



Screening for acetylcholinesterase inhibitory activity of methanolic extract of *Cassia fistula* roots

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Abstract

Inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of acetylcholine, is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis. Alzheimer's disease (AD) is the most common cause of memory impairment and dementia in the elderly. AD is pathologically characterized by extracellular deposits of beta-amyloid (A β) peptide, neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau, neuronal loss, and neurotransmitter dysfunction. Clinically, AD is characterized by progressive cognitive decline that usually starts with memory impairment and progresses to cause a more generalized cognitive dysfunction, behavioral dysregulation, and neuropsychiatric symptoms. A potential source of AChE inhibitors is certainly provided by the abundance of plants in nature. A treatment to the Alzheimer's disease consists of inhibition of the acetylcholinesterase, which is responsible for the acetylcholine control in the synapses. A microplate assay and a thin-layer chromatography (TLC) "in situ" assay based on the Ellman assay was used to screen for acetylcholinesterase inhibitors from ethyl acetate and methanol extracts of *Cassia Fistula* roots of family *leguminosae*.

Key-Words: Plant Extract, Acetylcholinesterase inhibition, Alkaloids, Alzheimer's disease

Introduction

Cholinesterase inhibitors are the only approved drugs for treating patients with mild to moderately severe Alzheimer's disease, a disorder associated with progressive degeneration of memory and cognitive function. The memory impairment in patients with Alzheimer's disease results from a deficit of cholinergic function in the brain. The most important changes observed in the brain are a decrease in cortical levels of the neurotransmitter acetylcholine and associated enzyme choline transferase. Acetylcholinesterase inhibitors can restore the level of acetylcholine by inhibiting acetylcholinesterase. ¹Principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease.² There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-based rivastigmine for treatment of cognitive dysfunction and memory loss associated with AD.

These compounds have been reported to have their adverse effects including gastrointestinal disturbances and problems associated with bioavailability, which necessitates the interest in finding better AChE inhibitors from natural resources. In traditional practices numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and different neuro pharmacological disorders. ³The plants used in these remedies were screened for their AChE inhibitory activity using Ellman's colorimetric method in 96-welled microplate (Ellman et al., 1961; Ingkaninan et al., 2000).

Hence an effort has been made to establish the scientific validity to investigate and screening for acetylcholinesterase inhibitory activity of methanolic extract of *cassia fistula* roots. . The result suggests that the AChE inhibitors should be alkaloids and these typical indole alkaloids have not been studied for their AChE activity before. Thus we proved that the roots of cassia fistula plant can be used for the isolation of acetylcholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease.

Material and Methods

Plant materials

The plant materials belonging to the genus *Cassia* (Leguminosae) were collected from kerala. The plant

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materials were authenticated when they were collected and chemically studied for alkaloids by us for the first time. The specimens were used for the extraction and alkaloid analysis.

Chemicals

Acetylthiocholine iodide (ATCI), AChE, 5, 5'-dithiobis [2-nitrobenzoic acid] (DTNB), were obtained from Sigma (St. Louis, MO). All organic solvents (analytical-reagent grade) were purchased from Merck (Darmstadt, Germany). AChE used in the assay was from electric eel (type VI-S lyophilized powder, 480 U/mg solid, 530 U/mg protein). DTNB was dissolved in the buffer containing 0.1M NaCl and 0.02M MgCl₂. ATCI was dissolved in deionized water. Acetylthiocholine iodide (ATCI), Acetylcholinesterase (AChE) from electric eels (type VI-S lyophilized powder), and 5, 5'-dithiobis [2-nitrobenzoic acid] (DTNB) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Methanol was purchased from Scharlab Chemie (Barcelona, Spain).

Extractions⁴

The plant materials were cut into small pieces and dried in a hot air oven at 55 °C. The dried materials were macerated in methanol for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. The residue from the filtration was macerated in methanol again for 3 days and filtered. The filtrate was evaporated with the same procedure and combined with the extract from the first extraction.

Microplate assay for AChE activity^{5,6}

The assay for measuring AChE activity was modified from the assay described by Ellman et al. (1961) and Ingkaninan et al. (2000). Briefly, 125 μ l of 3mM DTNB, 25 μ l of 15mM ATCI, and 50 μ l of buffer, 25 μ l of sample dissolved in buffer containing not more than 10% methanol were added to the wells followed by 25 μ l of 0.28 U/ml AChE. The microplate was then read at 405 nm every 5 s for 2 min by a CERES UV 900C microplate reader (Bio-Tek Instrument, USA). The velocities of the reactions were measured. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer without any inhibitor. Inhibitory activity was calculated from 100 subtracted by the percentage of enzyme activity. Every experiment was done in triplicate.

Thin layer chromatography (TLC) with bioassay detection for AChE inhibition⁷

The TLC with bioassay detection for AChE inhibition was modified from the study of Rhee et al. (2001). A 2.5mm silica gel plate F254 no. 5554 (Merck, Darmstadt, Germany) was used as a stationary phase. Two mobile phases, i.e. dichloromethane: ethanol: water 4:4:0.5 (v/v/v) and chloroform: methanol 9:1

(v/v) were used. Three microliter of plant extracts dissolved in methanol at concentration of 5 mg/ml was applied to the plate. After the plate had been developed, it was dried at room temperature and then sprayed with 30mM ATCI followed by 20mM DTNB. The plate was dried at room temperature for 45 min, and then sprayed with 10.17 U/ml AChE. After 20 min, the plate was observed under visible light. A positive spot indicating AChE inhibitor was a colorless spot on the yellow background. The result was compared to that from the TLC analysis of the same sample after spraying with anisaldehyde and dragendorff Reagents (Stahl, 1961).

Table. 1. The inhibitory activity of acetylcholinesterase of the methanolic extract of *Cassia fistula* roots

| Plants | Family | Part Used | Percentage Inhibitory Activity |
|-----------------------|-------------|-----------|--------------------------------|
| <i>Cassia fistula</i> | Leguminosae | Roots | 60–65% inhibitory activity |

Results and Discussion

The methanolic extracts of these plant roots were tested for AChE inhibitory activity using Ellman's colorimetric method in 96-welled microplate. The result shows that at the concentration of 0.1 mg/ml, the extracts from roots of *Cassia fistula* had showed 60–65% inhibitory activity on AChE (Table. 1). By means of TLC with bioassay detection for AChE inhibition, the extracts from *Cassia fistula* showed more than two spots of AChE inhibitors which were also positive with dragendorff reagent. This result suggests that the AChE inhibitors should be alkaloids. These typical indole alkaloids have not been studied for their AChE activity before. The roots of cassia fistula plant can be used for the isolation of acetylcholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease.

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