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Phytochemical screening and *in vitro* acetylcholinesterase inhibitory activity of seven plant extracts

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Abstract
Recent studies have shown the effectiveness of plants as enhancers of memory activity. This study sought to investigate the inhibitory effect of seven plants on acetylcholinesterase and its phytochemical contents. The in vitro acetylcholinesterase inhibitory effect by the seven plants and their phytochemical contents each, were examined in this study, using standard methods. Data was analyzed by ANOVA test and the value of $p \leq 0.05$ was considered significant. The plant extracts inhibition of acetylcholinesterase showed the following decreasing trend; *Carica papaya*-67.9%, *Solanum incanum* leaf – 66.9%, *Musa sapientum* -54.7%, *Solanum incanum* fruit -51.93%, *Persia americana* 46.60%, and *Kola acuminata* – 44.2% with the least inhibitory activity. All the seven plants showed remarkably the presence of all the phytochemicals especially flavonoids and the absence of steroids. The result of this study indicated that the seven chosen plants all showed acetylcholinesterase inhibitory activity, which may be the reason for its folkloric usage in Nigeria.

Keywords: Medicinal plants; Acetylcholinesterase; Acetylcholine; Inhibition; Phytochemicals

INTRODUCTION

Plants have played a major role in the history of drug discovery. It is a well-known fact that plants contain active compounds that have become new sources to investigate for, in the pharmaceutical industry. Plants constituents have the ability to enhance the activity of compounds or counteract toxic effects of compounds from other plant species (Howes and Houghton, 2003). In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuropharmacological disorders (Mukherjee *et al*., 2007a). These medicinal plants are readily affordable, available and with little or no side effects, when compared with the western pure and synthetic drugs.

Acetylcholinesterase is an enzyme which primarily inhibits acetylcholine, a neurotransmitter, considered to play a role in the pathology of Alzheimer's disease (AD) (Jann 1998). The pathological features identified in the central nervous (CNS) in AD are amyloid plaques, neurofibrillary tangles,

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inflammatory processes and disturbances of neurotransmitters (Bossy-Wetzel *et al*., 2004).

One of the main approaches to enhance cholinergic function in AD is the inhibition of acetylcholinesterase (AChE), a key enzyme in the degradation of acetylcholine. The availability of acetylcholine will mean that more of it is stored in the nerve terminals, and released for the nerve endings when the nerve terminal is depolarized, thereby entering the synapse and binding to the receptor. However, in patients with AD, the Ach released has a very short life span, due to the presence of large amounts of the enzyme, acetylcholinesterase (Houghton *et al*., 2006, Henrich and Teoh, 2004). Existing anticholinesterase drugs, for example, donepezil, tacrine and phygostgmine, used for the treatment of dementia are reported to have several dangerous adverse effects such as hepatotoxicity, short duration of biological action, low bioavailability, adverse cholinergic side effects in the periphery and a narrow therapeutic window (Burns and O'Brien, 2006, Sancheti *et al*., 2009, Sonkusare, *et al*., 2005).

Phytochemicals are plant secondary metabolites and have bioactive constituents with properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Kennedy and Wightman, 2011).

Since these plants have been used in the treatment of memory dysfunction in some folk traditional medicine, this present study was taken to evaluate the major phytochemical constituents and inhibitory activity of acetylcholinesterase potential of a few selected Nigerian medicinal plants.

EXPERIMENTAL

Materials. Fresh samples of the seven plants to be used were collected early in the morning from the Federal College of Forestry, Jos, Nigeria. Authentication of the plants was done at the Department of Plant Science and Technology, University of Jos, Nigeria. A UV-Visible spectrophotometer (Model 6305; Jenway, Barlow world Scientific, Dunmow, United Kingdom) was used to measured absorbance. All the chemicals used where of analytical grade while the water was glass distilled.

Preparation of crude acetylcholinesterase. Crude acetylcholinesterase enzyme used was freshly prepared from two male Albino rats, weighing average weight of 250g each. The two rats were humanely sacrificed. The rats were anesthetized using chloroform and the brain was extracted with the aid with the aid of a dissecting kit. The brain was immediately placed in cold phosphate buffer at pH of 7.1. The brain was homogenized and the resulting mixture was centrifuged at 10,000rpm for 5 minutes. The supernatant was collected and used as crude enzyme.

Preparation of ethanolic plant extract. The freshly collected plant samples were shade dried to a constant weight. The dried samples were then pulverized using electric blender (model MS-223, Taipei, Taiwan). The powered form was stored in an airtight container, until required for use. 10g of the sample was extracted with ethanol, then, the extract was filtered with whatman filter paper and the filtrate was concentrated under reduced pressure to give a solid extract. The concentrated extract was further lyophilized by leaving it to dry over night at room temperature. Then 0.05g of the plant extract

were reconstituted with 20ml of distilled water and used for subsequent analysis.

Phytochemical screening. Simple chemical tests to detect the presence of alkaloids, tanins, saponins, carbohydrates, glycosides, and flavonoids were done in accordance to standard methods (Evans, 2002; Sofowora, 1993; and Harborne, 1973).

***In vitro* acetylcholinesterase inhibition assay.** The plant extracts were examined for their AChE inhibitory activities at concentrations of 100 mg/l and were dissolved in 0.1 M phosphate buffer, following the spectrophotometric method developed by Ellman *et al*. (1961). To a 1 cm path length glass cell, were added in order, 200µl of acetylthiocholine iodide (15mM), 1000µl of DTNB (3mM), and 200µl of each test extract solution at the different concentrations evaluated, which were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 5 times, each 10 s. After that, 200µl of AChE solution were added to the initial mixture, to start the reaction and then the absorbance was determined. Control contained all components except the tested extract. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

$$\% IA = [(Cc. Ce)/Cc] \times 100$$

where: Cc is the control kinetic (containing all reactants, except the AChE enzyme) and Ce is the experimental kinetic for each sample concentration.

All treatments were performed in triplicate with two replicates.

Statistical analysis. The concentrations of the tested extracts that inhibited the hydrolysis of substrate (acetylthiocholine) at 50% (IC_{50}) were determined by a linear regression analysis between the inhibition percentages against the extract concentrations by using the Microsoft Excel program.

RESULTS AND DISCUSSION

Acetylcholine breakdown in the brain is prevented through the inhibition of AChE activity and the consequent increased concentration of acetylcholine in the brain leads to increased communication between the nerve cells that use acetylcholine as a chemical messenger and therefore a therapeutic effect in patients with Alzheimer's disease (Singhal *et al*., 2012). Figure 1, shows the effect of the ethanolic extracts of seven selected plants on acetylcholinesterase activity.

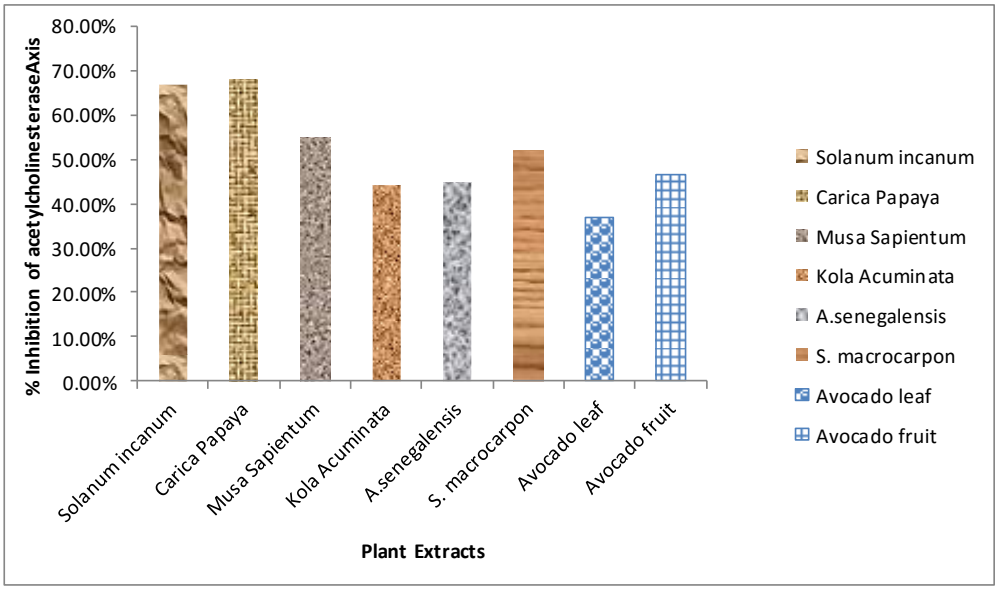

Figure 1: Plant extracts % Inhibition of Acetylcholinesterase

Table 1: IC_{50} of the effect of ethanolic extracts of seven selected plants

Extracts	IC_{50}
Solanum incanum leaf	98.5
Carica papaya	92.7
Musa sapientum	115.0
Kola acuminata	244.6
Annona senegalensis	244.5
Solanum incanum fruit	129.4
Persea americana	215.0

Table 2: Phytochemical screening of seven (7) plant extracts

Constituents	*Solanum incanum leaf*	*Carica papaya*	*Musa sapientum*	*Kola acuminata*	*Annona senegalensis*	*Solanum incanum fruit*	*Persea americana*
Alkaloids	+++	+	-	-	+	+++	-
Saponins	-	+	-	-	-	-	+
Tannins	-	+	+	-	+	-	+
Flavonoids	+++	+++	+++	+	+	+++	+++
Steroids	-	-	+	++	+	+	+
Anthraquinones	+	+	-	-	+	-	-
Cardiac glycosides	+	+++	++	++	+	++	++
Balsam	+	+++	++	++	+	++	++

-: absent; +: weak content; ++: moderate content; +++: strong content.

All the plants had an acetylcholinesterase percentage inhibition, with the *Carica papaya* having the highest percent inhibition with 67.9% and *Annona senegalensis* with the lowest percent inhibition with 44.2%. Nevertheless, the determined acetylcholinesterase activity of the chosen plants agreed with some earlier reports by Orhan *et al*. (2004) showed that *Fumaria Judaica* plant extract displayed a noticeable inhibition (54.44 %) against AChE. Ademosun and Oboh (2013) in which orange, grapefruit, and shaddock peels had anticholinesterase activity; the orange peels had the highest acetylcholinesterase inhibitory activity with 90%, which was higher than the highest percentage of AChE of the seven plants (67.9%).

The IC_{50}, is the concentration of an inhibitor (plant extract) that is required for 50 percent inhibition of an enzyme *in vitro*. The IC_{50} values are very dependent on conditions under which they are measured. In general, the higher the concentration, the more agonist activity will be lowered (Lazareno and Birdsall, 1993). Table 1, showed the IC_{50} for the selected seven plant extracts. *Carica papaya* had the least IC_{50}, with 92.7 and the highest IC_{50} was that of *Kola acuminata* with 244.5. Thus indicating that *Carica papaya* extract needed just 1/3 of the concentration of *Kola acuminata*, to cause a 50% inhibition. There is a correlation between the percentage inhibition and the $IC_{50.}$ This can be deduced to be an inverse relationship; the higher the percent inhibition, the lower the $IC_{50.}$

Different phytochemicals have been found to possess a range of activities, which may help in protecting degenerative diseases. A major role of the phytochemicals is protection against oxidation. Humans, and all animals, have complex antioxidant defence systems, but they are not perfect and oxidative damage will occur (Hollman and Katan, 1997, Liu, 2003). The polyunsaturated fatty acid-rich phospholipids in the brain membranes are easily attacked by free radicals, which ultimately leads to the development of Alzheimer's disease through oxidative damage to the brain phospholipids

(Breinholt *et al*., 2003). From Table 2, the phytochemical screening done showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, and cardiac glycosides. *Solanum incanum* leaf, *Carica papaya, Musa sapientum, Solanum incanum* fruit *and Persea americana* had heavy content of flavonoid, while *Kola acuminata* and *Annona senegalensis* had the weakest content of Flavonoid. This result also agrees with the phytochemical screening we did (Johnson and Olatoye, 2011).

Flavonoids are plant pigments that are synthesized from phenylalanine, they are the major component of many herbal preparations for medical use. They have proven ability to inhibit specific enzymes, to stimulate some hormones and neurotransmitters, and to scavenge free radicals (Ghasemzadeh and Ghasemzadeh, 2011).

Conclusion. This study has shown for the first time a correlation between the phytochemical constituents and its inhibitory activity on AChE. *Carica papaya* showed the heavy presence of flavonoid which may suggest the reason for the increase in percentage inhibition of Acetylcholinesterase, a key enzyme in the management of Alzheimer's disease.

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