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Research paper

In vitro anticholinesterase potential of some spices consumed in Cameroon and their protective effects on hydrogen peroxide-mediated oxidative stress damage in SK-N-SH cells

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ABSTRACT

Background: Many neurodegenerative such as Alzheimer's disease (AD) are characterized by cholinergic dysfunction and oxidative stress which is a key event in neuronal death process. Thus, anticholinesterase and anti-oxidation compounds are two promising strategies in the development of AD drugs. Beyond their culinary use, spices are today studies for health purpose. In this study, some spices consumed in Cameroon were evaluated for their anticholinesterase and neuroprotective effects.

Methods: Colorimetric methods were used to determine total flavonoid and alkaloid content of a combinated extract (hydroethanolic + ethanolic extracts) of different selected spices. Aftermaths, anti-cholinesterase activity of spice extract was carried out using Ellman's method. Finally, neuroprotective effects performed on human SK-N-SH cells stressed with H_2O_2 by assessing neuronal survival (resazurin assay) and neuronal death (LDH assay).

Results: Flavonoid content of spices extract were ranged from 22.94 to 32.01 mg EQ/g DM and alkaloid content were ranged from 320 to 896 mg EQu/g DM. Among the spices studied, *Xylopia parviflora* presented the greatest acetylcholinesterase inhibition with an IC50 = 14 µg/mL. In Cell culture experiments, pre-incubation of SK-N-SH cell with the selected spices at different concentrations were improved neuronal survival and reduced the percentage of neuronal cells dead.

Conclusion: The present results reveal that selected spices consumed in Cameroon have good anticholinesterase activity as well as neuroprotective effect on SK-N-SH which may provide new natural compounds that could help in the management of Alzheimer's disease.

1. Introduction

Neurodegenetative disease include a lot of disorders characterized by a progressive loss of neuronal cells, leading to cognitive impairments and dementia (Ramanan and Saykin, 2013). Alzheimer disease (AD) is the most common form of dementia and affects over 46 million people worldwide number that is expected to double because of increase of aging (Prince et al., 2015). In patients with AD, there is a progressive loss of cholinergic cells which is associated with a significant decrease in the amount of acetylcholine (ACh) a key neurotransmitter implicated in memory process. One of the mechanisms that contribute to the damage and death of brain cells in AD is oxidative stress (OS) (Millichap et al., 2021). OS is an imbalance intracellular redox status due to an excessive production of oxygen reactive species (ROS) and a reduced capacity of

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AchE, acetylcholine esterase; AD, Alzheimer disease; DPPH, 1, 1-Diphenyl-2-Picrilhydrazyl; EQ, equivalent; FDA, food and drug administration; IC, inhibitory concentration; IV, intravenous; LDH, lactate dehydrogenase; NAD, nicotinamide adenosine dinucleotide; OS, oxidative stress; ROS, reactive oxygen species, TAC, total antioxidant capacity.

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antioxidant defense system. ROS such as hydrogen peroxide are produced during aerobic respiration or cellular metabolism in different cellular organelles including mitochondria (Millichap et al., 2021). They are highly reactive compounds that able to induce modifications in the structure and function of cell membranes by oxidizing proteins, lipoproteins, enzymes, and genetic material. These events can lead to cellular death (Dos Santos Picanco et al., 2018). Cells contain antioxidant defense mechanisms to cope these highly reactive compounds, including enzymatic antioxidant such as catalase and non-enzymatic antioxidant such as vitamin E and natural flavonoids. However in AD patients, ROS generation overwhelms the cellular antioxidant capacity, therefore antioxidant defenses are no longer able to prevent ROS from causing oxidative damage (Niedzielska et al., 2016).

The most commonly approved drugs to improve AD cognitive function are acetylcholinesterase (AChE) inhibitors such as donepezil, rivastigmine, or galantamine (Atri, 2019). They act by blocking the hydrolyzation of acetylcholine (ACh) which is a neurotransmitter that transmits signal in the synapse. Two of these anticholinesterase drugs were isolated from plants. It concerns galantamine an alkaloid isolated from perce-neige and rivastigmine alkaloid derived from physostigmine (Heinrich and Teoh, 2004; Kalauni et al., 2002). One of the therapeutic limitations of these drugs is the fact that their target only one hypothesis of the disease ("cholinergic hypothesis") (Francis et al., 1999). Furthermore, all of these drugs have seriously side effects ranging from nausea and headaches, to amyloid-related imaging abnormalities. Thus, identified new anticholinesterase molecules with few or no sides effects and capable to protect neurons against neurotoxins or free radicals and promote neuronal survival are interest (Sharifi-Rad et al., 2020).

Recently, attention has focused on herbs and spices as source of anticholinesterase, antioxidants and neuroprotective compounds, which can protect neuronal cells against oxidative stress damage (Sharma et al., 2021). It is now known that spices contain several secondary metabolites include polyphenols, flavonoids and alkaloids which are responsible for their effects on the health (Pritam et al., 2022; Sharma et al., 2021). Some of these spices such as curcumin were shown good neuroprotective effects and ability to reduce cholinesterase inhibition (Sahebkar et al., 2015; Akinyemi et al., 2015). Despite the highly diversity of spices consumed in Cameroon, few studies on their neuroprotective and anticholinesterase potential were done. Because of their pluripotency, oral safety, long history of use, and low cost, spices may have great potential for the prevention of multiple neurological conditions. Thus, the current study aimed to evaluate the *in vitro* anticholinesterase potential of spices consumed in Cameroon and their protective effects on hydrogen peroxide-mediated oxidative stress damage in SK-N-SH cells.

2. Material and methods

2.1. Chemicals and solvents

The chemicals used in the experiments were provided by Sigma Aldrich, Germany. Alcohol and distilled water were used as solvents.

2.2. Plant material and preparation of extracts

2.2.1. Plant material

The plant material consisted of fruits and barks of spices purchased in the West Region of Cameroon (Bafoussam market) in 2016 and identified at the National Herbarium by comparison to standard reference specimen:

- *Xylopia parviflora*: F.J. Breteler N° 2018 Herbarium 9203/SRF/CAM
- *Tetrapleura tetraptera*: B. Mpom N° 15 Herbarium 1727/SRFK
- *Afrostyrax lepidophyllus*: J.J. Bos N° 3579 Herbarium HNC 29845
- *Zanthoxylum xanthoxyloides*: R. Letouzey N° 9704 Herbarium 21793/ SRF/CAM
- *Piper guineense*: R. Letouzey N° 1448 Herbarium 3615/ SRFK
- *Aframomum daniellii*: R Letouzey N° 845 Herbarium 5846/SRF/CAM

The fruits, barks and roots of spices collected were washed with tap water, oven dried (50 °C) and crushed to obtain powder which was kept in the opaque flasks. This powder has been used to prepare the extracts.

2.2.2. Preparation of extracts

The fruit powders obtained were subjected to two types of extractions: hydroethanolic and ethanolic. Concerning hydroethanolic extraction, the powders have been dissolved in a 1:4 (w/v) water-ethanol mixture. After homogenization, the mixture was macerated for 48 h at room temperature and the supernatant was then filtered with Whatman filter paper N°2.

Concerning ethanolic extraction, the residues obtained after hydroethanolic extraction were dissolved in ethanol in the proportions 1:4 (w/v). The mixture was macerated for 24 h at room temperature and the supernatant was then filtered.

The two filtrates (hydroethanolic and ethanolic) obtained were mixed to form the combined filtrate and it was the latter that was concentrated at 50 °C for 72 h. The mixture obtained after concentration was stored at 4 °C for further handling.

2.3. Determination of phytochemical content of spice extract

2.3.1. Flavonoid content. The flavonoids contained in the sample react with aluminum trichloride and potassium acetate to form a pinkish-colored complex that absorbs at 420 nm and the intensity of the coloring is proportional to the content of polyphenols present in the sample (Bahorun et al., 1996). Total flavonoids content was recorded in triplicate from the calibration curve of quercetin and expressed as mg of quercetin equivalent/g of dry sample.

2.3.2. Alkaloid content. The alkaloids in the sample react with iron chloride, hydrogen chloride, and 1,10-Phenanthroline to form a red solution that absorbs at 510 nm. The color intensity is proportional to the number of alkaloids present in the medium (Singh et al., 2001). Total alkaloids content was recorded in triplicate from the calibration curve of quinin and expressed as mg of quinin equivalent/g of dry sample.

2.4. Acetylcholinesterase (AChE) inhibitory activity assay

AChE belongs to the cholinesterase family. Enzyme activity was evaluated according to the procedure of Ellman et al. (1961). Acetylthiocholine was hydrolyzed by AChE to acetic acid and thiocholine. Catalytic activity was measured by following the increase in the yellow anion, 5-thio-2-nitrobenzoate, produced from thiocholine when it reacted with DTNB at 410 nm.

$$\textbf{Inhibition percent } (\%) = \frac{\textbf{Abs of control} - \textbf{Abs of sample}}{\textbf{Abs of control}} * \boldsymbol{100}$$

The IC50 value corresponding to the concentration of the extract required to inhibit 50 % of enzyme activity was calculated based on graph plotted with the concentration of extract on the x-axis and percentage inhibiting activity on the y-axis.

2.5. Antioxidant assay

The antioxidant activity was evaluated using methods involving synthetic oxidant DPPH, ABTS, TAC, and Lipid peroxidation *ex-vivo*.

2.5.1. 1, 1-Diphenyl-2-Picrilhydrazyl (DPPH) radical scavenging activity assay. The DPPH• free radical is trapped by antioxidants to form a stable DPPH-H. The color of the mixture changes from brown to yellow and absorbance is read at 517 nm (Katalinić et al., 2004). An aliquot of 50 µL of the test samples of different concentrations (0.5–10 mg/mL) was added in 1.95 mL of methanolic DPPH solution (0.3 mM) freshly

prepared. The mixture was shaken and placed in the dark for 30 min at room temperature. The absorbance of the mixture was then recorded spectrophotometrically at 517 nm. The decrease in absorbance was correlated with the radical scavenging potential of test samples. The percentage of inhibition was assessed as follow:

$$\%\ \text{of DPPH radical Scavenging}^{\cdot} = [A_0\text{-}(A_1\text{-}a_0)/A_0]$$

Where A_0 is the DPPH solution absorbance, A_1 is the absorbance of solution containing test sample and DPPH solution, and a_0 is the absorbance sample solution without DPPH. The inhibition Concentration 50 (IC50) value corresponding to the concentration of antioxidants required for a 50 % reduction of the DPPH radical, was calculated based on graph plotted with the concentration of extract on the x-axis and percentage inhibiting activity on the y-axis. As the IC50 value of an extract is low, the extract is considered as active.

2.5.2. ABTS radical scavenging activity. By reacting with potassium permanganate, ABTS forms the blue to green ABTS+ radical, the addition of an antioxidant reduces this radical and causes the mixture to discolor. This discoloration is measured by spectrophotometry at 734 nm and is proportional to the activity of the antioxidants present in the sample (Re et al., 1999). ABTS radical cations solution was obtained by combining 8 mM of ABTS with 3 mM of potassium persulfate in 25 mL of distillated water and kept to dark at room temperature for 16 h before to be used. For the assay, the solution was diluted 10 times with 95 % ethanol. Then, an aliquot of 20 µL of the sample (0.5–10 mg/mL)), was added in 1 mL ABTS working solution and incubated for 30 min at room temperature. The control was prepared as above, without the plant extract or ascorbic acid and methanol was taken as blank. The absorbance was recorded at 734 nm. The following formula was applied to calculate percentage inhibition:

$$\%\ \text{ABTS radical } \textit{Scavenging} = \frac{\textbf{Abs control} - \textbf{Abs sample}}{\textbf{Abs control}} * \boldsymbol{100}$$

Each sample was analyzed in thrice and the absorbance sample solution without ABTS working solution was removed to the absorbance of sample with ABTS working solution. The inhibition Concentration 50 (SC50) value corresponding to the concentration of antioxidants required for a 50 % reduction of the ABTS+ radical was calculated based on graph plotted with the concentration of extract on the x-axis and percentage inhibiting activity on the y-axis. As the IC50 value of an extract is low, the extract is considered as active.

2.5.3. Total Antioxidant capacity (TAC). The method used was described by (Prieto et al., 1999) based on the reduction of molybdenum, Mo(VI) into Mo(V) in the presence of extracts to form a green complex (phosphate/Mo(V)) in an acid medium. An aliquot of 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was added in 100 µL of sample (10 mg/mL)). The mixture was coated and incubated in a boiling water bath at 95 °C for 90 min then, the mixture was cooled in room temperature and the absorbance was recorded at 695 nm. Each sample was analyzed in thrice and the absorbance sample solution without reagent solution was removed to the absorbance of sample with reagent solution. Ascorbic acid was used as a standard and total antioxidant capacity was expressed as milligrams of ascorbic acid equivalent (AAE) per grams of extract of dry matter (mg AAE/g DM).

2.5.4. Anti-Lipid peroxidation analysis. Poly-unsaturated fatty acids contained in brain homogenates undergo peroxidation when put in the presence of iron sulphate. The generated malondialdehyde reacts with thiobarbituric acid to form a pink chromophore that absorbs at 532 nm (Kumar et al., 2000). An aliquot of 300 µL of extract sample (10 mg/mL) or distilled water (control) was added to 500 µL of brain homogenate. A volume of 100 µL of KCl (0.15 M) was added to the mixture and lipid peroxidation was initiated by adding 100 µL $FeSO_4$ (15 mM). Then, the reaction medium was incubated for at 37 °C for 30 min. An equal volume (1:1) (1 mL) of TBA (1 % w/v) and HCl (10 % v/v) was added to the media following by a solution of ascorbic acid (6 mM, 1 mL). The final mixture was heated at 80 °C for 20 min in water bath, cooled and then centrifugated. Absorbance of pink solution was noticed at 532 nm using spectrophotometer. Percentage inhibition was measured according to following formula:

$$\text{Lipid peroxidation impediment}(\%) = \frac{\textbf{Abs of control} - \textbf{Abs of sample}}{\textbf{Abs of control}} * \boldsymbol{100}$$

The inhibiting Concentration 50 (IC50) value corresponding to the concentration of antioxidants required for a 50 % reduction of the MDA was calculated based on graph plotted with the concentration of extract on the x-axis and percentage inhibiting activity on the y-axis. As the IC50 value of an extract is low, the extract is considered as active.

2.6. Neuroprotective activity assay

2.6.1. SK-N-SH cell culture. SK-N-SH cells are derived from Human neuroblastoma line and were obtained from American Type Culture Collection cells (ATCC).

SK-N-SH cells were grown in RPMI 1640 (Roswell Park Memorial Institute 1640) supplemented with glutamine 2 mM and fetal bovine serum (FBS 10 %). In order to limit microbial contamination, a mixture of antibiotic (antifongic 2.5 mg/mL; penicillin 1.25 U/mL and streptomycin 0.00125 U/mL) was added to the media. The cells were kept at 37ºC in an incubator under humidified atmosphere containing 5 % CO_2.

2.6.2. Cell Treatment. SK-N-SH cells were seeded in 96 well plate at the density of 20,000 cells per well in 200 µL of media. The plates were incubated during 48 h to reach 70–80 % of confluency. SK-N-SH cells were pre-incubated with spice extracts at concentrations 5–50 µg/mL following by 24 h exposure to H_2O_2 (250 µM). Subsequently, the cells were subjected to the measurement of cell viability (resazurin assay), and membrane integrity (LDH release assay). The H_2O_2 solution, and diluted concentrations of spice extract were prepared freshly in distilled water. All agents were added to the media at the indicated concentrations. Experimental set of the control cultures was supplemented with the appropriate vehicles.

2.6.3. Lactate dehydrogenase (LDH) (cytotoxicity test). The LDH release assay was done to evaluate the loss of membrane integrity related to cellular death. The level of lactate dehydrogenase (LDH) released into culture media after 24 h of particular treatments was measured with cytotoxicity detection kit (LDH Assay Kit, Abcam) as described previously. For the test, cells were pre-treated with various concentrations of different spice extracts for 2 h before treated with H_2O_2 (250 µM) for 24 h. Then, 50 µL of media of each well of 96 well were taken and introduced in a new plate. A volume of 50 µL of working solution was added. The blank was achieved with 100 µL of working solution. Plate was incubated for 40 min at room temperature and away from the light. Absorbance of plate was readied at 490 nm (Decker and Lohmann-Matthes, 1988). The LDH release percentage was calculated using the following formula:

$$\text{Percentage of LDH release } (\%) = [(\text{Abs sample} - \text{Abs control}) / \text{Abs control} \times 100]$$

2.6.4. Cell viability assay. The level of cell viability after 24 h of particular treatments was quantified using resazurin assay. The protocol is based on the reduction of oxidized non-fluorescent blue resazurin to a

red fluorescent dye (resorufin) by the mitochondrial respiratory chain in live cells. Cells were pre-treated with various concentration of spices extracts for 2 h before treated with H_2O_2 (250 μM) for 24 h. Then, media was replaced with 100 μL of fresh media containing 10 % of resazurin. The blank was achieved by 100 μL of solution without cells. After 3 h of incubation at 37 °C, the plate was readed at an excitation wavelength of 530 nm and emission of 590 nm (O'Brien et al., 2000). Cell viability percentage was calculated using the following formula

$$\text{Percentage of viability}(\%) = \frac{\mathbf{A}-\mathbf{B}}{\mathbf{C}-\mathbf{B}} * \boldsymbol{100}$$

Where: **A** is the OD at 570 nm of the tested sample (cells in the presence of spice extract); **B** is the OD at 570 nm of the culture medium; **C** is the OD at 570 nm of the negative control (cells treated with the culture medium) corresponding to 100 % viability. The data were normalized to the vehicle-treated cells (100 %) and expressed as a percentage of the control ± SEM.

2.7. Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 20.1 for Windows was used to analyse the results. Significant differences were detected at 95 % confidence interval and the results obtained were expressed as Mean ± Standard Deviation. The analysis was performed with GraphPad Prism version 5.00, GraphPad Software, Inc (San Diego, CA, USA).

3. Results

3.1. Total flavonoid and total alkaloid content

As shown in Table 1, the flavonoid content of different spices was ranged from 22.94 ± 0.73–32.01 ± 0.78 mg EQ/g DM respectively for *Aframomum daniellii* and *Zanthoxylum xanthoxyloides*. No significant was observed among the alkaloid content of the spices ranged from 320 ± 20–896 ± 14 mg EQ/g DM respectively for *Afrostyrax lepidophyllus* and *Xylopia parviflora*.

3.2. Inhibition of acetylcholinesterase

Table 2 shows AChE inhibitory activity of spice extracts. All the spice extracts were shown an AChE inhibitory activity but lower than donepezil. Among spices, the highest activity was observed in ***Xylopia parviflora*** with an IC_{50} = 14.00 ± 2.21 μg/mL.

3.3. Antioxidant activities of spices

The antioxidant capacity of natural products should be evaluated by combining several different *in vitro/ ex-vivo* tests to obtain conclusive results. DPPH, ABTS, Total Antioxidant Capacity (TAC) and lipid peroxidation methods are commonly used to determine antioxidant potential.

3.3.1. Effect on free radical scavenging (DPPH and ABTS). The DPPH and ABTS assay results of spice extracts are shown in Table 3. All the spice extracts were shown strong radical scavenging activities better than those of ascorbic acid. Among the spice extracts, highest scavenging activities were observed in *Xylopia parviflora* (DPPH SC_{50} =12.95 and ABTS SC_{50} =14.69 mg/mL) and *Zanthoxylum xanthoxyloides* (DPPH SC_{50} =15.37 and ABTS SC_{50} =9.440).

Table 1
Alkaloid and Flavonoid content of spices.

Spices extracts	Alkaloids (mg EQu/g DM)	Flavonoid (mg EQ/g DM)
Aframomum daniellii	493 ± 13	22.94 ± 0.73
Afrostyrax lepidophyllus	320 ± 20	30.92 ± 1.04
Piper guineense	464 ± 13	27.65 ± 0.78
Tetrapleura tetraptera	427 ± 19	26.78 ± 2.13
Xylopia parviflora	896 ± 14	30.23 ± 0.47
Zanthoxylum xanthoxyloides	369 ± 20	32.01 ± 0.78

Values are expressed as mean ± standard deviation EQ: Quercetin Equivalent; EQu: Quinin Equivalent

3.3.2. Evaluation of total antioxidant capacity (TAC). For the total antioxidant capacity of spices, it can be seen from Table 4 that it tended to be consistent with the results of DPPH and ABTS assays. The TAC activity of various spices species are in order of *Zanthoxylum xanthoxyloides* > *Afrostyrax lepidophyllus* > *Xylopia parviflora* > *Tetrapleura tetraptera* > *Piper guineense* > *Hypodaphnis zenkeri.*

3.3.3. Effect of spice extracts on lipid peroxidation. The results of lipid peroxidation assay are shown in Table 5. As for ABTS and DPPH results, spices extract showed good anti lipid peroxidation activities but lower than vitamin E. *Afrostyrax lepidophyllus* and *Zanthoxylum xanthoxyloides* had the best anti lipid peroxidation activities among spices with IC_{50} of 0.75 and 1.02 mg/mL respectively. The anti lipid peroxidation activity of various spices species is in order of ***Afrostyrax lepidophyllus*** > ***Zanthoxylum xanthoxyloides*** > ***Xylopia parviflora*** > *Tetrapleura tetraptera* > *Piper guineense* > *Hypodaphnis zenkeri.*

3.4. Effects of spice extracts on cell death (percentage of lactate dehydrogenase) in the presence of hydrogen peroxide in SK-N-SH cell cultures

For the purpose to confirm the neuroprotective potential of spice extracts, we evaluated the effect of spices on neuronal cells integrity in presence of H_2O_2 (250 μM) using LDH released assay. The results are revealed in Table 6. We observed that treatment of SK-N-SH cells with H_2O_2 (250 μM) for 24 h led to a significant increase (41 ± 3.4) % of LDH released percentage in cells culture medium compared to control cells indicating a loss of neuronal integrity or cells death. Pre-treatment of SK-N-SH cells with different concentrations of spices extracts significantly decreased the percentage of LDH released or percentage of dead cells.

3.5. Effects of spice extract on cell viability (resazurin test) in the presence of hydrogen peroxide in SK-N-SH cell cultures

In order to evaluate the effect of spices on viability of neuronal cells in presence of H_2O_2 (250 μM), metabolic activity of cells was assessed using resazurin test. The results are revealed in VI. We observed that treatment of SK-N-SH cells with H_2O_2 (250 μM) for 24 h led to a significant decrease (50 ± 1.18) of metabolic activity of neuronal cells indicating a decrease in neuronal survival or neuronal viability compared to control. Pre-treatment of SK-N-SH cells with different concentrations of spices extracts significantly increased the percentage of survival cells. The percentage of survival cells were ranged from 61 to 85 and were not significantly different by increasing spices extract concentrations (Table 7).

4. Discussion

One of the changes that occur in AD is an increase in acetylcholinesterase (AChE) activity, the enzyme responsible for acetylcholine (ACh) hydrolysis, from both cholinergic and non-cholinergic neurons of the brain (Konrath et al., 2013). Preventing breaking down of ACh is responsible for the elevation of ACh level in the synaptic cleft. In the present study AChE inhibitory activity of spice extract was assessed by Ellman's method. All the spices were shown AChE inhibition capacity. However, *Xylopia parviflora* was shown the highest activity (IC_{50} = 14 μg/mL) but less than the reference drug Donepezil (IC_{50} = 6.85 μg/mL). According to the literature reports, majority of AChE inhibitors belong to the alkaloid group, including indole, isoquinoline, quinolizidine, piperidine, and steroidal alkaloids (Dage et al., 2016). Thus, the highest activity of *X. parviflora* may be due to its high content in total alkaloids. In fact, studies have shown that alkaloids possess in

Table 2
IC_{50} spice extracts on acetylcholinesterase inhibition.

Spices	*Xylopia parviflora*	*Tetrapleura tetraptera*	*Dichrostachys glomerata*	*Aframomum daniellii*	*Zanthoxylum xanthoxyloides*	*Piper guineense*	Donepezil
IC_{50} (μg/mL)	14 ± 2.21^{b}	1150 ± 9.07^{f}	74 ± 3.90^{c}	831 ± 8.52^{e}	1230 ± 10.15^{f}	199 ± 4.91^{d}	6.85 ± 1.78^{a}

IC_{50} = inhibitory concentration 50 (50 % inhibition). Results are presented as mean ± standard deviation. Columns with different letters are significantly different at $P < 0.05$

Table 3
SC_{50} spice extracts on DPPH, ABTS radicals and vitamin C.

	Xylopia parviflora	*Tetrapleura tetraptera*	*Hypodaphnis zenkeri*	*Afrostyrax lepidophyllus*	*Zanthoxylum xanthoxyloides*	*Piper guineense*	*Vit C*
SC_{50} (mg extract/mL DPPH)	15.37 ± 0.27^{a}	86.75 ± 2.57^{c}	116.26 ± 1.37^{d}	29.34 ± 0.77^{b}	12.95 ± 1.64^{a}	80.58 ± 0.70^{c}	141.15 ± 1.69^{e}
SC_{50} (mg extract/mL ABTS)	14.69 ± 0.78^{a}	41.99 ± 0.78^{b}	40.65 ± 0.63^{b}	13.68 ± 0.79^{a}	9.440 ± 0.68^{a}	24.62 ± 0.95^{c}	80.25 ± 1.98^{d}

SC_{50} = scavenging concentration 50 (50 % inhibition of ABTS and DPPH). Results are presented as mean ± standard deviation. Lines with different letters are significantly different at $P < 0.05$.

Table 4
Total Antioxidant Capacity (TAC) of spice extracts.

Spices	*Xylopia parviflora*	*Tetrapleura tetraptera*	*Hypodaphnis zenkeri*	*Afrostyrax lepidophyllus*	*Zanthoxylum xanthoxyloides*	*Piper guineense*
Concentration (mg AAE/g DM)	36.61 ± 4.92^{a}	32.75 ± 3.78^{b}	$14{,}72 \pm 1{,}75^{c}$	$40.02 \pm 5{,}69^{a}$	43.18 ± 7.16^{a}	16.85 ± 2.58^{c}

AAE: Ascorbic Acid Equivalent; DM: Dry Matter. Results are presented as mean ± standard deviation. Lines with different letters are significantly different at $P < 0.05$

Table 5
Inhibition of lipid peroxidation in brain homogenates using Vitamin E and extract of spices.

Spices	*Xylopia parviflora*	*Tetrapleura tetraptera*	*Hypodaphnis zenkeri*	*Afrostyrax lepidophyllus*	*Zanthoxylum xanthoxyloides*	*Piper guineense*	Vit E
IC_{50}(mg/mL)	2.52 ± 1.78^{b}	3.87 ± 0.98^{b}	$6{,}50 \pm 1.02^{c}$	0.75 ± 0.01^{a}	1.02 ± 0.12^{a}	6.10 ± 1.14^{c}	0.47 ± 0.02^{a}

IC_{50} = inhibitory concentration 50 (50 % inhibition). Results are presented as mean ± standard deviation. Lines with different letters are significantly different at $P < 0.05$

Table 6
Percentage of dead cells after pre-incubation of SK-N-SH with various concentrations of spice extracts and futher treatment with hydrogen peroxide for 24 h (%).

Spices	*Afrostyrax lepidophyllus*	*Hypodaphnis zenkeri*	*Xylopia parviflora*	*Zanthoxylum xanthoxyloides*	*Pipper guineense*	*Tetrapleura tetraptera*
Control	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
H_2O_2 (250 μM)	41.00 ± 3.40 **	41.00 ± 3.40 **	41.00 ± 3.40 **	41.00 ± 3.40 **	41.00 ± 3.40 **	41.00 ± 3.40 **
5 μg/mL	20.00 ± 7.24 ***	15.00 ± 3.88 ***	21.00 ± 3.43 ***	12.00 ± 5.73 ***	08.00 ± 1.17 *	22.00 ± 7.50 ***
12.5 μg/mL	19.00 ± 5.88 ***	14.00 ± 7.80 ***	23.00 ± 5.62 ***	13.00 ± 5.05 ***	24.00 ± 2.77 ***	17.00 ± 2.36 *** *
25 μg/mL	24.00 ± 0.41 ***	14.00 ± 5.96 ***	19.00 ± 1.73 ***	18.00 ± 3.67 ***	26.00 ± 6.14 ***	16.00 ± 1.38 *** *
50 μg/mL	23.00 ± 3.31 ***	18.00 ± 1.83 ***	24.00 ± 3.80 ***	14.00 ± 2.11 ***	19.00 ± 2.08 ***	28.00 ± 5.97 ***

Results are expressed as a percentage of the control ± SEM. Columns with different number of stars* are significantly different at $P < 0.05$.

Table 7
Percentage of survival cells after pre-incubation of SK-N-SH with various concentrations of spice extracts and futher treatment with hydrogen peroxide for 24 h.

Spices	*Afrostyrax lepidophyllus*	*Hypodaphnis zenkeri*	*Xylopia parviflora*	*Zanthoxylum xanthoxyloides*	*Pipper guineense*	*Tetrapleura tetraptera*
Control (%)	100.00 ± 0.85^{a}	100.00 ± 0.85^{a}	100.00 ± 0.85^{a}	100.00 ± 0.85^{a}	100.00 ± 0.85^{a}	100.00 ± 0.85^{a}
H_2O_2 (250 μM)	50.00 ± 1.18^{b}	50.00 ± 1.18^{b}	50.00 ± 1.18^{b}	50.00 ± 1.18^{b}	50.00 ± 1.18^{b}	50.00 ± 1.18^{b}
5 μg/mL	74.67 ± 10.94^{c}	64.95 ± 9.49^{c}	80.09 ± 12.11^{c}	80.75 ± 11.92^{c}	61.57 ± 8.99^{c}	82.80 ± 12.54^{c}
12.5 μg/mL	72.98 ± 10.63^{c}	67.36 ± 9.55^{c}	77.55 ± 11.33^{c}	83.56 ± 12.09^{c}	69.54 ± 10.07^{c}	78.47 ± 11.21^{c}
25 μg/mL	66.67 ± 9.01^{c}	64.29 ± 8.59^{c}	81.02 ± 11.28^{c}	85.85 ± 12.03^{c}	67.53 ± 9.33^{c}	80.03 ± 11.30^{c}
50 μg/mL	68.72 ± 8.45^{c}	67.33 ± 8.06^{c}	83.27 ± 10.72^{c}	83.66 ± 10.52^{c}	72.29 ± 9.17^{c}	82.14 ± 10.61^{c}

Results are expressed as a percentage of the control ± SEM. Columns with different number of stars* are significantly different at $P < 0.05$.

their structure one or more nitrogen atoms able to interact with the active site of the enzyme (acetylcholinesterase) and competitively inhibit the fixation of acetylcholine (substrate) (Konrath et al., 2013).

The role of oxidative stress in neuropathogenesis of AD is well established, therefore antioxidant agents that are capable of targeting intracellular ROS in order to prevent oxidative-associated damage may be beneficial in the treatment or prevention of this disorder. In the current study, the spices extracts were shown an interesting *in vitro* antioxidant effects. In fact, they scavenged DPPH and ABTS radicals with SC_{50} ranging from 12.95 to 116.26 (mg extract/mL DPPH) and 9.44–41.99 (mg extract/mL ABTS). ScaInhibition concentrations of spices were lower than reference antioxidant vitamin C (141.15 mg/mL DPPH and 80.25 mg/mL ABTS). This may be due to higher content of flavonoids in these spices. In fact, flavonoids are able to neutralize free

radical due to the presence of hydroxyl group which can give a hydrogen or electron (Xie et al., 2022). It may be the reason that *Xylopia parviflora* and *Zanthoxylum xanthoxyloides* showed the higher radical scavenging capacity due to their high flavonoids content. As lipid peroxidation produces a lot of degradation products and is found to be an important cause of cell membrane destruction and cell damage. For the effectiveness of spices extract to act as antioxidant agent against brain lipid peroxidation, *Afrostyrax lepidophyllus* and *Zanthoxylum xanthoxyloides* were more effective. As shown in this study these two spices contained less alkaloids than other. Thus, it is plausible that alkaloids present in our spices reduce their anti-lipid peroxidation activity.

In vitro neuroprotective activities of the spices were achieved in neuroblastoma SK-N-SH cells. The ability of these cell lines to respond at variety of neuronal induced disorders has led to the use of neuroblastoma cell lines as model systems to study neuroprotective or neurotoxicity of a compounds. Hydrogen peroxide (H_2O_2) has been used extensively as an inducer of OS in *in vitro* models (Uberti et al., 2002). In fact, hydrogen peroxide can cross cells membrane due to its high membrane permeability and induce rapid increase of production of ROS which in turn induce cytotoxicity (Franco et al., 2010). In current study, the spices were shown good protective effect on SK-N-SH cells against H_2O_2-induced oxidative stress by significantly increasing neuronal survival and reducing neuronal death. The most effective spice was *X. Parviflora*. This effect may be due to its flavonoids content. Antioxidants like flavonoids or other polyphenols can protect against oxidative stress-induced neuronal cytotoxicity by several mechanisms (Choi et al., 2021). For this instance, flavonoids such as epigallocatechin-3-gallate (EGCG) can target mitochondria where it acts locally as a free radical trap to protect neurons. Furthermore, flavonoids can activate transcription factors such as Nuclear factor erythroid 2-related factor 2 (Nrf2), which are implicated in the expression of antioxidant enzymes, and can directly regulate the mitochondrial apoptosis system and promote neuronal survival (Naoi et al., 2019; Teixeira et al., 2018). They can also act by stimulating neural function and promoting neurogenesis (Spencer, 2010). Finally, several experimental studies have suggested that curcumin, a polyphenol extracted in curcuma spice can act simultaneously in multiple molecular pathways, and in consequence protecting the cells from oxidative damage (Lindholm et al., 2016; Pannu and Bhatnagar, 2018).

5. Conclusion

The current study demonstrated clearly that the selected spices have high content in flavonoids and alkaloids with good *in vitro* anticholinesterase, radical scavenging activities, and neuroprotective effects against H_2O_2-induced neuronal stress. Therefore, these spices, particular *X. Parviflora* may have promising compounds which could be tested as potential therapeutic agent for the treatment or prevention of dementia like AD. However, the effects of *X. Parviflora* must be tested in *in vivo* models.

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Data Availability

The data of this study are available on request from the corresponding author.

Consent for publication

Not applicable.

CRediT authorship contribution statement

All authors participated in the conception and design of the study. REDK, FAE, JVNK, and BDAA conducted the study and prepared the first draft of the manuscript. FAE, DJNN, DEM, FM, PVH performed the statistical analysis and revised the manuscript. NJL, DEM revised the final form of the manuscript. All the authors read and approved the final version of the manuscript.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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