



INHIBITORY ACTION OF CORCHORUS OLITORIUS ON KEY ENZYME LINKED TO ALZHEIMER'S DISEASE AND Fe^{2+} -INDUCED LIPID PEROXIDATION IN RAT BRAIN.

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ABSTRACT

This study sought to investigate the inhibitory effect of different extracts of Corchorusolitorius(jute leaf) on butyrylcholinesterase (BChE) and Fe^{2+} -induced lipid peroxidation in rat brain in vitro. Free phenolic, bound phenolic, methanolic and aqueous extracts of this vegetable were prepared and used subsequently for analysis. Total phenol, total flavonoid contents, Fe-chelating ability, lipid peroxidation and butyrylcholinesterase in rat brain in vitro were determined. The results showed that free phenolic extract had the highest total phenol and flavonoid contents while aqueous extract had the lowest. All the extracts chelated iron and inhibited Fe-induced lipid peroxidation as well as butyrylcholinesterase in rat brain in a dose-dependent manner. Furthermore, free phenolic extract also exhibited the highest ability to chelate iron and also to inhibit lipid peroxidation in rat brain. Methanol extract showed the highest inhibitory effect on butyrylcholinesterase activity. Consequently, the mechanism by which Corchorusolitorius exerts its neuroprotective action might be through the inhibition of BChE activity and prevention of lipid peroxidation in the brain. Considering the potentials of this vegetable in the treatment of Alzheimer's disease, further investigations on its active constituents should be carried out.

Key words: Corchorusolitorius, butyrylcholinesterase, phenolic, lipid peroxidation, brain.

INTRODUCTION

Indigenous vegetables play important roles in human diets and health. They have been reported to be more nutritive and less expensive than the exotic ones. *Corchorusolitorius* Linn. (Tiliaceae), commonly called "ewedu" in the south western Nigeria is an annual herb with a slender stem. It is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asia such as Phillipine and Malaysia, as well as in tropical Africa, Japan, the Caribbean and Cyprus (Samraet al., 2007). The plant is widely grown in the tropics for the viscosity of its leaves. The leaves (either fresh or dried) are cooked into a thick

viscous soup or added to stew or soup (Tindall, 1983). In West African countries including Ghana, Nigeria and Sierra Leone, the vegetable is cultivated for the stem bark which is used in the production of fibre (Jute) and for its mucilaginous leaves which are also used as food vegetable (Zakariaet al., 2006). The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhoea, pain, fever and tumor (Ndlovu and Afolayan, 2008). Its leaves and roots are eaten as herbal medicine in South East Asia (Zeghichiet al., 2003). The seeds are used as a purgative and have been found to contain cardenolide glycosides on preliminary analysis (Gupta et al., 2003). The methanolic extracts of the

seeds have also been reported to possess a broad spectrum of antibacterial activity (Pal et al., 2006). *Corchorusolitorius* rich in beta-carotene for good eyesight, iron for healthy red blood cells, calcium for strong bones and teeth, and vitamin C for smooth, clear skin, strong immune cells, and fast wound-healing (Jute,2008). Jute leaf as a vegetable contains an abundance of antioxidants that have been associated with protection from chronic diseases such as heart disease, cancer, diabetes, hypertension and brain diseases as well as other degenerative diseases (Obohet al., 2012).

Butyrylcholinesterase (E.C. 3.1.1.8) also known as pseudocholinesterase, non-specific cholinesterase or simply cholinesterase. BChE is of pharmacological and toxicological importance, because it hydrolyzes ester-containing drugs and scavenges cholinesterase inhibitors including potent organophosphorus nerve agents before they reach their synaptic targets (Ravehet al, 1997). BChE has an important role in the development and progressing of Alzheimer's disease. It has peptidase activity besides of esterase activity (Chattonet and Masson, 1986). However, there is a dearth of information on the role of jute leaf (*Corchorusolitorius*) in neurodegenerative diseases such as Alzheimer disease therefore, this study sought to evaluate the inhibitory effect of different extracts of jute leaf on butyrylcholinesterase as well as their *in vitro* antioxidant properties in rat's brain.

MATERIALS AND METHODS

Sample preparation

Fresh green leafy vegetables (*Corchorusolitorius*, Jute leaves) were bought from Ibaka market, in Akungba town, Ondo State, Nigeria. Authentication of samples was carried out at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The leaves of *Corchorusolitorius*

were removed from the stalk and sun dried. The dried leaves were homogenized with a dry blender.

Extraction of free soluble phenols

The free soluble phenols of *Corchorusolitorius* were extracted using the method reported by Chu et al., (2002). 20g of the dried and blended sample was weighed and 80% (W/V) acetone solution, prepared by measuring 500 ml of acetone solvent and making up to 1000 ml with distilled water, was added in the ratio 1:10. This was homogenized for 3 minutes to obtain a thoroughly homogenized sample and then left overnight. The homogenates were then filtered (using Filter paper Whatman No. 2) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45°C until about 90% of the filtrate had evaporated. The phenolic extracts were then frozen at -40°C until further analysis and the residues were kept for the extraction of bound phenolics.

Extraction of bound phenols

The residue from the extraction above were flushed with nitrogen and hydrolyzed with about 20 ml of 4M NaOH solution at room temperature for 1 hour with shaking. The pH of the mixture was then adjusted to pH 2 with concentrated HCl and the bound phytochemicals were extracted using ethylacetate (6 times). The ethylacetate fractions were then evaporated at 45°C (Chu et al., 2002).

Methanolic extract preparation

200 ml of dilute HCl and 200 ml of methanol was added to 20 g of blended sample (ratio 1:1). The mixture was then allowed to stand for 2 days.

Aqueous extract preparation

20 g of the blended sample was weighed and soaked in 200 ml distilled water for 24 hours. This was then decanted and centrifuged at 4000 rpm for 10 minutes to

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obtain a clear supernatant which was then used for subsequent analysis (Obohet al., 2007).

Determination of total phenol content

The total phenolic content was determined according to the method of Singleton et al., (1999). Appropriate dilutions of the extracts were oxidized with 2.5 ml 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm in the JENWAY UV-Visible spectrophotometer 752S. The total phenol content was subsequently calculated, using gallic acid as standard phenol.

Determination of total flavonoid content

The total flavonoid content of *Corchorus olitorius* 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 μ l 10% $AlCl_3$, 50 μ l 1M potassium acetate and 1.4 ml of water. The mixture was allowed to incubate at room temperature for 30 minutes. The absorbance was subsequently measured in the spectrophotometer at 415nm. The total flavonoid content was then calculated using quercetin as standard.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the extracts were determined using the method reported by Puntelet al. (2005). Freshly prepared 500 μ M $FeSO_4$ (150 μ l) was added to a reaction mixture containing 168 μ l 0.1M Tris- HCl (pH 7.4), 218 μ l saline and the extracts (0-25 μ l). The reaction mixture was incubated for 5 minutes and thereafter, 13 μ l 0.25% 1, 10-phenanthroline (w/v) was added. The absorbance of the mixture was then measured at 510 nm in a spectrophotometer and the Fe^{2+} chelation ability was subsequently calculated.

Lipid peroxidation assay

Preparation of tissue homogenates

The rats were sacrificed by cervical dislocation and the brains were quickly isolated and placed on ice and weighed. These tissues were subsequently homogenized in cold saline (1/10 w/v) with about 10 up- and -down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 10 minutes at 3000 g to yield a pellet that was discarded and the low speed supernatant (S1) is kept for lipid peroxidation and butyrylcholinesterase assays.

Lipid peroxidation and Thiobarbituric acid reactions

The lipid peroxidation assay was carried out using a modified method of Ohkawa et al., (1979). 100 μ l of S1 fraction of rat's brain homogenates prepared in 150 mM NaCl solution (1:10 w/v) was mixed with a reaction mixture containing 30 μ l of 0.1 M Tris-HCl buffer (pH 7.4) and phenolic extract solution (0-100 μ l). The volume was then made up to 300 μ l with water before incubation at 37°C for 1 hour. The color reactions were developed by adding 300 μ l, 8.1% SDS to the reaction mixture containing S1. This was subsequently followed by addition of 600 μ l of acetic acid / HCl (pH 3.4) and 600 μ l, 0.8% TBA (thiobarbituric acid). This mixture was then incubated at 100°C for 1 hour and the absorbance of the thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm with spectrophotometer. Malondialdehyde was used as standard.

Butyrylcholinesterase (BChE) inhibition assay

The BChE inhibitory activity was determined using the modified colorimetric method of Ellman et al., (1961) with a reaction mixture containing 200 μ l of BChE solution in 0.1M phosphate buffer, pH 8.0,

100 µl of a solution of 5, 5- dithio- bis (2-nitrobenzoic) acid (DTNB 3.3 mM in 0.1 M phosphate buffer solution, pH 7.0, containing NaHCO₃ 6mM), 100 µl of a solution of the extracts and 500 µl of phosphate buffer, pH 8.0. After incubation for 20 minutes at 25°C, butyrylthiocholine iodide (100 µl of 0.05mM water solution) was added as the substrate. BChE activity

was then determined using the UV-Visible spectrophotometer, measuring absorbance changes at 412 nm for 3 minutes at 25°C. The BChE inhibitory activity was expressed as percentage inhibition. % Inhibition = $(Abs_{ref} - Abs_{sam}) / Abs_{ref} * 100$ where, Abs_{ref} = Absorbance of Reference, Abs_{sam} = Absorbance of Sample.

Table 1: Total phenolic and flavonoid contents of the bound, free, methanolic and aqueous extracts of Corchorusolitorius

Plant extract	Total phenol (mg/g)	Total flavonoid (mg/g)
COB	24.63 ± 0.46 ^c	3.44 ± 1.42 ^e
COF	57.83 ± 4.81 ^a	74.25 ± 8.32 ^a
COM	49.19 ± 3.59 ^b	37.06 ± 0.67 ^b
COA	19.60 ± 2.83 ^d	0.9 ± 0.23 ^d

Values represent means of 3 determinations ± SD. Values with different superscripts are statistically significantly different from one another (P<0.05). COB: bound extract of *Corchorusolitorius*, COF: free extract of *Corchorusolitorius*, COM: methanolic extract of *Corchorusolitorius*, COA: aqueous extract of *Corchorusolitorius*.

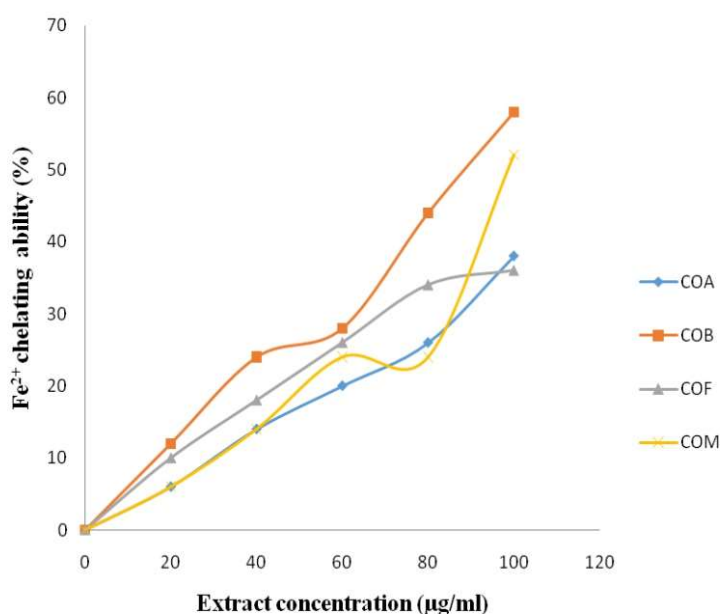


Figure 1. Fe²⁺ chelating activity of the aqueous, bound, free and methanolic extracts of Corchorusolitorius (µg/ml)

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KEY

COA: Aqueous extract of *Corchorusolitorius*

COB: Bound extract of *Corchorusolitorius*

COF: Free extract of *Corchorusolitorius*

COM: Methanolic extract of *Corchorusolitorius*

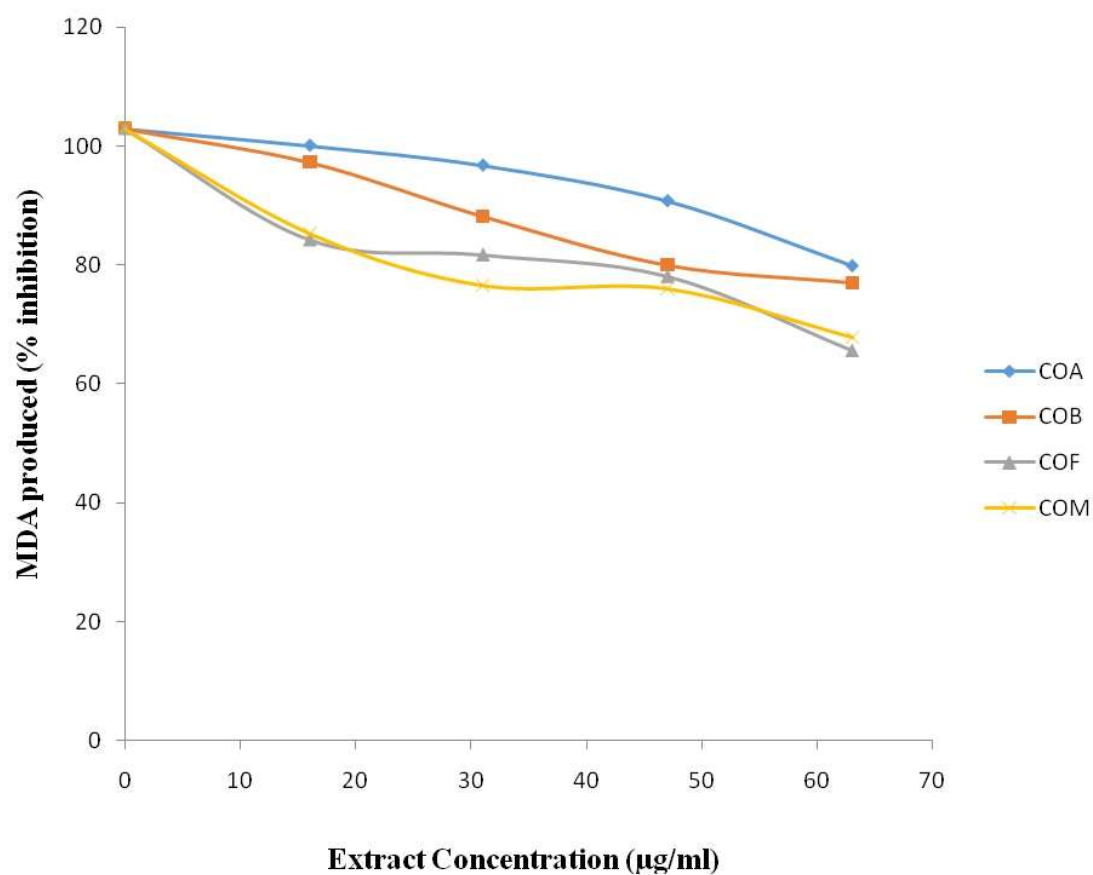


Figure 2. Inhibition of Fe^{2+} induced lipid peroxidation by *Corchorusolitorius*(µg/ml)

KEY

COA: Aqueous extract of *Corchorusolitorius*

COB: Bound extract of *Corchorusolitorius*

COF: Free extract of *Corchorusolitorius*

COM: Methanolic extract of *Corchorusolitorius*

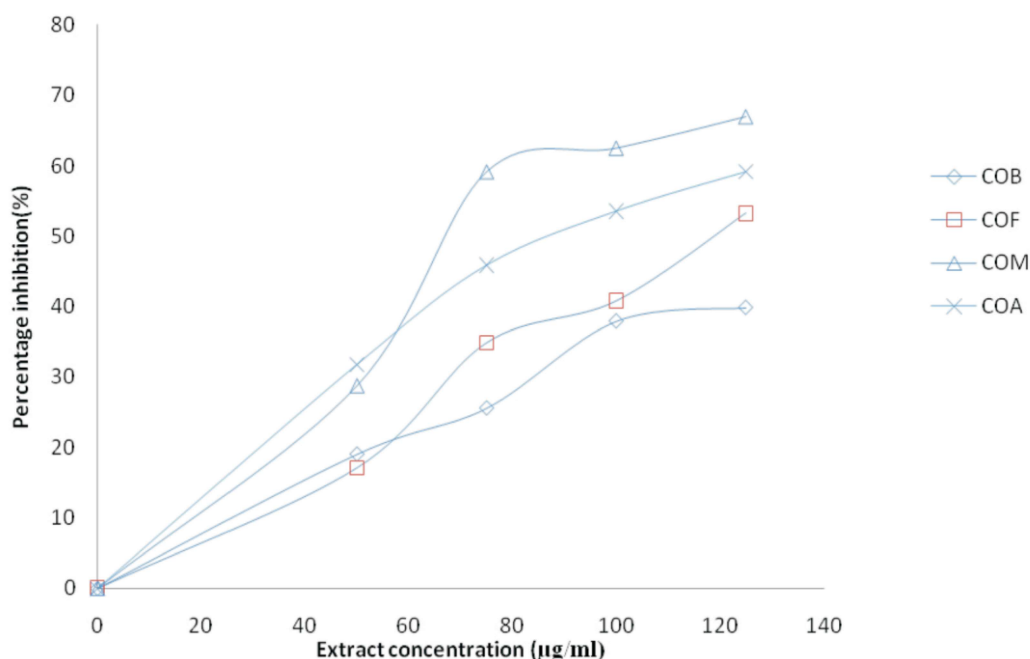


Figure 3. Brain butyrylcholinesterase inhibition by *Corchorusolitorius* ($\mu\text{g/ml}$)

KEY

- COA: Aqueous extract of *Corchorusolitorius*
 COB: Bound extract of *Corchorusolitorius*
 COF: Free extract of *Corchorusolitorius*
 COM: Methanolic extract of *Corchorusolitorius*

DISCUSSION

Several plants and their constituent phytochemicals with antioxidant activity have been shown to play important roles in the central nervous system and as such may be useful as therapeutics for management of Alzheimer's disease (AD). It is therefore believed that diets rich in such plants and their constituent phytochemicals as supplements may be relevant in slowing the progression of the disease (Howeset al., 2003). The antioxidant effects of this plant has been attributed to the redox properties of the hydroxyl groups of its phenolics (Perucka and Materska, 2005) achieved through several mechanisms including the scavenging of free radicals, chelation of transition metals serving as prooxidants, activation of antioxidant enzymes and inhibition of oxidases (Amicet al., 2003; Oboh, 2008).

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (Obohet al., 2007). Polyphenols are also considered to be strong antioxidants due to the redox properties of the hydroxyl groups of its phenolic constituents (Perucka and Materska, 2005). As shown in Table 3.1, the free extracts of *Corchorusolitorius* exhibited higher phenolic content than the aqueous, bound and methanolic extracts. Table 3.2 also shows that the free extract exhibited higher flavonoid content than the aqueous, bound and methanolic extracts. These findings agree with earlier reports by Chu et al., (2002) on the phenolic distribution in some common vegetables. Free phenolics are more readily absorbed and thus, exert beneficial bioactivities in early digestion. The significance of bound phytochemicals to human health is not clear (Amicet al., 2003; Sun et al., 2002).

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However, it is possible that different plants with different amounts of bound phytochemicals can be digested and absorbed at different sites of the gastrointestinal tract and play unique roles in maintaining human health.

The finding that Fe²⁺ caused an increase in MDA content in brain tissues incubated with Fe²⁺ could be attributed to the fact that Fe²⁺ can catalyze one- electron transfer reactions which generate reactive oxygen species, such as the reactive OH which is formed from H₂O₂ through the Fenton reaction(Zagoet al., 2000). Iron also decomposes lipid peroxides, thus generating peroxy and alkoxy radicals which favor the propagation of lipid peroxidation (Zagoet al., 2000). As shown in Figure 2 the free phenolic extract of *Corchorusolitorius*exhibited the highest inhibition of Fe²⁺ induced lipid peroxidation while the aqueous extract again showed the least inhibitory effect. This conforms with the levels of extractable phytochemicals present in *Corchorusolitorius*as the free extracts also showed the highest phenol and flavonoid content.

The results of the Fe²⁺ chelation assay did not agree with that of the inhibition of Fe²⁺ induced lipid peroxidation. This may suggest that Fe²⁺ chelation may not be the main mechanism through which antioxidants in *Corchorusolitorius*prevent lipid peroxidation in the brain. Antioxidants carry out their protective role on cells either by preventing the production of free radicals or by scavenging free radicals produced in the body or by reducing/chelating the transition metal composition of foods (Alia et al., 2003;

Amicet al., 2003; Obohet al., 2007).

The inhibition of cholinesterases has proven to be the most effective approach in clinical management of Alzheimer's disease (AD). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are the two cholinesterases present in the brain. Plants and their products have so far been found useful as butyrylcholinesterase inhibitors(Mukherjee et al., 2007). The determined butyrylcholinesterase activity showed that the methanol extract was the most potent. Inhibition of BChE is considered as a therapeutic strategy. Studies have shown that selective inhibition of BChE elevates acetylcholine levels and correlates more strongly with cognitive improvement (Casademont et al., 2003).

CONCLUSION

Inhibition of butyrylcholinesterase and Fe²⁺ induced lipid peroxidation in rat brain could be part of the mechanism by which green leafy vegetables such as *Corchorusolitorius* delay the onset of Alzheimer's disease. The cholinesterase inhibitory activity of the methanolic extract as well as the inhibition of Fe²⁺ induced lipid peroxidation by the free extract of *Corchorusolitorius* may be attributed to their phenolic and flavonoid contents. Their antioxidant properties may also contribute to their ability to protect the brain against oxidative damage induced by several pro-oxidants especially in the brain in AD where amyloid deposits result in brain damage, primarily exerting their neurotoxicity by oxidative damage of neurons.

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