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Effect of Blanching on Inhibition of Cholinesterases and Antioxidative Properties of Phenolic Extracts of African Lettuce *(Launaea taraxacifolia)*

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ABSTRACT: This study sought to assess the influence of blanching on the inhibition of key enzymes linked to neurodegeneration and the antioxidative properties of phenolic extracts of African lettuces. The phenolic extracts of blanched and unblanched African lettuces leaves were prepared using the mixture of absolute 1 M HCl and Methanol (1:1 v/v). Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) *in vitro* inhibitory properties, antioxidant properties (Fe^{2+} chelating ability, DPPH* radical scavenging ability), total phenol and flavonoid contents of the extract were determined. The results showed that unblanched African lettuces (AChE= 0.25 mg/ml, BChE=0.27 mg/ml) had significantly ($p<0.05$) higher inhibitory effect on AChE and BChE activities than blanched African lettuces (AChE= 0.34 mg/ml, BChE= 0.28 mg/ml). The result of the study revealed that blanching caused a decrease in the AChE and BChE inhibitory properties, antioxidant capacity, total phenol and total flavonoid contents of African lettuces. Therefore, the increased antioxidant and anticholinesterase properties of the unblanched African lettuce extract could be linked to its higher concentrations of the phenolic constituents compared to the blanched African lettuce.

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Neurodegenerative diseases are incurable and debilitating conditions that result in progressive degeneration and/or death of nerve cells. Both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for the breakdown of acetylcholine in the synaptic region and low levels of acetylcholine has been found to be related to age- related disorders that leads to loss of cognitive ability (Fusco *et al.*, 2007). In Alzheimer's disease (AD), cholinesterase (ChE) inhibitors, such as rivastigmine and galantamine, are employed to restore the acetylcholine levels and positively influence the AD patient (Wszelaki *et al.,* 2010). Most of these synthetic drugs used in the treatment/management of AD are selective AChE inhibitors. However, these AChE inhibitors come with several side effects, such as anorexia dizziness, nausea, vomiting, hepatotoxicity, and dyspepsia (Chaiyana and Okonogi, 2012; Schneider, 2004; De-Paula *et al.*, 2009). Phytochemicals are naturally, occurring bioactive non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have linked to reducing the risk of major degenerative diseases (Liu, 2004). It is defined as plant-derived chemicals which are beneficial to human health and disease prevention (Agte *et al.*, 2000). The availability of these phytochemicals in plants is not unrelated to the antioxidant potential and medicinal properties of the plants and their extracts. African Lettuce (*Launaea taraxacifolia)* also known as wild lettuce occurs mainly in the tropics. It is an erect perennial herb with leaves at the base of the stem in a rosette form. Apart from its use as a common vegetable, it is also eaten by some people as salad or cooked in soups and sauces (Adebooye *et al.*, 2003). African lettuce are usually blanched, a process whereby the vegetable is treated with hot water, before preparation in soups or sauces, in order to increase its palatability and acceptability. Hence, this study sought to assess the effect of blanching on the inhibitory activities of African lettuce plant extracts on enzymes linked to neurodegenerative diseases *in-vitro*.

MATERIALS AND METHODS

Sample Collection: African Lettuce (*Launaea taraxacifolia*) leaves were collected from a local farm in Akure Ondo State. Identification of the sample was carried out in Crop Soil and Pest Department, Federal University of Technology Akure, Ondo State. Chemicals and reagents used were of analytical grade.

Preparation of the phenolic-rich extracts: Two grammes of each powdered sample were extracted

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with the mixture of 100 mL of 1 M HCl and Methanol (1:1 w/v). The filtrate was then evaporated to dryness. *Determination of Acetylcholinesterase and Butyrylcholinesterase Inhibitory assays:* Inhibition of AChE was assessed by a modified colorimetric method of Perry *et al.,* 2000. The AChE activity was determined in a reaction mixture containing 200 µL of a solution of AChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 µL of 5, 5'-dithiobis (2-nitrobenzoic) acid solution (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mM), the extracts (0 - 100 µL), and 50 µL phosphate buffer, pH 8.0. After incubation for 20 min at 25ºC, 100 µL of 0.05 mM acetylthiocholine iodide solution was added as the substrate, and AChE activity was determined as changes in absorbance reading at 412 nm for 3 min at 25°C using a spectrophotometer. 100 µL of butyrylthiocholine iodide was used as a substrate to assay butyrylcholinesterase activity, while all other reagents and conditions were the same. The AChE and BChE inhibitory activities were expressed as percentage inhibition.

Determination of free radical scavenging ability: The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhdrazyl) free radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, appropriate dilution of the extracts (0 – 500 µl) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

*Iron (Fe^{2+}) chelation assay***:** The Fe^{2+} chelating ability of the ripe and unripe pepper fruit extracts were determined using a modified method of (Minotti and Aust, 1987) with a slight modification by (Puntel *et al.,* 2005). Freshly prepared 500 µmol L^{-1} $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl of 0.1mol l^{-1}Tris-HCl (pH 7.4), 218µl saline and the extracts (0 – 100 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1; 10-phenanthroline (w/v).The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated.

Determination of Total Phenol Content: The total phenol content was determined according to the method of Singleton *et al.,* (1999). Briefly, appropriate dilutions of the extracts (200 µl) were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by the addition of 2.0 ml of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45ºC and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of Total Flavonoid Content: The total flavonoid content was determined using a slightly modified method reported by Meda *et al.,* (2005). Briefly 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl 10% $A1C1_3$, 50 µ1 of 1 M Potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm; the total flavonoid content was subsequently calculated.

Data Analysis: The results of replicate readings were pooled and expressed as means ± standard deviation. Significance was accepted at $P \leq 0.05$. The IC_{50} (phenolic-rich extract concentration causing 50% enzyme inhibition/antioxidant activity) was determined using non-linear regression analysis.

RESULTS AND DISCUSSION

The ability of the phenolic-rich extracts of the blanched and unblanched African lettuces to inhibit acetylcholinesterase and butyrylcholinesterase activities *in vitro* was investigated and the results are presented in Figure 1 and 2 respectively with their IC_{50} in Table 1. The result revealed that both extracts inhibited acetylcholinesterase in a concentration-dependent manner (0.19 – 0.57 mg/ml). However, the unblanched African lettuces (IC_{50}= 0.25 mg/ml) had the highest acetylcholinesterase inhibitory activity than the blanched African lettuces (IC_{50}= 0.34 mg/ml). Also, the result of the inhibition of butyrylcholinesterase revealed that both extracts inhibit butyrylcholinesterase in a concentration dependent manner (0.19 – 0.57 mg/ml). However, there was no significant difference in the butyrylcholinesterase inhibitory activity of the blanched African lettuces (IC_{50}= 0.27 mg/ml) and that of the unblanched African lettuces (IC_{50}= 0.28 mg/ml).

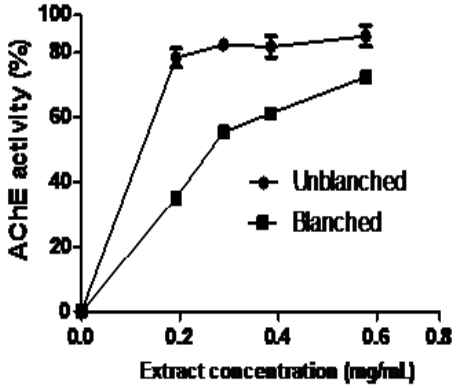

Fig 1. Acetylcholinesterase inhibitory activities of phenolic extracts of African lettuce

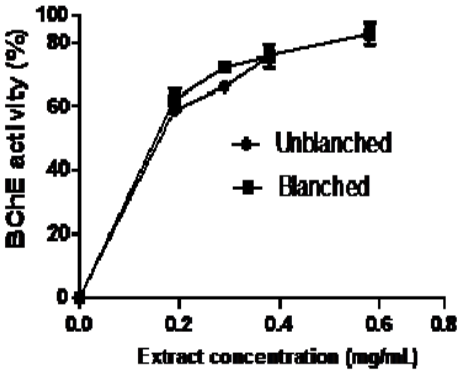

Fig 2. Butyrylcholinesterase inhibitory activities of phenolic extracts of African lettuce

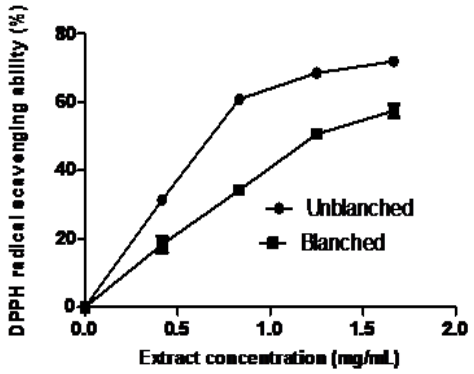

Fig 3. DPPH free radical scavenging ability of phenolic extracts of African lettuce

The DPPH* free radical scavenging ability of the phenolic extracts of both blanched and unblanched African lettuces is presented in Figure 3 as revealed by their IC_{50} value in Table 1, both blanched and unblanched African lettuces scavenged DDPH* radical in a concentration-dependent manner (0 – 1.66 mg/ml) with unblanched African lettuces (IC_{50}= 0.96 mg/ml) had significantly higher ($p<0.05$) DPPH* scavenging ability than the blanched African lettuces (IC_{50}= 1.33 mg/ml). Also, Fe^{2+} chelating ability of the phenolic extracts of both blanched and unblanched African lettuces as presented in Figure 4 revealed that both extracts of the African lettuces were able to chelate Fe^{2+} in a concentration-dependent manner. However, the unblanched African lettuces (IC_{50}= 0.40 mg/ml) had the highest Fe^{2+} chelating ability than blanched African lettuces (IC_{50}= 0.56 mg/ml). Blanching decreased the antioxidant activity of the leaves; this is also in agreement with several earlier studies that revealed that blanching reduces the antioxidant activity of vegetables (Oboh, 2005; Irondi *et al.,* 2016; Adedayo *et al.,* 2015; Bamidele *et al.*, 2017). This effect has been attributed to the loss in the phenolics and other water soluble phytoconstituents due to leaching and thermal degradation during blanching (Oboh, 2005).

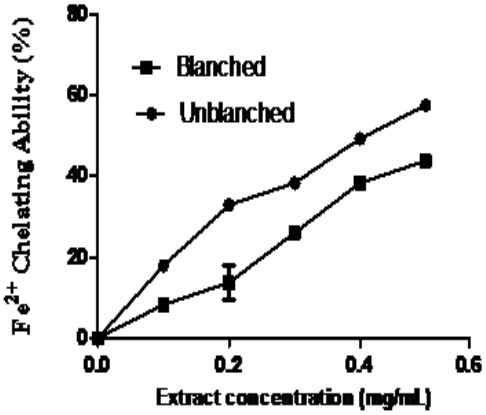

Fig 4. Fe^{2+} chelating ability of phenolic extracts of African lettuce

Table 1. IC_{50} of AChE, BChE, DPPH* radical scavenging ability, and Fe^{2+} chelating ability of the phenolic extracts of both unblanched and blanched African lettuce

	Sample IC_{50} (mg/mL)	
	Unblanched	**Blanched**
AChE	0.25 ± 0.14^a	0.34 ± 0.28^b
BChE	0.27 ± 0.07^a	0.28 ± 0.05^b
DPPH	0.96 ± 0.58^a	1.33 ± 0.68^b
Fe^{2+} Chelation	0.40 ± 0.19^a	0.56 ± 0.19^b

The values represent the means ± standard deviation of replicate readings. The values on the same row with the same superscript letter are not significantly different (P > 0.05).

Table 2. The total phenol (mg/100gGAE) and total flavonoid (mg/100g QE) of the phenolic extract of unblanched and blanched African lettuce

Samples	Total phenol (mgGAE/100g)	Total flavonoid (mgQE/100g
Unblanched	1.31 ± 0.61^b	0.19 ± 0.01^a
Blanched	0.72 ± 0.20^a	0.33 ± 0.09^b

The values represent the means ± standard deviation of replicate readings. The values on the same column with the same superscript letter are not significantly different (P > 0.05).

The results of the total phenol, and total flavonoid of the phenolic-rich extract of blanched and unblanched African lettuces are presented in Table 2, unblanched African lettuces (1.31 mg/ml) has significantly ($p<0.05$) higher total phenol content than blanched African lettuces (0.72 mg/ml), while the flavonoid content of blanched African lettuces (0.33 mg/ml) is higher than unblanched African lettuces (0.19 mg/ml). This result agrees with the findings of Oboh (2005), who reported a decrease in the phytochemicals of some green leafy vegetables; Adedayo *et al.* (2015), in fireweed and Bamidele *et al.* (2017), who reported decrease in the total phenolic with increase in blanching time.

Conclusion: The results of this study revealed that unblanched and blanched African lettuce leaf extracts exhibited both anticholinesterase and antioxidant properties. However, unblanched African lettuce leaf extract is more potent. This probably could be linked to its higher concentrations of the phenolic

constituents compared to blanched African lettuce that have lost most of the phenolic constituents due to the blanching process.

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