ANTICHOLINESTERASE, ANTIOXIDANT AND COGNITIVE ENHANCING PROPERTIES OF ESSENTIAL OILS FROM BLACK PEPPER (*Pipernigrum*L.)AND CALABASH NUTMEG (*Monodoramyristica*(GAERTN.) DUNAL).

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APPROVAL

This research projectwas supervised by me and approved in accordance with the requirements for the award of Master of Science (M.Sc.) degree in Biochemistry, ObafemiAwolowo University, Ile-Ife, Nigeria.

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Dr. E. M. Obuotor Date

Supervisor

DEDICATION

The Research work isDedicated

To God Almighty,

The Alpha and Omega,

for

Giving the Grace to finish the work

and to

entire OLAWUNI Family.

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TABLE OF CONTENT

LIST OF TABLES

Activity in Mice. 91

LIST OF FIGURES

LIST OF ABBREVIATION

AA	Alzheimer Association
AASA	Aminoadipicsemialdehyde
Aβ	amyloid β
ABTS	2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)
Ach	Acetylcholine
AChE	Acetylcholinesterase
AChEIs	Acetylcholinesterase Inhibitors
AD	Alzheimer's disease
ATChI	Acetylthiocholine Iodide
BuChCl	Butyrylthiocholine Chloride
BuChE	Butyrylcholinesterase
ChAT	Choline acetyltransferase
ChE	Cholinesterase
ChEIs	Cholinesterase Inhibitors
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DPPH	2,2-diphenyl-1-picrylhydrazyl hydrate
ELT	Escape Latency Time
FRAP	Ferric Reducing Antioxidant Power
GC-MS	Gas Chromatography and Mass Spectrometry
GSA	Glutamic Semialdehyde
GSH	Reduced Glutathione
GSH-Px	Glutathione peroxidases
GSSH	Oxidized Glutathione
HAChT	High Affinity Choline Transporter
NMDA	N-methyl-D-aspartate

NOS	Nitric Oxide Synthases
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
LTM	Long-Term Memory
LTP	Long Term Potentiation
MCI	Mild Cognitive Impairment
MM	*Monodoramyristica*
MTL	Medial Temporal Lobe
MWM	Morris Water-Maze
PABA	Paraaminobenzoic Acid
PAV	Passive Avoidance
PN	*Piper nigrum*
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SDL	Step-Down Latency
STDP	Spike-Timing-Dependent Plasticity
STLStep-Through Latency	
STM	Short-Term Memory
VAChT	Vesicular Acetylcholine Transporter
VD	Vascular Dementia

ABSTRACT

This study determined the chemical composition of the essential oils of the seeds of *Monodoramyristica* and ***Piper nigrum***and evaluated its*in vitro* anticholinesterase, antioxidant and antiamnesic potentials. This was with a view to using these oils in the management of neurodegenerative diseases.

Fifty grams (50 g) of the powdered seeds of *M. myristica* and ***P. nigrum*** were separately subjected to hydrodistillation in a Clavenger-type apparatus to isolate their essential oils. The oils were dried with anhydrous sodium sulphate (Na_2SO_4) and their compositional profiles were analysed by gas chromatography and mass spectrometry(GC-MS). Radical scavenging activities were tested using 2,2′-diphenyl-1-picrylhydrazyl hydrate (DPPH),2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$), nitric oxide (NO) inhibition, while the antioxidant capacities were investigated with the ferric reducing antioxidant power (FRAP) and total antioxidant capacities (TAC). The inhibitory effects on acetylcholinesterase (AChE) andbutyrylcholinesterase(BuChE) were investigated by standard method. In the *in vivo*antiamnesic studies, adult mice were used for the test in the cognitive behavioural paradigms. The animals were assessed for performance in the Passive Avoidance and Morris Water-Maze Tasks by measuring the Step-Through Latency Time (SLT) and Escape Latency Time (ELT) respectively. The animals were then sacrificed, whole brain excised, homogenized (10% weight/volume) and then assayed for brain acetylcholinesterase activity.

The result showed that *M. myristica* and *P.nigrum* oils were characterised by the presence of 51 and 61 components, representing 94.76% and 90.65% respectively of the total oil. The oils were dominated by α-phellandrene (18.13%), 1-methyl-4-1(1-methylethyl) benzene (16.26%) and α-pinene (7.16%) in *M.myristica* while β-pinene (5.92%), 2,4-quinolinediol

(4.74%) and caryophyllene (4.55%) were the major compounds in *P.nigrum*. *M.myristica* demonstrated free radical scavenging effects on DPPH (IC_{50} = 3.01 ± 0.04 mg/ml), NO inhibition (IC_{50} = 0.372 ± 0.013 mg/ml), $ABTS^{\cdot+}$ (IC_{50} = 0.347 ± 0.019 mg/ml). In terms of antioxidant capacities, FRAP (11.66 ± 0.26 µg AAE/ml) and TAC (274.41 ± 14.40 µg AAE/ml). *P. nigrum* also elicited free radical scavenging and antioxidant capacity on DPPH (IC_{50} = 9.89 ± 0.66 mg/ml), NO inhibition (IC_{50} = 0.384 ± 0.013 mg/ml), $ABTS^{\cdot+}$ (IC_{50} = 0.436 ± 0.012 mg/ml), FRAP (5.27 ± 0.06 µg AAE/ml) and TAC (266.90 ± 4.66 µg AAE/ml). Both oils at 416 µg/ml elicited remarkable and significant ($p<0.05$) inhibitory activity with *M. myristica* eliciting IC_{50} of 0. 205 ± 0.06 mg/ml and 0. 178 ± 0.016 mg/ml against AChE and BuChE respectively. *P. nigrum* inhibited AChE with IC_{50} = 272.66 ± 18.05 µg/ml and BuChE with IC_{50} = 223.75 ± 20.82 µg/ml. Kinetic studies revealed that the mode of inhibition exhibited by *M. myristica* oil against AChE and BuChE was competitive while *P. nigrum* on the other hand caused a competitive type of inhibition towards AChE and a mixed type towards BuChE. In the Morris Water-Maze Task, the Escape Latency Time (ELT) were significantly ($p<0.05$) decreased by both *M.myristica* and *P.nigrum* treated group compared to scopolamine treated group. Similarly, in the Passive Avoidance Task, the Step-Through Latency were significantly increased by the administration of the oils when compared to scopolamine treated group.

The study concluded that the seeds of *M. myristica* and *P. nigrum* were potential sources of active metabolites with anticholinesterase and antioxidant properties with *M. myristica* showing a higher activity.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a complex, multifaceted and heterogenous etiology. Pathologically it is characterized by an age-dependent atrophy of neurons as a result of degeneration of synapses and death of neurons, formation of β-amyloid plaques (senile plaques) and neurofibrillary tangles, oxidative and inflammatory processes and neurotransmitter disturbances in brain regions involved inlearning and memory processes(Perry *et al*., 2003; Giacobini, 2004; Matchynski *et al*., 2013). The main symptoms associated with AD involve cognitive dysfunction, primarily memory loss (Grosse *et al*., 1991). Other features associated with the later stages of AD include language deficits, depression, behavioural problems including mood disturbances and psychosis (Howes and Houghton, 2003). It has been estimated that between 50 to 60% of people over 65 years old suffer from this condition (Costa *et al*., 2013).

Like many other chronic degenerative diseases and conditions related to the aging processes such as cardiovascular disorders, diabetes mellitus, arthritis, cancer, etc, there is increasing evidence to suggest that oxidative stress is a major factor involved in the development and progression of AD (Ustun *et al.,* 2012). A number of studies have indicated free radical oxidative damage, particularly of neuronal lipids, proteins and nucleic acids is extensive in the brains of AD patients (Lyras *et al*., 1998; Markesbery, 1998; Syad *et al.,* 2012)

Most of the currently license drugs for AD therapy such as tacrine, donepezil, rivastigmine, galantamine actby countering the cholinergic deficit associated with cognitive dysfunction and are based on theinhibition of the brain cholinesterase enzymes (Savelev *et al*, 2003; Wetwitayaklung *et al*., 2007; Chaiyana *et al*., 2012).Though these drugs contributeto slow down the progress of the disease, they however, cause adverse side–effects including hepatotoxicity, gastrointestinal disturbances as well as problems associated with bioavailability (Schulz, 2003). Consequently, owing to the multifactorial nature of the Alzheimer's disease it is increasingly evident that a preventive and therapeutic intervention strategies would require a multitarget drug agent. In this regard, thesearch for lead compounds as drug candidates with multipotent biological properties and in particular, anti-cholinesterase, antiinflammatory and antioxidant activities, may hold some potential in the managementof the whole disorder of AD as opposed to symptomatic approach of increasing the half-life of the neurotransmitter acetylcholine in cholinergic synapsis (Tundis *et al.,* 2012; Majlessi *et al*., 2012).

The use of plant essential oils to improve health and well-being of humans have been an age-long practice. Aromatherapy,non-pharmacological therapy, is currently used worldwide in the management of chronic pain, depression, anxiety as well as cognitive, sleep and stress – related disorders (Savelev *et al.,* 2004; Anekonda and Reddy, 2005; Hritcu *etal*., 2012; Natarajan *et al.*, 2013). In a recent study, Hritcu *et al.* (2012) reported that dementia patients of the AD type showed significant improvement in cognitive performance following aromatherapy consisting of rosemary and lemon essential oils. Numerous studies have shown that essential oils from a variety of plant species such as Pinus spp (Ustun *et al*., 2012), *Geidiella acerosa* (Syad *et al*., 2012), *Hedychium gardnerianum* (Arruda *et al*., 2012) do possess both anticholinesterase and antioxidant properties. These properties have been demonstrated to be as a result of

synergistic properties of constituents in the essential oils which were mainly monoterpenes and sesquiterpenes (Arruda *et al*., 2012).

In the Southern part of Nigeria and perhaps in the Southern part of the West African sub-region, the use of aromatic plants as spices for culinary purposes is quite popular. *Piper nigrum* (black pepper) and *Monodora myristica* (calabash nutmeg) are common spices used for the preparation of the traditional cuisine known as "pepper soup" which is often recommended, owing ostensibly for the health promoting benefits, for elderly persons, nursing mothers shortly after delivery and persons suffering from discomfort associated with cold-weather induced conditions. These benefits includes: longevity, anti-aging, antifatigue, cognitive enhancement, antidiabetic properties. However, this claim has not yet been subjected to rigorous experimental and clinical investigation for the purpose of validation. Thus, there is the need to carry out experimental studies with a view to give scientific backing and possibly elucidate the biochemical mechanism of action of the phytoconstituents in the seed essential oils. Hence, the aim of this study is to determine the chemical composition as well as evaluate the antioxidant,anticholinesterase and possible cognitive enhancing properties of the essential oils obtained from the seeds of *P.nigrum* and *M. myristica.*

1.2 STATEMENT OF RESEARCH PROBLEM

Compounds with anti-cholinesterase and antioxidant activities are known to enhance the level of acetylcholine in cholinergic synapse, thereby enhancing cognitive function of individuals suffering from neurodegenerative disorders such as Alzheimer's disease (AD). Presently, the drugs available for the management of these disorders have been shown to possess significant side effects owing to the nature of the inhibition profile. Ethnomedical claims have implicated *P. nigrum* and*M. myristica* with central nervous system (CNS) related properties. Preliminary studies have shown that essential oils from these plants have potent

anticholinesterase and antioxidant properties i*n vitro*, hence there is the need to investigate these plants for phytoconstituents with reversible inhibition characteristics and possibly anti-amnesic properties.

1.3 SPECIFIC OBJECTIVES OF THE RESEARCH

The specific objectives of the research are to