Acetylcholinesterase Inhibitory and Antioxidant Activities of *Caesalpinia Bonduc* L. Bark

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Abstract— *The growth in the aging population has increased the number of patients with Alzheimer's disease (AD) worldwide. The naturally occurring enzyme inhibitors and antioxidants play an important role in a drug discovery program for such diseases. Caesalpinia bonduc L. (Fabaceae) is a medicinal plant used widely in the traditional system of medicine in the Asian region of the world. In the present study, the total ethanolic extract of bark of C. bonduc was evaluated for Acetylcholinesterase (AChE) enzyme inhibitory activity and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, ferrous iron chelating (FICA) and ferric reducing antioxidant potential (FRAP). Ethanolic extract of bark of C. bonduc exhibited moderate AChE inhibitory activity with an IC_{50} value of 190.76 ± 2.8 µg/mL while the IC_{50} of the Galanthamine, a clinically used inhibitor was 0.46 µg/mL. Bark extract showed good DPPH radical scavenging activity with an IC_{50} value of 83.69 ± 0.1 µg/mL, in comparison with that of Trolox (IC_{50} - 4.50 ± 0.3 µg/mL). Lower ferrous ion chelating effect was detected for the bark extract with an IC_{50} value of 3450.6 ± 235.19 µg/mL with comparison to that of EDTA (12.74 ± 0.2 µg/mL). The FRAP assay resulted the mg Trolox equivalent/g of extract of C. bonduc as 233.6 ± 0.2 mg. The results indicated that the ethanol extract of bark of C. bonduc showed AChE inhibitory, DPPH radical scavenging, FICA and FRAP activity. Therefore the in vitro assay data indicates the potential of the extract for further AChE inhibitory and antioxidant bioactive studies including activity-guided fraction of bioactive compounds.*

Keywords—Caesalpinia bonduc, acetylcholinesterase, antioxidant

I. INTRODUCTION

Neurodegenerative diseases such as Alzheimer's disease (AD) typically begin with subtle recognition failure and memory lapses. It slowly becomes more severe and eventually, incapacitates the individual's mental abilities. The drugs available for AD function by increasing the acetylcholine levels in the brain, which in turn enhances the signal transfer at the synapses (Ferreira *et al.,* 2006). Therefore cholinesterase enzyme (ChE) inhibitors are among the drugs most widely used in the treatment of AD. Currently drugs such as donepezil, galanthamine together with antioxidants derived from herbal extracts such as from *Ginggo biloba* are being used in the clinical practice for AD treatment (Orhan *et al*., 2006).

Lead compounds of many western drugs have originated from bioactive plant extracts. Owing to this factor in recent times there is a growing focus on plant-based research worldwide. Furthermore medicinal plants have been known as sources of therapeutics for thousands of years.

Caesalpinia bonduc L. (Fabaceae) is a medicinal plant widely distributed in the tropical regions of Asia and the Caribbean. The plant is being used in the traditional system of medicine in countries such as Sri Lanka, India, Nicobar Islands (Singh and Raghav, 2012). In Sri Lanka it is commonly called as "Kumburu". Pharmacological studies of the seeds and leaves have reported antioxidant, anti-inflammatory, antimalarial, antimicrobial, antidiarrheal, antidiabetic, antitumor, antihelmintic, antifilarial, hepatoprotective, antirheumatic and antipyretic activities (Singh and Raghav, 2012). Ata *et al.,* (2009) has recorded anti glutathione S-transferase assay guided isolation of a sterol namely 17–hydroxy-campesta-4, 6-dien-3-one from the ethanolic bark extract of *C. bonduc*. Previous phytochemical studies have also reported the isolation of diterpenoids such as neocaeslpin H, cordylane A, caesalpinin B, bonducellpin E, caesalpinolide A, 17-methylvouacapane8 (14), -9(11)-diene and neocaesalpin P, homoisoflavonoids namely, caesalpinianone, 6'-*O*-methylcaesalpinianone and other compounds such

as, hematoxylol, stereochenol A,6'-*O*-acetylloganic acid, 4'-*O*- acetylloganic acid and 2-*o*-ß-D-glucosyloxy-4-methoxybenzenepropanoic acid from the ethanolic extract of bark (Ata *et al.,* 2009*;* Ata *et al.,* 2009; Udenigwe *et al.,* 2007).

The present study was undertaken to evaluate the acetylcholinesterase inhibitory activity of the ethanol extract of bark of *C. bonduc* and its ability to function as an antioxidant.

II. MATERIAL AND METHODS

A. Collection, preparation and extraction of plant material

Bark of *C. bonduc* was collected from Chilaw, Sri Lanka. The voucher specimen of *C. bonduc* was deposited at the Herbal Technology Section at the Industrial Technology Institute, Sri Lanka. The collected bark was shade-dried. The 100 grams of the powdered bark was extracted with ethanol (250 mL) using cold extraction technique (Wu *et al.,* 2003). The plant material was extracted three times with ethanol. The filtrates were combined and concentrated to dryness under vacuum using a rotary evaporator to obtain the crude extract.

B. Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibition was determined using a modified method of Ellman *et al.,* (1961). A total reaction volume of 200 µl containing 0.002 U/mL of AChE (10 µl), different concentrations of ethanolic extracts and 0.1 M sodium phosphate buffer (pH 8.0), was pre incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 0.71 mM acetylthiocholine and 0.5 mM DNTB in 20 µl of 0.1 M sodium phosphate buffer. The hydrolysis of acetylthiocholine was monitored by the formation of yellow colour 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine for a period of 10 min at λ = 412 nm. Galanthamine was used as the positive control. The kinetic parameter *Vmax* was used to calculate the % inhibition and IC_{50} value (1).

% =[*Vmax* (Control) – *Vmax* (Test)]/*Vmax* (Control)]× 100 Inhibition (1)

C. Antioxidant activity

1) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: Free radical scavenging activity was measured by the Blois (1958), method with some modifications. The reaction mixture contained different concentrations of the ethanol extract and 200 µl of 40 µg/ ml solution of DPPH in methanol. Reaction volume was made up to 300 µl by using analytical grade methanol. The mixture was left to stand for 10 minutes in the dark. The absorption was measured at λ = 517 nm against a corresponding blank (methanol). Trolox was used as the positive standard. The capability to scavenge the DPPH radical by 50% (IC_{50}) was calculated using the equation (2).

% = [Abs (Control) – Abs (Test)]/Abs (Control)]× 100 Inhibition (2)

2) Ferrous iron chelating activity: Metal ion-chelating effect of the bark extract for ferrous ions was measured according to the method by Carter (1971) with some modifications. Reaction mixture of 200 µl containing distilled water, 20 µl of Ferrous sulphate, different concentrations of the ethanolic bark extract and 40 µl of Ferrozine in distilled water was added and the plate was incubated at room temperature for 10 minutes. The absorbance was measured at λ = 562 nm. EDTA was used as the positive standard. Percentage chelating effect was calculated using the following equation (3) and IC_{50} value was calculated.

% = [Abs (Control) – Abs (Test)] / Abs (Control)] × 100 Chelation (3)

3) Ferric reducing anti oxidant activity (FRAP): A modified protocol of Benzie and Szeto's (1999) was adopted for the FRAP assay. The fresh working solution of FRAP was prepared by mixing 25 mL acetate buffer, 2.5 mL of 2, 4, 6-tripyridyl-striazine (TPTZ) and 2.5 mL of $FeCl_3.6H_2O$. Different concentrations of the ethanol extract of bark was allowed to react with 150 µl of FRAP solution. The plate was vortexed and left to stand for 8 minutes. Absorbance of the Ferrous tripyridyltriazine complex was measured at λ = 593 nm. The standard curve was linear between 10 µg/mL and 120 µg/mL Trolox. Results are expressed in mg Trolox equivalent/g of extract using the standard curve of Trolox.

III. STATISTICAL ANALYSIS

All experiments were performed in triplicates using a 96 well micro plate reader Spectra Max 340 (molecular devices, CA, USA). The results are

presented as in mean ± Standard Error (SE). The IC_{50} values were calculated by linear regression analysis. Results were calculated by employing the Soft Max Pro program and Microsoft Office Excel for Mac 2008.

IV. RESULTS AND DISCUSSION

Pharmacological studies have discovered a vast range of bioactivities of phytochemicals, which has lead to a growing interest in the exploitation of plants for their therapeutic principles. Oxidative stress is known to be a causative agent for the development of degenerative diseases such as AD, cancer and cardiovascular diseases (Ames *et. al.,* 1993). In the present study *C. bonduc* was evaluated for the first time for its AChE inhibitory activity along with antioxidant activity *in vitro*.

A. AChE inhibitory activity

The IC_{50} values obtained for the ethanol extract of bark of *C. bonduc* and the standard Galanthamine are given in Table 1. The inhibitory activity of different concentrations is summarized in Figure 1. In comparison with the standard, Galanthamine (IC_{50} 0.46 ± 0.02 µg/mL), the bark extract exhibited moderate *in-vitro* acetylcholinesterase enzyme inhibitory activity.

Table 1. AChE inhibitory activity of ethanolic extract of *C. bonduc* bark

Plant/ Compound	IC_{50} (µg/mL)
C. bonduc	190. 76 ± 2.8
Galanthamine	0.46 ± 0.02

N=3 Data represented as mean ± SE

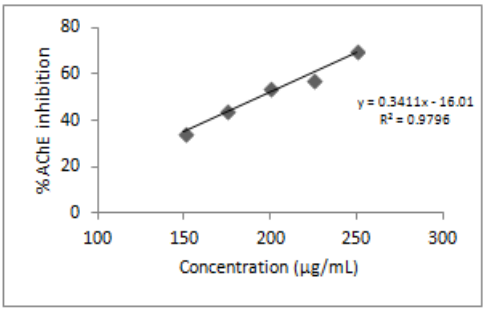

Figure 1. AChE inhibition potential of ethanolic extract of *C. bonduc* bark

B. Antioxidant activity

The anti oxidant activity of the ethanolic bark extract was evaluated by three methods: DPPH, FRAP and FICA. Results for all three assays are presented in Table 2.

1) DPPH radical scavenging activity: The DPPH test intends to measure the capacity of the extract to scavenge the DPPH free radical by donating a hydrogen atom or electron in solution (*Tepe* et al., *2005*). The anti-oxidant concentration required for 50% radical scavenging per unit DPPH exerted by the ethanolic bark extract of *C. bonduc* and positive standard Trolox is summarized in Table 2. The DPPH radical scavenging activity increased with the increasing of the sample concentration. The IC_{50} value of the extract was 83.69 ± 0.1 µg/mL in comparison to that of Trolox (IC_{50} 4.50 ± 0.3 µg/mL) which is a known antioxidant.

Table 2. Antioxidant activity of ethanolic extract of *C. bonduc* bark

Plant/ Compound	IC_{50} (µg /mL)		mg TE/g of extract
	DPPH	FICA	FRAP
C. bonduc	83.69 ± 0.14	3450.6 ± 235.19	233.6 ± 0.14
Trolox	4.50 ± 0.0	-	-
EDTA	-	12.74 ± 0.2	-

N=3 Data represented as mean ± SE

2) FICA assay: Transition metals catalyse oxidation reactions and therefore in the presence of chelating agents, complex formation with these transition metals are disrupted (Gordon, 1990). Through FICA assay the antioxidant activity of a plant extract is measured by how effectively the chelating compounds in it can compete with ferrozine for ferrous ion. The presence of chelating compounds in the extracts can disrupt the formation of ferrozine-Fe^{2+} complex. Ion chelating capacity of the ethanolic bark extract and the metal chelator EDTA were evaluated and the values are given in Table 2. The IC_{50} value of the ethanolic bark extract was found to be 3450.6 ± 235.19 µg/mL where as the IC_{50} value of standard EDTA was observed as 12.74 ± 0.2 µg/mL. Therefore this indicates that the ethanolic bark extract of *C. bonduc* is a very weak chelator of iron (II) ions.

3) FRAP assay: The reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity. The mg Trolox equivalent/g of extract of *C. bonduc* was found to be 233.6 ± 0.2 mg (Table 2).

Broad spectrum of compound classes such as alkaloids, tannins, terpenoids and flavonoids are known to exhibit AChE inhibitory and antioxidant activities (Adewusi *et al.,* 2011). Therefore previously isolated diterpenoids (Ata *et al.,* 2009), flavonoids (Ata *et al.,* 2009) and sterols (Udenigwe *et al.,* 2007) from the bark of *C. bonduc* may account for the observed AChE inhibitory and antioxidant activity in the present study. Hence, further studies are required to identify the acetylcholinesterase inhibitory and antioxidant active compounds from the ethanolic bark extract.

V. CONCLUSIONS

In summary, the crude ethanolic bark extract of C. *bonduc* possess moderate levels of AChE inhibitory activity and antioxidant activity. Plant extract should be further subjected to bioassay guided isolation of compounds by chromatographic techniques to identify the potential chemical entities for therapeutic use in the treatment of AD.

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