BIOSENSOR FOR MEASURING THE ANTI-DIABETIC POTENTIAL OF MEDICINAL PLANTS

by

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LIST OF ABBREVIATIONS

AD	Amperometric Detection
AG	α-glucosidase enzyme
CV	Cyclic voltammetry
DM	Diabetes Mellitus
DNS	3, 5-dinitrosalicylic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass spectroscopy
HPLC	High-performance liquid chromatography
MES	2-(N-Morpholino) ethane sulfonic acid
MWCNTs	Multi-walled carbon nanotubes
MWCNTs-NH_2	Amine functionalized multi-walled carbon nanotubes
NHS	N-hydroxysuccinimide
PB	Phosphate buffer
p-NP	para-nitrophenol
p-NPG	para-nitrophenyl-α-D-glucopyranoside
PVA	Poly (vinyl alcohol)
RSD	Relative standard deviation
SEM	Scanning Electron Microscopy
SPCE	Screen-printed carbon electrode
SP-CNTs	Screen-printed Carbon nanotubes

TCA	Trichloroacetic acid
WHO	World Health Organization

LIST OF SYMBOLS

I_{pa}	Anodic peak current
I_{pc}	Cathodic peak current
E_{dep}	Deposition potential
t_{dep}	Deposition time
ΔE	Difference of peak potential
µA	micro ampere
mM	Milli-molar
mV	Milli-volt
E	Potential

BIOSENSOR UNTUK MENGUKUR POTENSI TUMBUH-TUMBUHAN PERUBATAN SEBAGAI ANTI DIABETIS

ABSTRAK

Tiga alternatif tekniks elektro-enzim tekniks telah dibangunkan untuk mengukur potensi tumbuh-tumbuhan perubatan sebagai antidiabitis. Ketiga-tiga teknik tersebut adalah berdasarkan kepada perencatan enzim alfa glucosidase (AG) di dalam pertukaran para-nitrofinil –alfa-D-glukopiranosaid (PNPG) kepada para-nitrofenol (PNP) yang dimangkinkan oleh enzim AG. Teknik yang pertama, pes elektrod tiub nano karbon pelbagai dinding (MWCNTs) adalah terdiri daripada campuran seragam serbuk MWCNTs dan minyak mineral di dalam nisbah 60:40 dan telah digunakan bersama dengan enzim bebas dan larutan PNPG. Technik yang kedua, elektrod skrin tercetak tiub nano karbon (SP-CNTs) adalah berdasarkan kepada elektrod skrin tercetak (SPE) komersial dan telah digunakan bersama dengan enzim bebas dan larutan PNPG. Teknik yang ketiga, biosensor pakai buang, adalah berdasarkan kepada elektrod pertama dan kedua di mana enzim AG telah dipegun (immobilized) secara kovalen ke atas amina terfungsi tiub nano karbon pelbagai dinding (MWCNTs-NH_2) diikuti dengan pemerangkapan PNPG sebagai substrat dengan menggunakan poly(vinil alcohol) (PVA) terawat secara pembekuan-nyahbekuan ke atas SP-CNTs. PNPG telah diperangkap pada pH yang rendah untuk mengelakkan tindakbalas awal di antara PNPG dan enzim sekat gerak. Enzim AG yang terpegun dan PNPG di atas MWCNTs-NH2 telah dicirikan oleh Spektroskopi Inframerah Penukaran Fourier (FTIR) dan Mikroskop Imbasan Elektron (SEM). Kebolehgunaan setiap teknik untuk pengukuran antidiabitis telah diuji menggunakan tiga jenis tumbuhan ubatan iaitu Tebengau (*Ehretia laevis*), Cemumar (*Micromelum pubescens*), Kedondong (*Spondis dulcis*) dan ubat komersial Acarbose melalui spektrofotometri, voltametrik berkitas (CV) dan kaedah amperometrik. Hasil kajian menunjukkan perencatan daripada ekstak tumbuhan Tebengau lebih banyak daripada Acarbose, Cemumar dan Kedondong. Kinetik enzim sekat gerak dan tidak sekat gerak telah diukur dengan menggunakan persamaan Lineweaver –Burk. Tindakbalas CV untuk perencatan aktiviti enzim AG didalam biosensor oleh ekstrak tumbuhan Tebengau telah menunjukkan hubungan linear diantara julat 0.23 – 8.29 uA dan pengesan tahap perencatan adalah 0.253uA. Biosensor telah menunjukkan sensitiviti yang baik (0.422 uA/mg ekstrak tumbuhan Tebengau) dan tindakbalas yang cepat (22s). Biosensor mengekalkan lebih kurang 79.16 % aktiviti awalnya sehingga selepas 30 hari ianya disimpan pada suhu 4^{o}C. Kebolehulangan dan kebolehhasilan teknik dan biosensor pakai buang telah berjaya. Oleh itu, teknik dan biosensor pakai buang boleh digunakan untuk mengukur potensi tumbuhan-tumbuhan herba sebagai antidiabitis selain daripada mengukur aktiviti ubat antidiabitis komersial.

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ABSTRACT

Three alternative electro-enzyme techniques were developed for measuring antidiabetic potential of medicinal plants. All three techniques are based on the inhibition of α-glucosidase (AG) enzyme in the conversion of para-nitrophenyl-α-D-glucopyranoside (PNPG) into para-nitrophenol (p-NP) which is catalyzed by AG enzyme. The first technique, multi-walled carbon nanotubes (MWCNTs) paste electrode comprised of a uniform mixture of MWCNTs powder and mineral oil at the ratio of 60: 40 and used with free enzyme and PNPG solution. The second technique, screen printed carbon nanotubes (SP-CNTs) electrode was based on commercial screen printed electrode (SPE) and used with free enzyme and PNPG solution. The third technique, disposable biosensor, was based on the extension of the first and second electrodes where AG enzyme was covalently immobilized onto amine functionalized multi-walled carbon nanotubes (MWCNTs-NH_2) followed by entrapment of PNPG as a substrate using freezing–thawing treated poly(vinyl alcohol) on the SP-CNTs. The PNPG was entrapped at low pH to prevent the premature reaction between PNPG and immobilized enzyme. The immobilized AG enzyme and PNPG on MWCNTs-NH_2 was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The applicability of each technique for measuring antidiabetic was tested using three types medicinal plants namely Tebengau (*Ehretia laevis*), Cemumar (*Micromelum pubescens*), Kedondong (*Spondias dulcis*) and a commercial antidiabetic drug Acarbose via spectrophotometric, cyclic voltammetry (CV) and amperometric methods. The results showed that the inhibition obtained in the presence of Tebengau plant extracts is higher than that obtained with Acarbose, Cemumar and Kedondong. The kinetic of immobilized and non-immobilized enzyme was measured using Lineweaver-Burk equation. The CV response for inhibition of AG enzyme activity within the biosensor by Tebengau plant extracts showed a linear relationship in the range from 0.5 – 3.5 mg/mL and an inhibition detection limit was 0.5 mg/mL. The biosensor exhibited good sensitivity (1.037 µA/mg Tebengau plant extracts) and rapid response within 22 seconds. The biosensor retains about 79.16 % its initial activity even after 30 days when stored at 4^0C. The repeatability and reproducibility of the technique and disposable biosensor was satisfactory. Therefore, the techniques and disposable biosensor could be used for measuring the anti-diabetic potential of medicinal plants as well as to monitor the activity of commercial antidiabetic drugs.

CHAPTER 1

INTRODUCTION

1.1. Research Background

Diabetes mellitus (DM) is one of the major lives threatens diseases in the world and caused by the absolute or relative absence of insulin (Amos et al., 1997). The burden of diabetes is increasing globally and 346 million people are suffering worldwide by diabetes according to World Health Organization (WHO, 2011). Usually, DM is classified as Type 1 and Type 2 in which most of diabetes patients are suffering by Type 2. Type 2 DM can be controlled by various synthetic antidiabetic drugs, however, these synthetic drugs have some side effect such as weight gain, pain at the site of injection, a feeling of fullness in the abdomen, hypoglycemia, and poorly controlled blood glucose levels (Fujimoto et al., 2013). As a result, there is an increasing demand for antidiabetic drugs produced from natural resources with a relatively low cost and fewer side effects (Liu et al., 2013)

In fact, many medicinal plants found around the world exhibit a significant potential for the treatment of DM. Currently, the antidiabetic potential of medicinal plants is measured in the laboratory through the inhibition of the AG enzyme reaction. In human body, carbohydrate is digested through AG and α-amylase enzyme reaction to produce glucose. This AG enzyme reaction can be inhibited by the medicinal plant extracts. The inhibition is defined as the antidiabetic potential of medicinal plants. This inhibition is usually determined through a number of conventional methods, such as the

colorimetric method [Kumar et al., 2011], the titration method [Goldberg et al., 2012) high-performance liquid chromatography (HPLC) [Shivanna et al., 2013]. However, these approaches carry some drawbacks, such as time-consuming nature and the need of expensive devices and well-trained operators. Moreover, these methods require elaborate sample pre-treatment [Malode et al., 2012]. As a result, the development of a convenient and highly sensitive quality control method is an urgent requirement for the screening of medicinal plants for the treatment of DM.

For this purpose, MWCNTs was used to fabricate the MWCNTs paste electrode as sensor to measure the antidiabetic potential of medicinal plants. Because, the recent development of carbon nanomaterials have provided many new advantages for electroanalysis. In particular, MWCNTs are new types of carbon nanostructure materials which is widely using for modification of electrodes due to their electronic, chemical and mechanical properties, such as electrocatalytic outcome, rapid electron transfer rate, broad working surface area (Jeykumari et al., 2007), and chemical functionalization, make them particularly fascinating for electrochemical sensing (Shahrokhian and Zare-Mehrjardi, 2007). Moreover, the paste electrodes are easy to modify and they have renewable surfaces, stable response, low ohmic resistance and wide operational window (Shahrokhian et al., 2010; Zhou et al., 2009).

Carbon nanotubes (CNTs) based screen-printed carbon nanotubes (SP-CNTs) electrodes were also used electrochemically to measure the antidiabetic potential of medicinal plants due to their many advantages: these are low-cost disposable devices that are mass produced, easy to use, designed to work with micro-volumes of samples, portable and require low amounts of reagents and samples (Moreno et al., 2010; Metters

et al.,2013). Finally, MWCNTs based enzymatic disposable biosensor was developed to determine the antidiabetic potential of medicinal plants. In this case, AG enzyme was immobilized by covalent immobilization with amine functionalized multi-walled carbon nanotubes (MWCNTs-NH_2) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as coupling reagent associated to N-hydroxysuccinimide (NHS) in order to improve immobilization efficiency. The immobilized AG enzyme was again entrapped in poly (vinyl alcohol) (PAV) together with PNPG as substrate and dropped on the screen-printed carbon (SPC) electrode to develop the disposable biosensor. The developed biosensor was used electrochemically to measure the antidiabetic potential of medicinal plants. Because in recent years, the electrochemical techniques are becoming popular due to their high sensitivity, low cost, and short analysis time (Martín-Yerga et al., 2012).

PNPG hydrolyses by AG enzyme to release para-nitrophenol (p-NP) as the following reaction in Figure 1.1 (Timur and Anik, 2007). This reaction is used to measure the AG enzyme activity as well as to detect the antidiabetic potential of medicinal plants

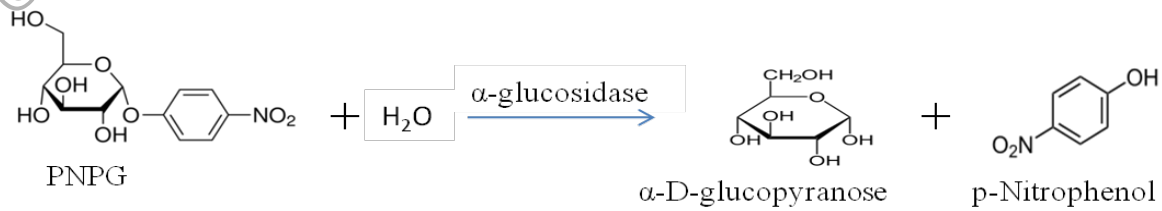

Figure 1.1: Enzymatic hydrolysis of PNPG

The resulting disposable biosensor, MWCNTs paste electrode and SP-CNTs electrode were used for measuring the AG enzyme activity to detect the liberated p-NP by CV method. The activity of the biosensor and sensors were investigated by detecting