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BIOACTIVE COMPOUNDS OF PALM KERNEL CAKE

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BIOACTIVE COMPOUNDS OF PALM KERNEL CAKE

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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan projek "BIOACTIVE COMPOUNDS OF PALM KERNEL CAKE" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Khairunisa Amira binti Ahmad (162213) sebagai syarat untuk kursus BCH 4999 Projek.

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LIST OF ABBREVIATIONS

Abs	Absorbance
CO_2	Carbon dioxide
DCM	Dichloromethane
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl
EBV	Epstein-Barr virus
GC-MS	Gas chromatography mass spectrometry
H_2O_2	Hydrogen peroxide
HBV	Hepatitis B virus
HPV	Human papillomavirus
IC_{50}	Half maximal inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO	Nitric oxide
NO_3	Nitrate
O_2	Oxygen
$^1O_2$	Singlet oxygen
O_2^-	Superoxide
OD	Optical densities
•OH	Hydroxyl radical
PBS	Phosphate-buffered saline
PKC	Palm kernel cake
ROS	Reactive oxygen species
RNS	Reactive nitrogen species

ABSTRACT

In Malaysia, the incidence of cancer is increasing and it is ranked number four as a disease that caused death. One of the causes of cancer is the presence of free radicals especially reactive oxygen species (ROS). Antioxidants are able to scavenge free radicals and inhibit their formation. The research of finding antioxidants in natural products such as from plant has been increasing because the use of synthetic antioxidants may be toxic to humans. Plant contains secondary metabolites that are considered as bioactive compounds because they have pharmacological potentials which give benefits to human health. In this study, palm kernel cake (PKC) was used as a source of bioactive compounds for antioxidant and anticancer activities. The results showed that PKC extracts possessed antioxidant activity as the compounds present were able to scavenge 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical. Extracts prepared from freeze-dried extract were better when compared to that prepared from oven dried PKC. The IC_{50} value obtained was 487±0.02 µg/ml. PKC extracts also showed anti-cancer property significantly ($P < 0.05$) when they were used to treat cancer cell lines *in vitro*, in a dose dependent manner. PKC extracts were able to reduce the cell viabilities of human breast cancer cell line (MCF-7), human hepatoma cancer cell line (HepG2), human lung cancer cell line (A549) and human cervical carcinoma cancer cell line (HeLa). The PKC extracts were observed to contain high phenolic compounds. For freeze-dried PKC extract, the total phenolic content was 15.26±1.18 mg GAE/g of PKC extract and 7.61±0.87 mg GAE/g for oven-dried PKC extract. Most of the compounds analysed by gas chromatography mass spectrometry (GCMS) were fatty acids and phenols and their derivatives. The phenolic compounds were known to have antioxidant and anticancer activities.

ABSTRAK

Di Malaysia, penyakit kanser semakin meningkat dan berada di kedudukan ke-empat sebagai penyakit yang menyebabkan kematian. Salah satu punca kanser adalah kehadiran radikal bebas terutamanya spesies oksigen reaktif. Antioksidan mampu menghapuskan radikal bebas dan menghalang pembentukannya. Penyelidikan dalam mencari antioksidan daripada sumber semulajadi seperti tumbuhan telah meningkat kerana penggunaan antioksidan sintetik dikhuatiri toksik kepada manusia. Tumbuhan mengandungi metabolit sekunder yang dianggap sebagai sebatian bioaktif kerana berpotensi untuk farmakologi dan memberi manfaat kepada kesihatan manusia. Dalam kajian ini, hampas isirong palma atau *Palm kernel cake* (PKC) telah digunakan sebagai sumber sebatian bioaktif untuk aktiviti antioksidan dan anti kanser. Hasil kajian menunjukkan bahawa ekstrak PKC mempunyai aktiviti antioksidan kerana sebatian yang hadir mampu menghapuskan radikal bebas, 2,2-diphenyl-1-picryl-hydrazyl (DPPH). Ekstrak disediakan daripada beku-kering adalah lebih baik daripada PKC ketuhar kering. Nilai IC_{50} yang diperolehi ialah 487 ± 0.02 μg / ml. Ekstrak PKC juga menunjukkan aktiviti anti-kanser secara signifikan ($P < 0.05$) apabila ia telah digunakan untuk merawat garisan sel kanser *in vitro*, dan bergantung kepada dos. Ekstrak PKC dapat mengurangkan peratus sel yang hidup pada garisan sel kanser payudara (MCF-7), garisan sel kanser hati (HepG2), garisan sel kanser paru-paru (A549) dan garisan sel kanser serviks (HeLa). Ekstrak PKC mengandungi sebatian fenolik yang tinggi. Bagi ekstrak PKC beku-kering, jumlah kandungan fenolik adalah 15.26 ± 1.18 mg GAE / g ekstrak PKC dan 7.61 ± 0.87 mg GAE / g bagi ekstrak PKC ketuhar-kering. Kebanyakan sebatian dianalisis oleh *gas chromatography mass spektrometry* (GCMS) adalah asid lemak dan fenol serta terbitannya. Sebatian fenolik telah diketahui mempunyai aktiviti antioksidan dan anti kanser.

CHAPTER 1.0

INTRODUCTION

1.1 Introduction

The increasing number of cases involving cancer has become a major problem for most of the countries in the world. GLOBOCAN is a project that estimated the incidence, prevalence and mortality of cancer and estimated that in 2008, the number of people died of cancer was about 7.6 million people. World Health Organization (WHO) also estimated that 12 million people will die of cancer in 2030 (Farooqui *et al.*, 2013). This problem is also affecting Malaysia as cancer is ranked number four as the cause of death of the patient. The types of cancer common among men are lung, nasopharynx and stomach cancer while for women are breast, cervix and ovary cancer (Chin & Lim, 2002).

One of the causes of cancer is due to the activity of free radicals in the form of reactive oxygen species (ROS) that is abundant in human body due to oxidative stress (Wong *et al*., 2006). ROS like hydroxyl, superoxide and peroxyl radicals that formed in human tissue cell can caused damaged to the DNA, protein and lipids which will lead to cancer and other human diseases (Ganesan *et al*., 2008). Compounds with antioxidant property will prevent oxidation of cell contents by scavenging ROS or inhibiting their formation (Gupta & Sharma, 2006). In food industry, synthetic antioxidant like butylated hydroxytoluene (BHT) is added to control lipid oxidation but it may be toxic and harmful to the human health. Therefore, antioxidants from natural products are more favourable (Wong *et al*., 2006).

Plants contained secondary metabolites that are not used for the main growth and development of plants, but are usually used for plant protection and signalling activities. These metabolites are considered as bioactive compounds because they affect human health as they have pharmacological potentials (Bernhoff, 2010). There are three main categories of bioactive compounds in plants which are i) terpenes and terpenoids, ii) alkaloids and iii) phenolics compounds (Croteau *et al.*, 2000). The most abundant bioactive compound in plants is phenolic compounds that generally possess antioxidant activity to scavenge free radicals (Dai & Mumper, 2010). Therefore, it is considered that plants have metabolites that contain antioxidant and anti-cancer properties with pharmaceutical potentials.

Palm kernel cake (PKC) is a plant material that is believed to have antioxidant property as it is a by-product of palm fruit (*Elaeis guineensis*). In 2014, about 2.5 million tonnes of PKC is produced in Malaysia covering the area of 4.917 hectares of oil palm (MPOB, 2015). PKC has been used as animal feed, but it should be explored as a source of bioactive compounds. Therefore, the main purpose of this project was to evaluate whether PKC could be a potential source of metabolites with biological activities.

1.2 Objectives

The specific objectives were:

1) To determine the effects of different drying methods and different solvents for the extraction of bioactive compounds in PKC.
2) To conduct antioxidant and anti-cancer assay using PKC extracts.
3) To determine the bioactive compounds present in PKC extracts.

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