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EXTRACTION AND SCREENING OF ANTIOXIDANTS IN *Metroxylon sagu*

NOOR IDAYU MHD TAHIR

THIS THESIS IS SUBMITTED TO FULFILL THE REQUIREMENT IN OBTAINING THE DEGREE OF SCIENCE BACHELOR WITH HONOURS

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March 2004

DECLARATION

I, hereby declare that this thesis is of my own work and effort, except the quotations, excerpts and facts, for which each source has been clearly stated.

March 12, 2004

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VERIFICATION

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ABSTRACT

Antioxidants were extracted from sago pith, sago processing effluent and waste sago pith obtained from a small-scale sago processing site in Beaufort with methanol to be further studied with phytochemical and biological activity tests while hexane was used to extract vitamin A and its precursors. The extracts were submitted to four phytochemical tests which detected flavonoids, tannin and phenolic compounds in the extracts. The concentration of flavonoids was contained highest in sago pith, followed by its waste water and the waste pith. Tannin presence was observed highest in the processing waste water. Biological activities of the extracts were assessed trough linoleic acid autoxidation test, peroxidation value determination test and brine shrimp lethality test. Sago pith showed the longest induction period of 5 days which indicates the highest antioxidant activity. The peroxidation value determination test showed that extract of waste water possess the highest capability of preserving the unsaturated fat from oxidizing, followed by sago pith extract and waste pith extract. Mortality percentage in brine shrimps incubated with different concentration of extracts was rather low, indicating that the extracts do not cause lethality in brine shrimps. Several phenolic compounds were observed after running silica gel 60 F_{254} column chromatography (acetone: water: acetic acid, 75:20:5, v/v) with subsequent thin layer chromatography (n-propanol:water, 64:36, v/v). Vitamin C was determined at 264 nm by using a C18 reversed-phase column HPLC with a mobile phase of acetonitrile: water (75:25, v/v) in which the highest concentration was observed in waste water, followed by sago pith and waste pith. Carotene was detected at 450 nm using UV/Vis spectrophotometer where sago pith contain the highest concentration, followed by waste pith and waste water extract. Sago pith and its by-products (wastes) contain antioxidants and are non-toxic.

ABSTRAK

Bahan-bahan antioksida diekstrak dari pokok sagu, sisa cecair dan sisa pepejal pemprosesan sagu yang diperolehi dari tapak pemprosesan sagu kecil-kecilan di Beaufort. Pengekstrakan dilakukan menggunakan metanol untuk analisis fitokimia, aktiviti biologi dan kromatografi. Heksana pula digunakan untuk mengekstrak vitamin A dan prekursornya. Analisis fitokimia yang dijalankan ke atas ekstrak menunjukkan ia mengandungi flavonoid, tanin dan bahan-bahan fenolik. Kepekatan flavonoid adalah tertinggi di dalam ekstrak pokok sagu, diikuti dengan sisa cecair dan seterusnya sisa pepejal pemprosesan. Kandungan tanin tertinggi di dalam ekstrak sisa cecair pemprosesan. Aktiviti biologi ekstrak-ekstrak dikaji melalui ujian autoksida asid linoleik, ujian penentuan nilai peroksida dan ujian kemautan udang laut. Ekstrak pokok sagu memberikan tempoh induksi terpanjang iaitu 5 hari di mana aktiviti antioksidanya bertahan paling lama. Ujian penentuan nilai peroksida menunjukkan ekstrak sisa cecair mempunyai kebolehan tertinggi untuk mengelakkan pengoksidaan lemak tak tepu (asid linoleik), diikuti oleh ekstrak pokok sagu dan sisa pepejal. Peratus kemautan udang laut adalah rendah sama sekali di mana lebih tinggi kepekatan ekstrak yang digunakan, lebih tinggi peratus udang yang hidup. Kromatografi Lapisan Nipis (TLC) menggunakan gel silica 60 F_{254} dengan fasa mobil n-propanol: air (64:36, v/v) selepas penulenan dengan kromatografi turus gel silica 60 F_{254} dengan fasa mobil aceton: air: asid asetik (75:20:5, v/v) memperlihatkan kehadiran bahan-bahan fenolik dalam ekstrak-ekstrak. Vitamin C dapat dikesan pada 254 nm menggunakan Kromatografi Cecair Berkeupayaan Tinggi (HPLC) kolum C18 fasa berbalik dengan fasa mobil acetonitril: air (75:25, v/v) di mana kepekatan tertinggi terkandung dalam ekstrak sisa cecair pemprosesan, diikuti oleh ekstrak pokok sagu dan sisa pepejal. Karoten dikesan pada 450 nm menggunakan spektrofotometer UV/Vis di mana ekstrak pokok sagu mempunyai kepekatan tertinggi diikuti dengan sisa pepejal dan sisa cecair pemprosesan. Sagu dan bahan buangannya mempunyai bahan antioksida dan tidak toksik.

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$HO^{\cdot}$	hydroxyl radicals
$O_2^{\cdot -}$	superoxide ion
$^{\cdot}NO$	nitric oxide
$O^{\cdot}$	singlet oxygen
$ROO^{\cdot}$	peroxyl radicals
$HO_2^{\cdot}$	hydroperoxyl radical
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
TBHQ	tertiary butylated hydroquinone
PG	propyl gallate
cm	centimetre
GSH	glutathione
Q_{10}	coenzyme Q
SOD	superoxide dismutase
LDL	low density lipoprotein
CH_3	methyl group
AMP	adenosine monophosphate
UV/Vis	Ultra Violet/Visible
RDA	Recommended Dietary Allowance
HPLC	High Performance Liquid Chromatography
PV	Peroxidation Value
TLC	Thin Layer Chromatography
H_2O	water
NH_3	ammonia
g	gram
°C	degree Celcius
ml	mililitre
%	percentage
$FeCl_3$	ferum chloride (III)
nm	nanometre
N	Normality
ppm	part per million
DMSO	dimethyl sulphoxide
(w/v)	weight upon volume
HCl	hydrochloride acid
NaCl	natrium chloride
Abs_{500}	absorbance at 500 nm
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
SCN^-	thiocyanate ion
$Fe(SCN)_6^{3-}$	ferum hexacyanoferat ion
$Na_2S_2O_3$	sodium thiosulphate
Abs_{450}	absorbance at 450 nm

CHAPTER 1

INTRODUCTION

1.1 Free radicals and antioxidant

Free radicals are atoms or group of atoms with odd electron number or unpaired electron that are produced after oxygen react with certain molecules (by-products of oxygen metabolism). When these reactive free radicals are formed, it will begin a chain reaction like a domino effect, resulting more destructive reaction. Free radicals promote oxidation that result in high energy and kills bacterial invaders by stealing electron from the nearest stable mode. However, danger comes when they react with main cellular component such as DNA or the cell membrane. Cell function will be disrupted or the cell can even die. Reactive radical species such as hydroxyl radicals ($HO^{\cdot}$), superoxide ion ($O_2^{\cdot-}$), nitric oxide ($^{\cdot}NO$), singlet oxygen ($O^{\cdot}$), peroxyl radicals ($ROO^{\cdot}$) and hydroperoxyl radical ($HO_2^{\cdot}$) need to be controlled and prevented from damaging the lipids, proteins and other complexes, for examples; unsaturated fatty acid, hyaluronic acid, DNA and RNA. These free radicals are also associated with diseases of ageing process.

To avoid the damaging effect of free radicals, human body gets protection from antioxidant defence system. Scientific research has shown that antioxidant has roles in protecting human from various diseases. There are plenty of antioxidant resources that can be consumed to decrease the action of free radicals against human such as carrots, tomatoes, yam, green vegetables, blueberries, ginko biloba, garlic, green tea and etcetera.

Antioxidant can be defined as substance that slow down or even stop oxidative damage on attacked molecules by free radicals. Oxidative damage is generally known as effects of free radicals reaction onto biological molecules. Antioxidant protects the body by removing the catalysts for example the enzyme in food oxidation or substituting the component or compound that are oxidize-prone or even repairing the damage made by free radicals.

Vitamin A, vitamin C, vitamin E, flavonoids, carotenoids, and tannins are some of the natural occurring antioxidant, just to name a few. While examples of synthetic antioxidant are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinone (TBHQ) and propyl gallate (PG) which are commercially used as food additives.

Vitamin A is fat-soluble vitamin which is found in animal product such as meat and milk. It can also be made by our body by its precursor, β-carotene. As an antioxidant, vitamin A protects cell membrane and fat tissue, helps to repair damage of the body by pollutants in air and increase body defence system.

Vitamin C was firstly renowned in 1970s when a scientist that won the Nobel Prize, Linus Pauling recommended a high daily dosage (8-10 gram) of vitamin C to avoid or to treat cold. As an antioxidant, vitamin C helps to fight against cardiovascular diseases by protecting the artery track from oxidative damage. There are also research that state vitamin C as a body protection from cigarettes smoke and other air pollutants.

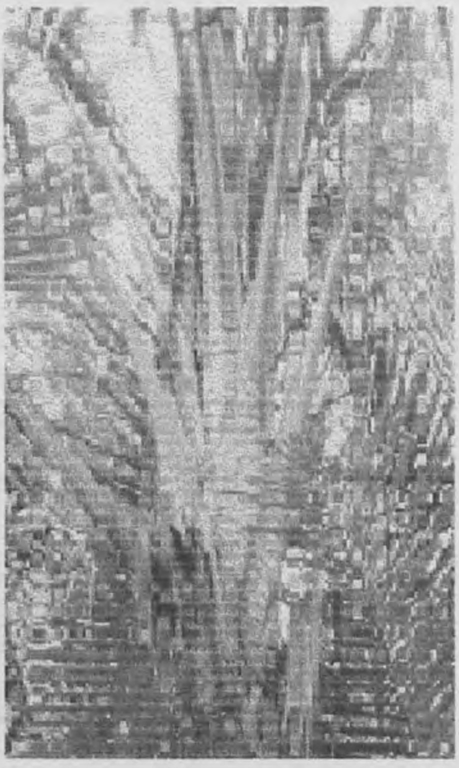
Vitamin E is an effective chain breaking antioxidant in protecting poly-unsaturated lipid from harmed by free radicals. However, it interact better if taken along with other antioxidant especially vitamin C, selenium and β-carotene. Vitamin E can also increase cardiovascular health. It acts as vasodilator that expands the vein and prevents the hardening of arteries and blood clotting.

Bioflavonoids consist of 5000 substances that react as antioxidant. It can be found in fruits, vegetables, green tea, soy products, herbs and spices. Bioflavonoid activities are more effective if combined with vitamin C. this combination is used in allergy treatment, diabetes, hypertension and virus infection. One of the many examples in the flavonoid family is procyanidins that helps in regenerating other antioxidants that neutralize free radicals in the blood and body tissue.

1.2 *Metroxylon sagu*

Sago palm is known as 'rumbia' plant amongst locals. It is said to be originated from Papua New Guinea. Sago palm are planted widely especially in Sarawak in swampy area near rice fields and settlement area. It has a few local names like 'mulong' for the Ibans and Malays, 'dulo' (for the non-spiny types) and 'balau' (for the spiny types) for the Kenyah people.

Picture 1.1: Spiny type of sago palm



Picture 1.2: Non-spiny type of sago palm

Sago palm has various usages. Its leaves are made into house thatches, hats, baskets and blowpipe darts. When the plant reaches twelve years old, the sticky sap is used as gum and starches. Ripe fruits and palm cabbage are eaten. There are also the

Rhynchophorus schach larvae (sago grubs) which live in the trunks of palms that have been cut down for 2-3 days. These grubs are edible and highly prized by the locals.

Sago palm grows in natural peat swamps with minimal drainage and more environmental friendly compared to other crops requiring complete drainage of peat basin (MARDI report in The Planter (78) 2002). Sago starch production is centered in the Oya and Mukah river area in Sibu division, where the swamps extend as much as 32 km inland. In Peninsular Malaysia, sago palm planting is concentrated in Batu Pahat, Johor. Table 1.1 below shows the area of palm cultivation in Malaysia for the year 1986.

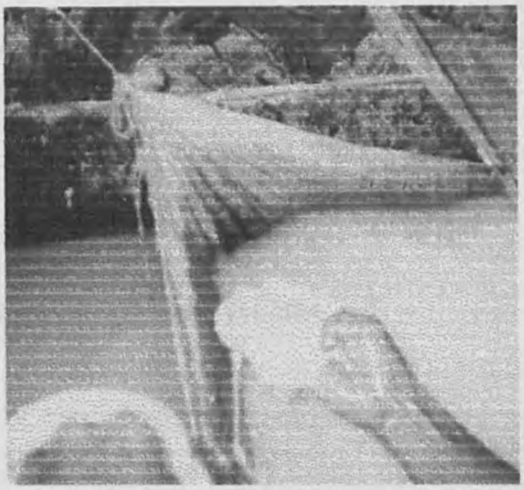
Table 1.1: Table of palm cultivation area in Malaysia for the year 1986.

(Source: Ministry of Agriculture)

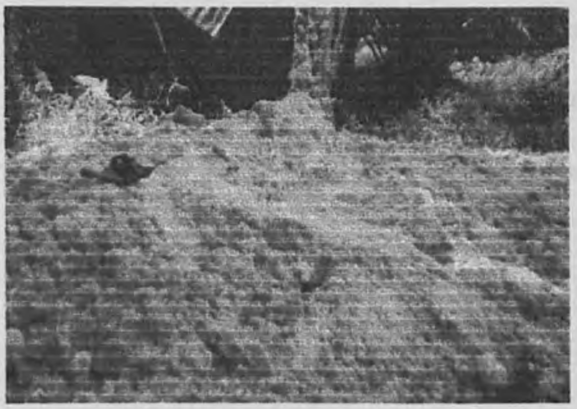
Crop	**Hectares under cultivation**
African oil palm	1359671
Coconut palm	182776
Sago palm	647
Betel nut palm	445
Salac palm	41
Sugar palm	33

Pictures 1.3 (a), 1.3 (b), 1.3 (c) and 1.3 (d) shows a route of sago utilization into a product (sago flour) and in the same time producing a big amount of waste (solid and liquid).

Picture 1.3 (a): A sago palm ready to be harvested



Picture 1.3 (b): Sago flour to be collected and dried



Picture 1.3 (c): Waste sago pith

Picture 1.3 (d): Sago processing effluent

1.3 Objectives of the project

Extraction and screening of antioxidant in *Metroxylon sagu* are done to study the biochemical composition that are classified as antioxidants for examples; vitamin A, vitamin C, vitamin E, flavanoids, tannin, and other phenolic compounds qualitatively.

The main objectives of this research are;

1) Extracting antioxidant in *Metroxylon sagu* and its waste products.

2) Screening and determining the types of antioxidant in the extract of *Metroxylon sagu* and its waste products.

3) Studying the biological activity of antioxidant agent in the extract of *Metroxylon sagu* and its waste products.

There are two types of waste, the solid and the liquid waste. Both of these wastes are considered to possess no commercial value to the sago starch producer and farmers. In a biological point of view, these wastes can be utilized into beneficial and valuable materials and further studies on their biological properties should be carried out.

CHAPTER 2

LITERATURE REVIEW

2.1 Sago as a source of food

After ten to twelve years old, the sago palm reaches its optimum maturity and can be cut down for use. The trunk of the plant is the main part to be utilized and processed. The starch extraction rate for each section of sago trunk (90 cm) is about eighteen percent (Tan *et al.*, 2002). Sago is extracted by a crude method of crushing or macerating the pith, washing it thorough, allowing the starch to settle at the bottom and then collected, dried and stored for use.

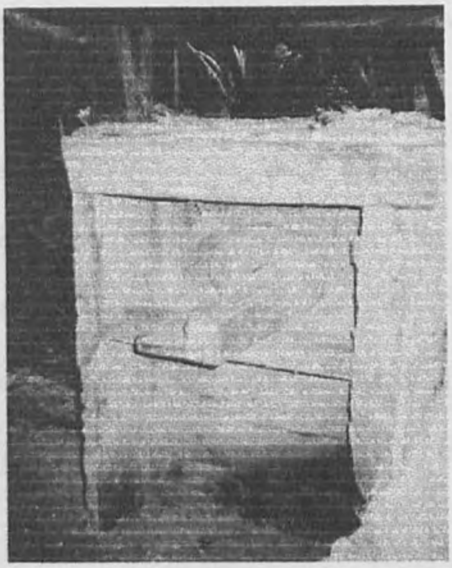
The sago starch processing in Sabah is considered only a cottage industry where factories using modern technology imported from Europe that enables production of higher quality of sago starch are located in Sarawak. Picture 2.1 shows the trunk of a sago tree in which the pith is later processed.

Picture 2.1: The pith of sago palm

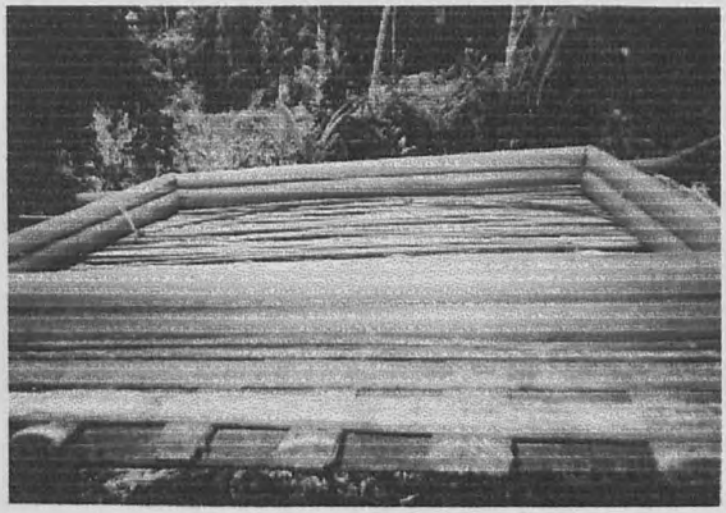
The photos below show a traditional technique used by villagers in Beaufort to process sago pith into sago flour.

Picture 2.2: The pith is inserted into the inlet of the macerating machine.

There is another method used in small scale sago processing. The sago pith is chopped using a simple mechanical cutter shown in Picture 2.3 and then brought to a platform made of sago trunk (Picture 2.4) to be crushed to obtain the pith extract by stepping on the blended pith. The liquid extract are collected under the platform, filtered and dried to produce starch flour.



Picture 2.3: Traditional mechanical cutter



Picture 2.4: Stepping platform

Starch and cellulose are the most commonly found carbohydrate, where carbohydrates constitute the greatest portion of food consumed in the world by man and

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