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

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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 potentials 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 brain with L. nobilis extract exhibiting significantly (p<0.05) higher inhibitory activity (IC50: 67.83±13.53 µg/mL) than R. officinalis (IC50: 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 IC50 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, 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. 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, a number of 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). In view of the adverse effects that characterize the usage of the current AD drugs, it is of necessity to explore natural resources for new cholinesterase inhibitors with minimal side effects.

Culinary spices and herbs have shown promising medicinal properties as majority 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 flavour soups and different dishes, particularly, it is used in Nigeria to flavour 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; Rukhkhyan et al., 2013; Algabri et al., 2018). However, there is limited data on its neuroprotective potentials.

Similarly, rosemary (R. officinalis) is a potent herb of Mediterranean origin and serves as a source of flavouring 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 antioxidant 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. 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 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 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 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 μM acetylthiocholine 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 sec at 15 sec intervals using an ultraviolet-visible spectrophotometer (Jenway 6305 model, Staffordshire, UK). The cholinergic enzymes activities were therefore expressed as percentage inhibition.

Inhibition of Lipid Peroxidation

The inhibitory effects of the extracts on iron-induced lipid peroxidation in rat brain were assessed using the method of Okawa et al. (1979). The volume of the reaction mixture, which comprised 100μL of rat brain homogenate, 30 μL of 0.1M Tris-HCl buffer (pH 7.4), extracts (25-100μL) and 30μL of 250μM freshly prepared FeSO4 was made up to 270μL with distilled water. Thereafter, it was incubated at 37°C for 1 h. Subsequently, the colour developed by adding 300μL 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 K2S2O8 (2.45 mM, final concentration) in the absence of light for 16 h. Afterward, the A734nm 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 A734 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 Fe3+ to Fe2+ 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% Na2CO3 was added to neutralize the reaction. After the mixture had been incubated (45°C, 40 min), the A765nm was read and the TPC was expressed as ‘milligram GAE (Galllic 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% aluminium 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 A415nm 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

Prior to 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 (Chipitti 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 quadrupole temperature 150°C). One micro litre of the silylated samples was injected with split mode (10:1) using N2 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 National Institute of Standard and Technology MS (Gaithersburg, U.S.A) library.

Preparation of Protein and Ligand for in 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 http://www.pubchem.ncbi.nlm.nih.gov. Open babel program (O’Boyle et al., 2011), was used to convert the compounds into the mol2 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, 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). IC50 values: Concentration of the extract that causes 50% inhibition) and EC50 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 IC50 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 IC50 values (Table 1) revealed no significant difference (p>0.05) in the BChE inhibitory activities of both extracts (*L. nobilis* IC50: 4.76±0.36 µg/mL; *R. officinalis* IC50: 5.60±0.40 µg/mL).

The effects of the extracts on Fe2+ induced lipid peroxidation in rat brain as presented in Fig. 3 indicated that incubation of rat’s brain homogenate with FeSO4 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 Fe2+ 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 IC50 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 EC50 of FRAP (Table 1) indicated that *L. nobilis* had a better FRAP as it had a significantly (p<0.05) lower EC50 (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 Fe2+ in a dose-dependent manner. Based on the IC50 values of the extracts depicted in Table 1, there was no significant difference (p>0.05) in the Fe2+-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). 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-saliclyc acid, homovanillic acid, γ-tocopherol, biphenyl-4-carboxylic acid, chlorogenic acid, ethyl-trans-cinnamate, cinnamaldehyde, sinapinic acid, transcinnamyl acetate, elenemic, 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,
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: EC50 of FRAP and IC50 values of cholinesterase inhibition, lipid peroxidation inhibition and Fe2+-chelation abilities of aqueous extracts of L. nobilis and R. officinalis

<table>
<thead>
<tr>
<th>Samples</th>
<th>AChE (µg/mL)</th>
<th>BChE (µg/mL)</th>
<th>Lipid peroxidation inhibition (µg/mL)</th>
<th>FRAP (µg/mL)</th>
<th>Fe2+-chelation (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. nobilis</td>
<td>4.21±0.50a</td>
<td>4.76±0.36a</td>
<td>67.83±13.53b</td>
<td>149.05±8.35a</td>
<td>10.93±0.42a</td>
</tr>
<tr>
<td>R. officinalis</td>
<td>5.55±0.84a</td>
<td>5.60±0.49a</td>
<td>96.96±15.63b</td>
<td>167.00±9.60a</td>
<td>10.12±0.40a</td>
</tr>
</tbody>
</table>

Values represent mean ± SD. Mean values with the same superscript letter along the same column are not significantly different (p>0.05). IC50 values: Extract concentration that caused 50% inhibition. EC50 of FRAP indicates extract concentration that exhibited 50% of ferric reducing property

Table 2: Total phenol and total flavonoid contents

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenol (mg GAE/g)</th>
<th>Total flavonoid (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. nobilis</td>
<td>0.90±0.10a</td>
<td>0.48±0.03a</td>
</tr>
<tr>
<td>R. officinalis</td>
<td>2.24±0.15b</td>
<td>0.85±0.09a</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound detected</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak Area (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L. nobilis</td>
</tr>
<tr>
<td>1.</td>
<td>p-Salicylic acid</td>
<td>C7H6O3</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>*Methyl eugenol</td>
<td>C11H10O2</td>
<td>178</td>
<td>12.90</td>
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<td>3.</td>
<td>*Vanillic acid</td>
<td>C8H8O4</td>
<td>168</td>
<td>9.64</td>
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<tr>
<td>4.</td>
<td>*p-Coumaric acid</td>
<td>C9H6O3</td>
<td>164</td>
<td>6.46</td>
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<td>5.</td>
<td>*Ferulic acid</td>
<td>C10H8O4</td>
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<td>6.</td>
<td>Homovanillic acid</td>
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<td>γ-Tocopherol</td>
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<td>*Coumarin</td>
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<td>Biphenyl-4-carboxylic acid</td>
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<td>C10H9O2</td>
<td>164</td>
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<td>C7H6O4</td>
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<tr>
<td>23.</td>
<td>Elemicin</td>
<td>C13H16O3</td>
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<tr>
<td>24.</td>
<td>*Quercetin</td>
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<td>1.43</td>
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<td>26.</td>
<td>*Protocatechuic acid</td>
<td>C10H8O4</td>
<td>154</td>
<td>1.20</td>
</tr>
<tr>
<td>27.</td>
<td>Eugenol</td>
<td>C10H12O2</td>
<td>164</td>
<td>1.09</td>
</tr>
<tr>
<td>28.</td>
<td>*Estragole</td>
<td>C10H7O3</td>
<td>148</td>
<td>1.07</td>
</tr>
<tr>
<td>29.</td>
<td>*1,2-Benzenediol</td>
<td>C9H8O2</td>
<td>110</td>
<td>0.84</td>
</tr>
<tr>
<td>30.</td>
<td>Carnosic acid</td>
<td>C29H29O4</td>
<td>332</td>
<td>0.60</td>
</tr>
<tr>
<td>31.</td>
<td>Rosmanol</td>
<td>C28H28O5</td>
<td>346</td>
<td>13.81</td>
</tr>
<tr>
<td>32.</td>
<td>Hispidulin 7-glucoside</td>
<td>C29H39O11</td>
<td>462</td>
<td>8.16</td>
</tr>
<tr>
<td>33.</td>
<td>Gamma-eudesmol</td>
<td>C10H10O3</td>
<td>222</td>
<td>5.64</td>
</tr>
<tr>
<td>34.</td>
<td>Rutin</td>
<td>C27H26O19</td>
<td>664</td>
<td>5.06</td>
</tr>
<tr>
<td>35.</td>
<td>Hesperetin 7-O-rutinoside</td>
<td>C25H24O17</td>
<td>609</td>
<td>2.58</td>
</tr>
<tr>
<td>36.</td>
<td>Luteolin</td>
<td>C13H10O6</td>
<td>286</td>
<td>2.13</td>
</tr>
<tr>
<td>37.</td>
<td>Isohamnetin</td>
<td>C16H20O7</td>
<td>316</td>
<td>1.59</td>
</tr>
<tr>
<td>38.</td>
<td>Apigenin</td>
<td>C14H10O5</td>
<td>270</td>
<td>1.23</td>
</tr>
<tr>
<td>39.</td>
<td>Chetochrysin</td>
<td>C16H12O4</td>
<td>268</td>
<td>0.92</td>
</tr>
</tbody>
</table>

ND: Not Detected; *Detected in both samples
Table 4: Binding affinities of most potent cholinesterase inhibitors detected in *L. nobilis* and *R. officinalis*

<table>
<thead>
<tr>
<th>S/N</th>
<th>Detected compounds</th>
<th>Binding affinity (kcal/mol)</th>
<th>AChE</th>
<th>BChE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Galantamine</em></td>
<td>-10.8</td>
<td>-10.1</td>
<td>-10.1</td>
</tr>
<tr>
<td>2.</td>
<td><em>Rutin</em></td>
<td>-11.7</td>
<td>-12.0</td>
<td>-12.0</td>
</tr>
<tr>
<td>3.</td>
<td><em>Chlorogenic acid</em></td>
<td>-12.4</td>
<td>-10.5</td>
<td>-10.5</td>
</tr>
<tr>
<td>4.</td>
<td><em>Rosmarinic acid</em></td>
<td>-11.8</td>
<td>-10.9</td>
<td>-10.9</td>
</tr>
<tr>
<td>5.</td>
<td><em>Hesperetin 7-O-rutinoside</em></td>
<td>-11.8</td>
<td>-10.6</td>
<td>-10.6</td>
</tr>
<tr>
<td>7.</td>
<td><em>Luteolin</em></td>
<td>-11.2</td>
<td>-10.2</td>
<td>-10.2</td>
</tr>
<tr>
<td>8.</td>
<td><em>Carnosic acid</em></td>
<td>-10.1</td>
<td>-10.6</td>
<td>-10.6</td>
</tr>
<tr>
<td>9a.</td>
<td><em>Quercetin</em></td>
<td>-10.4</td>
<td>-10.4</td>
<td>-10.4</td>
</tr>
</tbody>
</table>

*: 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 with 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 rosmarinol, 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 include 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; Mollicia 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. Enzyme inhibition strategy has been employed for 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 enzymes 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 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 IC₅₀ value of 229.14±3.86 mg/mL for AChE inhibition while we reported 5.55±0.84 µg/mL as the IC₅₀ 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...
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 be helpful in filling the research gap in literature (biochemical basis for neuroprotection) as it identified inhibition of cholinergic enzymes activities and lipid peroxidation, as well as 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.

Conclusion

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Author Contributions

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

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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 Okaigbeto: Writing-review and editing.

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

Ohuwafemi 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


