2050 retrieved on February 21, 2023, from

https://www.statista.com/statistics/738332/malaysia-projected-number-of-people-with-dementia/ (Published by Statista Research Department on 7, 2014). Dementia has substantial societal and economic effects on the direct expenses of medical and social care. The overall economic cost of dementia care globally was US$ 1.3 trillion in 2019, and it is anticipated that this cost would rise to US$ 2.8 trillion by 2030 due to the increasing number of persons with dementia and their cost of care (Xu et al., 2017).

Increasing age is the most prominent known risk factor for developing all dementias and AD, although it is not an inherent determinant of biological ageing. However, dementia not only affects the elderly, but it can also affect young people. The term "young onset dementia" indicates dementia with symptom beginning before the age of 65 years old (Chiari et al., 2021). According to WHO young onset dementia accounts for up to 9% of overall dementia cases annually. The number of people with young-onset dementia is growing as the disease is becoming more recognized with clinical significance (Vieira et al., 2013). Based on a 5-year age band meta-analyses done in 2021, the age-standardized prevalence of worldwide young onset dementia reported that 3.9 million people aged 30 to 64 years are living with young onset dementia (Hendriks et al., 2021). However, low levels of awareness and the difficulties of diagnosing young onset dementia at working age cause inaccuracy in the exact number of people living with young onset dementia. Thus, there is a scarcity of appropriate support for people living with young onset of dementia due to a lack of knowledge and understanding of young onset dementia by professionals including healthcare experts and the public (Cartwright et al., 2021).

2.1.2 Behavioral and Psychological Symptoms of Dementia

Behavioral and psychological symptoms of dementia (BPSD) such as agitation, psychosis, depression and apathy are known as the spectrum of non-cognitive and non-neurological symptoms of dementia. Studies reported that BPSD affects up to 90% of those diagnosed with dementia at any given point in the duration of their illness (Tible et al., 2017).

There is no existing cure for dementia. However, there are some medications approved by the Food and Drug Administration (FDA) for symptomatic management of AD such as acetylcholinesterase inhibitors (AChEI) and memantine. In June 2021, FDA approved the first disease-modifying drug, aducanumab for the management of AD. In addition, for the treatment of BPSD, antipsychotic drugs, antidepressants and mood stabilizers are recommended (Forester & Vahia, 2019). However, concerns are growing over the serious adverse effects of the treatments for BSPD such as extrapyramidal reactions, cardiac arrhythmias, and urinary retention (Ballard et al., 2002; McShane et al., 2019). As such, non-pharmacological approaches are becoming the frontline treatment to manage BPSD due to their cost-effectiveness and fewer side effects. List of non-pharmacological interventions have been applied to people with dementia and their caregivers in homes or community settings (McDermott et al., 2019; Vasse et al., 2012). Music therapy, art therapy, art activities, aromatherapy, reminiscences, animal-assisted therapy, massage therapy, poetry workshops, cognitive-behavioral therapy, mediation, and group therapy are several non-pharmacological interventions that provide some beneficial effects in reducing BPSD among persons with dementia (Vasse et al., 2012).

2.2 Biomarkers Associated with Dementia

According to WHO, the term biomarker refers to “almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or molecular interaction.’(Robinson, 1993). Biomarkers are defined as reliable indicators that are used to evaluate responsiveness to medical interventions, diagnose diseased or normal processes, and anticipate effects (Kamtchum-Tatuene & Jickling, 2019). Present clinical diagnosis of dementia requires a series of examinations including medical history, neuropsychological assessment, and several radiological investigations such as computerized tomography (CT), Magnetic resonance imaging (MRI) and Positron emission tomography (PET) (Yao et al., 2019).

Biomarkers for dementia are used to recognize distinctive aspects of the underlying pathology, identify early phases of pathological changes, anticipate the drop or transformation between clinical disease conditions and monitor disease progression as well as reaction to treatment (Ahmed et al., 2014). In recent years, research on various clinical biomarkers and combinations of biomarkers has been undertaken (Park et al., 2022).

2.3.1. Salivary biomarkers

Salivary analysis has been commonly used in recent days for the diagnosis of a disease. This is because, salivary samples are easy to collect, non-invasive and inexpensive with minimal discomfort to patients (Lee et al., 2009). Previous studies have used salivary samples for the clinical diagnosis of dementia. A cross-sectional study on the salivary flow rate (SFR) found an independent association with cognitive

impairment among Korean elders. Low SFR (< 0.3 mL/min) indicate cognitive impairment by 1.5 times more than among participants with normal SFR (Do et al., 2021). In another study, 15 AD patients with a mean age of 77.8 ± 1.8 years and mean Mini-Mental State Examination (MMSE) score of 19.0 ± 1.3 and 7 controls with a mean age of 60.4 ± 4.7 years and mean MMSE of 29.0 ± 0.4 reported the difference of salivary $A\beta_{42}$ among these two groups. It was found that salivary $A\beta_{42}$ levels were significantly higher in AD patients than in controls (51.7 ± 1.6 pg/mL for AD and 21.1 ± 0.3 pg/mL for controls, $p < 0.001$) (Sabbagh et al., 2018). However, concentrations of certain biomarkers in salivary samples are 10–1,500 times lesser or diluted than in the plasma because saliva is an ultrafiltrate of blood where it does not carry larger molecules or charged biomolecules (Chiappin et al., 2007; Miočević et al., 2017).

2.3.2. Blood biomarkers

Blood biomarker analysis is a well-established analytical and reliable technique to detect the progression of disease in clinical studies. Detecting the specific blood biomarker is essential to get a deeper and proper understanding of dementia. Evaluating blood biomarkers in clinical practice may support the diagnosis and development of treatments for dementia. Neuroinflammatory (Li et al., 2019; Nguyen et al., 2007; Schuss et al., 2018), neurodegenerative (Dong et al., 2019; Weinstein et al., 2014), stress hormones (Ouanes & Popp, 2019), oxidative stress and antioxidative markers (Serrano & Klann, 2004), are the common biomarkers used in the diagnosis of dementia. However, there is a lack of evidence on the changes in blood biomarkers upon intervention in persons with dementia.

Neurodegeneration is a dementia biomarker which indicates the progressive damage of nerve cells in the brain. Aβ peptide is produced from the Aβ precursor protein (APP). Beta (β)-secretase and gamma (λ) -secretase degrades APP to create amyloid peptides containing 40 ($A\beta_{1-40}$) or 42 ($A\beta_{1-42}$) amino acids (Rukmangadachar & Bollu, 2021). Moreover, Aβ peptides containing between 38 and 43 amino acids are also detected, but $A\beta_{42}$ is the most fibrillogenic and the predominant component of amyloid plaques in AD (Masters et al., 2015). The accumulation of Aβ in the brain causes memory loss and gives rise to cognitive impairment over time (Chen et al., 2017).

A post-mortem cross-sectional study among 79 nursing homes in Manhattan, New York examined residents with Clinical Dementia Rating (CDR) scale scores from 0 to 5 in different cortical regions of the brain including middle frontal gyrus, superior temporal gyrus, entorhinal cortex, inferior parietal lobule, and primary visual cortex. It was found that $A\beta_{1-40}$ and $A\beta_{1-42}$ were higher in the CDR 5 group compared to CDR 0 group. For example, the average value of $A\beta_{1-40}$ and $A\beta_{1-42}$ at the frontal cortex region were 475 ± 162 µg/mL and 121±36 µg/mL for CDR 0 group. Meanwhile, the average value of $A\beta_{1-40}$ and $A\beta_{1-42}$ for CDR 5 was higher at 2180 ±276 µg/mL and 823±215 µg/mL respectively. This finding suggests that $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides show a systematic increase in the severity of dementia (Näslund et al., 2000). Besides that, plasma concentrations of $A\beta_{1–40}$ and $A\beta_{1–42}$ among 32 persons with dementia were analyzed based on the severity of dementia. Findings revealed that $A\beta_{1–40}$ was higher among persons with severe dementia 369.5 ± 168.5 ng/mL than persons with mild dementia 201.3 ± 152.7 ng/mL significantly (p=0.010). However, the plasma concentration of $A\beta_{1–42}$ reduced non-significantly (Yang et al., 2020).

Nutritional deficiencies (folate, vitamin B12, and vitamin B6) are another risk factors that probably accelerate the progress of dementia and lead to neurodegeneration. Homocysteine is broken down by vitamins B12 and B6 to produce other essential substances for the body. Therefore, high homocysteine levels indicate a vitamin deficiency in the body. Recently, a panel of researchers presented a consensus statement after reviewing the literature evidence over the past 20 years. They concluded that moderately elevated plasma total homocysteine which is above 11 μmol/L, is one of the causes of age-related cognitive decline and dementia (Smith et al., 2018). Similarly, an eight year follow-up study examined the level of plasma total homocysteine measured at baseline and eight years before the diagnosis of dementia. They found that plasma homocysteine level more than 14 μmol/L, doubles the risk of AD (Seshadri et al., 2002). Correlation analyses among 21 healthy adults with a mean age of 46.21 ± 7.99 showed that increased plasma homocysteine was associated with lower memory scores (Çebi et al., 2022). An animal study which evaluated the effectiveness of palm oil-derived tocotrienol-rich fraction showed an increase in the plasma homocysteine levels of vascular dementia rats compared to the control group (Visioli et al., 2022).

Folate or folic acid which is one of the B vitamins is important for the formation of red blood cells and the growth and function of healthy cells. A deficiency of folate in the brain causes neurodegenerative complications associated with dementia (Hagnelius et al., 2008; Robinson et al., 2018; Tian et al., 2016). A lower level of folate is associated with memory deficit and psychomotor speed which is linked with dementia (De Lau et al., 2007; Nurk et al., 2005). A 6 years-follow up test on memory performance was conducted using plasma analysis among 2,189 older adults (age, 65-67 years at baseline). Participants with memory deficit had lower folate concentration

in plasma at follow-up [6.7 nmol/L (95% CI, 6.2 to 7.1)] compared with those without memory deficit [7.6 nmol/L (95% CI, 7.5 to 7.8)] at $p < 0.001$ (Nurk et al., 2005). Another 1 year follow-up study on a huge national cohort of older adults aged between 60 and 75 years (n=27 188) showed lack of serum folate was associated with higher risks of dementia (Hazard Ratio =1.68; 95% CI 1.32 to 2.13) and all-cause mortality (Hazard Ratio=2.98; 95% CI 2.52 to 3.52) significantly at $p<0.001$ (Rotstein et al., 2022).

Brain-derived neurotrophic factor (BDNF) is a part of the neurotrophic family, and a key protein in charge for the survival and growth of neurons ((Mattson et al., 2004). BDNF plays a crucial part in neural plasticity aspects, such as memory, neurogenesis, learning, and mood alterations (Gorski et al., 2003). Several studies have found a low level of BDNF in the serum and plasma of persons with AD and persons with mild cognitive impairment (MCI) (Jung et al., 2009; Martínez-Iglesias et al., 2021; Numakawa et al., 2010; Porritt et al., 2005; Ng et al., 2019). A meta-analysis conducted on 15 studies with 2067 older adults found that serum BDNF concentrations were significantly lower in patients with AD compared to healthy adults ($p = 0.030$) (Numakawa et al., 2018).

Another study was conducted among the Korean population to examine the difference in serum BDNF levels between older adults over 65 years with and without dementia. Among 132 participants, the serum BDNF levels were lower in the samples of MCI and AD participants compared to the samples of healthy participants with a significance of $p<0.01$. The serum BDNF level of the healthy group was 27.9±6.9 ng/mL, while the serum BDNF levels of AD and MCI were 22.9±5.0 ng/mL and 22.8±6.3 ng/mL respectively (Jung et al., 2009).

Cytokines act as key mediators of communication between the brain and the immune system, impacting neurodevelopment such as neurogenesis, migration, differentiation, and synaptogenesis. Cytokines play a fundamental role in neuroinflammatory processes which is a primary marker for dementia (Paul-Samojedny et al., 2013). In dementia, cleavage, or cell division of the APP causes interleukin-6 (IL-6) production in immune cells, which upregulates APP and hyperphosphorylated tau in neurons (Hampel et al., 2005). Significant elevation of IL-6 was detected in the plasma of 16 AD patients and 18 control individuals resulting in a correlation between brain inflammation and cognitive impairment (Lyra e Silva et al., 2021).

Similarly, interleukin-2 (IL-2) secretion was higher in vascular dementia groups (1558 ± 279 pg/mL) and moderately severe AD groups (1999 ± 262 pg/mL) significantly at $p < 0.01$ compared to healthy control (1026 ± 262 pg/mL). These results indicate that increased levels of IL-2 secretion may correspond to the severity of dementia due to increased neuronal damage (Huberman et al., 1995). In another study, the plasma cytokine profile of 38 persons with mild cognitive impairment and dementia with Lewy bodies and 20 healthy volunteers with a mean age of 75.9±7.3 was characterized. Significant differences were seen in IL-2 in healthy control (0.38±0.42 pg/L) and persons with mild cognitive impairment and dementia with Lewy body (3.68±0.85 pg/L). It was stated that IL-2 was higher in persons with dementia suggesting neuroinflammation (King et al., 2018).

With regard to alpha-tumor necrosis factor (TNF-α) concentrations, previous longitudinal studies have found higher levels of TNF-α, in peripheral blood samples of patients with AD compared to a control group (Kim et al., 2018; Lai et al., 2017).

Cytokines multiplex assay was done to observe the difference in the level of cytokines in the blood among persons with Lewy body dementia and Parkinson's disease dementia compared to controls. The median (range) of TNF-α was reported as 15.49 (5.41–29.45) ng/mL for Lewy body dementia and 15.31 (6.22–30.02) ng/mL for Parkinson's disease dementia compared with control [8.89 (1.53–21.92)] ng/mL. Thus, increased production of TNF-α refers to the progression of neuroinflammation and cognitive deterioration (Usenko et al., 2020).

Indication of stress markers is essential for dementia because persons with dementia tend to undergo stress and depression. Adrenocorticotropic hormone (ACTH) is a hormone made by the pituitary gland, a small gland at the base of the brain. Another hormone called cortisol is produced under the direction of ACTH (Allen & Sharma, 2018). Neurotransmitters, peptides, immunological factors, and other stimulatory and inhibitory substances influence the release of ACTH. Psychiatric disorders are one of the many factors that lead to dysregulation of ACTH release (Rhodes, 2017). Prior research involving 15 dementia patients revealed that their ACTH levels were considerably lower than those of age-matched healthy volunteers (n=13) (Facchinetti et al., 1984). According to a case study of a person with modest brain atrophy, the blood sample's ACTH levels were low (<1.5) compared with the baseline range (Ikenouchi et al., 2022).

Cortisol is widely known as the hormone with the most prominent effects on brain function and has been associated with effects on cognition, sleep, mood, appetite, stress, and anxiety (Copinschi & Caufriez, 2013; Lupien et al., 2007). Cortisol regulates several mechanisms, including immunological and metabolic reactions (Lupien et al., 2007). Most severe dementia patients had the highest cortisol

concentrations and have been associated with dementia progression (Ouanes & Popp, 2019; Schrijvers et al., 2011; Zvěřová et al., 2013). Both ACTH and cortisol concentrations exhibit significant diurnal variation, which can also be due to external factors such as stress derived events (Tsigos et al., 2020).

Oxidative stress is an essential factor associated with dementia. Malondialdehyde (MDA) is a type of lipid peroxidation product and an important marker in measuring the oxidative damage of the body cell membrane of the human body. It is reported that the abnormal increase of MDA correlates with memory impairment which can be tested using a thiobarbituric acid reactive substance (TBARS) assay. Over the decades, strong relationships between oxidative stress and cognitive dysfunction during aging and age-related neuronal illnesses have been discovered in cellular, biochemical, and molecular research. Several animal and human studies had shown a higher oxidative stress level associated with dementia (Kamsler et al., 2006; Keller et al., 2005; Serrano & Klann, 2004). In preclinical studies, MDA in dementia-induced mice including AD and MCI models showed a significant increase compared with the control group (Yu-ping Xiong & Ya-Jie Tian, 2016; Zhang et al., 2019). Similarly, MDA level was found to be high in the serum of dementia patients in human trials (Gustaw-Rothenberg et al., 2010; Guven et al., 2020).

A sufficient amount of antioxidants prevents oxidative stress by scavenging oxidants when they are created in excess by various sources (Gupta et al., 2021). A key antioxidant marker called glutathione guards the brain against reactive oxygen species (ROS), which are created when oxygen is metabolized by the brain. Total antioxidant capacity (TAOC) is another marker of DNA oxidation produced by the

oxidation of DNA bases (El-Edel et al., 2020). Clinical and preclinical studies indicate GSH levels were significantly lower in dementia subjects compared to controls (Croisile et al., 2012; Gustaw-Rothenberg et al., 2010; Mandal et al., 2012; Walia et al., 2021). A lower level of reduced GSH and TAOC in clinical studies indicates a reduced antioxidant capacity in persons with dementia (Keller et al., 2005).

Many studies have been conducted to analyze changes in blood biomarkers of persons with dementia, but they only studied certain biomarkers. The need to analyse major parameters involving all biomarkers that impact dementia is cardinal to have a deep understanding of the mechanism of this disease.

2.3 Aromatherapy

Aromatherapy is one of the complementary non-pharmacological approaches used in the management of BPSD due to its negligible side effects (Cohen-Mansfield, 2013; de Oliveira et al., 2015; Scales et al., 2018). The term "essential oils" refers to a class of volatile molecules that are complex, natural, and created by aromatic plants. These compounds possess distinct fragrances. Natural aromatic plants and essential oils have been used to promote psychological and physical well-being in the treatment of diseases like AD, cardiovascular disease, cancer, and pregnancy labor discomfort (Ali et al., 2015). Aromatherapy works by stimulating olfactory receptors that in turn stimulate the limbic system that is linked to the regulation of emotions (Figure 2.1).

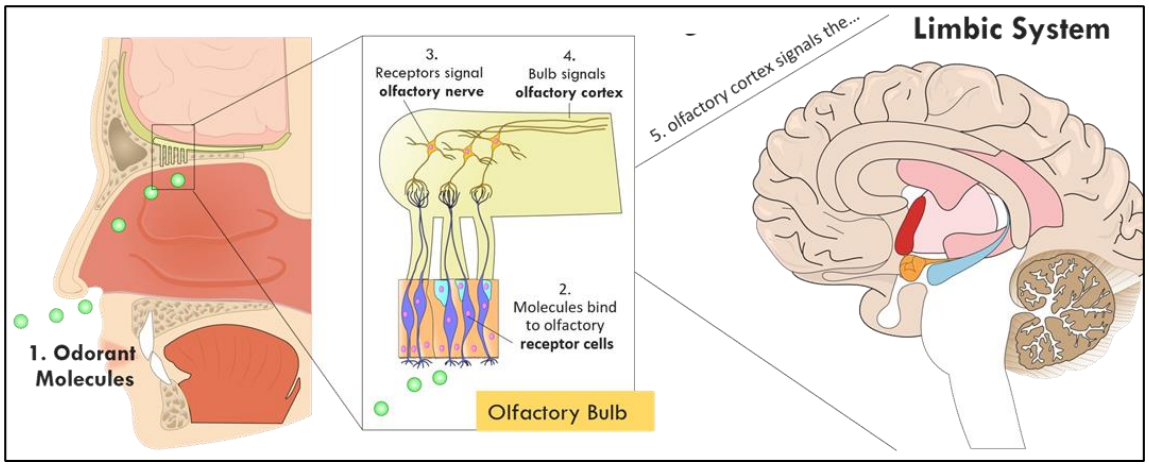

Figure 2.1 Mechanism of aromatherapy stimulation of the brain, Adapted from Limbic System - Jodi McKenna (2012)

Essential oils are administered through inhalation or diffusion, massage, or basic applications on the skin surface and seldom, they are taken ingested. Through inhalation, these essential oils work on the olfactory nerves that connect the nose to the brain. The essential oil can be diffused using diffusers which will break down essential oils into smaller molecules and spread them in the air. These tiny molecules stay in the air for a long time which means the surrounding area benefits from the aroma for a long time (Ali et al., 2015). According to studies, terpenes and terpenoids, which are tiny, lipid-soluble organic compounds, are the main components of essential oils. These molecules can be taken up by the skin or nasal mucosa and enter systemic blood circulation. The blood-brain barrier can also be crossed by plenty of terpenes (Agatonovic-Kustrin et al., 2019, 2020).

Essential oils of lavender, lemon balm, rosemary, orange, and eucalyptus have been used for managing BPSD in persons with dementia. Among these oils, lavender was the most studied in clinical trials (Fujii et al., 2008; Gray & Clair, 2002; Holmes et al., 2002; Jimbo et al., 2009; Mascherona et al., 2020; Moorman Li et al., 2017; Pamela Wan-ki et al., 2007; Smallwood et al., 2001; Watson et al., 2019; Yang Lee,

et al., 2016). Table 2.1 shows the type of essential oils used and their benefits in managing BPSD in persons with dementia. Most of the study has been conducted in Japan and no studies have been conducted in Malaysia. Most studies had a smaller sample size of fewer than 30 subjects. A cohort pre- and post-study had a larger sample size of 102 persons with dementia from 10 long term facilities. The inhalation method was used in most studies to avoid direct exposure to essential oils on the skin (Ali et al., 2015).

A pilot study was conducted using lavender in an adult day care center amongst 23 persons with dementia. Lavender aromatherapy was provided to participants two times every day for 20 minutes per session over two months during the clinic days using an advanced diffuser. The behavioral issues of each participant were recorded using a monthly flow record during the pre-and post-intervention periods. There was a significant reduction in the total number of agitation occurrences of participants between the pre-intervention and post-intervention of which 129 agitation occurrences during the pre-intervention phase were reduced to 25 after lavender aromatherapy was provided (Moorman Li et al., 2017). In another study, 32 persons with BPSD who were admitted to the Geriatrics Department of the Clinica Luganese Moncucco, Switzerland were subjected to psychotropic drug therapy combined with diffused lavender essential oil therapy for 48 hrs of admission. Results of this study suggest standard psychotropic drug therapy combined with diffused lavender essential oil therapy was more effective at treating BPSD than standard psychotropic drug therapy alone (Mascherona et al., 2020).

A randomized control trial among older people with (n=50) and without (n=15) dementia assessed the effectiveness of lavender and lemon balm essential oils on agitation based on Cohens Mansfield Agitation Inventory (CMAI) and Neuropsychiatric Inventory (NPI) domains. Two drops of oil were poured onto a cotton patch and attached to the cloth to the participant's collar area two hours daily. Post-hoc analysis on physical nonaggressive behavior was reduced by lavender (-1.26) when compared to lemon balm (-0.10) and placebo (-0.23) for people with dementia at p=0.04. On the other hand, at p=0.01, lemon balm (0.31) was more effective in reducing irritability among persons with dementia compared to lavender (-1.84) and placebo (-0.77) (Watson et al., 2019). Recently, a clinical trial protocol was published to assess the effect of bergamot essential oil on agitation in persons with severe dementia (Scuteri et al., 2021). A total of 134 subjects with severe dementia aged ≥65 years were expected to participate in this study. About 80mg of bergamot essential oil cream for the treatment group or placebo cream for the control group will be transdermally applied. These creams will be applied on both arms of the participants twice a day for 4 weeks along with a 4-week follow-up period. The findings of this study have not been published yet.

There are several assessment methods used in previous studies (See Table 2.1). Among them, NPI and CMAI were used commonly in many countries. Agitation is one of the major behavioral symptoms manifested by persons with dementia (Chang, 2019) therefore, CMAI has been widely used to observe the difference upon an intervention. Moreover, CMAI has been translated and validated in Chinese, Dutch and French languages to captivate researchers to use this instrument in their trial (Barry et al., 2013; Choy et al., 2001; Zuidema et al., 2007). With regards to NPI, it is an easy-to-use inventory that consists of all the neuropsychiatric symptoms that may be

manifested by an individual with dementia (Cummings, 1994). Moreover, the NPI has 4 versions established for diverse clinical applications and has been translated into about 40 different languages (Cummings, 2020). Gottfries, Brane, Steen scale (GBSS), a revised version of Hasegawa's Dementia Scale (HDS-R), Functional Assessment Staging of Alzheimer's disease (FAST) and the Touch Panel-type Dementia Assessment Scale (TDAS) has also been used but mostly in Japan (Goto et al., 2020; Jimbo et al., 2009). Other assessment tools were used based on the objectives and setting of the study.

Survey instruments are the predominant methods used in previous studies. However, analysis of biological specimens is essential to provide quantitative evidence of the effectiveness of essential oil. Several studies on saliva, urine and blood analysis have been assessed previously on aromatherapy. Previously, a study evaluated the effects of 4 aromatic plants, *Lavandula angustifolia* and *Salvia sclarea* L. from China, *Santalum album* from India, and *Citrus sinensis* from the United States using a urine sample. These essential oils were diffused in a closed room for about 45 minutes a day for 10 continuous days among 31 female volunteers. Urine samples were collected at baseline (pre-exposure to essential oil inhalation) and on the 10^{th} day (post exposure to essential oil inhalation). Results found increased levels of homocysteine at $p=0.0024$ (Zhang et al., 2013).

With regards to salivary analysis, subjects in a randomized crossover study were exposed to bergamot essential oil with saturated water vapor for 15 minutes to measure salivary cortisol levels. It was found that the average salivary cortisol level was 0.16 ± 0.01 for the control group and a significantly lower level for the treatment group (0.12 ± 0.01) at $p=0.003$ (Watanabe et al., 2015). In another randomized

controlled study, the effect of orange essential oil was assessed among 30 children with anxiety during dental treatment. The difference in mean between the salivary cortisol of the control and treatment group was 1.047 ± 2.198 nmol/L (p-value = 0.014) (Jafarzadeh et al., 2013).

Blood analysis was also reported in previous studies of aromatherapy's impact on the body. In an animal study, a mixture of lemon and rosemary oil was given at night time and a mixture of lavender and orange essential oils was given in the daytime to mice. Enzyme-linked immunosorbent assay (ELISA) shows a reduction in $A\beta_{1-42}$ (control group: 271.8 ± 5.2 pmol/g-tissue vs aromatherapy group: 218.9 ± 11.6 pmol/g-tissue) and $A\beta_{1-40}$ (control group: 415.6 ± 35.6 pmol/g-tissue vs aromatherapy group: 330.3 ± 20.2 pmol/g-tissue) concentrations in the hippocampus at p-value =0.046 (Okuda et al., 2020). In addition, ACTH levels were assessed on experimental menopausal female rats using plasma samples upon lavender oil and linalool vapour inhalation. Treatments were given 20 minutes twice daily for three days. ACTH levels were significantly decreased from 459.2 ± 45.44 pg/mL before treatment to 402.5 ± 62.25 pg/mL using the lavender essential oil and 358.2 ± 43.47 pg/mL using linalool vapor (Yamada et al., 2005).

With regards to human trials, a new pilot randomized controlled study comparing the impacts of board game training and olfactory-based sensory stimulation on memory, emotion, and blood biomarkers was carried out. The intervention was done for 30 min sessions twice a week for 12 weeks among 28 participants with dementia. A total of 15 essential oils including lavender, sweet orange, lemongrass, rosemary, mint and hinoki were diffused during olfactory-based sensory stimulation and board game training. Plasma $A\beta_{1-42}$ level and plasma Tau level were measured but

no significant finding was reported (Lin & Li, 2022). Apart from this, blood-based analysis in human trials evaluating aromatherapy efficacy is still scarce.

Table 2.1 Studies of essential oils efficacy in persons with dementia

Essential oils	Human studies	Assessment method	Sample size	Study setting	Administration method	Findings
Lavender	Gray & Clair, 2002	Videotaping	13	Two midwestern residential care facilities	Inhalation	Improvement in cognitive functions
	Jimbo et al., 2009	Japanese version of the Gottfries, Brane, Steen scale (GBSS-J), Functional Assessment Staging of Alzheimer's disease (FAST), a revised version of Hasegawa's Dementia Scale (HDS-R), and the Touch Panel-type Dementia Assessment Scale (TDAS)	28	Japan	Inhalation	
	Holmes et al., 2002	Pittsburgh Agitation Scale (PAS)	25	Not stated	Inhalation	Reduction in anxiety
	Fujii et al., 2008	Neuropsychiatric Inventory (NPI)	28	Japan	Inhalation	Improvement in motivational behavior
	Mascherona et al., 2020	Italian version of the NPI	32	Not stated	Inhalation	Reduction in agitation and depressive mood
	Moorman Li et al., 2017	Behavior Intervention Monthly Flow Record	23	Adult Day Care Centre in Jacksonville, Florida	Inhalation	