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Research Article

# EVALUATION OF ACETYLCHOLINESTERASE ACTIVITY AND CYTOTOXICITY OF DIFFERENT PARTS OF *NELUMBO NUCIFERA* GAERTN ON HUMAN NEUROBLASTOMA CELL LINE (SH-SY5Y)

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**ABSTRACT**

**Objective:** Cognitive deterioration occurring in patients with probable Alzheimer's disease is associated with a progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine in the brain. This study aimed to evaluate the acetylcholinesterase (AChE) inhibitory effects and cytotoxicity in SH-SY5Y cells of different parts of three lotus extracts.

**Methods:** AChE activity was quantified by spectrophotometry and cytotoxicity by flow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in SH-SY5Y cells exposed to extracts.

**Results:** All of the extracts had inhibitory effects to AChE at $p<0.05$, but Roseum Plenum stem extract could inhibit AChE more than 30% ($p<0.05$). The all of the extracts could be an increase SH-SY5Y cell proliferation, while Album Plenum flower extract could be cytotoxic on SH-SY5Y cells.

**Conclusion:** The extracts of lotus could be supplemented compound for cognitive deterioration or Alzheimer's patients.

**Keywords:** *Nelumbo nucifera* Gaertn, Acetylcholinesterase activity, Human neuroblastoma cell line.

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## INTRODUCTION

The most common cause of dementia in the elderly associated with probable Alzheimer's disease (AD) is one of the most common types of dementia, a chronic, progressive, disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity, and language [1]. Patients with AD often have cholinergic deficits in association with the disease [2]. The symptoms of all types of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the brain areas affected [3]. Cognitive deterioration occurring in patients with probable AD is associated with a progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine (ACh) in the brain.

The neurons can be subdivided into subclasses based on location, function, or chemistry. Neurons express a wide array of molecular components that may result in lethality if they, or their function, are adversely affected. To survive, a functioning nervous system is crucial. Therefore, neurons, synapses, signal molecules, and brain are needed. The endogenous signal molecules in the nervous system are called neurotransmitters, and their functions are chemically induced or attenuate the activity of other neurons or other organs in the body [4]. The storage of neurotransmitter in the vesicles has two main functions to protect the neurotransmitter from degradation and the fast release of the neurotransmitter. Neuromuscular transmission, parasympathetic signaling and cholinergic signaling in the central nervous system are dependent on ACh. ACh is rapidly hydrolyzed in the brain by acetylcholinesterase (AChE). Although AChE is found in higher concentrations in the brain tissue of patients with AD, there is evidence that known to receive cholinergic innervations [5].

The expression of both muscarinic and nicotinic ACh receptors has been reported in SH-SY5Y cells. The G-protein coupled muscarinic receptors are present on membranes of both undifferentiated and differentiated cells, though the levels and binding properties are differentially regulated according to the method of differentiation.

Lotus (*Nelumbo nucifera* Gaertn, *N. nucifera*) is considered as an important Thai traditional herb. All parts of *N. nucifera* are used as medicine for various medicinal purposes [6,7]. It has antidiabetic, antipyretic, anticancerous, anti-inflammatory, antimicrobial, antiobesity, antiviral, antioxidant, hepatoprotective, anti-thrombotic, anti-inflammatory, immunomodulatory, antidiarrheal, hypocholesterolaemic, anti-arrhythmic, and antifertility activities properties [6,8-21]. The rhizome can be cooked and eaten as a common vegetable. The extract from rhizome showed antidiabetic and antiobesity attributes. The leaf is used for diverse applications for diarrhea, high fever, hemorrhoids, leprosy stopped bleeding [22], and stopping bleeding [23]. The seed is used as a diuretic, antivomiting, anti-inflammation, and cancer [24]. The numerous researches were reported that the extract of part lotus exhibits high antioxidative capacity [25], and the main compositions are phenolics including dopa, catachol, gallic acid, catechin, and epicatechin [26]. The major constituents that separate from the lotus plant are alkaloids (liensinine, neferine, nuciferine, remrefidine, and isoliensinine) and flavonoids (coclaurine, norcoclaurine, and quercetin) [27]. The aim of this study was to evaluate the AChE activity, and cytotoxicity on human neuroblastoma SH-SY5Y cell line was evaluated to assess dementia activity.

## METHODS

### Plant materials and extraction

The *N. nucifera* materials including of *N. nucifera* cv. Roseum Plenum (RP), *N. nucifera* cv. Album Plenum (AP), and *N. nucifera* cv. Hindu Lotus were collected from lotus museum, Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand. The different parts of lotus (leaf, stem, and flower) were macerated in 95% ethanol for 7 days. The ethanolic extracts were evaporated and dried in a rotary evaporator.

### Reagents

Dulbecco's Modified Eagle Medium (DMEM), F12 medium, penicillin/streptomycin, trypsin/EDTA, and 3-(4,5-dimethythiazol-2-yl)-2,5-

diphenyltetrazolium bromide (MTT) were purchased from Invitrogen (Invitrogen, USA). DMSO, phosphate-buffered saline (PBS), and dithiobisnitrobenzoic acid (DTNB) were purchased from ThermoFisher (Thermo Scientific, USA).

**Determination of AChE activity**

The activity of AChE in red blood cell (RBC) was determined according to slightly modified Ellman's method as previously described [28-30]. The mixture was prepared by mixing 10 µL in each aliquot of 1:10 RBC and extracts added in 1.0 mL of 0.25 mM DTNB in phosphate buffer pH 7.4. This was pre-incubated for 5 min at room temperature, and the reaction was started with the addition 25 µL of acetylthiocholine iodide. The absorbance per minute (ΔA/min) of thiocholine product was determined by spectrometric absorption at 405 nm. The data were converted into the standardized units of nanomoles substrate hydrolyzed/min x mL, using the extinction coefficient for the yellow product (ε= 13.6 $mM^{-1}cm^{-1}$) to find the concentration. The AChE activity was calculated and expressed as U/L (AChE factor = 76,838). The experiment was run in triplicate. The AChE activities and AChE inhibitions were calculated by the following:

AChE activity = ΔA/min × factor

$$\%\text{AChE inition} = \frac{Ac - As}{Ac} \times 100$$

Where ΔA = the absorbance per minute of thiocholine product
Ac = the delta absorbance per minute of control
As = the delta absorbance per minute of sample

**Cell culture and treatment**

Human neuronal SH-SY5Y cells were cultured in DMEM/F12 with 10% fetal bovine serum (DMEM/F12) (Invitrogen, USA), and penicillin/streptomycin (100 IU/mL) at 37°C in a humidified incubator with 5% $CO_2$ atmosphere. SH-SY5Y cells were allowed to adhere at the bottom of a culture dish (60 mm) for 24 h before treatment with fresh medium containing 1 mg/mL of initial stock extracts for 24 h. SH-SY5Y cells treated with a vehicle that served as a control. For all experiments were performed in five replicates.

**MTT reduction assay**

SH-SY5Y cells were seeded in 6-well culture plate (2.5 × $10^4$ cells per well) for 24 h. After SH-SY5Y cells were pre-treated with extracts. The end of the treatment, SH-SY5Y cells were incubated with 100 µL of MTT solution (5 mg/mL in PBS) for 2 h. Then, the medium was discarded and added formazan crystal products. The formazan crystal products were dissolved in 200 µL DMSO and stirred for 10 min. Absorbance was measured at 570 nm using a microplate reader. The results were compared with the untreated control, expressed as the percentage of cell viability. The data were obtained from five replicates. The percentage of cell viability was calculated from the following;

Cell viability (%) = (As/Ac) × 100

Where Ac = the absorbance of SH-SY5Y treated with vehicle medium
As = the absorbance of SH-SY5Y treated with sample medium

**Statistical analysis**

All data were analyzed with a mean ± standard deviation of three independent experiment, descriptive statistics, *t*-test, and one-way analysis of variance using GraphPad Prism 6 version 6.01 (GraphPad Software Inc. La Jolla, CA. USA). p=0.05 was considered a statistically significant difference.

## RESULTS AND DISCUSSION

**AChE inhibitory activities**

The results of the AChE activity are shown in Fig. 1. The baseline AChE activity in 1:10 dilution of packed RBC was 10,321.94±379.03 U/L. At

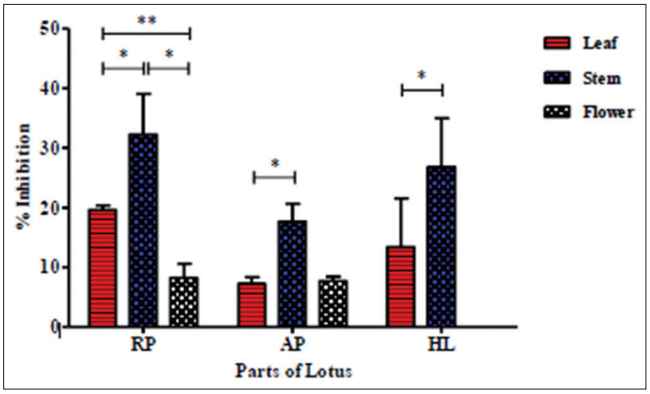


**Fig. 1: AChE inhibitory activities of *Nelumbo nucifera* extracts in packed red blood cell at a concentration of 10 mg/mL. Values were done in triplicate and represented mean±SD. *p<0.05, **p<0.01**

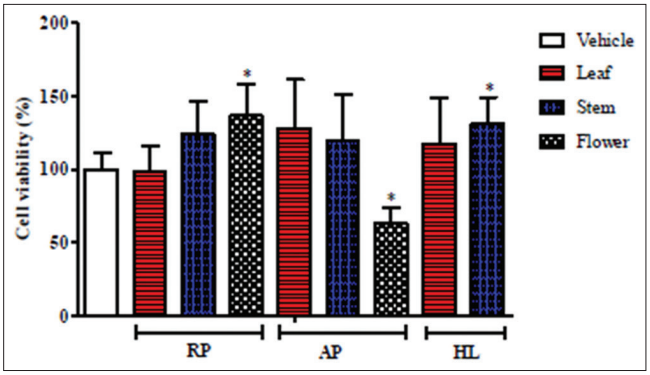


**Fig. 2: SH-SY5Y cell viability percentage of *Nelumbo nucifera*. Values are expressed as mean±SD. *p<0.005, compared with phosphate-buffered saline**

the 5 mg/mL concentration of leaf, stem, and flower ethanolic extracts of RP showed that ChE activity was 8,298.53±76.84, 6,480.02±319.90, and 9,476.72±247.00, respectively. The leaf, stem, and flower ethanolic extracts of AP showed that ChE activity was 9,579.17±117.37, 8,759.56±277.04, and 9,527.94±76.84, respectively. The leaf and stem ethanolic extracts of HL showed that ChE activity was 9,451.10±203.29 and 8,068.02±203.29, respectively. The extracts of *Nelumbo nucifera* (RP, AP, and HL) are shown mild AChE inhibition (14.36–27.64%) while the flower extract showed no symptoms of intoxication on AChE inhibition (10.57–28.86%), as shown in Fig. 1. The AChE activity decreased approximately to the one-third of the baseline activity (less than 33%), consistent with no symptoms of intoxication.

**Cytotoxicity of SH-SY5Y cells**

SH-SY5Y cells were incubated with extracts for 48 h. And then, MTT assay was carried out to determine the cell growth in response to extract treatment. The result showed that the growth of the cells was markedly inhibited from the extracts of flowers of AP and displayed a toxic response to the treatment, while the other samples could be increased SH-SY5Y cell proliferation, as shown in Fig. 2.

## CONCLUSION

The extracts promoted a moderate cytotoxic effect against SH-SY5Y cell and did not show a significant AChE inhibitory activity. According to the study, we found that the ethanolic extracts of *N. nucifera* could be supplemented compound for cognitive deterioration or Alzheimer's patients. However, further study on the effect of phytochemical composition in the different parts of *N. nucifera*. on the activity of AChE and cytotoxicity on SH-SY5Y cell.

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**CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.

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