DEVELOPMENT OF VOLTAMMETRIC BIOSENSOR FOR THE SCREENING OF PLANT EXTRACT WITH ANTI ALZHEIMER ACTIVITY

Moch. Amrun Hidayat[*#], Bayu Tri Murti[*], Athika Darumas Putri[*], Bambang Kuswandi[*]

[*]Chemo & Biosensor Research Group, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Jember, Indonesia
[#]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Jember, Indonesia

INTRODUCTION

- 5% of men and 6% of woman of above the age of 60 years are affected with Alzheimer's type dementia worldwide (Fratiglioni *et al*., 1999). World Health Organization (WHO) has estimated that 35.6 million people are currently living with dementia worldwide which will be further increased to 65.7 million by 2030 and 115.4 million by 2050 (Wimo & Prince, 2010).
- Alzheimer's is characterized by involvement of neurotransmitter acetylcholine in Alzheimer's disease resulting into disproportionate deficiency of acetylcholine by degradation activity of acetylcholinetransferase and acetylcholinesterase (AChE).
- The clinical response of few drugs namely Donepezil, Rivastigmine, Galantamine, and Memantine, approved by Food and Drug Administration (FDA), USA for the treatment of Alzheimer's disease, available presently, have been often found to be unsatisfactory.
- Ethnopharmacological approach and bioassay guided isolation have provided a lead in identifying potential AChE inhibitors from plant sources, including those for memory disorders (Singh *et al*., 2011).
- An electrochemical biosensor which use small amount of enzyme have been developed for detecting AChE inhibitory activity. The biosensor used acetylcholine chloride (AChCl) as substrate for the enzyme-modified electrode, on which AChE is co-immobilized with inhibitor. AChE is sensitive for the inhibitor (including herbals). As a result, the current generated is inversely proportional to the amount of inhibitor present in the solution.

METHODS

Chemicals

Acetylcholine chloride (AChCl) and lyophilized acetylcholine esterase powder (EC3.1.1.7, from *Electrophorus electricus*, 518 U/mgsolid) were purchased from Sigma Aldrich (Singapore). Rivastigmine (RVT) were extracted from Exelon® capsule (Novartis Ltd., Australia) using ethanol by ultrasonication, and calculated as Rivastigmine Hydrogen Tartrate (MW = 400.42). KCl, NaCl, Phosphate buffer solution (PBS) were purchased from Brataco Chemika (Bandung, Indonesia). PBS was adjusted at pH 7.2 using NaOH solution.

Plant materials

Both of *Curcuma longa* rhizome and *Piper nigrum* fruit were collected in Sleman, Indonesia in January, 2013. The plants were authenticated, and voucher specimens of these samples are stored in Herbarium Jemberiesense, University of Jember. Fresh samples of the plant materials were air dried and ground at ambient temperature. Ten grams of powdered sample were extracted with70% ethanol by maceration for 72 hours. The liquid extract (menstruum) were collected, filtered, and concentrated in a vacuo. The extract was kept and stored at -20^{o}C until prior use.

Preparation of enzyme based SPCE

Acetylcholinesterase (0.5 mg) was dissolved in 2 ml 0.01 M PBS. Five microliters of the solution (25.9 U) was mixed with carbon paste. The mixing paste was then adsorbed onto the surface of working electrode (Ag:AgCl) by screen printed technology to obtain enzyme based Screen Printed Carbon Electrode (Enzyme-SPCE).

Instrumentation

All of electrochemical analysis were recorded using Potensiostat® version 2.1.5, EmStat PalmSens® version 1.8 and SPCE from EDAQ® Instrument, USA. The potential and current signals were recorded using a PSLite® and E-Chem® Software, USA. The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were employed for electrochemical analysis.

Analytical condition

The condition of DPV method were described as follows: scan rate at 20 mV/s, E begin -0.5 V, E end +0.5 V, E step 0.005 V, E pulse 0.025 V, E condition -1.2 V, E deposition -1 V, and t pulse 0.07 s. The condition of CV method were described as follows: potential range 1 V, scan rate 50 mV/s, sample width 20ms, and sample period 5 ms. The potential signal of sample solution against 3M KCl in enzyme-SPCE at +500 mV was used for all of measurements. AChCl solutions were used as substrate for acetylcholinesterase. All of samples were dissolved in PBS for electrochemical analysis.

Optimization of AChCl concentration

Initially, the DPV and CV profile of AChCl solutions (10-80 mM) were made to determine optimum concentration for electrochemical analysis. In CV profile, the concentration of AChCl which exhibit the highest current was selected as working concentration. In contrast, the concentration of AChCl which give the lowest current signal was selected as working concentration in DPV method.

AChE inhibition

Fifty microliters of test solution containing sample solution (AChE inhibitor) and AChCl solution at same ratio (1:1) were dropped onto enzyme-SPCE surface prior to electrochemical analysis. After single measurement, the electrode was thoroughly rinsed with PBS. PBS was also used to replace sample solution as blank. The test solution without addition of inhibitor was used as control. RVT (1-10 µM) was used as positive control. Both of DPV and CV profiles were made for all of testing samples. The percentage of enzyme inhibition by samples (I) were calculated with following formula (Pohanka *et al*., 2008).

$$I = \frac{i_i - i_b}{i_0 - i_b} \, x \, 100$$

RESULTS & DISCUSSION

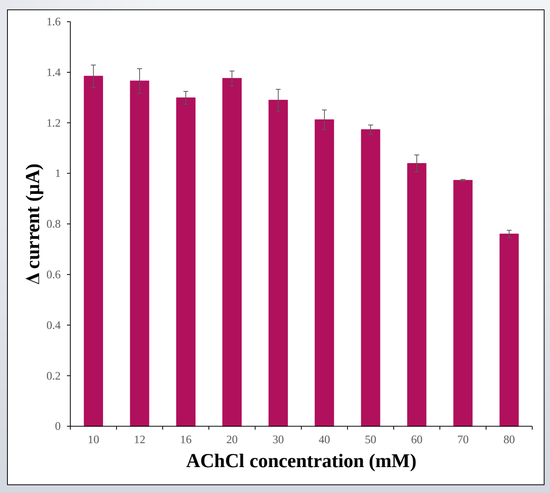

Figure 1. The current response of various AChCl concentration at potential 0.5 mV using CV method.

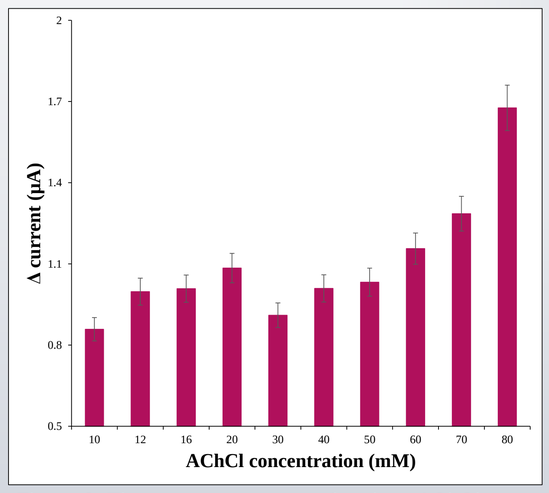

Figure 2. The current response of various AChCl concentration at potential 0.5 mV using DPV method.

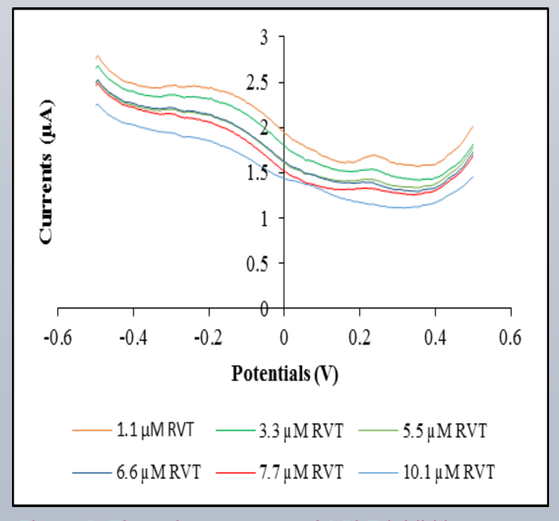

Figure 3. The voltammogram of AChE inhibition activity by RVT using DPV method at 30 mM AChCl.

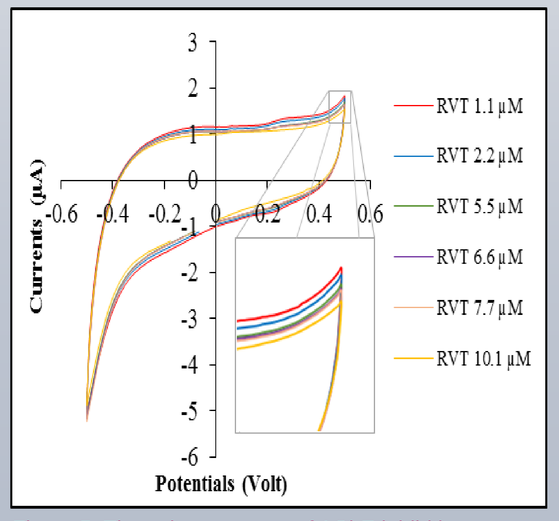

Figure 5. The voltammogram of AChE inhibition activity by RVT using CV method with 30 mM AChCl

From the figure of enzyme inhibition profiles of both of the extracts below (Figure 7 and Figure 8) showed that higher extract concentrations, higher enzyme inhibitions. The *Piper nigrum* extract (PNE) showed higher of enzyme inhibitions than the *Curcuma longa* extract (CLE) in all of the same concentrations in DPV method (Figure 7). In CV method, the PNE had only one of the same concentration that showed higher of enzyme inhibition than the CLE (Figure 8). More than this, the IC_{50} of both of the extracts, showed that PNE had the enzyme inhibition ability better than CLE (Table 1) in DPV and CV methods. Despite, it was presented that the inhibition activities of CLE was interesting because the 50% inhibition was showed in the range of the low concentrations.

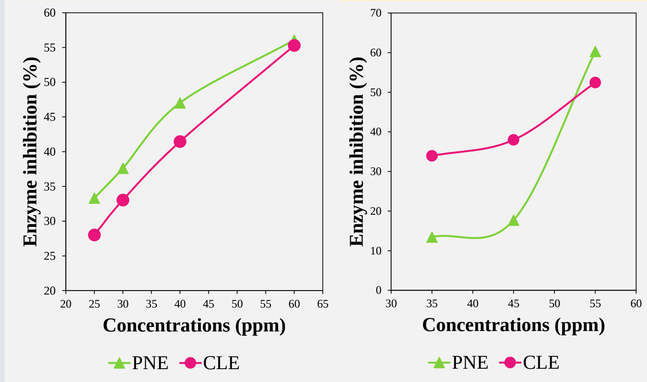

Figure 7. Enzyme Inhibition profile of Piper nigrum (PNE) and Curcuma longa (CLE) in DPV method

Figure 8. Enzyme Inhibition profile of Piper nigrum extract (PNE) and Curcuma longa extract (CLE) in CV method

Table 1. IC_{50} values of AChE inhibition activity of Piper nigrum extract (PNE) and Curcuma longa extract (CLE)

Methods	Materials	IC_{50} (ppm)
DPV	PNE	48.8386
	CLE	52.4739
CV	PNE	53.3268
	CLE	54.2397

CONCLUSIONS

In this study, the enzyme-SPCE-based optical biosensor was developed to determine the AChE inhibition activity of plant extract samples. The voltammetric DPV and CV method were optimized for the determination of IC_{50} value. The developed biosensor can be used as screening tool for plant with anti Alzheimer activity.

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