American Journal of Biochemistry and Biotechnology

Original Research Paper

Aqueous Extracts of Bay Leaf (*Laurus Nobilis*) and Rosemary (*Rosmarinus Officinalis*) Inhibit Iron-Induced Lipid Peroxidation and Key-Enzymes Implicated in Alzheimer's Disease in Rat Brain-*In Vitro*

[1]Ayodeji O. Falade, [1]Goodness I. Omolaiye, [1]Kayode E. Adewole, [2]Odunayo M. Agunloye, [3]Ahmed A. Ishola, [4]Kunle Okaiyeto, [2]Ganiyu Oboh and [4]Oluwafemi O. Oguntibeju

[1]Department of Biochemistry, Computational Biochemistry and Phytomedicine Research Group, University of Medical Sciences, P. M. B. 536, Ondo State, Nigeria
[2]Functional Foods, Nutraceuticals, and Phytomedicine Unit, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria
[3]Central Research Laboratories Limited, 132B, University Road, Ilorin, Kwara State, Nigeria
[4]Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7535, South Africa

Article history
Received: 29-07-2021
Revised: 13-10-2021
Accepted: 16-10-2021

Corresponding Author:
Ayodeji O. Falade
Department of Biochemistry, Computational Biochemistry and Phytomedicine Research Group, University of Medical Sciences, P. M. B. 536, Ondo State, Nigeria
Email: afalade@unimed.edu.ng; ayodeji.falade@yahoo.com

Abstract: Neurological disorders remain a global health challenge, with Alzheimer's Disease (AD) as the most famous cause of dementia in old people. Nevertheless, culinary spices and herbs have shown promising potential in the management of neurological diseases. This study aimed at investigating the neuroprotective property of aqueous extracts of bay leaf (*Laurus nobilis*) and rosemary (*Rosmarinus officinalis*) by assessing the antioxidant activity of the extracts and the effects on key enzymes implicated in AD: Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) and lipid peroxidation *in vitro*. Bioactive constituents of the samples were characterized using GC-MS and the interaction of the identified compounds with AChE and BChE was determined through molecular docking. Both extracts exhibited remarkable inhibitory activities against AChE and BChE, with no significant ($p>0.05$) difference in their inhibitory activities. Similarly, aqueous extracts of both samples inhibited iron-induced lipid peroxidation in rat brains with *L. Nobilis* extract exhibiting significantly ($p<0.05$) higher inhibitory activity (IC_{50}: 67.83±13.53 µg/mL) than *R. Officinalis* (IC_{50}: 96.96±15.63 µg/mL). Also, *L. Nobilis* extract displayed a better radical scavenging ability and Ferric Reducing Antioxidant Power (FRAP). However, no significant difference ($p>0.05$) in their iron-chelating abilities as reflected in the IC_{50} values (*L. Nobilis*: 10.93±0.42 µg/mL; *R. Officinalis*: 10.12±0.40 µg/mL). Furthermore, GC-MS analysis confirmed the presence of 39 phenolic compounds in both samples, with chlorogenic acid, rosmarinic acid, rosmanol, rutin, and hesperetin 7-O-rutinoside and luteolin predicted to be stronger cholinesterase inhibitors compared to galantamine in an *in silico* study. Hence, *L. nobilis* and *R. officinalis* may be considered promising sources of nutraceuticals in the management of AD. Future research would consider *in vivo* studies on the neuroactive properties of the extracts and the potent cholinesterase inhibitors identified *in silico*.

Keywords: Acetylcholinesterase, Antioxidant, Butyrylcholinesterase, Cholinesterase Inhibitors, Herbs, Phenolic Compounds

Introduction

Alzheimer's Disease (AD) is a prominent causative factor of dementia in old people, typified by impairment in cognitive function and neuronal loss (Kinney *et al*., 2018). The number of patients suffering from dementia worldwide is projected to be 65.7 million by 2030 (Duthey, 2013), therefore, AD has become a main public health concern as the global population continues to age. The further projection indicates that by 2050, people above 60 years of age will

account for twenty-two percent of the global population with four-fifths living in Asia, Latin America, or Africa (Duthey, 2013). With these projections and the increased occurrence of neurodegenerative disorders in the world, there is a need for proactive interventions in the prevention and management of AD.

The etiology of AD is multifaceted, with oxidative stress, inflammation, degradation, and aggregation of protein as some of the proposed causative factors. AD is also associated with the depletion of acetylcholine in the brain, which is due to the catalytic activities of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) (Adedayo *et al*., 2020), thereby making AChE and BChE targets for drug design in the management of AD. Besides, several studies have implicated cholinergic enzymes in the early stages of amyloid plaque formation (Majdi *et al*., 2020). Consequently, inhibition of AChE and BChE tends to increase acetylcholine in the brain, thereby causing a decrease in plaque formation (Ehab *et al*., 2019). Moreover, most of the drugs currently being used in the treatment of AD patients are accompanied by adverse outcomes (Ehab *et al*., 2019). Given the adverse effects that characterize the usage of the current AD drugs, it is a necessity to explore natural resources for new cholinesterase inhibitors with minimal side effects.

Culinary spices and herbs have shown promising medicinal properties as a majority are being used in traditional medicine for the treatment of various diseases (Sachan *et al*., 2018). Bay leaf (*Laurus nobilis*) and rosemary (*Rosmarinus officinalis*) plants are selected for this study because of their availability in the market; wide usage across the globe and potential medicinal properties. Bay leaf (*L. nobilis*) is an aromatic leaf usually used as culinary spices globally. Although it originated from the Southern Mediterranean, it is used in other parts of the world to flavor soups and different dishes, particularly, it is used in Nigeria to flavor jollof rice. Apart from the culinary uses of bay leaf, it has been reported that the aromatic leaf may have some health benefits such as antimicrobial, antioxidant, anticonvulsive, and wound healing properties (Ozcan *et al*., 2010; Rukhkyan *et al*., 2013; Algabri *et al*., 2018). However, there is limited data on its neuroprotective potential.

Similarly, rosemary (*R. officinalis*) is a potent herb of Mediterranean origin and serves as a source of flavoring in the preparation of a variety of cuisines. It is also famous for its aptitude to enhance memory and cognition (Ahmed and Babakir-Mina, 2020), but there is a need to establish the biochemical basis for its hypothesized neuroprotection. This study therefore aimed at investigating the neuroprotective property of aqueous extracts of *L. nobilis* and *R. officinalis* and the mechanisms of action by answering the following research questions: Firstly, what is the effect of aqueous extracts of *L. nobilis* and *R. officinalis* on AChE and BChE? Secondly, can aqueous extracts of *L. nobilis* and *R. officinalis* inhibit iron-induced lipid peroxidation? Lastly, do *L. nobilis* and *R. officinalis* extracts possess antioxidative properties?

Materials and Methods

Study Design

This study was carried out using the research process map presented in Fig. 1. The study began with sample collection followed by authentication of samples. Then, aqueous extraction of the samples was performed and the following *in vitro* assays were carried out on the extracts: Cholinesterase inhibition, lipid peroxidation, and antioxidant assays. Subsequently, the samples were derivatized and subjected to GCMS analysis for identification of the bioactive constituents, which were used for molecular docking studies. A detailed description of the research steps is presented in the succeeding section.

Culinary Herbs Collection, Authentication, and Preparation

Dried bay leaf (*L. nobilis*) and fresh rosemary shrub (*R. officinalis*) were purchased from Shoprite Mall, Akure, Ondo State, Nigeria. Thereafter, the samples were authenticated by MrMomoh, a Forest Biologist at the Federal University of Technology, Akure (FUTA), and deposited at FUTA herbarium with voucher numbers 0299 and 0300 for *L. nobilis* and *R. officinalis*, respectively. The rosemary sample was oven-dried at 40°C. Then, the two samples were milled into a fine powder using an electric blender. Subsequently, 500 mg of each sample was dispersed in 250 mL of distilled water and vigorously shaken for 24 h using the HY-4 speed governing multipurpose oscillator (HINOTEK, Ningbo, China). The mixture was initially filtered using a sieving mesh while subsequent filtration was carried out with Whatman filter paper and the ensuing filtrate was utilized for the in vitro assays.

Animals and Preparation of Brain Homogenate

Six male Wistar rats (200-250 g) "were obtained from the Department of Biochemistry, FUTA, Nigeria. After overnight fasting, the animals were sacrificed and the brain tissues harvested. Then, the harvested brain tissues were cleaned in cold normal saline and homogenized in phosphate buffer (0.1 M; pH 6.9). The homogenates were centrifuged at 12,000 x g for 15 min at 4°C and the collected supernatants were immediately utilized as the enzyme source for the cholinesterase inhibition assay. The animal protocol was approved by the Health Research Ethics Committee of the University of Medical Sciences, Ondo, Ondo State, Nigeria.

In Vitro Cholinesterase Inhibition Assay

The inhibitory activities of the aqueous extracts from bay leaf and rosemary against AChE and BChE were evaluated using the method of Oboh *et al.* (2017). The reaction mixture [rat brain homogenate; 3.3 mm 5,5' -dithio-bis (2-nitrobenzoic) acid; appropriate dilutions of sample extracts and phosphate buffer (pH 8.0)] was incubated

at 25°C for 20 min. The mixture without the sample extracts was used as the control. Subsequently, the respective substrates (50 µMacetylthiocholine iodide and butyrylthiocholine iodide) for AChE and BChE were added. Then, the catalytic activities of the enzymes were monitored by reading the absorbance at 412 nm for 180 at 15-sec intervals using an ultraviolet-visible spectrophotometer (Jenway 6305 model, Staffordshire, UK). The cholinergic enzyme activities were therefore expressed as percentage inhibition.

Inhibition of Lipid Peroxidation

The inhibitory effects of the extracts on iron-induced lipid peroxidation in rat brains were assessed using the method of Ohkawa *et al*. (1979). The volume of the reaction mixture, which comprised 100 µL of rat brain homogenate, 30 µL of 0.1 M Tris-HCl buffer (pH 7.4), extracts (25-100 µL), and 30 µL of 250 µm freshly prepared $FeSO_4$ was made up to 270 µL with distilled water. Thereafter, it was incubated at 37°C for 1 h. Subsequently, the color developed by adding 300 µL sodium dodecyl sulphate, 500 µL acetic acid HCl (pH 3.4), and 500 µL 0.8% thiobarbituric acid. The mixture was subsequently, incubated at 100°C for 1 h. Thereafter, the Thiobarbituric Acid Reactive Species (TBARS) generated were determined by measuring the absorbance at 532 nm and expressed as percentage control.

Antioxidant Assays

The ability of the extracts to scavenge 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical (ABTS*) was evaluated using the method of Re *et al.* (1999). ABTS* was produced by reacting 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) aqueous solution (7 mm) with $K_2S_2O_8$ (2.45 mm, final concentration) in the absence of light for 16 h.

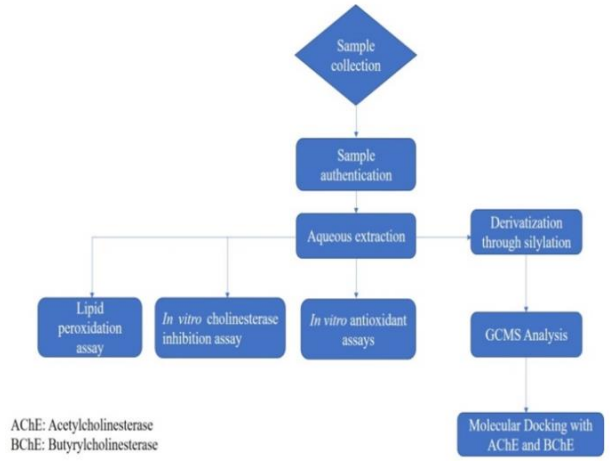

Fig. 1: Research process map

Afterward, the A_{734nm} was adjusted to 0.700 with ethanol. About 200 µL of appropriately diluted sample extract was subsequently mixed with the ABTS solution (2000 µL) and the A_{734} nm was read after 15 min. Subsequently, the ABTS* scavenging ability was calculated and expressed as percentage inhibition. Meanwhile, the ability of the extracts to reduce Fe^{3+} to Fe^{2+} was evaluated through the method of Oyaizu (1986) as earlier reported by Oboh *et al.* (2017). Moreover, the iron-chelating property of the extracts was evaluated by the method of Puntel *et al.* (2005) described elsewhere (Oboh *et al*., 2017).

Polyphenol Content of the Extracts

The polyphenol content of the extracts was determined by the methods of Singleton *et al.* (1999) and Meda *et al.* (2005a), for total phenol and flavonoid, respectively. For the Total Phenol Content (TPC) determination, the extracts were added to 10% Folin-Ciocalteau's reagent (v/v), which served as an oxidizing agent, after which, 7.5% Na_2CO_3 was added to neutralize the reaction. After the mixture had been incubated (45°C, 40 min), the A_{765}nm was read and the TPC was expressed as 'milligram GAE (Gallic Acid Equivalent)/g dry weight of the sample. Meanwhile, to determine the total flavonoid, suitable dilutions of the extracts were added to methanol (0.5 mL), 10% aluminum chloride (500 µL), 1 m potassium acetate (50 µL), and distilled water (1.4 mL). After incubation of the mixture (25°C, 30 min), the A_{415}nm was read. The total flavonoid content was expressed as Milligram (QE) Quercetin Equivalent/g dry weight of the sample.

Characterization of Bioactive Constituents Using GC-MS

Before the GC-MS analysis, samples were derivatized through silylation using the method described by Proestos and Komaitis (2013). Then, the silylated samples were analyzed through GC-MS (Chipiti *et al*., 2015). The analysis was performed using a Varian 3800 gas chromatograph (Agilent Technology, USA) equipped with an Agilent fused silica capillary CP-Sil 5 CB column (30 m × 0.25 mm i.d.) connected to a Varian 4000 mass spectrometer operating in the EI mode (70 eV; m/z 30-800 amu; source temperature 230°C and a quadruple temperature 150°C). One microliter of the silylated samples was injected with split mode (10:1) using N_2 as a carrier, with a flow rate of 0.8 mL/min and a total run time of 40-55 min. Identification of phytochemical components of the samples was conducted using the database of the National Institute of Standard and Technology MS (Gaithersburg, U.S.A) library.

Preparation of Protein and Ligand for Silico Study

The structures of AChE and BChE with respective PDB IDs 6O4W (Gerlits *et al*., 2019) and 4B0P (Wandhammer *et al*., 2013) were downloaded from http://www.rcsb.org (Protein Databank). These crystal

structures were processed by eliminating 'attached water and ligands' molecules. Meanwhile, the required hydrogen atoms were added with the aid of Scripps Research Institute's Autodock v4.2 program. The search grid was extended beyond the subject proteins and the characteristics of the atomic solution were resolved. Gasteiger type-polar hydrogen charges were assigned; the non-polar ones were merged with the carbons while the internal degrees of freedom and torsion were formed. The proteins were then saved as pdbqt entities for molecular docking. For the ligand preparation, the Structure Data File (SDF) format of galantamine and 39 phenolic compounds detected in the samples were downloaded from www.pubchem.ncbi.nlm.nih.gov. Open babel program (O'Boyle *et al*., 2011), was used to convert the compounds into the mol 2 chemical form. The α-carbons of the ligands were identified after the torsion and internal degrees of freedom were zeroed. Thereafter, the compounds were saved as pdbqt files with Autodock tools."

Molecular Docking

Docking and evaluation of binding tendencies of the compounds to AChE and BChE were carried out by employing Vina GUI (Trott and Olson, 2010). The prepared proteins and the compounds were placed in the columns assigned for them. The grid center for docking was detected as X = 91.89, Y = 84.37, Z = -15.94 with the dimensions of the grid box, 80.71 × 78.89 × 59.62 for AChE; X = 31.98, Y = 18.61, Z = 25.95 with the dimensions of the grid box, 64.67 × 65.76 × 82.29 for BChE. Subsequently, Vina GUI was run, and the software assigned the energy values using Root Mean Square Deviation (RMSD), with the pose having the highest affinity for a particular cluster chosen as the representative. The compounds were then, graded by their affinity figures. The amino acid interactions (molecular interactions) of the subject proteins (AChE and BChE) and the ligands with remarkable binding energy were processed with Discovery Studio Visualizer, 2020.

Data Analysis

Data from the study were statistically analyzed using unpaired t-tests in Graph Pad Prism version 5.0. Significance was accepted at p values less than 0.05 ($p<0.05$). IC_{50} values: Concentration of the extract that causes 50% inhibition) and EC_{50} of FRAP (concentration of the extract that can demonstrate 50% of FRAP were also determined using Graph Pad Prism 5.0.

Results

The inhibitory effect of aqueous extracts of *L. nobilis* and *R. officinalis* on cholinesterase activity is presented in Fig. 2. The result showed that aqueous extracts of *L. nobilis* and *R. officinalis* demonstrated remarkable inhibitory activities against AChE as both extracts exhibited about 100% inhibition at the highest concentration of 0.3 µg/mL (Fig. 2a). However, there was no significant difference ($p>0.05$) in the AChE inhibitory activities of both extracts as reflected in their respective IC_{50} values (*L. nobilis*: 4.21±0.50 µg/mL; *R. officinalis*: 5.55±0.80 µg/mL) (Table 1). Similarly, aqueous extracts of both samples demonstrated exceptional inhibitory activities against BChE as the extracts exhibited about 100% inhibition at 0.30 µg/mL (Fig. 2b) but the IC_{50} values (Table 1) revealed no significant difference ($p>0.05$) in the BChE inhibitory activities of both extracts (*L. nobilis* IC_{50}: 4.76±0.36 µg/mL; *R. officinalis* IC_{50}: 5.60±0.40 µg/mL).

The effects of the extracts on Fe^{2+}-induced lipid peroxidation in rat brain as presented in Fig. 3 indicated that incubation of rat's brain homogenate with $FeSO_4$ resulted in increased generation of TBARS, which was inhibited by the introduction of the extracts from both samples at all concentrations (Fig. 3). Although both samples exhibited inhibitory ability against Fe^{2+}-induced lipid peroxidation in rat brain, *L. nobilis* had a better ability to inhibit lipid peroxidation as it had a significantly ($p<0.05$) lower IC_{50} value (67.83±13.53 µg/mL) than *R. officinalis* (96.96±15.63 µg/mL).

The ability of the extracts to scavenge ABTS radicals as presented in Fig. 4a showed that *L. nobilis* extract had a significantly ($p<0.05$) higher radical scavenging ability than *R. officinalis*. Interestingly, there was a significant increase ($p<0.05$) in the ABTS radical scavenging ability of both samples with an increase in sample concentration.

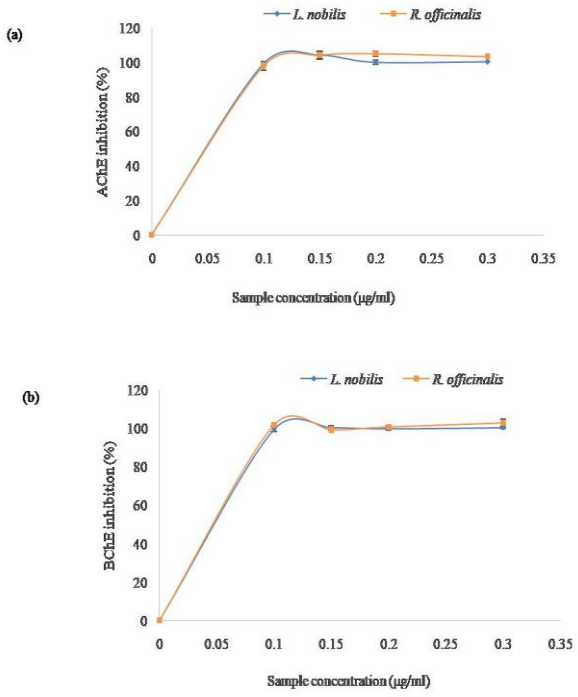

Fig. 2: Inhibitory effect of aqueous extracts of *L. nobilis* and *R. officinalis* on AChE and BChE activities. (a) AChE inhibition (b) BChE inhibition

At higher concentrations (200 µg/mL) of the extracts, both samples demonstrated remarkable ABTS scavenging abilities of over 80%, with *L. nobilis* exhibiting 86% inhibition while *R. officinalis* extract displayed 82% inhibition (Fig. 4a). The FRAP result presented in Fig. 4b showed that both extracts displayed increased ferric reducing properties in a dose-dependent manner, with *R. officinalis* extract exhibiting about 51 mg AAE/g while *L. nobilis* extract had approximately 46 mg AAE/g at the highest concentration (500 µg/mL). However, the EC_{50} of FRAP (Table 1) indicated that *L. Nobilis* had a better FRAP as it had a significantly ($p<0.05$) lower EC_{50} (149.05±8.35 µg/mL) than *R. officinalis* (167.00±9.60 µg/mL). The iron-chelating ability of *L. nobilis* and *R. officinalis* aqueous extracts as presented in Fig. 4c revealed that both extracts chelated Fe^{2+} in a dose-dependent manner. Based on the IC_{50} values of the extracts depicted in Table 1, there was no significant difference ($p>0.05$) in the Fe^{2+}-chelating ability of both samples.

Moreover, the total phenol and flavonoid contents of *L. nobilis* and *R. officinalis* aqueous extracts are presented in Table 2. The result indicated that extract from *R. officinalis* contained a significantly higher ($p<0.05$) total phenol (2.24±0.15 mg GAE/g) than *L. nobilis* extract (0.90±0.10 mg GAE/g). However, there was no significant difference ($p>0.05$) in the total flavonoid content of both samples. Table 3 and Fig. 5a and b showed the results obtained on the phenolic characterization of the samples. A total of seventeen phenolic compounds were detected in both samples, which include: Methyl eugenol, vanillic acid, p-coumaric acid, ferulic acid, gentisic acid, coumarin, p-anisic acid, gallic acid, p-hydroxybenzaldehyde, trans-isoeugenol, 3-hydroxybenzoic acid, rosmarinic acid, caffeic acid, quercetin, protocatechuic acid, estragole, and 1,2-benzenediol. However, carnosic acid, rosmanol, hispidulin 7-glucoside, gamma-eudesmol, rutin, hesperetin 7-O-rutinoside, luteolin, isorhamnetin, apigenin, and techtochrysin were peculiar to rosemary while p-salicylic acid, homovanillic acid, γ-tocopherol, biphenyl-4-carboxylic acid, chlorogenic acid, ethyl-trans-cinnamate, cinnamaldehyde, sinapinic acid, trans-cynnamyl acetate, elemicin, syringic acid and eugenol were detected in bay leaf only.

Nevertheless, molecular docking evaluation showed that six of the phenolic compounds have notable binding affinities for AChE relative to galantamine, a typical cholinesterase inhibitor. As shown in Table 4, these compounds include chlorogenic acid, rosmarinic acid, rosmanol, rutin, hesperetin 7-O-rutinoside, and luteolin with the respective binding energy of -12.4, -11.8, -11.1, -11.7, -11.8 and -11.2 kcal/mol compared to -10.8 kcal/mol binding energy of galantamine. For BChE, eight compounds: Chlorogenic acid, rosmarinic acid, quercetin, carnosic acid, rosmanol, rutin, hesperetin 7-O-rutinoside, and luteolin were outstanding in their respective binding energy of -10.5, -10.9, -10.4, -10.6, -10.7, -12.0, -10.6 and -10.2 kcal/mol compared to -10.1 kcal/mol for galantamine (Table 4). However, a closer look revealed that all the six compounds (chlorogenic acid, rosmarinic acid, rosmanol, rutin, hesperetin 7-O-rutinoside, and luteolin) that have higher binding propensities for AChE relative to galantamine also exhibited a higher binding affinity for BChE, relative to galantamine.

The binding interactions of the six compounds with AChE are shown in Fig. 6a-g. Galantamine, chlorogenic acid, and rutin bound to a notable region in AChE (Fig. 6a-c) while interacting with key amino acid residues; specifically, galantamine via a hydrophobic mode interacted with Trp 86, Tyr 337, and Phe 338, with Glu 202 by a single hydrogen bond and Asn87 and Tyr 124 by carbon-hydrogen bond (Fig. 7a), chlorogenic acid interacted with Trp286 via a hydrophobic mode and interacted with Tyr341 through a hydrogen bond (Fig. 7b), while the means of interface between rutin and AChE was mainly by a hydrogen bond with Pro 368, Gln 369, His 405 and Asn533 (Fig.7c). For rosmanol, hydrophobic interaction was observed with Trp286 and Tyr341, hydrogen bond interaction with Asp74 and Tyr124 (Fig. 7d) while for hesperetin 7-O-rutinoside, hydrophobic interaction was observed for the same amino acids as for rosmanol (Trp286 and Tyr341) plus three additional interactions with Tyr337, Phe338, and His447 whereas a single hydrogen bond interaction was seen with Tyr72 (Fig. 7e). The interaction observed between luteolin and AChE includes hydrophobic interaction with Trp286, Tyr337, and Tyr341 as well as hydrogen bond with Tyr124 and Phe295 (Fig.7f), while rosmarinic acid forms hydrophobic bonds with Trp286 and His447, hydrogen bond with Tyr124 and Tyr 341, as well as a carbon-hydrogen bond with Trp86 (Fig.7g).

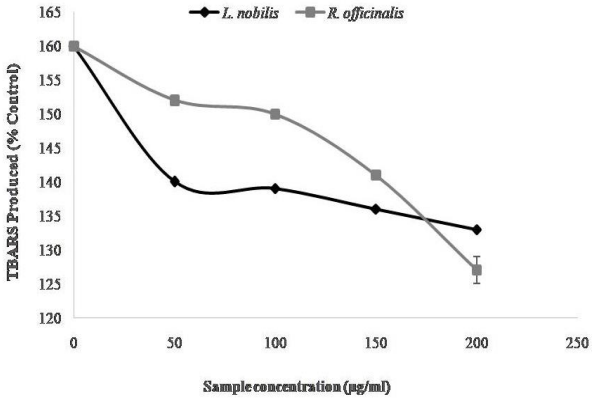

Fig. 3: Inhibitory effects of aqueous extracts of *L. nobilis* and *R. officinalis* on Fe^{2+}-induced lipid peroxidation in rat brain

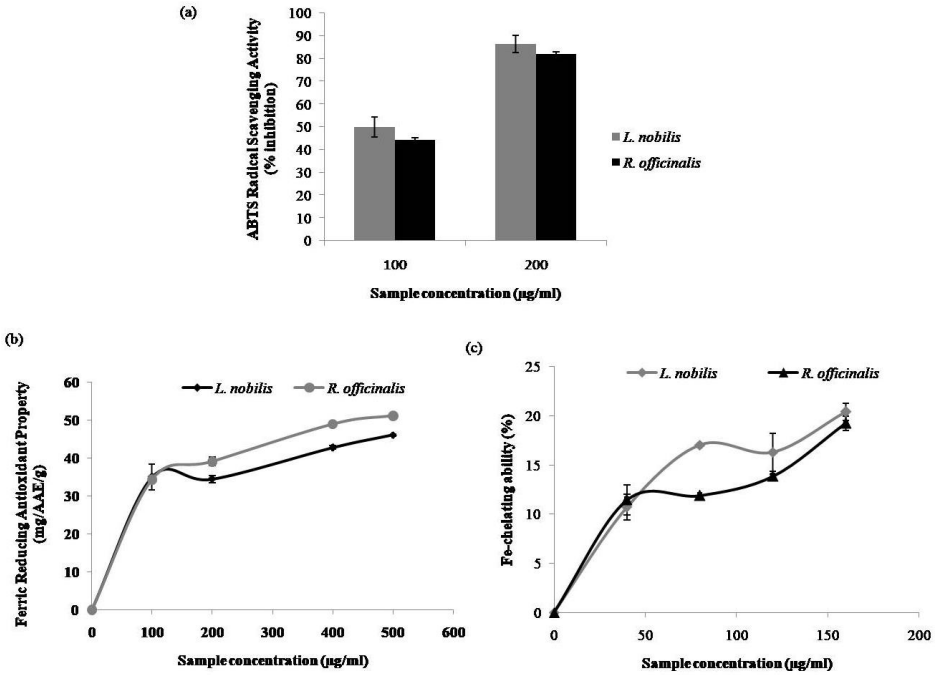

Fig. 4: Antioxidant properties of aqueous extracts of *L. nobilis* and *R. officinalis* (a) ABTS* scavenging ability (b) Ferric reducing antioxidant property (c) Fe-chelating ability

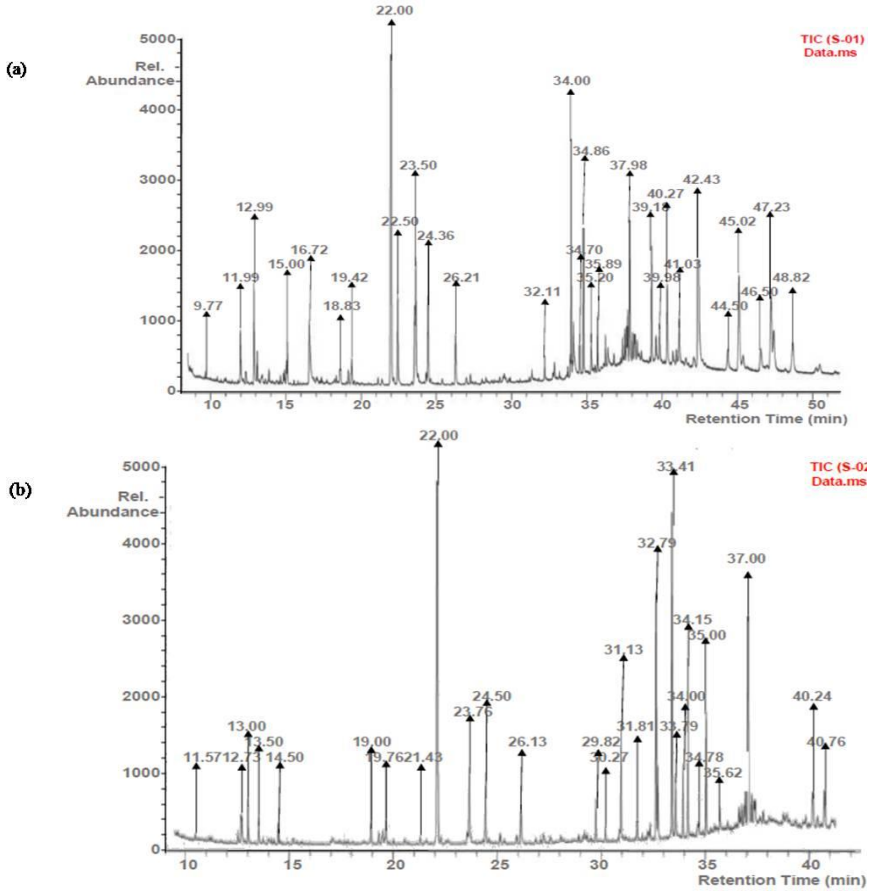

Fig. 5: Chromatograms of bioactive compounds detected in (a) *L. nobilis* and (b) *R. officinalis*

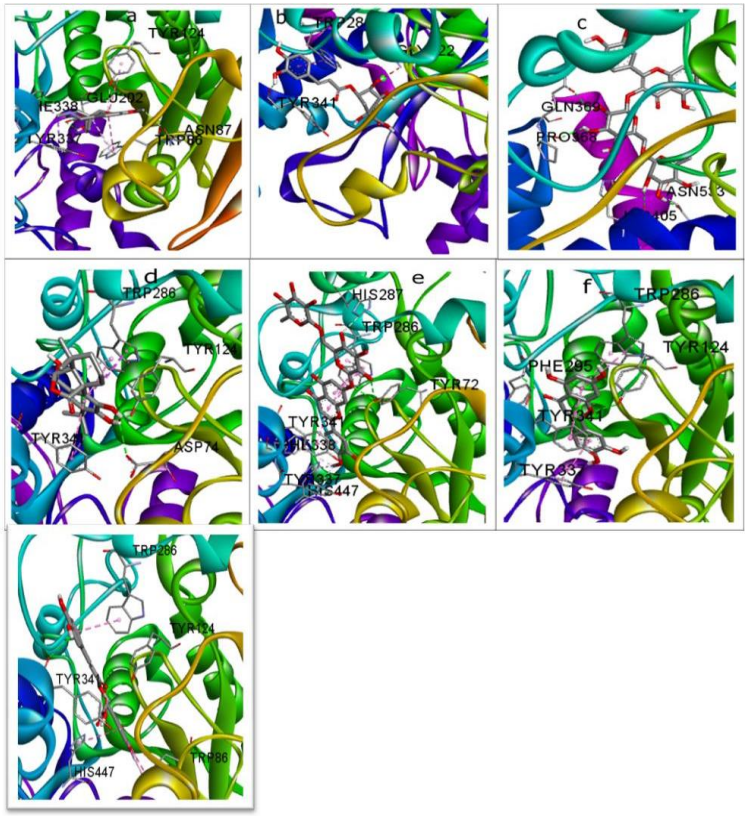

Fig.6: 3D view of the binding of selected phenolic compounds identified in *L. nobilis* and *R. officinalis* to acetylcholinesterase. (a) galantamine (b) chlorogenic acid (c) rutin (d) rosmanol (e) hesperetin 7-O-rutinoside (f) luteolin (g) rosmarinic acid

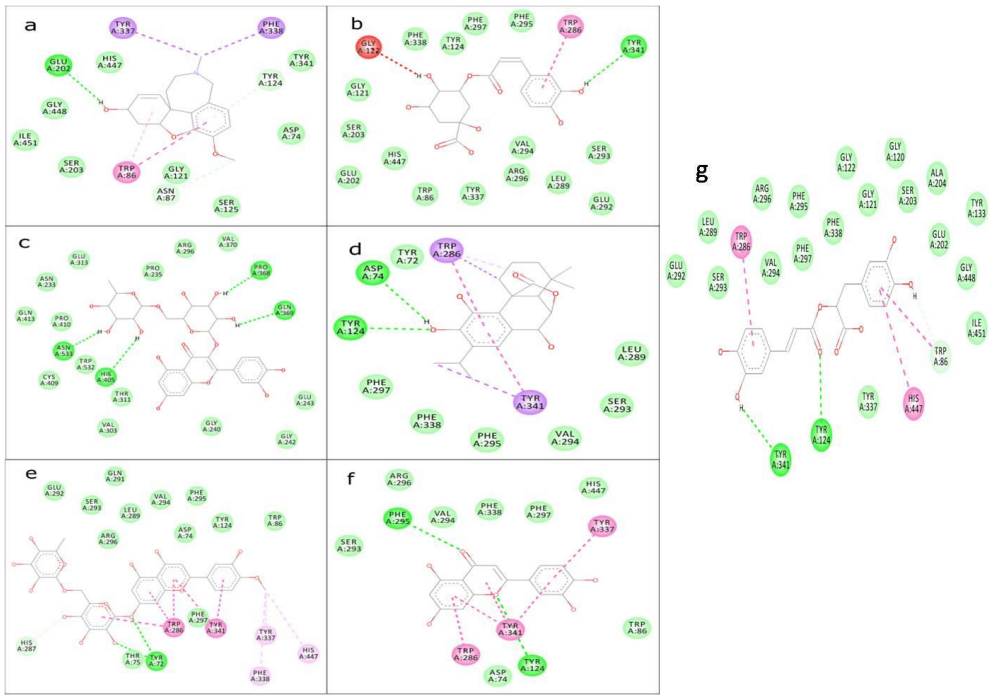

Fig. 7: 2D view of the binding of selected phenolic compounds identified in *L. nobilis* and *R. officinalis* to amino acids in the binding site of acetylcholinesterase. (a) galantamine (b) chlorogenic acid (c) rutin (d) rosmanol (e) hesperetin 7-O-rutinoside (f) luteolin (g) rosmarinic acid

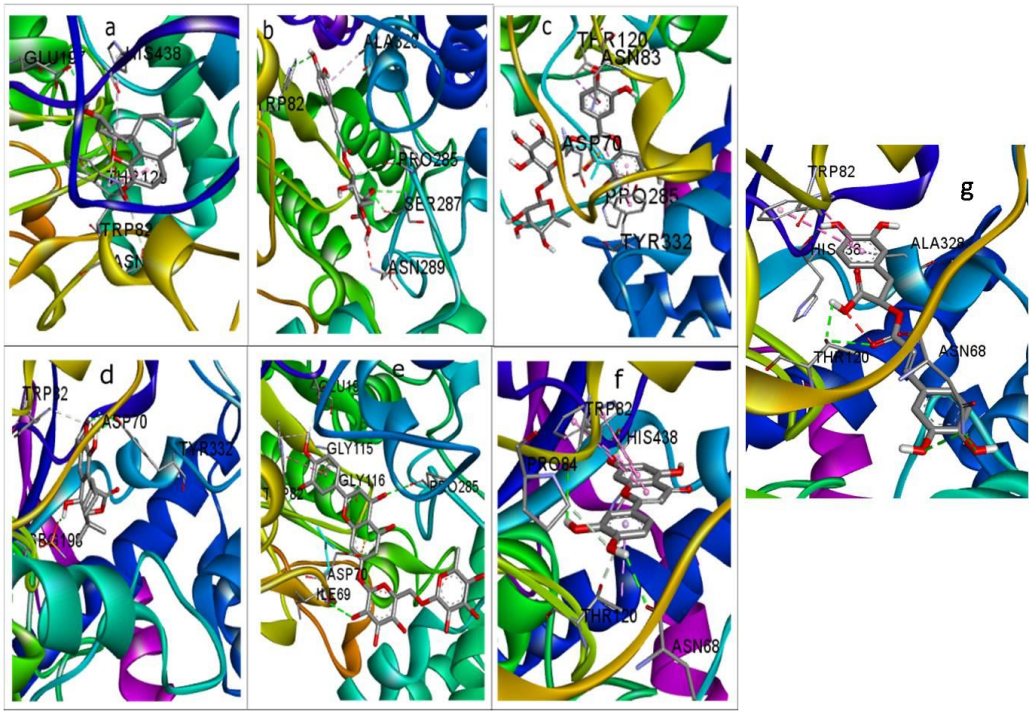

Fig. 8: 3D view of the binding of selected phenolic compounds identified in *L. nobilis* and *R. officinalis* to butyrylcholinesterase. (a) galantamine (b) chlorogenic acid (c) rutin (d) rosmanol (e) hesperetin 7-O-rutinoside (f) luteolin (g) rosmarinic acid

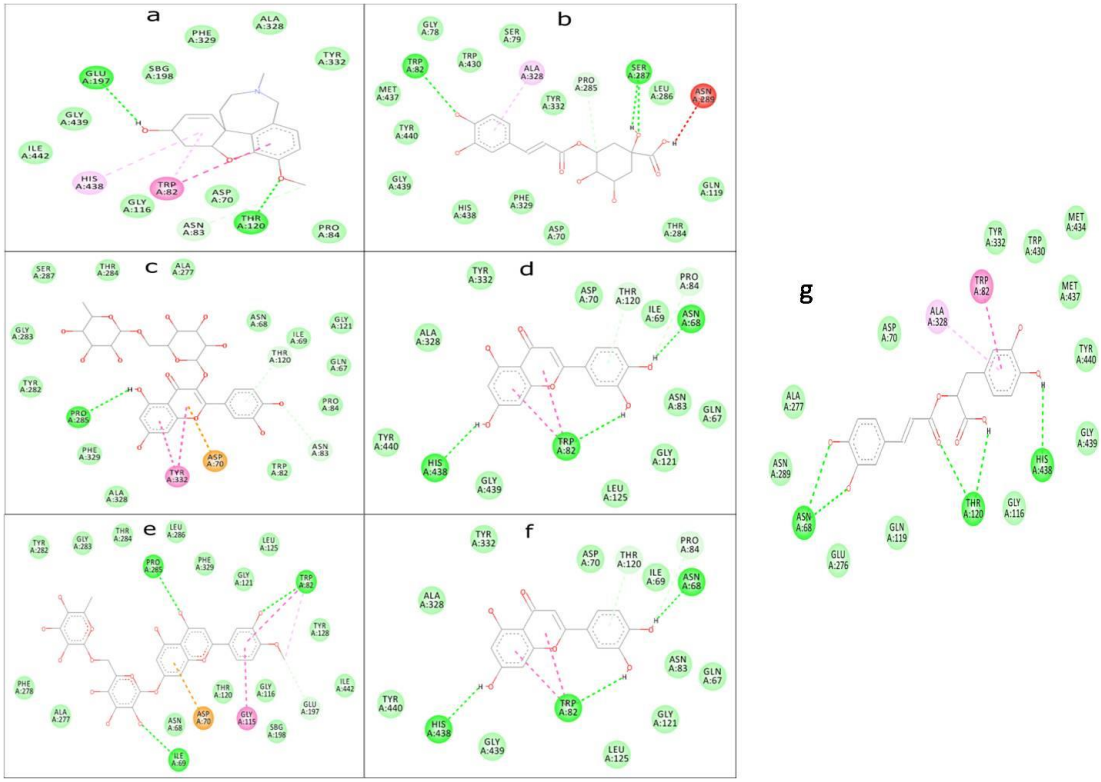

Fig. 9: 2D view of the binding of selected phenolic compounds identified in *L. nobilis* and *R. officinalis* to amino acids in the binding site of butyrylcholinesterase. (a) galantamine (b) chlorogenic acid (c) rutin (d) rosmanol (e) hesperetin 7-O-rutinoside (f) luteolin (g) rosmarinic acid

Table 1: EC_{50} of FRAP and IC_{50} values of cholinesterase inhibition, lipid peroxidation inhibition, and Fe-chelation abilities of aqueous extracts of *L. nobilis* and *R. officinalis*

Samples	AChE (µg/mL)	BChE (µg/mL)	Lipid peroxidation inhibition (µg/mL)	FRAP (µg/mL)	Fe^{2+}-chelation (µg/mL)
L. nobilis	4.21±0.50[a]	4.76±0.36[a]	67.83±13.53[a]	149.05±8.35[a]	10.93±0.42[a]
R. officinalis	5.55±0.84[a]	5.60±0.49[a]	96.96±15.63[b]	167.00±9.60[b]	10.12±0.40[a]

Values represent mean ± SD. Mean values with the same superscript letter along the same column are not significantly different ($p>0.05$). IC_{50} values: Extract concentration that caused 50% inhibition. EC_{50} of FRAP indicates extract concentration that exhibited 50% of ferric reducing property

Table 2: Total phenol and total flavonoid contents

Samples	Total phenol (mg GAE/g)	Total flavonoid (mg QE/g)
L. nobilis	0.90±0.10[a]	0.48±0.03[a]
R. officinalis	2.24±0.15[b]	0.85±0.09[a]

Values represent mean ± SD. Mean values with the different superscript letters along the same column are significantly different ($p<0.05$)

Table 3: Phenolic composition of *L. nobilis* and *R. officinalis*

				Peak Area (%)	
S/N	Compound detected	Molecular formula	Molecular weight	*L. nobilis*	*R. officinalis*
1.	p-Salicylic acid	$C_7H_6O_3$	138	12.90	ND
2.	*Methyl eugenol	$C_{11}H_{14}O_2$	178	9.64	15.13
3.	*Vanillic acid	$C_8H_8O_4$	168	6.46	0.64
4.	*p-Coumaric acid	$C_9H_8O_3$	164	5.86	2.57
5.	*Ferulic acid	$C_{10}H_{10}O_4$	194	5.36	3.14
6.	Homovanillic acid	$C_9H_{10}O_4$	182	4.85	ND
7.	*Gentisic acid	$C_5H_{11}NO_2$	117	4.66	0.61
8.	γ-Tocopherol	$C_{28}H_{48}O_2$	416	4.36	ND
9.	*Coumarin	$C_9H_6O_2$	146	4.28	1.91
10.	Biphenyl-4-carboxylic acid	$C_{13}H_{10}O_2$	198	4.07	ND
11.	*p-Anisic acid	$C_8H_8O_3$	152	3.75	0.65
12.	Chlorogenic acid	$C_{16}H_{18}O_9$	354	3.22	ND
13.	Ethyl-trans-Cinnamate	$C_{11}H_{12}O_2$	176	3.21	ND
14.	Cinnamaldehyde	C_9H_8O	132	2.95	ND
15.	*Gallic acid	$C_7H_6O_5$	170	2.68	10.04
16.	Sinapinic acid	$C_{11}H_{12}O_5$	224	2.66	ND
17.	*p-hydroxybenzaldehyde	$C_7H_6O_2$	122	2.33	1.70
18.	*Trans-Isoeugenol	$C_{10}H_{12}O_2$	164	2.14	1.69
19.	*3-hydroxybenzoic acid	$C_7H_6O_3$	138	1.93	1.23
20.	Trans-Cynnamyl acetate	$C_{11}H_{12}O_2$	176	1.61	ND
21.	*Rosmarinic acid	$C_{18}H_{16}O_8$	360	1.53	9.42
22.	*Caffeic acid	$C_9H_8O_4$	180	1.46	1.88
23.	Elemicin	$C_{12}H_{16}O_3$	208	1.43	ND
24.	*Quercetin	$C_{15}H_{10}O_7$	302	1.29	3.45
25.	Syringic acid	$C_9H_{10}O_5$	198	1.20	ND
26.	*Protocatechuic acid	$C_7H_6O_4$	154	1.09	0.63
27.	Eugenol	$C_{10}H_{12}O_2$	164	1.07	ND
28.	*Estragole	$C_{10}H_{12}O$	148	0.84	0.62
29.	*1,2-Benzenediol	$C_6H_6O_2$	110	0.60	0.64
30.	Carnosic acid	$C_{20}H_{28}O_4$	332	ND	13.81
31.	Rosmanol	$C_{20}H_{26}O_5$	346	ND	8.16
32.	Hispidulin 7-glucoside	$C_{22}H_{22}O_{11}$	462	ND	5.64
33.	Gamma-eudesmol	$C_{15}H_{26}O$	222	ND	5.06
34.	Rutin	$C_{27}H_{36}O_{19}$	664	ND	2.58
35.	Hesperetin 7-O-rutinoside	$C_{28}H_{34}O_{15}$	609	ND	2.55
36.	Luteolin	$C_{15}H_{10}O_6$	286	ND	2.13
37.	Isorhamnetin	$C_{16}H_{12}O_7$	316	ND	1.59
38.	Apigenin	$C_{15}H_{10}O_5$	270	ND	1.23
39.	Techtochrysin	$C_{16}H_{12}O_4$	268	ND	0.92

ND: Not Detected; *Detected in both samples

Table 4: Binding affinities of most potent cholinesterase inhibitors detected in *L. nobilis and R. officinalis*

S/N	Detected compounds	Binding affinity (kcal/mol)	
		AChE	BChE
	[&]Galantamine	-10.8	-10.1
1.	[*]Rutin	-11.7	-12.0
2.	[#]Chlorogenic acid	-12.4	-10.5
3.	[**]Rosmarinic acid	-11.8	-10.9
4.	[*]Hesperetin 7-O-rutinoside	-11.8	-10.6
5.	[*]Rosmanol	-11.1	-10.7
6.	[*]Luteolin	-11.2	-10.2
7.	[*]Carnosic acid	-10.1	-10.6
8.	[**]Quercetin	-10.4	-10.4

[&]: Standard cholinesterase inhibitor; [*]: Detected in *R. officinalis* alone; [#]: Detected in *L. nobilis* alone; [**]: Detected in *L. nobilis* and *R. officinalis*

Furthermore, the binding interactions of these compounds with BChE are shown in Fig. 8a-g. Chlorogenic acid and rutin bind to a similar region as galantamine, relating to conserved amino acids at the active and anionic site of BChE (Fig. 8a-c). The interaction of galantamine with BChE was through hydrophobic interactions with His438 and conserved residue Trp82 as well as hydrogen bond with Thr120 and Glu197 (Fig. 9a). For chlorogenic acid, Trp82 and Ser287 were involved in hydrogen bond formation plus a π-alkyl interaction with Ala328 (Fig. 9b). Hydrogen bond with Pro285, π-anion interaction with Asp70, and hydrophobic bond with Tyr332 were involved in the binding interaction of rutin to BChE (Fig. 9c). For rosmanol, hydrogen bond interaction was observed with Asn68, Trp82, and His438, while carbon-hydrogen bond was observed with Pro84 and Thr120 (Fig. 9d). For hesperetin 7-O-rutinoside, hydrophobic interaction was observed with Gly115, hydrogen bond interaction was seen with Asn69, Trp82, and Pro285, carbon-hydrogen bond with Glu197 and π-anion interaction Asp70 (Fig. 9e). The interaction observed between luteolin and BChE includes hydrogen interaction with Asn68, Trp82, and His438 and carbon-hydrogen bond with Pro84 and Thr120 (Fig. 9f), while rosmarinic acid forms hydrophobic bonds with Trp82 and Ala328, hydrogen bond with Asn68, Thr120, and His428 (Fig. 9g).

Discussion

Neurodegenerative diseases are some of the prevalent health challenges around the world, with rising occurrences being observed among the elderly (Kaur *et al*., 2017; Mollica *et al*., 2018). As a result of the continuous increase in the prevalence of neurological disorders in society, researchers have continued to exploit bioactive compounds from natural resources in the development of novel drugs for effective treatment. The enzyme inhibition strategy has been employed for the development of various classes of FDA-approved drugs such as donepezil and galantamine, which are notable cholinesterase inhibitors. Therefore, the ability of the extracts of *L. nobilis* and *R. officinalis* to inhibit AChE and BChE activities could be of therapeutic importance in the management of AD, as cholinesterase inhibitors are some of the most effective classes of drugs currently being used in treating AD. Findings from this study agree with previous related studies that reported the ability of various extracts of *L. nobilis* and *R. officinalis* to inhibit cholinergic enzyme activities (Ferreira *et al*., 2006; Orhan *et al*., 2008). However, extracts in this study showed higher inhibitory capacity against AChE and BChE. While ethanolic extract of *L. nobilis* exhibited 64% inhibition against AChE activity at 1 mg/mL, essential oil from the sample (*L. nobilis*) did not inhibit AChE activity at the same concentration but exhibited 51% inhibition against the cholinergic enzyme activity at 0.5 mg/mL (Ferreira *et al*., 2006). Nevertheless, rosemary essential oil displayed about 64 and 74% inhibition against AChE and BChE activity, respectively at 1 mg/mL (Orhan *et al*., 2008). Also, different extracts of the rosemary plant studied by Orhan *et al*. (2008) showed a different level of inhibition against the two cholinergic enzymes. For instance, petroleum ether extract of *R. officinalis* exhibited lower cholinesterase inhibitory activity: 8.5 and 54% inhibition against AChE and BChE at 1 mg/mL, respectively (Orhan *et al*., 2008) whereas ethyl acetate and methanolic extracts of *R. officinalis* showed no inhibitory activity against AChE in the study but significantly inhibited BChE with approximately 34 and 84% inhibition, respectively. Furthermore, the AChE inhibitory activity of aqueous extract of fresh rosemary reported by Sharma *et al*. (2020) was lower than what we obtained in the present study. Sharma *et al*. (2020) recorded an IC_{50} value of 229.14±3.86 mg/mL for AChE inhibition while we reported 5.55±0.84 µg/mL as the IC_{50} for the inhibition of the same enzyme by rosemary aqueous extract. Nevertheless, the authors did not report on the BChE inhibitory activity of the extract (Sharma *et al*., 2020). It is worthy of note that the inhibitory potency of the studied

extracts is comparable to that of galantamine, a standard cholinesterase inhibitor, which showed about 100 and 80% inhibition against AChE and BChE activity, respectively at 1 mg/mL (Orhan *et al*., 2008). Similarly, the IC_{50} values of the extracts (*L. nobilis*: 4.21 µg/mL and *R. officinalis*: 5.55 µg/mL) are comparable to that of galantamine (6.33 µg/mL) reported by a previous related study (Sharma *et al*., 2020). The discrepancies in the inhibitory capacity of the various extracts may be attributed to varying bioactive constituents and plant varieties (Tural and Turhan, 2017). Findings from this study, therefore, suggest that aqueous extracts of *L. nobilis* and *R. officinalis* may be sources of more potent cholinesterase inhibitors, with a prospect in drug development for the management of AD and other neurodegenerative diseases.

The processes that are involved in the development of neurological diseases such as AD are complex, however, oxidative stress has been identified as a cardinal factor in the pathological pathway of such diseases (Adedayo *et al*., 2020). The oxidation hypothesis involves the generation of free radicals, which predispose the cell membrane to lipid peroxidation. More so, the brain is highly prone to attacks from Reactive Oxygen Species (ROS) due to their high Polyunsaturated Fatty Acids (PUFA) composition, which is susceptible to oxidation (Adefegha *et al*., 2016). It has thus, been established that the inhibition of lipid peroxidation in the brain is significant to the management of neurodegenerative diseases. Furthermore, bioactive compounds with antioxidant properties may be of significance in the prevention and treatment of neurological disorders since they can counteract the overproduction of ROS (Foyet *et al*., 2019). The abilities of *L. nobilis* and *R. officinalis* extracts to inhibit lipid peroxidation as evident in the inhibition of TBARS generation suggest their potential to protect the brain from the damaging effect of free radicals (Adedayo *et al*., 2020). Likewise, the abilities of the extracts to scavenge ABTS radical, reduce Fe^{3+} to Fe^{2+} and chelate Fe^{2+} confirm their antioxidant properties (Kivrak *et al*., 2017; Oudjedi *et al*., 2018).

The biological activity of *L. Nobilis* and *R. officinalis* aqueous extracts, as evident in their anti-cholinesterase, antioxidant and lipid peroxidation inhibitory properties may be attributed to the presence of polyphenols in the samples as dietary polyphenols are natural antioxidants, with diverse therapeutic potentials (Choi *et al*., 2012; Fiore *et al*., 2020). This claim is corroborated by previous similar studies, which confirmed the neuroprotective potentials of plant polyphenols as evident in their remarkable anticholinesterase activities (Oboh *et al*., 2012; Ademosun *et al*., 2016). Our findings on polyphenol content agree with earlier studies as Sharma *et al*. (2020) reported 2.18±0.25 mg GAE/g as the total phenol content of aqueous extract of fresh *R. officinalis* whereas 2.24±0.15 mg GAE/g was obtained as TPC for similar extract in the present study. On the other hand, Kaurinovic *et al.* (2010) reported 0.68 mg/g as the total flavonoid content of *L. nobilis* aqueous extract as against what was recorded in this study (0.48±0.03 mg QE/g).

Furthermore, the neurotherapeutic potential of bioactive compounds in the extracts was proven by the remarkable cholinesterase inhibitory activity demonstrated in the *in silico* study. From the perspective of the binding affinities of the bioactive compounds with both target proteins, chlorogenic acid, rosmarinic acid, rutin, rosmanol, hesperetin 7-O-rutinoside, and luteolin possibly contributed to the observed AChE- and BChE-inhibitory activities of the spices in the present study, which may be attributed to the hydrogen bond acceptance or hydrogen donor properties of the inherent hydroxyl groups in these compounds while interacting with amino acid residues in the active sites of these enzymes. These compounds may, therefore, be responsible for the cholinergic inhibitory activities exhibited by the aqueous extracts of *L. nobilis* and *R. officinalis* observed in the present study.

Conclusion

Aqueous extracts of *L. Nobilis* and *R. officinalis* displayed remarkable inhibitory activity against selected cholinergic enzymes (AChE and BChE) and lipid peroxidation in rat brain while exhibiting antioxidant properties *in-vitro*. The observed biological activity of the extracts may be attributed to the detected phenolic compounds in the samples. This was confirmed *in silico*, where rutin, chlorogenic acid, rosmarinic acid, hesperetin 7-O-rutinoside, rosmanol, and luteolin showed comparable cholinesterase inhibition to galantamine, a standard drug. More so, this study shall help fill the research gap in the literature (the biochemical basis for neuroprotection) as it identified inhibition of cholinergic enzyme activities and lipid peroxidation, as well as an antioxidant mechanism as some of the biochemical reasons for the neuroprotective potentials of the studied herbs. *L. nobilis* and *R. officinalis* may be potential sources of nutraceuticals in the management of AD. However, *in vivo* studies on the neuroactive properties of the extracts and the identified compounds are necessary.

Funding Information

The APC was funded by the Cape Pennisula University of Technology, Bellville, South Africa.

Author Contributions

Ayodeji O. Falade: Conceptualization, formal analysis, writing-original draft, writing-review and editing, supervision.

Goodness I. Omolaiye: Investigation, writing-original draft.

Kayode E. Adewole: Conceptualization, writing-original draft, writing-review, and editing.

Odunayo M. Agunloye: Conceptualization, writing-review and editing, supervision.

Ahmed A. Ishola: Data curation, investigation, formal analysis.

Kunle Okaiyeto: Writing-review and editing.

Ganiyu Oboh: Methodology, resources, writing-review, and editing.

Oluwafemi O. Oguntibeju: Writing-review and editing.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all the other authors approved the final version of the manuscript. The animal protocol was approved by the Health Research Ethics Committee (HREC) of the University of Medical Sciences, Ondo, Ondo State, Nigeria

References

Adedayo, B. C., Oyeleye, S. I., Okeke, B. M. & Oboh, G. (2020). Anti-cholinesterase and antioxidant properties of alkaloid and phenolic-rich extracts from pawpaw (*Carica papaya*) leaf: A comparative study. Flavour Fragr J 2020, 00, 1-8. doi.org/10.1002/ffj.3615.

Adefegha, S. A., Oboh, G., Molehin, O. R., Saliu, J. A., Athayde, M. L. & Boligon, A. A. (2016). Chromatographic fingerprint analysis, acetylcholinesterase inhibitory properties, and antioxidant activities of red flower rag leaf (*Crassocephalum crepidioides*) extract. Journal of Food Biochemistry, 40, 109-119. doi.org/10.1111/jfbc.12200

Ademosun, A. O., Oboh, G., Bello, F. &Ayeni, P. O. (2016). Antioxidative properties and effect of quercetin and its glycosylated form (rutin) on acetylcholinesterase and butyrylcholinesterase activities. Journal of Evidence-Based Complementary and Alternative Medicine, 21, NP11–NP17. doi.org/10.1177/2156587215610032

Ahmed, H. M. & Babakir-Mina, M. (2020). Investigation of rosemary herbal extracts (*Rosmarinus officinalis*) and their potential effects on immunity. Phytother Res, 34 (8), 1829-1837. doi.org/10.1002/ptr.6648

Algabri, S. O., Doro, B. M., Abadi, A. M., Shiba, M. A., & Salem, A. H. (2018). Bay Leaves have Antimicrobial and Antioxidant Activities. Journal of Pathogen Research, 1(1).

Chipiti, T., Ibrahim, M. A., Koorbanally, N. A., & Islam, M. S. (2015). *In vitro* antioxidant activity and GC-MS analysis of the ethanol and aqueous extracts of *Cissus cornifolia* (Baker) Splanch (Vitaceae) parts. ActaPoloniaePharmaceutica Drug Research, 72(1), 119-127. https://www.ptfarm.pl/pub/File/Acta_Poloniae/2015/1/acta1_2015.pdf#page=121

Choi, D-Y., Lee, Y-J., Hong, J. T. & Lee, H-J. (2012). Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Research Bulletin, 87, 144-153. doi.org/10.1016/j.brainresbull.2011.11.014

Duthey, B. (2013). Background paper 6.11: Alzheimer's disease and other dementias. A public health approach to innovation, 6, 1-74. https://www.who.int/medicines/areas/priority_medicines/BP6_11Alzheimer.pdf

Ehab, A., Ibrahim, M., Magdi, M., Ayman, M., Zidan, N., Osman, A. (2019). Alzheimer's disease and its current treatments; is there a possibility for a cure? Open J Chem, 5, 13-19. doi.org/10.17352/ojc.000012.

Ferreira, A., Proenca, C., Serralheiro, M. L. M. & Ara´ujo M. E. M. (2006). The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. Journal of Ethnopharmacology, 108, 31–37. doi.org/10.1016/j.jep.2006.04.010

Fiore, M., Messina, M. P., Petrella, C., D'Angelo, A., Greco, A, Ralli, M. (2020). Antioxidant properties of plant polyphenols in the counteraction of alcohol-abuse induced damage: Impact on the Mediterranean diet. Journal of Functional Foods, 71, 104012. doi.org/10.1016/j.jff.2020.104012

Foyet, H. S., Wado, E. K.,Abaissou, N., Assongalem, E. A. & Eyong, O. K. (2019). Anticholinesterase and antioxidant potential of hydroethanolic extract of *Ziziphus mucronata* (Rhamnaceae) leave scopolamine-induced memory and cognitive dysfunctions in mice. Evid Based Complimentary and Alternative Medicine, 4568401. doi.org/10.1155/2019/4568401

Gerlits, O., Ho, K. Y., Cheng, X., Blumenthal, D., Taylor, P., Kovalevsky, A. (2019). A new crystal form of human acetylcholinesterase for exploratory room-temperature crystallography studies. ChemBiol Interact, 309, 108698. doi.org/10.1016/j.cbi.2019.06.011

Kaur, N., Sarkar, B., Gill, I., Kaur, S., Mittal, S., Dhiman, M. (2017). Indian herbs and their therapeutic potential against Alzheimer's disease and other neurological disorders, in T. Farooqui and A. A. Farooqui (Eds). Neuroprotective Effects of Phytochemicals in Neurological Disorders, John Wiley & Sons Inc., pp. 79-112. doi.org/10.1002/9781119155195.ch4

Kaurinovic, B., Popovic, M. & Vlaisavljevic, S. (2010). *In vitro* and in vivo effects of *Laurus nobilis* L. leaf extracts. Molecules, 15, 3378-3390. doi.org/10.3390/molecules15053378

Kinney, J. W., Bemiller, S. M., Murtishaw, A. S., Leisgang, A. M., Salazar, A. M. & Lamb, B. T. (2018). Inflammation is a central mechanism in Alzheimer's disease. Alzheimer's & Dementia: Translational Research & Clinical Interventions, 4, 575-590. doi.org/10.1016/j.trci.2018.06.014

Kivrak, S., Göktürk, T. & Kivrak, I. (2017). Assessment of volatile oil composition, phenolics, and antioxidant activity of Bay (*Laurus nobilis*) leaf and usage in cosmetic Applications. International Journal of Science and Metabolite, 4, 148-161. doi.org/10.21448/ijsm.323800

Majdi, A., Sadigh-Eteghad, S., Aghsan, S. R., Farajdokht, F., Vatandoust, S. M., Namvaran, A. & Mahmoudi, J. (2020). Amyloid-β, tau and the cholinergic system in Alzheimer's disease: Seeking direction in a tangle of clues. Rev. Neurosci. 2020; aop. doi.org/10.1515/revneuro-2019-0089

Meda, A., Lamien, C. E. & Romito, M. (2005). Determination of the total phenolic, flavonoid, and proline contents in Burkina Faso honey, as well as their radical scavenging activity. Food Chem, 9, 571-577. doi.org/10.1016/j.foodchem.2004.10.006

Mollica, A., Stefanucci, A., Zengin, G., Locatelli, M., Macedonio, G., Orlando, G., Ferrante, C., Menghini, L. (2018). Polyphenolic composition, enzyme inhibitory effects ex-vivo and in-vivo studies on two Brassicaceaeof north-central Italy. Biomed Pharmacother, 107, 129-138. doi.org/10.1016/j.biopha.2018.07.169

O'Boyle, N. M., Banck, M., James, C. A., Morley, C. & Hutchison, G. R. (2011). Open babel: An open chemical toolbox. J Cheminformatics, 3, 33. doi.org/10.1186/1758-2946-3-33

Oboh, G., Ademiluyi, A. O., Ogunsuyi, O. B., Oyeleye, S. I., Dada, A. F. & Boligon, A. A. (2017). Cabbage and cucumber extracts exhibited anticholinesterase and antimonoamine oxidase and antioxidant properties. J Food Biochem, 00, e12358. doi.org/10.1111/jfbc.12358

Oboh, G., Agunloye, O. M., Akinyemi, A. J., Ademiluyi, A. O. & Adefegha, S. A. (2012). Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats 'brain-*in vitro*. Neurochem Res, 38, 413-419. doi.org/10.1007/s11064-012-0935-6

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Analytical Biochemistry, 95 (11), 351-358. doi.org/10.1016/0003-2697(79)90738-3

Orhan, I., Aslan S., Kartal, M., Sener, B., & Husnu Can Baser, K. (2008). Inhibitory effect of Turkish *Rosmarinus officinalis* L. on acetylcholinesterase and butyrylcholinesterase enzymes. Food Chem, 108, 663-668. doi.org/10.1016/j.foodchem.2007.11.023

Oudjedi, K., Manso, S., Nerin, C., Hassissen, N. & Zaidi, F. (2018). New active antioxidant multilayer food packaging films containing Algerian Sage and Bay leaves extracts and their application for oxidative stability of fried potatoes. Food Control. doi.org/10.1016/ j.foodcont.2018.11.018

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. JPNJ Nutr, 44, 307–315. doi.org/10.5264/eiyogakuzashi.44.307

Ozcan, B., Esen, M., Sangun, M. K., Coleri, A., & Caliskan, M. (2010). Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. Journal of Environmental Biology, 31(5), 637-641. http://jeb.co.in/journal_issues/201009_sep10/paper_14.pdf

Proestos, C. & Komaitis, M. (2013). Analysis of naturally occurring phenolic compounds in aromatic plants by RP-HPLC coupled to diode array detector (DAD) and GC-MS after Silylation. Foods, 2, 90-99. doi.org/10.3390/foods2010090

Puntel, R. L., Nogueira, C. W. & Rocha, J. B. T. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. Neurochem Res, 30, 225–235. doi.org/10.1007/s11064-004-2445-7

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free RadicBiol Med, 26, 1231-1237. doi.org/10.1016/S0891-5849(98)00315-3

Rukhkyan, M., Tiratsuyan, S., Zilfyan, A., & Vardapetyan, H. (2013). Wound healing activity of L. nobilis leaves extracts. Issues in Theoretical and Clinical Medicine, 16, 20-24.

Sachan, A. K., Kumar, S., Kumari, K., & Singh, D. (2018). Medicinal uses of spices used in our traditional culture: Worldwide. Journal of Medicinal Plants Studies, 6(3), 116-122.

Sharma, Y., Fagan, J. & Schaefer, J. (2020). *In vitro* screening for acetylcholinesterase inhibition and antioxidant potential in different extracts of Sage (*Salvia officinalis* L.) and Rosemary (*Rosmarinus officinalis* L.). Journal of Biologically Active Products from Nature, 10 (1), 59-69. doi.org/10.1080/22311866.2020.1729239

Singleton, V. L., Orthofer, R. & Lamuela, R. R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants utilizing Folin-Ciocalteu reagent: Methods in enzymology. In Oxidants and antioxidants, Part A, Vol. 299. San Diego, CA: Academic Press. doi.org/10.1016/S0076-6879(99)99017-1

Trott, O. & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J ComputChem, 31, 455-461. doi.org/10.1002/jcc.21334

Tural, S. & Turhan, S. (2017). Antimicrobial and antioxidant properties of Thyme (*Thymus vulgaris* L.), Rosemary (*Rosmarinus officinalis* L.), and Laurel (*Lauris nobilis* L.) essential oils and their mixtures. GIDA The Journal of Food, 42 (5), 588-596. doi.org/10.15237/gida.GD17030

Wandhammer, M., de Koning, M., van Grol, M., Loiodice, M., Saurel, L., Noort, D., & Nachon, F. (2013). A step toward the reactivation of aged cholinesterases-crystal structure of ligands binding to aged human butyrylcholinesterase. Chemico-biological interactions, 203(1), 19-23. doi.org/10.1016/j.cbi.2012.08.005