

Full Length Research Paper

Antioxidant and anticholinesterase activities of aqueous extract of *Uraria picta* (Jacq.) DC

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Numerous plants have been used in treating/managing Alzheimer's disease in folklore. Inhibition of cholinesterase enzymes is an alternative way used in treating/managing Alzheimer's disease. This study therefore sought to investigate the interaction of aqueous extract of *Uraria picta* with key enzymes (acetylcholinesterase and butyrylcholinesterase) linked with Alzheimer's disease *in vitro*. Inhibition of acetylcholinesterase and butyrylcholinesterase, the total phenolic content and radical scavenging abilities were assessed *in vitro*. The extract inhibited acetylcholinesterase and butyrylcholinesterase in a dose dependent manner. Present in the extract are phenol and flavonoids. The extract also scavenged 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical (ABTS*) and hydroxyl radical (OH*) in a dose-dependent manner. Inhibition of acetylcholinesterase, butyrylcholinesterase and the exhibited antioxidant properties could make *U. picta* extract a good means in treating/managing Alzheimer's disease.

Key words: Alzheimer's disease, acetylcholinesterase, butyrylcholinesterase, *Uraria picta*, antioxidant.

INTRODUCTION

Medicinal role of herbs is an important topic in plant research. Finding natural antioxidants and anticholinesterases in plants is of great interest lately. Antioxidants have the ability to inhibit oxidative damage, prevent inflammatory conditions (Khanna et al., 2007), and prevent neurodegenerative conditions (Fusco et al., 2007). Some of the commercially available synthetic drugs used in treating neurodegenerative diseases have their side effects on prolonged use (Johnson et al., 2000; Wentrup et al., 2008; Winblad et al., 2007).

Dementia is a disease marked by gradual loss of cognitive functioning which can also incorporate losses of motor, emotion, and social functioning. It is a permanent and progressive disease that eventually renders people unable to care for themselves. Older people with dementia exist in nearly every country in the world. Dementia rates are predicted to increase at an alarming

rate in the least developed and developing regions of the world despite mortality resulting from malnutrition, poverty, war, and infectious diseases. World Health Organization (WHO) projections suggest that by 2025, about three-quarters of the estimated 1.2 billion people of ages 60 years and older will reside in developing countries (WHO, 2002). Thus, by 2040, if growth in the older population continues, and there are no changes in mortality or burden reduction by preventive measures, 71% of 81.1 million dementia cases will be in the developing world (Ferri et al., 2005). Oxidative stress is critical to the pathologies associated with brain damage and cognitive abilities (Markberry and Lovell, 2007). Although multiple factors are involved in the development of neurodegenerative diseases, dysregulation in the inflammatory network and oxidative imbalance are key components in the pathogenesis of diseases such as

Alzheimer's disease, Parkinson's disease, brain tumors, and multiple sclerosis (Marchetti and Abbracchio, 2005; Kannappan et al., 2011). The brain and nervous system are thought to be particularly vulnerable to oxidative stress due to limited antioxidant capacity (Marksberry and Lovell 2007). Present in the senile plaque of Alzheimer's disease patients are iron, ferritin, and transferrin (Tuppo and Forman, 2001; Ademosun and Oboh, 2012). Alzheimer's disease (AD) is the major cause of dementia. Cognitive decline in Alzheimer's disease patients is related to progressive cholinergic degeneration since studies have shown increased levels of cholinesterase enzymes in postmortem brain samples of Alzheimer's disease patients (Farfara et al., 2008; Snyder et al., 2001). So a promising approach to treating Alzheimer's disease patient is to enhance the level of cholinergic neurotransmitters in the brain by cholinesterase inhibitors (Scarpini et al., 2003). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are the two major cholinesterase enzymes and they play an important role in decreasing choline levels in the body.

Uraria picta (Jacq.) DC is a woody herb, single-stemmed, fibrous, 1 m high, or more and of grass savanna. It belongs to the family Leguminosae-Papilionoideae. It is common throughout the region in all states and widespread in tropical Africa, India, Asian tropics and Australia. In Nigeria, *U. picta* is locally known as 'Alupayida' (Yoruba), 'Obuodo dumbwada' (Igbo), 'kaskaifi', 'dakushe', 'wutsiyarbera', 'wutsiyarkusu' (Hausa) (Adegoke et al., 1968). The leaves of *U. picta* have a trace of alkaloid and they are considered to be antiseptic. It is also used in treating malaria (Adegoke et al., 1968). The dried powder is used in treating gonorrhea and for contractions of the uterus leading to abortion (Anislie, 1937). Studies have shown that cognitive function can be enhanced traditionally by some plants (Howes and Houghton, 2003), and *U. picta* has promising potential. However, there is dearth of information on inhibition of cholinesterase enzymes by *U. picta*. This study therefore sought to investigate the plant as a medicinal intervention in the management of Alzheimer's disease.

MATERIALS AND METHODS

Plant

U. picta leaves were sourced locally in Akure town and authentication was done in the Department of Biology, Federal University of Technology Akure, Nigeria.

Extract preparation

The aqueous extract of the medicinal plant was prepared by homogenizing 1 g of the plant in 20 ml-distilled water and the homogenate was centrifuged at 2000 rpm for 10 min. The supernatant was subsequently freeze-dried and used for subsequent assays (Oboh et al., 2010).

Reagents

All chemicals used in this study were of analytical grade, and glass-distilled water was used.

AChE and butyrylcholinesterase (BChE) inhibition assay

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 μ l of a solution of AChE (0.415 U/ml in 0.1 M phosphate buffer, pH 8.0), 100 μ l of a solution of 5,5'-dithio-bis(2-nitrobenzoic) acid (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing NaHCO₃ (6 mM), extract dilutions (0 to 100 μ l), and 500 μ l of phosphate buffer, pH 8.0. After incubation for 20 min at 25°C, acetylthiocholine iodide (100 μ l of 0.05 mM solution) was added as the substrate, and AChE activity was determined with an ultraviolet spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C.

Hundred microliters of butyrylthiocholine iodide was used as a substrate to assay butyrylcholinesterase enzyme, while all the other reagents and conditions were the same. The AChE and BChE inhibitory activity was expressed as percentage inhibition.

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). In brief, appropriate dilution of the extract was oxidized with 2.5 ml of 10% (v/v) Folin-Ciocalteu reagent and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C, and the absorbance was measured at 765 nm in a spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalents (GAE).

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. (2005). In brief, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml of methanol, 50 μ l of 10% AlCl₃, 50 μ l of 1 M potassium acetate, and 1.4 ml of 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. The non flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content. Quercetin was used as standard.

ABTS radical scavenging ability

The ABTS* scavenging ability of the extract was determined according to the method described by Re et al. (1999). The ABTS* was generated by reacting an (7 mmol/L) ABTS aqueous solution with K₂S₂O₈ (2.45 mmol/L, final concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extract was added to 2.0 ml ABTS solution and the absorbance was measured at 734 nm after 15 min. Trolox was used as standard and trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Fenton reaction (degradation of deoxyribose)

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the extract to prevent Fe²⁺/H₂O₂-induced

Table 1. Total phenol content, total flavonoid content, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging ability and IC₅₀ values of aqueous extract of *Uraria picta*.

Extract	Value
Phenol content (mg/100 g)	25.24 ± 1.67
Flavonoid content (mg/100 g)	19.43 ± 0.91
ABTS*scavenging ability (mmol/TEAC g)	0.233 ± 0.02
IC ₅₀ AChE (mg/ml)	39.12 ± 1.01
IC ₅₀ BChE (mg/ml)	39.02 ± 1.08

Values represent means ± standard deviation of triplicate readings.

decomposition of deoxyribose. The extract (0 to 100 µl) was added to a reaction mixture containing 120 µl of 20 mM deoxyribose, 400 µl of 0.1 M phosphate buffer, and 40 µl of 500 µM FeSO₄. The volume was made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was then stopped by addition of 0.5 ml of 28% trichloroacetic acid. This was followed by addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer.

Data analysis

The results of triplicate experiments were pooled and expressed as mean ± standard deviation (SD) (Zar, 1984). Statistical significance was determined by Student's t-test and IC₅₀ (inhibitory concentration) was determined using linear regression analysis.

RESULTS

The AChE inhibitory potential of *U. picta* extract was investigated and the result is shown in Figure 1; the result revealed that the extract inhibited AChE activity in a dose-dependent manner, having an inhibitory activity of 80.56% at the highest concentration (57.69) tested [IC₅₀ (inhibitory concentration) = 39.12 mg/ml] as presented in Table 1. Also, the ability of the extract to inhibit BChE activity *in vitro* was also investigated, and the result shown in Figure 2 revealed that the extract inhibited BChE in a dose-dependent manner with inhibitory activity of 77.27% at the highest concentration (57.69) tested [IC₅₀ = 39.02 mg/ml] (Table 1).

The results of the total phenol, total flavonoid content and ABTS radical scavenging ability of the extract which was reported as TEAC are shown in Table 1. The extract contained 25.24 mg/100 g total phenolic content and 19.43 mg/100 g total flavonoid content. The extract was able to scavenge ABTS radical having a scavenging ability of 0.233 mmol/TEAC g. Furthermore, the result of the hydroxyl radical (OH*) scavenging ability of the extract is as shown in Figure 3; the extract scavenged OH* in a dose-dependent manner having a scavenging ability of 84.96% at the highest concentration (71.43 mg/ml) tested.

DISCUSSION

AChE and BChE inhibition have been accepted as an effective model for treating/managing AD (Howes et al., 2003). The aqueous extract of the *U. picta* studied was able to inhibit AChE and BChE in a dose dependent manner. Cholinesterases inhibition by the *U. picta* studied could be of great importance as it is an acceptable therapeutic way in the management/treatment of neurodegenerative conditions. Also, in some forms of Alzheimer's disease, BChE variant has been shown to increase brain susceptibility, thereby making BChE inhibition of the extract an alternative approach in managing neurodegenerative conditions.

Once AChE is inhibited, acetylcholine breakdown in the brain becomes impossible. The consequent increase in the brain acetylcholine concentrations facilitates communication between nerve cells that use acetylcholine as a chemical messenger, and this may improve or stabilize the symptoms of Alzheimer's disease temporarily (Howes et al., 2003). Aqueous extract of *U. picta* was able to inhibit AChE and BChE activities in a dose-dependent manner *in vitro*. This AChE and BChE inhibition is in agreement with some earlier reports where plant phytochemicals from *Citrus medica* inhibited AChE and plants extracts of *Ginkgo biloba* and *Salvia lavandulaefolia*, showed a significant improvement in cognitive performance and memory (Mazza et al., 2006; Maruyama et al., 2006; Akhondzadeh and Abbasi 2006). This also agreed with a work reported by Oboh et al. (2010) where red and white ginger inhibited AChE activity *in vitro*. The ability of the extract to inhibit AChE and BChE could be due to the antioxidant ability of the plant.

Free radicals produced in the body can be neutralized/scavenged by the help of antioxidants (Oboh et al., 2007). Phenolic compounds can protect the body against free radicals, whose formation is associated with the normal metabolism of aerobic cells. These phenolics have the ability to remove free radicals, activate antioxidant enzymes, chelate metal catalysts, reduce α-tocopherol radicals and inhibit oxidases (Amic et al., 2003). Many plants have flavonoids and studies have conclusively shown that the majority of the antioxidant activity maybe from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin rather than from vitamins C, E and β-carotene (Marin et al., 2004; Oboh et al., 2007). Antioxidant activity of flavonoid makes them useful in lowering cellular oxidative stress (Oboh et al., 2007). Also, the free radical scavenging ability of the extract was investigated using a moderately stable nitrogen-centred radical species (ABTS) (Re et al., 1999). The aqueous extract exhibited scavenging ability and this is in agreement with the flavonoid content and some reports where correlations were reported between flavonoid content and antioxidant capacity of some plant foods (Amic et al., 2003), this also agrees with previous work where methanolic extract of *U. picta* showed free radical

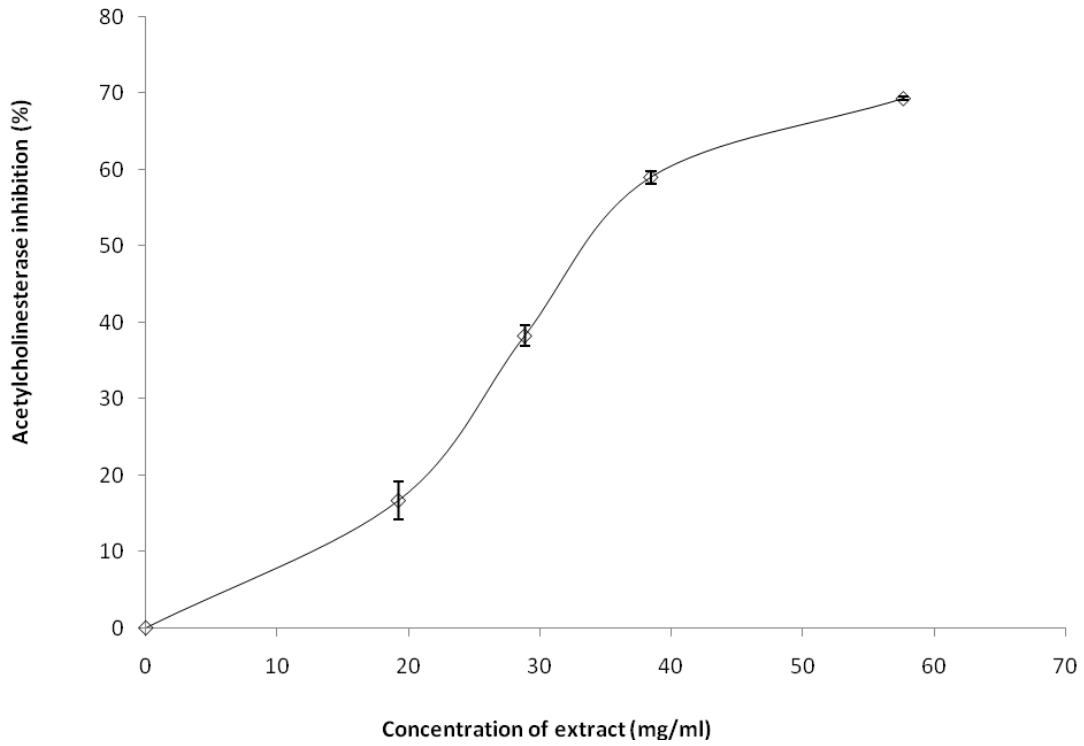


Figure 1. Acetylcholinesterase inhibitory activity of aqueous extract of *Uraria picta*.

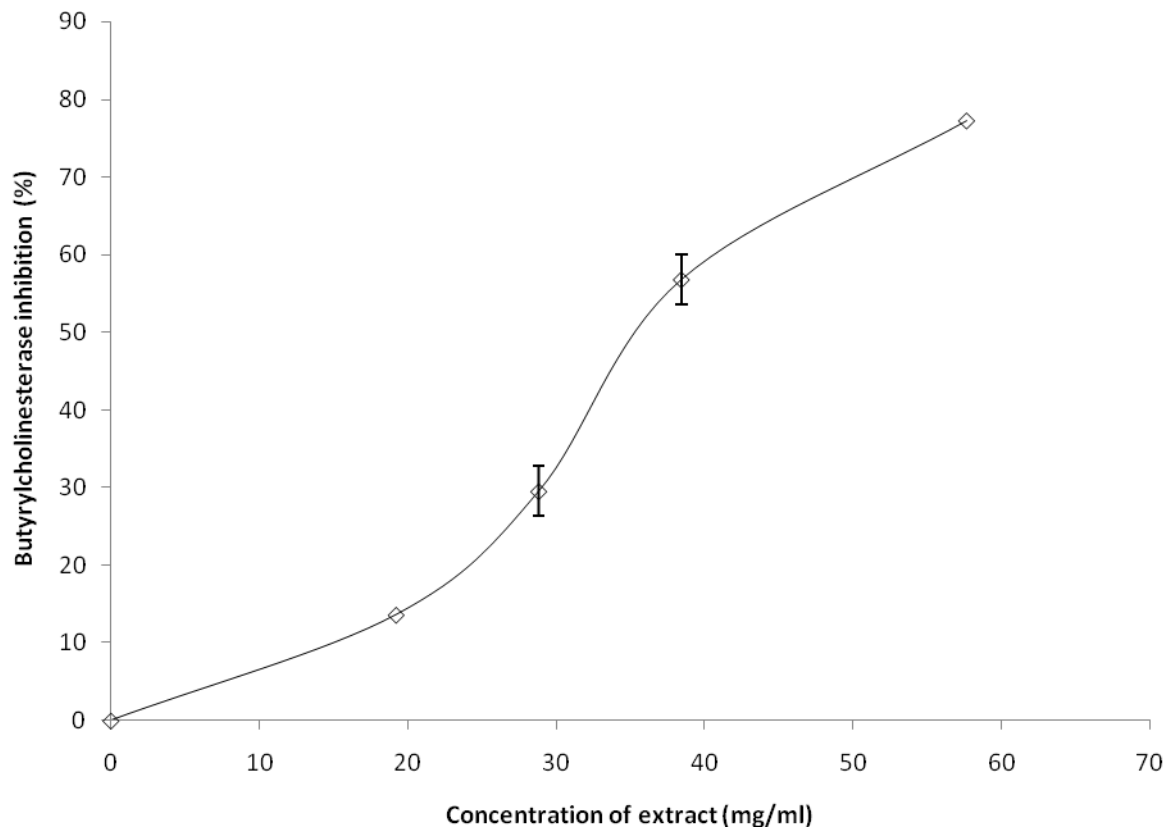


Figure 2. Butyrylcholinesterase inhibitory activity of aqueous extract of *Uraria picta*.

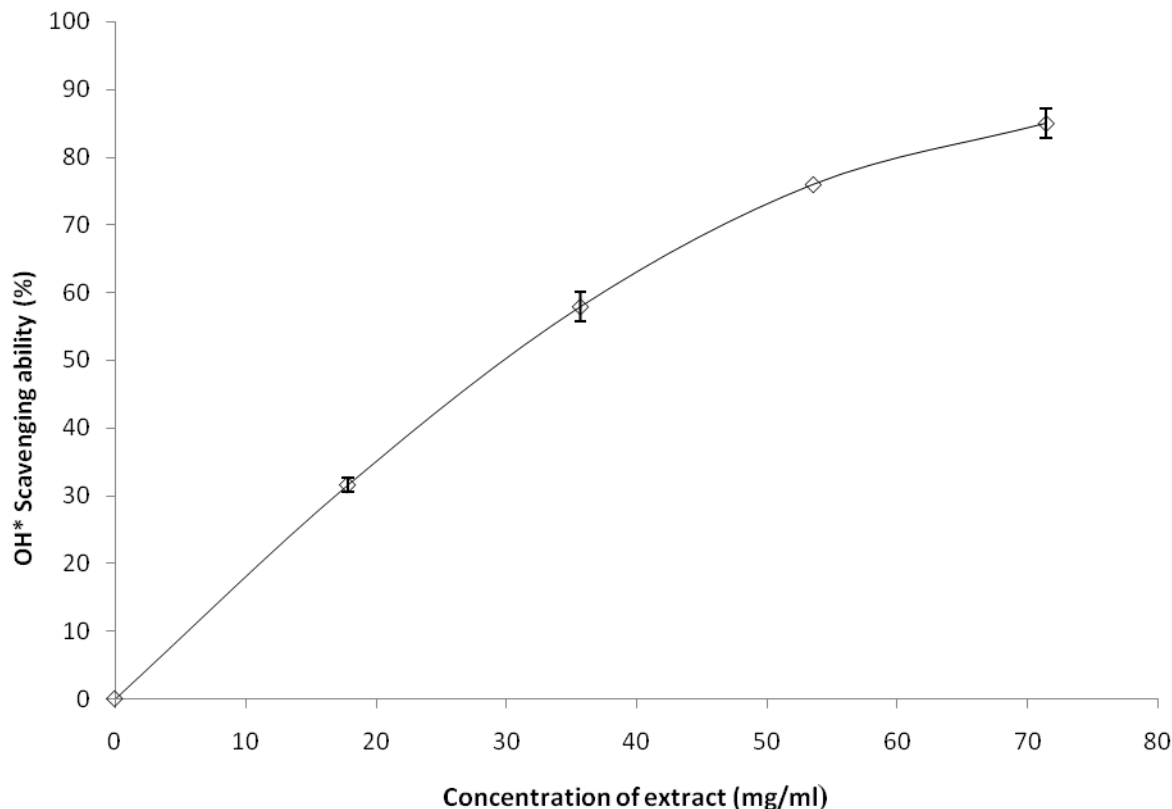


Figure 3. Hydroxyl radical (OH*) scavenging ability of aqueous extract of *Uraria picta*.

scavenging ability (Patel et al., 2011). Oxygen radicals are produced by iron in Fenton reaction. An electron is then donated to hydrogen peroxide by iron converting Fe^{2+} to Fe^{3+} , bringing about radicals. Present in the senile plaque of Alzheimer's disease patients are iron, ferritin, and transferrin (Tuppo and Forman, 2001; Ademosun and Oboh, 2012). Production of superoxide is catalyzed by a membrane-associated enzyme known as NADPH oxidase (Azumi et al., 1999). NADPH oxidase generates superoxide by transferring electrons from NADPH inside the cell across the membrane. Superoxide anion produced is a reactive free radical, which can lead to the production of hydrogen peroxide spontaneously, this will then undergo further reactions to generate reactive oxygen species, and it can also generate hydroxyl radicals. The extract, however, scavenged hydroxyl radicals in a dose-dependent manner. This result is important since oxidative damage is a pathogenesis in Alzheimer's disease and studies show that hydrogen peroxide mediates oxidative damage (Behl et al., 1994; Kim et al., 2003). Recent studies have shown that polyphenols have neuroprotection against damage caused by Fenton reaction (Heo and Lee, 2004; Ademosun and Oboh, 2012) and that they are able to cross the blood-brain barrier (Youdim et al., 2004; Abd El Mohsen et al., 2002; Ademosun and Oboh, 2012).

Conclusions

In these findings, it can be concluded that aqueous extract of *U. picta* inhibited AChE and BChE in a dose-dependent manner and exhibited radical scavenging ability due to the phytochemicals present in the extract and this could be part of the mechanism through which the extract helps in treating/managing Alzheimer's disease.

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