African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 16 No.1; pp. 69-80 (2024)

Original Research Article

GC-MS ANALYSIS, CHOLINESTERASE INHIBITORY AND ANTIOXIDANT POTENTIALS OF *ZAPOTECA PORTORICENSIS* (JACQ.) H.M. HERN (FABACEAE) ROOT EXTRACTS

JOEL OJOGBANE ONOJA[1,2,3,*], JULIUS IDOWU OLAWUNI[4], MALACHUKWU CHIBUNDU UMEOKOLI[1]

1. Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria.
2. Institute of Drug-Herbal Medicine-Excipient Research and Development, University of Nigeria, Nsukka, Nigeria.
3. Center for Drug Discovery, University of Buea, Buea, Cameroon
4. Department of Biochemistry and Molecular Biology, Faculty of Sciences, Obafemi Awolowo University Ile-Ife, Nigeria.

ABSTRACT

The age-related neurodegenerative illness known as Alzheimer's disease (AD) is typified by the unavoidable decline in cognitive function. Existing drugs has limitations, therefore there is an urgent need for new neurotherapeutic agents. There has been a lot of excitement in recent years about finding therapeutic herbs that can restore cognitive impairment due to their lower side effects. The aim of the study is to evaluate the cholinesterase inhibitory and antioxidant potentials of *Zapoteca portoricensis* (Jacq.) H.M. Hern root extracts and also identify the bioactive molecules present. Ellman colorimetric assay was used to evaluate the cholinesterase inhibitory potentials of various extracts at 1 mg/mL. The antioxidant potentials of extracts were evaluated using *in-vitro* chemical analysis. GC-MS was used to identify bioactive molecules from most active extract. One-way ANOVA at $\alpha_{0.05}$ was adopted. Ethyl acetate extract showed the highest acetylcholinesterase (IC_{50} = 0.149±0.00 mg/mL) and butyrylcholinesterase (IC_{50} = 0.393±0.02 mg/mL) inhibitory potential as compared to eserine for AChE (IC_{50} = 0.051±0.01 mg/mL) and for BuChE (IC_{50} = 0.049±0.00 mg/mL). Ethyl acetate extract also demonstrated its potential to chelate metals (IC_{50} = 0.106±0.02 mg/mL) as compared to EDTA (IC_{50} = 0.086±0.00 mg/mL). The DPPH radical scavenging potential was higher in ethyl acetate extract (IC_{50} = 0.082±0.05 mg/mL) when compared to vitamin C (IC_{50} = 0.008±0.00 mg/mL). The total antioxidant capacity of extracts revealed that ethyl acetate extract also has the highest concentration (125.5±14.3 mg/g AAE/g of extract (R^2=0.9276)). The GC-MS analysis of ethyl acetate extract revealed the presence of 62 compounds which includes n-Hexadecanoic acid (RT 14.81, Area % 10.03), 9,12-Octadecadienoic acid (Z,Z)- (RT 15.97, Area % 10.03), cis-9-Hexadecenal (RT 16.01, Area % 8.36). The ethyl acetate extract from the root of *Zapoteca portoricensis* has the aptitude to prolong the half-life of acetylcholine by its ability to inhibit cholinesterase enzyme.

ARTICLE INFO

Received 07 February, 2024
Accepted 21 March, 2024
Published 26 March, 2024

KEYWORDS

Fabaceae,
Zapoteca portoricensis,
Cholinesterase inhibition,
Antioxidant,
GC-MS

INTRODUCTION

Alzheimer's disease (AD) is a progressive brain ailment that leads to a slow and irreversible loss of memory, linguistic ability, spatial and temporal perception, and ultimately the capacity to take care of oneself. In those 65 years of age and beyond, late-onset Alzheimer's disease is now known to be the most common cause of mental function loss [1-2]. The

***Corresponding author:** joel.onoja@unn.edu.ng; +234-806 2872 407

https://doi.org/10.59493/ajopred/2024.1.8 ISSN: 0794-800X (print); 1596-2431 (online)

most common type of dementia is AD. Someone in the world develops dementia every 3 seconds. In 2020, there are over 55 million people in the world living with dementia. This number is expected to double every 20 years to 78 million in 2030 and 139 million in 2050 [3]. Alzheimer's sufferers' brains exhibit characteristic structures known as tangles and plaques, which are improperly structured proteins that are thought to be the disease's signature [4]. These distinctive formations are not present in every area of the brain. Memory-related areas are the most prominently affected.

The primary causes of deteriorating cognitive performance in AD patients are the degeneration of cholinergic neurons in the brain and loss of neurotransmission. The cholinergic hypothesis states that the primary cause of AD is a decrease in acetylcholine (ACh) synthesis, which is triggered by the cholinesterase enzyme. Consequently, cholinesterase (ChE) is an important therapeutic target for the treatment of AD [5-6]. One possible treatment strategy is to increase the cholinergic level of the brain by inhibiting the biological activity of cholinesterase using cholinesterase inhibitors which are able to increase the function of neural cells by increasing the concentration of acetylcholine [7]. A variety of ChE inhibitors have been produced which includes donepezil, galantamine, rivastigmine and memantine used to treat AD and currently accessible on the market [8-9]. Nevertheless, these medications have a limited window of effectiveness, and they have a variety of dose-related adverse effects, especially at larger dosages [10].

As of late, lecanemab and aducanumab (anti-amyloid monoclonal antibodies (MABs)) have been endorsed within the USA by FDA for AD treatment especially in patients with mild cognitive impairment or mild dementia stage of the progressive neurodegenerative ailment [11-13]. The first disease-modifying treatments for Alzheimer's disease are the anti-amyloid monoclonal antibodies; they reduce the disease clinical decline by interfering with its fundamental molecular mechanisms. Aducanumab is categorized as a monoclonal antibody of the human immunoglobulin gamma 1 (IgG1) class of immunotherapeutics. Via the blood-brain barrier, it specifically targets and binds aggregated soluble oligomers and insoluble fibril conformations of Aβ plaques in the brain to carry out its mechanism of action. [14-16]. On the other hand, monoclonal antibodies have adverse effects. Patients should be closely watched for the development of amyloid-related imaging abnormalities (ARIA) such as ARIA-edema (ARIA-E), ARIA-hemosiderin deposition (ARIA-H) microhemorrhage, ARIA-H superficial siderosis, and infusion reactions during the initial phase of treatment. [17-18]. Therefore, there is urgent need to continue to explore all therapeutic targets for Alzheimer's disease with the aim of discovering and development of new neurotherapeutic agents with high efficacy and little or no limitations.

It has been found that cholinesterase inhibitory activity is present in many therapeutic herbs. Substances separated from *Acorus tatarinowii* Schott (Acoraceae) rhizome were found to inhibit acetylcholinesterase [19]. Methanolic extract made from *Adhatoda vasica* (Acanthaceae) plant parts was discovered to be AChE inhibitor [20]. *Sesuvium portulacastrum* (Aizoaceae), methanolic leaf extract which is known to have a high concentration of alkaloids as shown by phytochemical analysis, was found to have dual cholinergic inhibitory activity [21]. A flavonoid included in *Silybum marianum* (Asteraceae) called silibinin has the ability to prevent the aggregation of AChE and Aβ peptides [22]. *Zapoteca portoricensis* (Jacq.) H.M. Hern (Fabaceae), commonly called white stickpea, is a perennial shrub with slender unarmed branches and with small oral green leaves. In Eastern Nigeria, its various plant components are utilized to treat conditions like skin infections, protracted labor, convulsions, and constipation. Several pharmacological activities of *Zapoteca portricensis* has been reported such as anti-ulcer activity [23], anti-inflammatory effect [24], anti-microbial effects [25], treatment of testosterone induced benign prostate hyperplasia [26], anti-trypanosoma potentials [27], hepatoprotective and antioxidant effects [28], and treatment and management of sexually transmitted infection [29].

Alzheimer disease prevention or treatment with natural compounds may be an approach that is capable of targeting a number of different molecular events implicated in its pathogenesis. The aim of the study is to evaluate the cholinesterase inhibitory activities and antioxidant potentials of *Zapoteca portoricensis* (Jacq.) H.M.Hern root extracts and also identify the bioactive molecules present using Gas Chromatography-Mass Spectrometry.

MATERIALS AND METHODS

Materials and Equipment

Reagents/Solvents

Methanol, n-hexane, ethyl acetate, ferric chloride ($FeCl_3$), sulphuric acid (H_2SO_4), acetylthiocholine iodide (ATChI), butyrylthiocholine chloride (BuChCl), acetylcholinesterase, butyrylcholinesterase, Tris-HCl buffer, eserine, ascorbic acid (AA), sodium phosphate ($NaH_2PO_4.2H_2O$), ammonium molybdate, Vitamin C, DPPH, $FeCl_2$, ferrozine are of Analytical grade obtained from Sigma Aldrich and Loba Chemie, India.

Equipment

Microplate reader (EPOCH 2, BioTek), Weighing balance (M411L, M-Metlar, Nigeria), water bath (HH-2), rotary evaporator (BUCHI, R-300), GC-MS-QP2010SE SHIMADZU JAPAN machine.

Plant Collection

The plant roots were collected in the month of February, 2023 from Obollo Afor, Enugu State, Nigeria. Mr. Felix Nwafor, a taxonomist at the University of Nigeria, Nsukka, Department of Pharmacognosy and Environmental Medicine, recognized the plant. A voucher sample (PCG/UNN/0318) was deposited in the herbarium. The roots (1.1 Kg) were shade dried for two weeks and then grinded. The powdered root was weighed and

found to be 658.2g and then stored in a clean air tight container prior to use.

Plant Extraction

Here, the extraction method is a sequential extraction method based on the eluent's rising polarity. For the extractions, n-hexane, ethyl acetate, and methanol were the three solvents utilized. The powdered plant material (658.2 g) was placed inside a maceration jar and macerated with 3 L of n-hexane and then filtered after 72 hours to get filtrate. After the marc had air dried, 3 L of ethyl acetate was added, and it was left to stand for an additional 72 hours while being constantly stirred every 8 hours. After filtering and drying the marc, two liters of methanol were added, let it rest for seventy-two hours, and then it was filtered again. The filtrates were subsequently concentrated at 40°C using a rotary evaporator set to low pressure.

Cholinesterase Inhibitory Assay

Spectrophotometric analysis was used to assess the inhibitions of acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) [30]. 240 µL of buffer (50 mM Tris-HCl, pH 8.0) and 20 µL of test sample concentrations (1, 0.5, 0.25, 0.125, 0.06255, 0.03125 mg/mL) were put to 96-well plates, along with 20 µL of the enzyme preparation (0.28 U/mL). Following a 30-minute incubation period at 37°C, 20 µL of 10 mM DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) was added to the reaction mixture. Then, 20 µL of 25 mM ATChI/BChCl was added to start the reaction. Afterwards, the absorbance change per minute (∆A/min) was measured spectrophotometrically at 412 nm to ascertain the rate of hydrolysis of ATChI/BChCl. As a negative control, a buffer solution was employed. Every assay was run in triplicate. The positive control utilized was eserine {(-) physostigmine}. Percentage inhibition was calculated using Equation 1.

$$I\% = [(Vo - Vi)/Vo] \times 100 \ldots\ldots\ldots\ldots Equation\ 1$$

where:
I (%) = Percentage inhibition
V_i = absorbance of test sample
V_o = absorbance of control sample

Metal Chelation Assay

Metal chelation potential of extracts was performed in accordance with Singh and Rajini's [31] methodology. Twenty times as much ferrozine (5 mM) as 2 mM FeCl2•4H2O was diluted in solutions. Briefly, an aliquot one milliliter (1 mL) of various extract concentrations was combined with one milliliter (1 mL) of $FeCl_2$•$4H_2O$. Ferrozine (1 mL) was added to start the reaction after it had been incubated for 5 minutes. After giving the combination a good shake and letting it sit for a further 10 minutes, the absorbance of the mixture was measured using a spectrophotometer set to 562 nm. Using the formula below (Equation 2), the percentage inhibition of the production of the ferrozine–Fe^{+2} combination was determined:

$$CE\ \% = [(A_c - A_s)/A_c] \times 100 \ldots\ldots. Equation\ 2$$

where
CE is the chelating effect
A_c is the absorbance of the control sample (which contains ferrozine and $FeCl_2$, complex formation molecules) and
A_s is the absorbance of the tested sample

Radical Scavenging Assay using DPPH Assay

According to Brand-Williams [32], the extracts' capacity to scavenge radicals was assessed using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate). DPPH is reduced when it reacts with an antioxidant that has the ability to donate hydrogen. Using spectrophotometry, the color shift from deep violet to light yellow was measured at 517 nm. One milliliter of 0.3 millimeter DPPH in methanol was added to one milliliter of various concentrations (1, 0.5, 0.25, 0.125, 0.06255, and 0.03125 mg/mL) of the extracts or standard (Vitamin C) in a test tube. After mixing the mixture and letting it sit in the dark for thirty minutes, the absorbance at 517 nm was measured and compared to a DPPH control that had only one milliliter of methanol instead of the extract. The percent of inhibition (*I*%) was calculated using Equation 3.

$$I\% = [(A_b - A_s)/A_b] \times 100 \ldots\ldots\ldots Equation\ 3$$

where:
A_s is the absorbance of the test compound and A_b is the absorbance of the control reaction, which contains all of the reagents except for the test compound.
The graph that plotted the percentage of inhibition against extract concentration was used to compute the sample concentration that provided 50% inhibition (IC_{50}).

Total Antioxidant Capacity

According to the method described by Prieto [33], one milliliter of the reagent solution, which contained 0.6 M sulfuric acid, 28 mM sodium phosphate ($NaH_2PO4.2H_2O$), and 4 mM ammonium molybdate, was added to 0.1 milliliter of the extracts or standard solutions of ascorbic acid (0.02, 0.04, 0.06, 0.08, 0.1 mg/mL). For ninety minutes, the tubes holding the reaction mixture were incubated at 95 degrees Celsius in a water bath. After allowing the combination to stand and settle to room temperature, the absorbance at 695 nm was measured in comparison to a blank which consisted of the reacting mixture containing distilled water in place of the extract. The antioxidant activities are expressed as mg of AAE/g of the extracts using Equation 4:

$$y = mx + c \ldots\ldots. Equation\ 4$$

where y is the absorbance of sample, m and c values from the straight line curve of AA.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GC-MS procedure was carried out using GCMS-QP2010SE Shimadzu, Japan machine. The chemicals included in the *Zapoteca portoricensis* ethyl acetate extract were analyzed using a Gas Chromatography Mass Spectrometer that was set to operate at a temperature range of 60.0°C to 300.00°C, with a hold duration of 1.5-4.6 minutes and a rate of 14 C/min. The chromatographic settings were as follows: split injection mode, helium 99.999% carrier gas, with a column flow rate of 3.22 mL/min. 60.0 °C for the column oven, 250.00 °C for the injection, a linear velocity flow control mode, 144.4 kPa of pressure, 22.6 mL/min for the total flow, 32.2 mL/min for the column, 46.3 cm/sec for the linear velocity, 3.0 mL/min for the purge, and 5.1 split ratio. Through the use of National Institute Standard and Technology (NIST) library software and GC-MS spectra, the compounds were identified along with their respective retention indices.

Statistical Analysis

Graphpad Prism 7 was used for the statistical analysis. The data was presented in triplicate parallel measurements and as mean ± S.D. One-way ANOVA was used for statistical analysis, and at α0.05, Dunnett's Multiple Comparisons test was used. At the 5% significance level ($P < 0.05$), differences in means were deemed noteworthy. Microsoft Excel was used to create standard curves and calculate the 50% inhibitory concentration (IC_{50}) values.

RESULTS AND DISCUSSION

Cholinesterase Inhibitory Activity of Extracts of *Zapoteca Portoricensis* Root

Colorimetric assay method was adopted to evaluate the cholinesterase inhibitory potential of extracts of *Zapoteca portoricensis* root. In principle, 5-thio-2-nitrobenzoate (TNB) is produced when acetylcholinesterase (AChE) hydrolyzes acethylcholine to thiocholine. This thiocholine then interacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Cholinesterase (AChE) activity is reflected in the absorbance of TNB, which is used to measure its concentration. The half-maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological/biochemical function. The lower the IC_{50} value, the higher the activity. Ethyl acetate extract had the highest acetylcholinesterase inhibitory activity, with an IC_{50} value of 0.149±0.00 mg/mL (74.60±2.25% inhibition) as compared to eserine inhibition on acetylcholinesterase (IC_{50}=0.050±0.01 mg/mL, 63.64±3.90%) at 0.1 mg/mL. Moreover, acetylcholinesterase was significantly inhibited by methanol extract at 61.05±1.64% (IC_{50} = 0.368±0.02 mg/mL), while n-hexane extract had the lowest average percentage of inhibition at 56.59±2.35% (IC_{50} = 0.444±0.06 mg/mL) (Figure 1). The extract derived from ethyl acetate exhibited the greatest inhibitory efficacy against butyrylcholinesterase (IC_{50} = 0.393±0.02 mg/mL, 60.80±1.54%) when compared to standard eserine (IC_{50} = 0.049±0.00 mg/mL, 61.33±2.37%) at 0.1 mg/mL, methanol extract also showed good butyrylcholinesterase inhibition (IC_{50} = 0.562±0.03 mg/mL, 59.25±2.86%) and n-hexane extract having a minimal butyrylcholinesterase inhibition (IC_{50} = 0.913±0.11 mg/mL, 50.79±3.69%) (Figure 2). Our findings suggest that, components found in ethyl acetate extract of *Z. portoricensis* could prevent catalytic hydrolyses of acetylcholine, thereby increasing its half-life in the brain. To further corroborate our findings, several medicinal plants in family Fabaceae have been reported for use in the treatment of neurological disorders. Physostigmine a reversible acetylcholinesterase inhibitor approved by FDA originally was isolated from the seed of calabar bean *Physostigma venenosum L.* (Fabaceae) [34, 35].

Metal ion Chelating Activity

The metal chelating abilities of plant extracts of *Zapoteca portoricensis* root was evaluated. The ethyl acetate extract of *Z. portoricensis* root at 1 mg/mL concentration showed good metal chelating activity (IC_{50} = 0.106±0.02 mg/mL) as compared to the EDTA (IC_{50} = 0.086±0.0005 mg/mL).The ethyl acetate extract of *Z. portoricensis* extract also had smaller IC_{50} value demonstrated higher metal chelation activity when compared to the n-hexane extract (IC_{50} =1.005±0.06 mg/mL) and methanol extract (IC_{50} = 0.230±0.1 mg/mL) (Figure 3). The result illustrated that ethyl acetate extract can effectively attenuate the ROS-mediated neuronal death in Alzheimer's disease due to bioactive compounds present in them. As a specialized organ, the brain depends on metal ions for several critical cellular functions. Because of this, the brain has a comparatively high concentration of transition metals, including iron, zinc, and copper, which are involved in synaptic neuronal activity and maintain the functionality of different metalloproteins. For this reason, cells have evolved highly developed machinery to regulate metal-ion homeostasis. But if these processes fail, or if metals that have no known biological purpose are absorbed, the ionic balance is upset, which can lead to a variety of diseases, including AD and other neurodegenerative conditions [36]. A number of research conducted in the past several years have demonstrated the crucial role that metal ions play in the pathophysiology of major brain illnesses including Alzheimer's. Potential treatments for illnesses involving metal ion imbalance have been proposed, including metal ion chelators. A great area to apply the metal chelator strategy to therapy is neurodegeneration. Restoring ionic equilibrium by more effective and nuanced manipulation of metal ion homeostasis appears to be the aim in neurodegeneration. Therefore, we require mild chelators that can coordinate harmful metals without upsetting metal homeostasis. Numerous natural compounds with diverse origins have been assessed preclinically and clinically for their neuroprotective properties in averting and reducing the multifactorial pathologies associated with Alzheimer's disease. Naturally occurring polyphenols, such as curcumin, epigallocatechin-3-

gallate (EGCG), quercetin, myricetin, and others, are considered candidate therapeutics for AD due to their metal chelating potentials [37-38].

DPPH Radical Scavenging Activity

According to the current findings, every extract that was tested exhibited moderate to strong antioxidant activity. Ethyl acetate

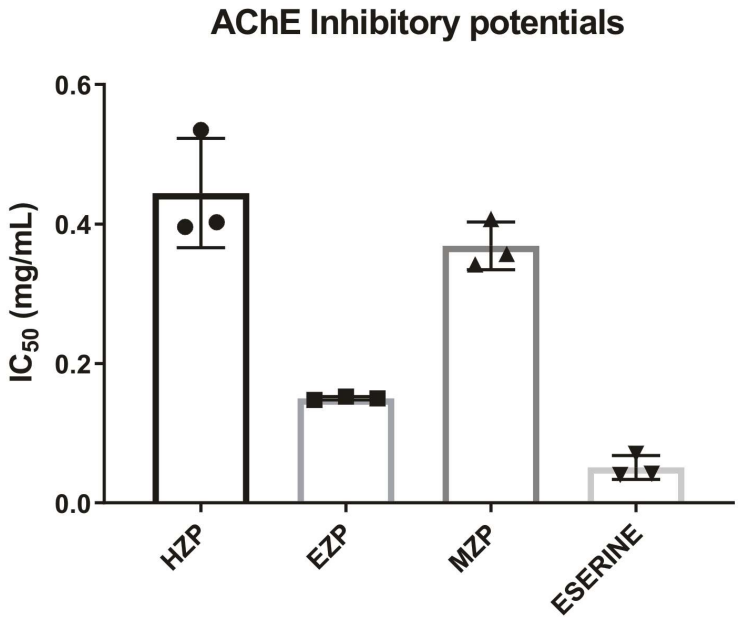

Figure 1: AChE inhibitory potentials of extract(s) of *Zapoteca portoricensis* root

Where HZP (hexane extract), EZP (ethyl acetate extract), MZP (methanol extract)

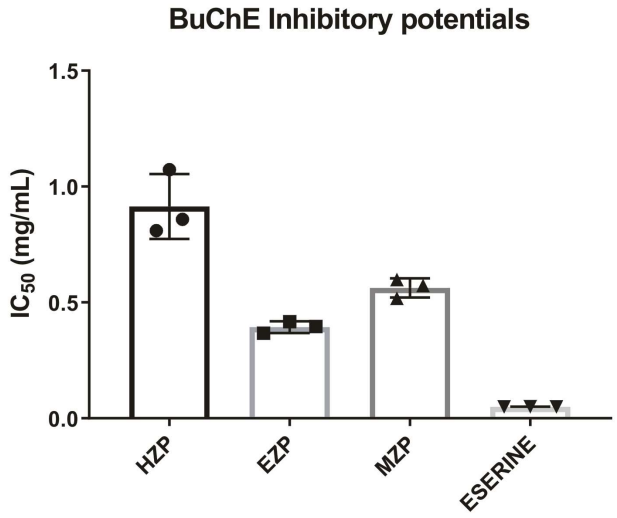

Figure 2: BuChE inhibitory potentials of extract(s) of *Zapoteca portoricensis* root

HZP (hexane extract), EZP (ethyl acetate extract), MZP (methanol extract)

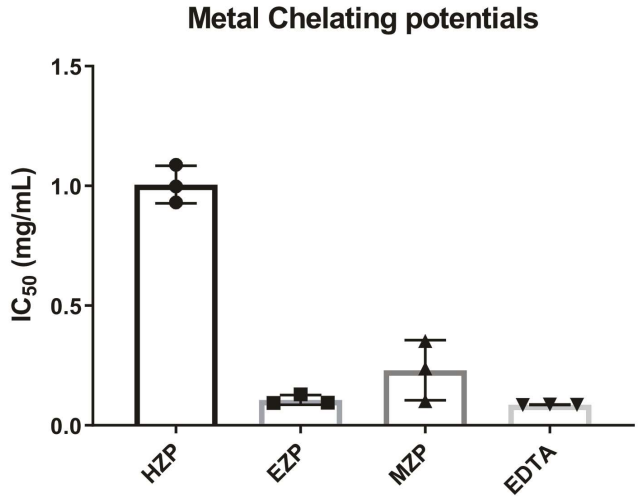

Figure 3: Metal chelating potentials of *Zapoteca portoricensis* extract(s)

Where HZP (hexane extract), EZP (ethyl acetate extract), MZP (methanol extract)

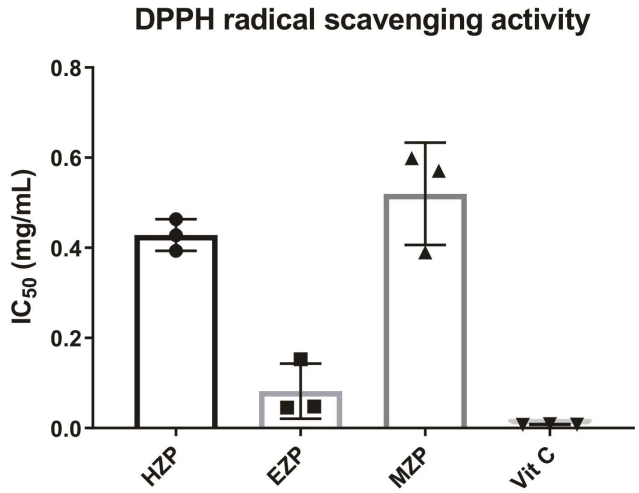

Figure 4: DPPH radical scavenging activity of *Zapoteca portoricensis* root extract(s)

Where HZP (hexane extract), EZP (ethyl acetate extract), MZP (methanol extract)

extract of *Zapoteca portoricensis,* at concentration of 1 mg/mL showed the highest percentage inhibition of 64.38±0.31% (IC_{50} = 0.082±0.05 mg/mL) when compared to vitamin C (IC_{50} of 0.008±0.00 mg/mL) at 0.01 mg/mL (Figure 4). Methanol extracts (IC_{50} = 0.519±0.1 mg/mL, 56.86±5.31% inhibition) and n-hexane extracts (IC_{50} = 0.428±0.02 mg/mL, 63.75±2.27% inhibition) also showed ability to scavenge the DPPH radical. Thus, one can say that ethyl acetate extract of *Z. portoricensis* has a very good DPPH scavenging activity due to the presence of anti-oxidant compounds such as flavonoids and tannins in them. A straightforward and accurate technique for assessing radical scavenging effect is to use DPPH, which is based on the stable free radical's capacity to decolorize in the presence of antioxidants [39]. Furthermore, it has been established that the primary cause of the antioxidant effect of plant products is the radical-scavenging activity of phenolic compounds, including tannins, flavonoids, polyphenols, and phenolic terpenes, which stabilize diphenyl-2-picrylhydrazyl (DPPH) free radicals by donating an electron or a hydrogen ion [40-41]. Free-radical scavenging antioxidant therapy may be used to prevent, treat, or mitigate a number of these conditions, including AD [42]. Also, Fabaceae are associated with a high antioxidant activity and a high total phenolic, total flavonoid contents [43-44].

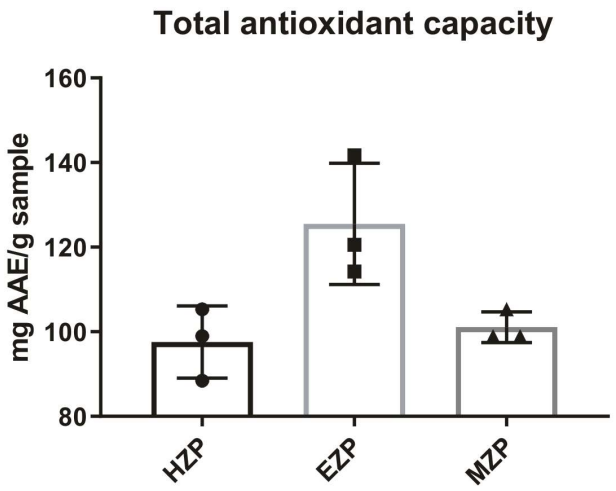

Figure 5: Total antioxidant capacity of *Zapoteca portoricensis* extract(s)

Where HZP (hexane extract), EZP (ethyl acetate extract), MZP (methanol extract)

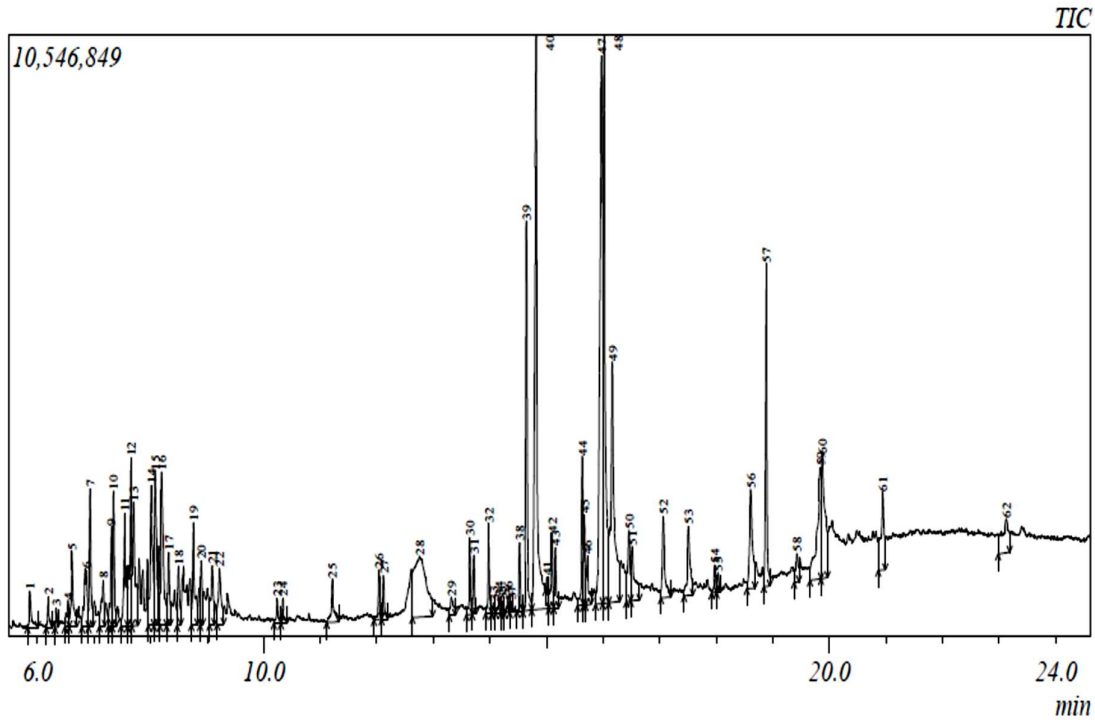

Figure 6: GC-MS chromatogram of ethyl acetate extract of *Zapoteca portoricensis* root

Table 1: Molecules identified from the ethyl acetate extract of *Zapoteca portoricensis* root using GC-MS

Peak#	RT	Area%	Molecular weight	Molecular formula	Compound name
1	5.869	0.54	120	C_9H_{12}	Benzene, 1,2,4-trimethyl-
2	6.193	0.36	120	C_9H_{12}	Benzene, 1,2,4-trimethyl-
3	6.333	0.20	118	C_9H_{10}	Benzene, 2-propenyl-
4	6.541	0.37	134	$C_9H_{10}O$	2-Indanol
5	6.616	1.09	134	$C_{10}H_{14}$	Benzene, 1-ethyl-2,3-dimethyl-
6	6.864	1.27	134	$C_{10}H_{14}$	Benzene, 1-ethyl-2,3-dimethyl-
7	6.933	1.33	134	$C_{10}H_{14}$	Benzene, 4-ethyl-1,2-dimethyl-
8	7.165	0.77	134	$C_{10}H_{14}$	Benzene, 1-ethyl-2,3-dimethyl-
9	7.308	0.95	134	$C_{10}H_{14}$	Benzene, 1,2,4,5-tetramethyl-
10	7.346	1.30	134	$C_{10}H_{14}$	Benzene, 1,2,3,4-tetramethyl-
11	7.542	1.63	132	$C_{10}H_{12}$	1H-Indene,2,3-dihydro-5methyl-
12	7.655	2.18	132	$C_{10}H_{12}$	1H-Indene,2,3-dihydro-4methyl-
13	7.701	2.03	164	$C_{11}H_{16}O$	6,7-Dimethyl-3,5,8,8a tetrahydro-1H-2-benzopyran
14	8.009	2.14	128	$C_{10}H_8$	Naphthalene
15	8.085	1.96	146	$C_{11}H_{14}$	1H-Indene,2,3-dihydro-1,3 dimethyl-
16	8.200	2.53	174	$C_{13}H_{18}$	Benzene,1-cyclopropylmethyl-4-(1-methylethyl)-
17	8.322	0.83	204	$C_{14}H_{20}O$	1-Hexene,2-(O-anisyl)-4-methyl-
18	8.497	0.75	162	$C_{12}H_{18}$	Benzene, 1,3,5-triethyl-
19	8.759	1.45	146	$C_{11}H_{14}$	Benzene,1-methyl-3-(1-methyl-2-propenyl)-
20	8.900	0.71	146	$C_{11}H_{14}$	1H-Indene,2,3-dihydro-4,7-dimethyl-
21	9.093	0.90	174	$C_{13}H_{18}$	Benzene,1-cyclopropylmethyl-4-(1-methylethyl
22	9.218	1.10	246	$C_{15}H_{18}O_3$	Butanoic acid, 3-[(1-phenylethyl-2-propynyl)oxy]
23	10.239	0.32	200	$C_{13}H_{28}O$	n-Tridecan-1-ol
24	10.339	0.28	268	$C_{19}H_{40}$	Nonadecane
25	11.214	0.76	206	$C_{14}H_{22}O$	Phenol,2,4-bis(1,1-dimethylethyl)-
26	12.036	0.49	242	$C_{16}H_{34}O$	1-Hexadecanol
27	12.119	0.43	296	$C_{21}H_{44}$	Heptadecane,2,6,10,15-tetramethyl-
28	12.759	5.08	194	$C_{17}H_{14}O_6$	3-O-Methyl-d-glucose
29	13.316	0.38	284	$C_{18}H_{36}O_2$	Octadecanoic acid
30	13.643	0.73	228	$C_{15}H_{32}O$	n-Pentadecanol
31	13.714	0.55	268	$C_{19}H_{40}$	Nonadecane
32	13.976	0.87	278	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
33	14.065	0.15	212	$C_{11}H_{16}O_4$	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
34	14.166	0.23	156	$C_9H_{16}O_2$	Cyclohexanone,2-ethyl-4-methoxy-
35	14.216	0.09	142	$C_9H_{18}O$	5-Methyl-5-octen-1-ol
36	14.315	0.14	376	$C_{23}H_{36}O_4$	Phthalic acid, butyl undecyl ester
37	14.379	0.08	496	$C_{27}H_{52}O_4Si_2$	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-
38	14.524	0.56	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
39	14.645	3.69	278	$C_{16}H_{22}O_4$	Dibutyl phthalate
40	14.814	10.03	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
41	15.006	0.09	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
42	15.095	0.87	284	$C_{19}H_{40}O$	n-Nonadecanol-1
43	15.157	0.67	306	$C_{16}H_{34}O_3S$	Sulfurous acid, 2-propyl tridecyl ester
44	15.632	1.43	284	$C_{19}H_{40}O$	n-Nonadecanol-1
45	15.669	0.80	334	$C_{22}H_{38}O_2$	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester
46	15.723	0.71	268	$C_{17}H_{32}O_2$	7-Hexadecenoic acid, methyl ester, (Z)-
47	15.974	10.03	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-

Table 1 contd.

48	16.017	8.36	238	$C_{16}H_{30}O$	cis-9-Hexadecenal
49	16.162	5.34	284	$C_{18}H_{36}O_2$	Octadecanoic acid
50	16.456	1.04	256	$C_{17}H_{36}O$	n-Heptadecanol-1
51	16.513	1.29	352	$C_{25}H_{52}$	2-methyltetracosane
52	17.060	1.35	284	$C_{19}H_{40}O$	n-Nonadecanol-1
53	17.507	1.28	281	$C_{18}H_{35}NO$	9-Octadecenamide, (Z)-
54	17.974	0.30	346	$C_{20}H_{39}ClO_2$	3-Chloropropionicacid, heptadecyl ester
55	18.033	0.21	328	$C_{20}H_{40}O_3$	2-Hydroxyhexadecyl butanoate
56	18.610	2.02	568	$C_{35}H_{68}O_5$	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl
57	18.882	3.25	390	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate
58	19.427	0.47	226	$C_{15}H_{30}O$	Z-11-Pentadecenol
59	19.836	2.93	310	$C_{20}H_{38}O_2$	Ethanol,2-(9,12-octadecadienyloxy)-, (Z,Z)-
60	19.873	3.03	212	$C_{13}H_{24}O_2$	Tridecanedial
61	20.942	1.77	244	$C_{14}H_{25}ClO$	13-Oxabicyclo[9.3.1]pentadecane, 15-chloro-
62	23.122	1.48	375	$C_{19}H_{31}C_{l2}NS$	2,6-Lutidine3,5-dichloro-4-dodecylthio-

Total Antioxidant Capacity

This is a quantitative way to measure the antioxidant capacity of extracts. The antioxidant chemical in the extracts was proven to reduce Mo (VI) to Mo (V), forming a green phosphate/Mo (V) complex at an acidic pH with a maximum absorption at 695 nm. In this research, the total antioxidant capacity was higher in ethyl acetate extract with value of 125.5±14.3 mg/g ascorbic acid equivalent (R^2=0.9276) amongst tested extracts when compared to methanol and hexane extract which had values of 101.07±3.7 and 97.6±8.5 mg/g ascorbic acid equivalent, respectively (Figure 5). The study revealed that ethyl acetate extract can effectively reduce the ROS-mediated neuronal death in Alzheimer's disease due to the presence of antioxidant chemicals in it. By fostering antioxidative defenses to neutralize reactive oxygen species, the antioxidant system plays a crucial role in protecting brain tissue's proper redox balance and saving neuronal cells from oxidative damage. The development of new or combination exogenous antioxidant supplements, maintaining the functional integrity of intrinsic antioxidant systems to prevent harmful disorders of the central nervous system, and finding novel therapeutic approaches to prevent and/or reduce brain injury are currently areas of growing interest in research [45]. Various studies have corroborated our findings which indicates that antioxidant compounds like luteolin, melatonin, resveratrol (3, 5, 40-trihydroxy-trans-stilbene) are found in a number of plants. It has been shown that resveratrol has antioxidant qualities and that it raises glutathione levels while lowering nitrite and malondialdehyde levels [46]. Research conducted on various cell lines harboring mutant AβPP695 revealed that resveratrol demonstrated antiamyloidogenic properties by lowering the amounts of released intracellular Aβ peptide [47], Anthocyanins (ANTs) are able to penetrate the blood-brain barrier and shield brain tissue from the deleterious effects of Abeta, mitochondrial malfunction, and OS-induced apoptosis [48].

GC-MS Analysis

GC-MS chromatogram of ethyl acetate extract of *Zapoteca portoricensis* root (Figure 6) showed the presence of sixty-two (62) bioactive compounds. The National Institute of Standards and Technology (NIST) database was used to interpret the mass spectra and identify the components based on their retention indices. The chemical constituents with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1. The following components with a high concentrations are n-Hexadecanoic acid (RT 14.81, Area % 10.03), 9,12-Octadecadienoic acid (Z,Z)-(RT 15.97, Area % 10.03), cis-9-Hexadecenal (RT 16.01, Area % 8.36), Octadecanoic acid (RT 16.16, Area % 5.34), Tridecanedial (RT 19.87, Area % 3.03). Some of the compounds (Table 1) present in the ethyl acetate extract of *Zapoteca portoricensis* has been reported in some medicinal plants such as *Cnidoscolus aconitifolius* and *Clerodendrum splendens* with anti-Alzheimer's potentials [49-50]. More so, n-Hexadecanoic acid have been reported to be an antioxidant compound [51], The octadecanoic acid (stearic acid) have also been reported with significant antioxidant potential and strong neuroprotective activities [52-53], Similarly, the *cis-cis*-9,12-Octadecadienoic acid which is commonly known as linoleic acid have already been confirmed to possess neuroprotective and antioxidant effect [54-55]. The cholinesterase and antioxidant activities of the extract may be attributed to the presence of these compounds.

CONCLUSION

The ethyl acetate extract of *Zapoteca portoricensis* root has shown antioxidant and cholinesterase inhibitory properties in

the management of Alzheimer's disease. The compounds found in the ethyl acetate extract that were identified by GC-MS may provide a promising starting point for the creation of novel medications intended to treat AD and other neurological conditions. We suggest conducting additional research on the isolation and characterisation of bioactive compounds with potentials for clinical use.

CONFLICTS OF INTEREST

There are no conflicts of interest.

ACKNOWLEDGEMENTS

Authors would like to acknowledge TETFund Institutional Based Research (IBR) (TETF/DR&D/CE/UNI/NSUKKA/BR/2020/VOL.I) for funding part of this research.

AUTHOR'S CONTRIBUTION

Onoja O. Joel designed the experiment, wrote the first draft of manuscript, supervised and partook in the experiment. Olawuni Julius Idowu and Umeokoli Malachukwu C. collected and extracted the plants and performed the bio-assay *in vitro*. All authors read and approve the manuscript.

REFERENCES

1. Rabinovici GD. Late-onset Alzheimer disease. Continuum: Lifelong Learning in Neurology, 25(1), 2019:14-33.
2. Gaugler J, James B, Johnson T, Reimer J, Solis M, Weuve J, Buckley RF, Hohman TJ. 2022 Alzheimer's Disease Facts and Figures. Alzheimers & Dementia, 18(4), 2022:700-89.
3. Long S, Benoist C, Weidner W. World Alzheimer Report 2023: Reducing dementia risk: never too early, never too late. London, England: Alzheimer's Disease International, 2023.
4. Walker LC. Prion-like mechanisms in Alzheimer disease. Handbook of Clinical Neurology, 153, 2018:303-19.
5. Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics. Molecular Medicine Reports, 20(2), 2019:1479-87.
6. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: targeting the cholinergic system. Current Neuropharmacology, 14(1), 2016:101-15.
7. Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current Neuropharmacology, 11(3), 2013:315-35.
8. Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease: Causes and treatment. Molecules, 25(24), 2020:5789.
9. Yiannopoulou KG, Papageorgiou SG. Current and future treatments in Alzheimer disease: an update. Journal of Central Nervous System Disease, 12, 2020:1179573520907397.
10. Ruangritchankul S, Chantharit P, Srisuma S, Gray LC. Adverse drug reactions of acetylcholinesterase inhibitors in older people living with dementia: A comprehensive literature review. Therapeutics and Clinical Risk Management, 17, 2021:927-49.
11. Dhillon S. Aducanumab: first approval. Drugs, 81, 2021:1437-43.
12. Cummings J, Aisen P, Apostolova LG, Atri A, Salloway S, Weiner M. Aducanumab: appropriate use recommendations. The Journal of Prevention of Alzheimer's Disease, 8, 2021:398-410.
13. Cummings J, Salloway S. Aducanumab: Appropriate use recommendations. Alzheimers Dement, 18(3), 2022:531-533.
14. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature, 537(7618), 2016:50-6.
15. Kastanenka KV, Bussiere T, Shakerdge N, Qian F, Weinreb PH, Rhodes K, Bacskai BJ. Immunotherapy with aducanumab restores calcium homeostasis in Tg2576 mice. Journal of Neuroscience, 36(50), 2016:12549-58.
16. Walsh S, Merrick R, Milne R, Brayne C. Aducanumab for Alzheimer's disease? British Medical Journal, 2021:374.
17. Ferrero J, Williams L, Stella H, Leitermann K, Mikulskis A, O'Gorman J, Sevigny J. First-in-human, double-blind,placebo-controlled, single-dose escalation study of aducanumab (BIIB037) in mild-to-moderate Alzheimer's disease. Alzheimers Dement (N Y), 2(3), 2016:169-176.
18. Inderbir SP, Mayur P. Aducanumab**,** NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health. Stat Pearls, Stat Pearls Publishing, Treasure Island (FL), 2023.
19. Gao E, Zhou ZQ, Zou J, Yu Y, Feng XL, Chen GD, He RR, Yao XS, Gao H. Bioactive asarone-derived phenylpropanoids from the rhizome of Acorus tatarinowii Schott. Journal of Natural Products, 22;80(11), 2017:2923-9.
20. Ali SK, Hamed AR, Soltan MM, Hegazy UM, Elgorashi EE, El-Garf IA, Hussein AA. In-vitro evaluation of selected Egyptian traditional herbal medicines for treatment of Alzheimer disease. BMC Complementary and Alternative Medicine, 13, 2013:1-0.
21. Suganthy N, Pandian SK, Devi KP. Cholinesterase inhibitory effects of Rhizophora lamarckii, Avicennia officinalis, Sesuvium portulacastrum and Suaeda monica: mangroves inhabiting an Indian coastal area (Vellar Estuary). Journal of Enzyme Inhibition and Medicinal Chemistry, 24(3), 2009:702-7.

22. Duan S, Guan X, Lin R, Liu X, Yan Y, Lin R, Zhang T, Chen X, Huang J, Sun X, Li Q. Silibinin inhibits acetylcholinesterase activity and amyloid β peptide aggregation: A dual-target drug for the treatment of Alzheimer's disease. Neurobiology of Aging, 36(5), 2015:1792-1807.
23. Ukwe CV, Ubaka CM, Adibe MO, Okonkwo CJ, Akah PA. Antiulcer activity of roots of Zapoteca portoricensis (fam. Fabiaceae). Journal of Basic and Clinical Pharmacy, 1(3), 2010:183.
24. Agbo MO, Okoye FB, Nwodo JN. In vivo anti-inflammatory effect of Zapoteca portoricensis (Jacq) HM Hernández. International Journal of Health Research, 3(1), 2010:29-35.
25. Agbafor KN, Akubugwo EI, Ogbashi ME, Ajah PM, Ukwandu CC. Chemical and antimicrobial properties of leaf extracts of *Zapoteca portoricensis*. Research Journal of Medicinal Plant, 5(5), 2011:605-12.
26. Joshua PE, Ezugwu CH, Chilaka FC, Nwodo OF, Dasofunjo K, Ezugwu MU. Effect of ethanol extract of *Zapoteca portoricensis* stem on testosterone-induced benign prostate hyperplasia (BPH) in adult male albino rats. Australian Journal of Basic and Applied Sciences, 12, 2018:9-18.
27. Nwodo NJ, Omeje EO, Brun R. In vitro-in vivo studies on anti-trypanosomal potentials of *Zapoteca portoricensis*. Asian Pacific Journal of Tropical Medicine, 2(1), 2009:25-9.
28. Agbafor KN, Ogbanshi ME, Akubugwo EI. Phytochemical screening, hepatoprotective and antioxidant effects of leaf extracts of *Zapoteca portoricensis*. Advances in Biological Chemistry, 10, 2014:2014.
29. Gbadamosi IT. Ethnobotanical survey of plants used for the treatment and management of sexually transmitted infections in Ibadan, Nigeria. Ethnobotany Research and Applications, 12, 2014:659-69.
30. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2), 1961:88-95.
31. Singh N, Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. Food Chemistry, 85(4), 2004:611-617.
32. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und-Technologie* (LWT)-Food science and Technology, 28(1), 1995:25-30.
33. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 269(2), 1999:337-41.
34. Natarajan S, Shunmugiah KP, Kasi PD. Plants traditionally used in age-related brain disorders (dementia): an ethanopharmacological survey. Pharmaceutical Biology, 51(4), 2013:492-523.
35. Fatima I, Safdar N, Akhtar W, Munir A, Saqib S, Ayaz A, Bahadur S, Alrefaei AF, Ullah F, Zaman W. Evaluation of potential inhibitory effects on acetylcholinesterase, pancreatic lipase, and cancer cell lines using raw leaves extracts of three fabaceae species. Heliyon, 9(5), 2023, e15909
36. Kozlowski H, Janicka-Klos A, Brasun J, Gaggelli E, Valensin D, Valensin G. Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation). Coordination Chemistry Reviews, 253(21-22), 2009:2665-85.
37. Lakey-Beitia J, Burillo AM, La Penna G, Hegde ML, Rao KS. Polyphenols as potential metal chelation compounds against Alzheimer's disease. Journal of Alzheimer's Disease, 82(s1), 2021:S335-57.
38. Chen X, Drew J, Berney W, Lei W. Neuroprotective natural products for Alzheimer's disease. Cells, 10(6), 2021:1309.
39. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal, 21(2), 2013:143-52.
40. Rahman MA, Moon SS. Antioxidant polyphenol glycosides from the plant Draba nemorosa. Bulletin of the Korean Chemical Society, 28(5), 2007:827-31.
41. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines, 5(3), 2018:93.
42. Delanty N, Dichter MA. Antioxidant therapy in neurologic disease. Archives of Neurology, 57(9), 2000:1265-70.
43. Polo-Olivella JA, Betancur-Pérez JF, Arboleda-Valencia JW. Fabaceae family from dry forest in Colombia: potential biotechnological use as a source of biocides and antioxidant compounds. Pharmacology Online, 2, 2019:355-73.
44. Mans DR, Pinas NM, Djotaroeno M, Friperson P, Pawirodihardjo J, Lichtveld MY. Insight into the antioxidant activities of ten Fabaceae plant species that are medicinally used by the Aucan Tribal Peoples from the Republic of Suriname (South America). GSC Biological and Pharmaceutical Sciences, 20(3), 2022:082-96.
45. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Current Biology, 24(10), 2014:R453-62.
46. Gomes BA, Silva JP, Romeiro CF, Dos Santos SM, Rodrigues CA, Gonçalves PR, Sakai JT, Mendes PF, Varela EL, Monteiro MC. Neuroprotective mechanisms of resveratrol in Alzheimer's disease:

role of SIRT1. Oxidative Medicine and Cellular Longevity, 2018:2018.
47. Singh SK, Srikrishna S, Castellani RJ, Perry G. Antioxidants in the prevention and treatment of Alzheimer's disease. In Nutritional Antioxidant Therapies: Treatments and Perspectives; Springer: Cham, Switzerland, 2017:523–553.
48. Belkacemi A, Ramassamy C. Innovative anthocyanin/anthocyanidin formulation protects SK-N-SH cells against the amyloid-β peptide-induced toxicity: relevance to Alzheimer's disease. Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents), 16(1), 2016:37-49.
49. Onoja JO, Ugwuanyi KC, Ugwoke CEC, Ezegbe CA. Identification of Cholinesterase Inhibitors and Antioxidant Molecules from Leaf of Clerodendrum splendens G. Don (Verbenaceae) Using GC-MS. Tropical Journal of Natural Product Research, 7(2), 2023: 2437–2448.
50. Onoja JO, Ugwueze JN. GC-MS Identification of Cholinesterase Inhibitory and Antioxidant Molecules from Leaves of Cnidoscolus aconitifolius (Miller) IM Johnston (Euphorbiaceae). Current Enzyme Inhibition, 19(1), 2023:19-37.
51. Starlin T, Prabha PS, Thayakumar BK, Gopalakrishnan VK. Screening and GC-MS profiling of ethanolic extract of Tylophora pauciflora. Bioinformation, 15(6), 2019:425.
52. Wang ZJ, Liang CL, Li GM, Yu CY, Yin M. Stearic acid protects primary cultured cortical neurons against oxidative stress. Acta Pharmacologica Sinica, 28(3), 2007:315-26.
53. Khabbush A, Orford M, Tsai YC, Rutherford T, O'Donnell M, Eaton S, Heales SJ. Neuronal decanoic acid oxidation is markedly lower than that of octanoic acid: a mechanistic insight into the medium-chain triglyceride ketogenic diet. Epilepsia, 58(8), 2017:1423-9.
54. Basiricò L, Morera P, Dipasquale D, Tröscher A, Bernabucci U. Comparison between conjugated linoleic acid and essential fatty acids in preventing oxidative stress in bovine mammary epithelial cells. Journal of Dairy Science, 100(3), 2017:2299-309.
55. Hunt WT, Kamboj A, Anderson HD, Anderson CM. Protection of cortical neurons from excitotoxicity by conjugated linoleic acid. Journal of Neurochemistry, 115(1), 2010:123-30.