SCREENING OF SELECTED POLYPHENOL COMPOUNDS IN PALM OIL MILL EFFLUENT (POME)

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To my beloved father and mother, Mr. Sharif and Mrs. Fouziah Ali,
sister, brother, friends and not to be forgotten Dr. Razauden Mohamed Zulkifli,
thanks for all supports and motivations given…

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ABSTRACT

Total antioxidant activities and phenolic contents of raw and treated POME extracts (rPOME and tPOME respectively) were investigated. The total antioxidant activity was estimated using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The total phenolic contents were measured using Folin-Ciocalteu assay. Thin layer chromatography and reversed-phase high-performance liquid chromatographic (RP-HPLC) separation methods with UV spectrophotometric detection were also used for the determination of compound of interest in POME extracts. Identification was based on retention times and UV spectra by comparison with commercial standards. The standards used were Gallic acid, Resveratrol, Quercetin and Kaempferol. The extracts were analyzed after extraction with ethanol. Resveratrol were identified in the rPOME while no detection of any four of the standards was showed in the tPOME. From the study, rPOME was clearly shown to have higher antioxidant activities and total phenolic contents than the tPOME. The highest percentage inhibition of the DPPH by rPOME was 89.5% and the total phenolic content measured was 6.5 mg of gallic acid equivalents (GAE). In conclusion, palm oil mill effluent could potentially become an alternative source of antioxidant.

ABSTRAK

Kajian mengenai aktiviti antioksidan dan kandungan bahan phenolik di dalam ekstrak bahan buangan kelapa sawit yang tidak dirawat dan dirawat (rPOME dan tPOME) telah dijalankan. Analisis DPPH (2,2-diphenyl-1-picrylhydrazyl) telah digunakan untuk menentukan aktiviti antioksidan yang terdapat di dalam ekstrak. Kandungan bahan phenolik di dalam ekstrak pula diukur menggunakan analisis Folin-Ciocalteu. Teknik Thin Layer Chromatography (TLC) dan teknik pemisahan Reversed-phase High Performance Liquid Chromatography (RP-HPLC) dengan pengesan spektrofotometri juga telah digunakan untuk mengenal pasti bahan-bahan yang terkandung di dalam ekstrak POME. Bahan-bahan yang terkandung di dalam ekstrak dikenalpasti berdasarkan perbandingan terhadap waktu tahanan dan spektrum UV bahan piawai. Bahan piawai yang digunakan sebagai perbandingan adalah Gallic acid, Resveratrol, Quercetin dan Kaempferol. Semua analisis dijalankan setelah sampel bahan buangan sawit diekstrak dengan pelarut ethanol. Resveratrol telah dikanalpasti terkandung di dalam rPOME manakala tPOME tidak mengandungi keempat-empat bahan piawai. Daripada kajian ini, nilai aktiviti antioksidan dan kandungan bahan phenolik didapati lebih tinggi di dalam rPOME berbanding tPOME. rPOME menunjukkan peratusan penghalangan aktiviti DPPH tertinggi oleh ekstrak sebanyak 89.5% dan jumlah keseluruhan kandungan bahan phenolik di dalam rPOME adalah 6.5 mg bersamaan piawai Gallic acid (GAE). Sebagai kesimpulan, bahan buangan kelapa sawit berpotensi sebagai sumber alternatif antioksidan.

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LIST OF ABBREVIATIONS

$AgSO_4$	-	Silver sulphate
ApcI	-	Atmospheric pressure chemical ionization
BOD	-	Biochemical oxygen demand
°C	-	Degree celcius
CID	-	Collision-induced dissociation
Cm	-	Centimeters
COD	-	Chemical oxygen demand
DAD	-	Diod array detector
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
ESI	-	Electrospray ionization
g	-	Gram
GAE	-	Gallic acid equivalent
g/kg	-	Gram per kilogram
GSH	-	Glutathione
GSSG	-	Glutathione disulfide
H_2O_2	-	Hydrogen peroxide
H_2SO_4	-	Sulphuric acid
$HgSO_4$	-	Mercury (II) sulphate
$K_2Cr_2O_7$	-	Potassium dichromate

L	-	Liter
LC/MS	-	Liquid chromatography coupled to mass spectrometry
m^3	-	Meter cubic
mg	-	Miligram
ml	-	Mililitre
mg/kg	-	Miligram per kilogram
mg/l	-	Miligram per liter
MS	-	Mass spectrometry
Na_2CO_3	-	Sodium carbonate
NADPH	-	Nicotinamide adenine dinucleotide phosphate
nm	-	Nanometer
NMR	-	Nuclear magnetic resonance
$O_2^{-\bullet}$	-	Superoxide anion radicals
$^{\bullet}OH$	-	Hydroxyl radical species
POME	-	Palm oil mill effluent
R_f	-	Retention factor
ROS	-	Reactive oxygen species
rpm	-	Rotary per minutes
RP-HPLC	-	Reversed-phase high performance liquid chromatography
rPOME	-	Raw palm oil mill effluent
S.E.M	-	Standard error mean
tPOME	-	Treated palm oil mill effluent
TLC	-	Thin layer chromatography
UV	-	Ultraviolet
UV-vis	-	Ultraviolet-visible
µl	-	Microliter

μM	-	Micromolar
μg/ml	-	Microgram per milliliter
v/v	-	Volume per volume
wt	-	Weight

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CHAPTER 1

INTRODUCTION

1.1 Background

The Malaysian palm oil industry is growing rapidly and is becoming a very important agriculture-based industry, where the country today is the world's leading producer and exporter of palm oil, replacing Nigeria as the chief producer since 1971 (Yusoff, 2006). However, wet process of palm oil milling consumes a large amount of process water. It is estimated that for 1 tonne of crude palm oil produced, 5-7.5 tonnes of water are required, and more than 50% of the water will end up as palm oil mill effluent (POME) (Ahmad *et al.*, 2003). Raw POME is a colloidal suspension containing 95-96% water, 0.6-0.7% oil and 4-5% total solids including 2-4% suspended solids that are mainly consisted of debris from palm fruit mesocarp generated from three main sources, namely sterilizer condensate, separator sludge and hydrocyclone wastewater (Borja and Banks, 1994). For a well-controlled conventional mill, about 0.9, 1.5 and 0.1m^3 wastewater are generated from sterilizer condensate, separator sludge and hydrocyclone wastewater, respectively, for each tonne of crude palm oil produced (Khalid and Wan, 1992).

In the year 2004, more than 40 million tonnes of POME was generated from 372 mills in Malaysia (Yacob *et al*., 2006). If the effluent is discharged untreated, it can certainly cause considerable environmental problems (Davis and Reilly, 1980) due to its high biochemical oxygen demand (25,000 mg/l), chemical oxygen demand (53,630 mg/l), oil and grease (8370 mg/l), total solids (43,635 mg/l) as well as suspended solids (19,020 mg/l) (Ma, 1995). Therefore, the palm oil mill industry in Malaysia is identified as the one that produces the largest pollution load into the rivers throughout the country (Hwang *et al*., 1978).

The discharge of untreated POME creates adverse impact to the environment. However the idea of nurturing POME and its derivatives as valuable resources should not be dismissed. This is because POME contains high concentrations of protein, carbohydrate, nitrogenous compounds, lipids and minerals (Habib *et al*., 1997) that may be converted into useful materials (Agamuthu and Tan, 1985). Several studies have been reported on the exploitation of POME and its derivative as fermentation media to produce antibiotic and bioinsecticide (Suwandi, 1991), solvents (Somrutai *et al*, 1996), polyhydroxyalkanoates (Hassan *et al*, 1996), organic acids (Yee *et al*, 2003) as well as enzymes. Furthermore it is also believed that POME contains polyphenolic compounds that play important role as antioxidant (Prasertsan *et al*, 1997).

Polyphenol is a group of chemical substances found in plant that can be characterized based on more than one phenol groups in the compound itself. Recently, polyphenol has been given a great attention due to huge market for food supplement but most importantly their ability to act as an effective antioxidant, anticancer properties, and beneficial cardiovascular effect and promotes long life (Cho *et al*., 2006, Pearson et al., 2008, Howitz *et al*., 2003, Valenzano *et al*., 2006). Example of polyphenol derived from plant are Syringic acid, Coumaric acid, Ferulic acid, Resveratrol, Quercetin, Kaempferol and Isorhamnetin (Li *et al*., 2009). Some of these polyphenol for instance already on the market for sale as a supplement such as Resveratrol. Resveratrol is found in red grape, red wines and onions in small quantity (Soleas *et al*., 2000). However, the studies of the polyphenol content in POME have yet to be discovered.

1.2 Justification Rational of the Research

Waste substances generated from industries have becoming a major problem to the environment. Some wastes are hazardous and need to be removed immediately to avoid it from entering the food chain and polluting the ecosystem. Biorecycling or the potential usage of industrial waste in particular Palm Oil Mill Effluents (POME) is one of the solution other than treated the waste directly. It is estimated that an average about ten percent of raw POME is generated for every fresh fruit bunch processed. POME consists of water soluble component as well as suspended materials like palm fiber and oil. It is believed that this waste carrying secondary metabolite that can be extract and are useful for commercialization in particular polyphenol and flavanoids. If the potential polyphenols, were found and characterized, the waste can be turned into supplement to human and farm animal hence increased our health, capital and at the same time reducing the waste.

1.3 Objectives

The objectives of the research are:

a) To measure the antioxidant activities of the POME extracts by DPPH (2,2 diphenyl-1-picrylhydrazyl) assay.
b) To measure the amount of total polyphenol extracted from the POME by Folin-ciocalteu assay.
c) To extract and identify different types of polyphenol present in the POME extracts by Thin Layer Chromatography (TLC) and Reversed-phase High Performance Liquid Chromatography (RP-HPLC).

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