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*Original Research Article*

# *In Vitro* Antioxidant and Anti-Cholinesterase Properties of Essential Oils from Pepper Fruits (*Dennettia Tripetala* G. Baker)

Bukola C. Adedayo[1], Adeniyi A. Adebayo[1,2], Ganiyu Oboh[1]

[1]*Functional Foods and Nutraceutical Research Unit, Biochemistry Department, Federal University of Technology, Akure, Nigeria*
[2]*Department of Chemical Sciences (Biochemistry Option), Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria*

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ABSTRACT

Essential oils from different plant species have been reported to possess various biological activities. Thus, this study is aimed at investigating the antioxidant and anti-cholinesterase properties of essential oils from ripe and unripe pepper fruits (*Dennettia tripetala* G. Baker). Essential oil was extracted from ripe and unripe pepper fruits (100 g each) using a Clevenger apparatus. Thereafter, the antioxidant activities of the essential oil were assessed through several antioxidant assays. In addition, the effect of the essential oil on cholinesterases; acetycholinesterase (AChE) and butyrylcholinesterase (BChE) was assessed using spectrophotometric method. The results revealed that both ripe and unripe pepper fruits essential oils showed strong antioxidant activities and there was no significant difference in their antioxidant activities except for ferric reducing antioxidant power where unripe pepper fruit essential oil showed higher antioxidant activity (16.33 mgAAE/g) than ripe pepper fruit essential oil (12.55 mgAAE/g). In addition, unripe pepper fruit essential oil had a stronger inhibitory effect on AChE ($IC_{50}$ = 1.67 mg/mL) than that of ripe pepper fruit ($IC_{50}$ = 2.69 mg/mL), whereas there was no significant difference in their BChE inhibitory properties. Essential oils from both ripe and unripe pepper fruits had antioxidant properties and inhibited AChE and BChE activities *in vitro*. However, findings in this study revealed that ripening could reduce the biological activities of pepper fruit essential oils, most especially ferric reducing antioxidant property and acetylcholinesterase inhibition.

***Keywords:*** Anticholinesterase, Essential oil, *Dennettia tripetala*, Antioxidant.

## Introduction

Essential oils are volatile liquids present in different parts of plants. Essential oils are known to contain varieties of compounds such as monoterpenes, sesquiterpenes, etc. Essential oils from different plant species has gained public interest for their applications in the food and cosmetic industries and as an active agent in disease prevention/management.[1-3] Previous studies have highlighted various biological activities of essential oils which include antimicrobial, anti-inflammatory, anticancer and antidiabetic activities.[4-6] Plants constitute a vital component of biodiversity as they play a key role in maintaining environmental balance and eco-stability.

Pepper fruit (*Dennettia tripetala* G. Baker), member of Annonaceae family, is commonly consumed in the southern part of Nigeria. Locally, it is known as "Nkarika" by the Efiks, "Nmimi" by Igbos and "Igbere" among the Yorubas.[7] Pepper fruit is a medicinal plant normally used in folk medicine to cure fever, cough, and toothache. In addition, the fruits are usually taken with garden egg, and palm wine among Nigerians especially in the southern part of Nigeria where it is normally served to entertain guests during the coronation ceremony, yam festival and wedding ceremony.[8] Several authors have reported various biological activities of *D. tripetala*, which include insecticidal and hypoglycemic properties among others.[9-11]

Neurodegeneration is a gradual decline in the activities of the brain and associated neurons (central nervous system) that eventually lead to cognitive dysfunction. Neurodegenerative diseases are pathologies with multiple aetiologies.[12,13] Although, the basis for the disease is not yet fully understood, however several reports have linked neurodegeneration to the decline in some neurotransmitters in the brain. In addition, oxidative stress is another culprit in the pathogenesis and progression of neurodegenerative diseases.[14] Impairment of cholinergic transmission has been reported as one of the factors contributing to the pathogenesis of most neurodegenerative diseases.[15] Although researchers have worked extensively on some biological activities of pepper fruits extracts,[10,16-19] necessary information on the effect of ripening on the biological activities of pepper fruits essential oil is missing from the literature. Therefore, the present study investigated the effect of ripening on the *in vitro* antioxidant and anti-cholinesterase properties of pepper fruits essential oils.

## Materials and Methods

### *Chemicals*

Acetylcholinesterase (from electric eel, type VI-S), butyrylcholinesterase (from equine serum), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide, butyrylthiocholine iodide were obtained from Sigma-Aldrich Co. (Steinheim, Germany). Potassium ferricyanide, potassium persulphate, quercetin, gallic acid, ascorbic acid, trichloroacetic acid, ferric chloride, and sodium carbonate were procured from BDH Chemicals Ltd. (Poole, England).

*Corresponding author. E mail: chrisadeb2013@yahoo.com
Tel: +2348039115201

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*Sample collection*

Ripe and unripe pepper fruits were purchased from Idanre market, Idanre, Nigeria during rainy season. The identification of samples was carried out at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure. Nigeria and the voucher number FUTA/0299 was allotted. Both ripe and unripe pepper fruits were washed to remove dirt, chopped into small pieces and air-dried at room temperature. After drying, samples were ground into coarse powder using electric blender and were kept in air-tight containers for extraction.

*Essential oil extraction process*

Essential oil extraction from the samples was carried out using a Clevenger apparatus. In brief, 100 g of powdered sample was weighed into a 1000 mL round bottom flask placed on the heating mantle. 500 mL of distilled water was added and mixed thoroughly. The Clevenger apparatus was then set up for essential oil extraction. The yields are 3.10 mL and 2.50 mL for ripe and unripe pepper fruit, respectively. The essential oils were collected inside sealed vials and stored in the refrigerator (4$^{o}$C) for further analyses.

*Total phenolic and flavonoid contents determination*

The total phenolic content of the essential oils was determined using the Folin-Ciocalteu method as described by Oboh *et al.*[20] with slight modification. Whereas, the total flavonoid content was determined using the standard method of Meda *et al.*[21] In total phenol content determination, gallic acid was used as standard and expressed as gallic acid equivalent (GAE) while quercetin was used as standard for total flavonoid content determination.

*1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay*

1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability of the essential oils was determined as described by Gyamfi *et al.*[22]

*$Fe^{2+}$ chelating assay*

The $Fe^{2+}$ chelating ability of the samples was determined in a reaction mixture containing 150 µL of freshly prepared ferrous sulfate (500 µM), 168 µL tris buffer (0.1 M; pH 7.4), 218 µL of 0.9% sodium chloride and 0 – 100 µL of essential oils. The mixture was incubated at 37$^{o}$C for 5 minutes. Colour was developed by adding 13 µL of 0.25% 1,10-orthophenanthroline. Absorbance was subsequently measured at 510 nm, and the Fe (II) chelating ability was subsequently calculated and expressed as percentage.[23]

*Ferric reducing antioxidant power*

The ferric reducing antioxidant power (FRAP) of the essential oils was determined by assessing their ability to reduce $Fe^{3+}$ to $Fe^{2+}$ as described by Oyaizu.[24]

*Cholinesterases inhibition assay*

The effect of the essential oils on cholinesterases (AChE and BChE) activities was determined using the method of Oyeleye *et al.*[25] The assay was carried out in a reaction mixture 200 µL of AChE solution, 100 µL of 5, 5' dithiobis(2-nitrobenzoic) acid, 0 – 100 µL of essential oil and 500 µL phosphate buffer (0.1 M; pH 8.0). The mixture was incubated at 25$^{o}$C for 20 minutes and 100 µL of substrate (acetylthiocholine iodide or butyrylthiocholine iodide) was added. AChE activities was monitored for 3 minutes using UV/Visible spectrophotometer. The inhibitory effect of the essential oils on cholinesterases (AChE and BChE) activities was subsequently calculated and expressed as percentage inhibition.

*Statistical analysis*

The results of the replicate readings were pooled and expressed as mean ± standard deviation (SD). Mean values were appropriately analyzed and compared using appropriate analysis of variance (ANOVA) followed by Duncan post-hoc test; significance level was accepted at $p < 0.05$. In addition, $IC_{50}$ was calculated using nonlinear regression analysis.[26] All statistical analyses were carried out using GraphPad Prism version 5.00 for windows.

## Results and Discussion

The results of total phenol and flavonoid contents of ripe and unripe pepper fruits essential oil are presented in Table 1. Ripening of the pepper fruits caused a significant ($p < 0.05$) decrease in the total phenolic content of the essential oil (unripe = 33.70 mgGAE/100 g) and (ripe = 31.04 mg/100 g). Whereas, there was no significant difference between total flavonoid content of essential oil of ripe (3.57 mgQE/100 g) and unripe (4.05 mgQE/100 g) pepper fruits. Phenolic compounds are generally known to exert beneficial effects especially in preventing certain diseases (such as diabetes, hypertension, and cancer), and as potent antioxidant and anticholinesterase agents.[27,28] Okoh *et al.*[29] reported that pepper fruit essential oils are rich in monoterpenoids and sesquiterpenoids which accounted for about 74.60% and 19.45% of the total oil contents in unripe pepper fruits and 75.10% and 20.10% in ripe pepper fruits. 2-methylphenyl formate, α-terpinene, and linalool were dominant monoterpenoids identified in ripe and unripe pepper fruits essential oil. Whereas, the analysis revealed an abundance of sesquiterpenoids which include; caryophyllene, α-farsenesene, *trans*-cadinol, caryophyllene oxide and L-ascorbic acid 2,6 dihexadecanoate.[29] However, some of these bioactive compounds were found to be present in a higher proportion in ripe pepper fruits essential oil than that of unripe pepper fruits, which could be a result of the effect of ripening on the bioavailability of these compounds.[17] The presence of functional groups such as "hydroxyl" in linalool, terpien-4-ol and *trans*-cadinol, "alkanoate" in 2-methylphenyl formate and ascorbic acid 2,6 dihexadecanoate and "ether" in safrole could contribute to the biological activities reported in this study.[30,31]

The antioxidants activities of ripe and unripe pepper fruits essential oils in this study as typified by their ferric reducing antioxidant power (FRAP), $Fe^{2+}$ chelating, ABTS and DPPH radicals scavenging activities. The ferric reducing antioxidant property presented as ascorbic acid equivalent revealed that unripe pepper fruits essential oil (16.33 ± 0.30 mgAAE/g) was significantly ($p < 0.05$) different from ripe pepper fruit essential oil (12.55 ± 0.45 mgAAE/g) (Table 2). Conversely, there was no significant difference in ABTS radical scavenging ability of ripe and unripe pepper fruits essential oils (Table 2). Furthermore, both ripe and unripe pepper fruits essential oils scavenged DPPH radical in a concentration-dependent manner (Figure 1). However, there was no significant difference in the DPPH radical scavenging ability of ripe and unripe pepper fruits essential oils (Table 3). Similarly, ripe and unripe pepper fruits essential oils chelated $Fe^{2+}$ in a concentration-dependent manner (Figure 2). As shown in Table 3, there was no difference in their $Fe^{2+}$ chelating activities. Oxidative stress remains one of the major causes of neurodegenerative diseases possibly due to the high content of polyunsaturated fatty acids in the brain.[32,33] Hence, antioxidants would be of immense benefit in the management/treatment of neurodegenerative diseases, because of the high utilization of metabolic oxygen by the brain, there is a tendency of increased production of reactive oxygen species (ROS). In the brain, ROS induced lipid oxidation due to its high content of phospholipids which are easily attacked by free radicals, thereby causing a gradual decline in neuronal and cognitive functions.[34] Homeostasis of iron is crucial for normal brain activity, and any alteration in the iron homeostatic mechanisms may lead to iron overload, which is harmful to the brain and neurons.[35] Iron catalyzes the formation of hydroxyl radicals via Fenton reaction, elevating lipid peroxidation noticed in the substantial nigra of patients with Alzheimer's disease.[36] Interestingly, $Fe^{2+}$ chelating activities of essential oils used in this study could be of therapeutic importance against neurodegenerative diseases by preventing iron overload in the brain. Oxidative stress has been implicated in the etiology of several neurodegenerative diseases.[37] Thus, antioxidant therapy is an important mechanism in the overall prevention and management of neurodegenerative diseases. Therefore, the *in vitro* antioxidant properties of the essential oils could be of therapeutic importance in the management and/or prevention of neurodegenerative diseases. Antioxidant property of plant samples is an important factor underlying their consistent usage in the management of human diseases.[38-40]

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The effects of ripe and unripe pepper fruits essential oils on cholinesterases (AChE and BChE) activities are presented in Figures 3 and 4, respectively. The results revealed that ripe and unripe pepper fruits essential oils inhibited both AChE and BChE activities in a concentration-dependent manner. However, unripe pepper fruits essential oil had higher AChE inhibitory effect ($p < 0.05$) ($IC_{50}$ = 1.67 mg/mL) than ripe pepper fruits essential oil ($IC_{50}$ = 2.69 mg/mL); whereas, there was no significant difference in their BChE inhibitory effect (Table 3). In combating neurodegenerative diseases, inhibition of AChE and BChE activities is important as this increases the level of neurotransmitters (acetylcholine and butyrylcholine), thereby enhancing communication between nerve cells.[41-43] The anticholinesterases effect of the essential oils could be attributed to the presence of some constituents of essential oils such as linalool, α-pinene, and caryophyllene which have been previously reported to possess anticholinesterase effects.[44,45] Findings in this study showed that the anticholinesterase potentials displayed by ripe and unripe pepper fruit essential oils could be of pharmacological importance in the management of neurodegenerative diseases (such as Alzheimer's and Parkinson's diseases) and cognitive impairment.[46] Moreover, BChE inhibition lowers the production of plaques that are toxic to the brain and increase brain susceptibility to neurodegeneration.[47,48]

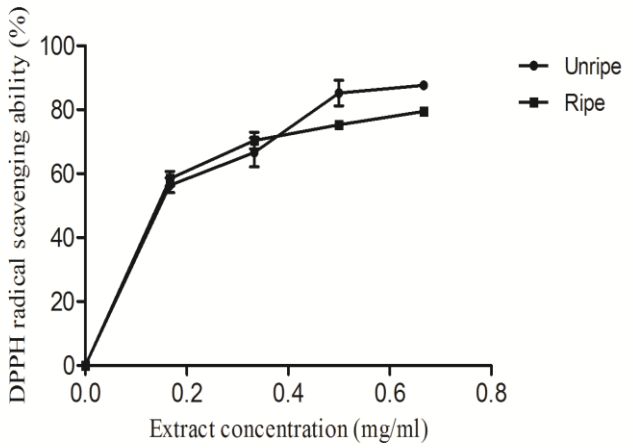


**Figure 1:** DPPH radical scavenging ability of ripe and unripe pepper fruits essential oil

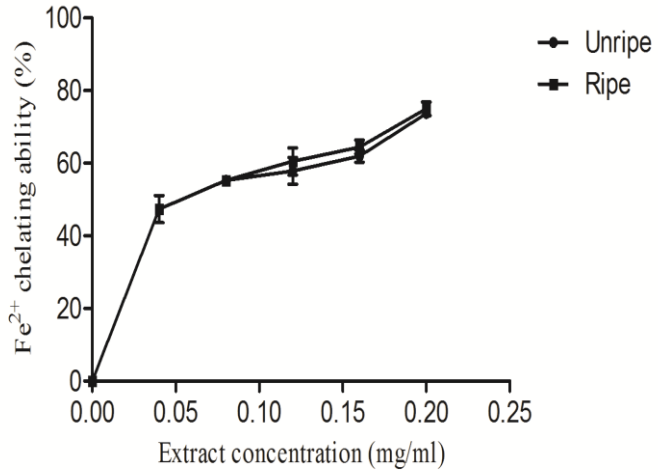


**Figure 2:** $Fe^{2+}$ chelating ability of ripe and unripe pepper fruits essential oil

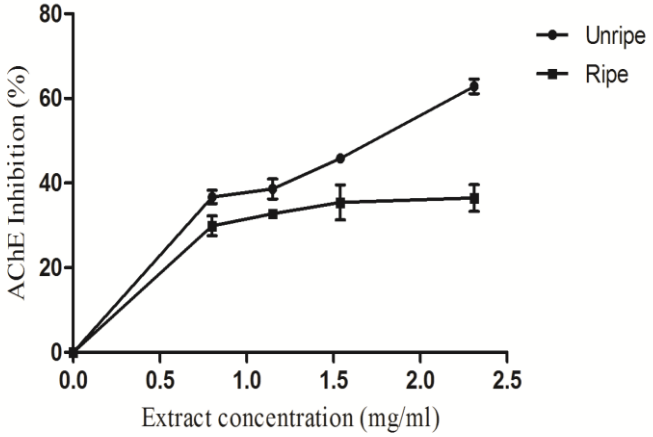


**Figure 3:** Acetylcholinesterase (AChE) inhibitory effect of ripe and unripe pepper fruits essential oil

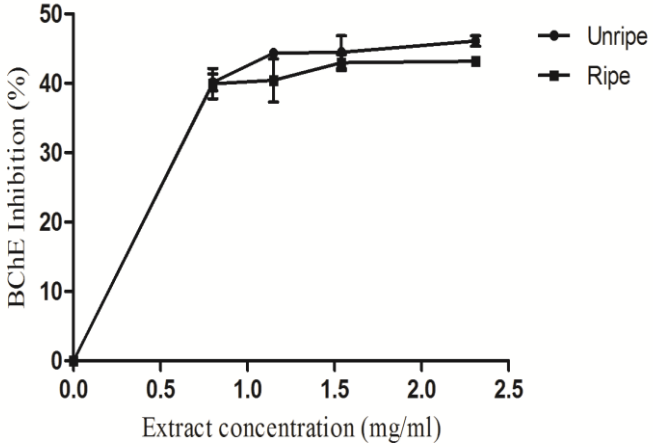


**Figure 4:** Butyrylcholinesterase (BChE) inhibitory effect of ripe and unripe pepper fruits essential oil

**Table 1:** Total phenol and flavonoid contents of ripe and unripe pepper fruits essential oil

| Sample | Total phenol (mgGAE/100g) | Total flavonoid (mgQE/100g) |
|---|---|---|
| Ripe pepper fruits | 31.04 ± 1.41[a] | 3.57 ± 0.30[a] |
| Unripe pepper fruits | 33.70 ± 6.36[b] | 4.05 ± 0.24[a] |

Values are expressed as mean ± standard deviation of replicate experiments (n = 3). Values with the same superscript alphabet along the same column are not statistically different at $p < 0.05$. GAE: Gallic acid equivalent; QE: Quercetin equivalent.

**Table 2:** Ferric reducing antioxidant property (FRAP) and ABTS radical scavenging activity of ripe and unripe pepper fruits essential oil

| Sample | FRAP (mgAAE/g) | ABTS (mmolTEAC/g) |
|---|---|---|
| Ripe pepper fruits | 12.55 ± 0.45[a] | 4.53 ± 0.63[a] |
| Unripe pepper fruits | 16.33 ± 0.30[b] | 4.32 ± 0.00[a] |

Values are expressed as mean ± standard deviation of replicate experiments (n = 3). Values with the same superscript alphabet along the same column are not statistically different at $p < 0.05$. AAE: Ascorbic acid equivalent; TEAC: Trolox equivalent antioxidant capacity.

**Table 3:** $EC_{50}$ values of DPPH radical scavenging and $Fe^{2+}$ chelating, and $IC_{50}$ values of cholinesterases (AChE and BChE) inhibitory activities of ripe and unripe pepper fruits essential oil

| Parameters (mg/mL) | Ripe pepper fruits | Unripe pepper fruits |
|---|---|---|
| DPPH radical scavenging | 0.27 ± 0.01a | 0.26 ± 0.01a |
| Fe2+ chelation | 0.10 ± 0.03a | 0.10 ± 0.00a |
| AChE inhibition | 2.69 ± 0.07b | 1.67 ± 0.04a |
| BChE inhibition | 2.12 ± 0.06a | 1.96 ± 0.05a |

Values are expressed as mean ± standard deviation of replicate experiments (n = 3). Values with the same superscript alphabet on the same row are not statistically different at $p < 0.05$.

## Conclusion

Pepper fruits essential oils possess antioxidant properties and inhibited AChE and BChE activities *in vitro*. However, findings in this study revealed that ripening could reduce the biological activities of pepper fruit essential oils most especially ferric reducing antioxidant property and acetylcholinesterase inhibition.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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