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ANTIOXIDANT AND WOUND HEALING PROPERTIES OF CASHEW AND SWEET POTATO SHOOT EXTRACTS IN RATS

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ANTIOXIDANT AND WOUND HEALING PROPERTIES OF CASHEW AND SWEET POTATO SHOOT EXTRACTS IN RATS

By

MEHDI JAVADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2009

Specially dedicated to My Parents, my brothers and sisters and last but not least to all my lecturers and friends……

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Suhaila Mohamed, PhD

Faculty : Food Science and Technology

Ipomoea batatas (sweet potato) and *Anacardium occidentale* (cashew) shoots are commonly consumed vegetables which may have health benefits. The present study aims to investigate the polyphenol content, antioxidant activity and wound healing properties of ethanolic extracts of *Ipomoea batatas* and *Anacardium occidentale* shoots. Total phenolic content of these herbs were assessed using Folin-Ciocalteau assay and the antioxidant activity was measured utilizing free radical scavenging properties against the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Identification of flavonoids was done utilizing high performance liquid chromatography (HPLC). The results showed that ethanolic extracts of *A. occidantale* shoots had higher amount of total polyphenol (304.7 mg/GAE/g) than that of *I. batatas* shoots (86.5 mg/GAE/g). However the free radical scavenging activity (DPPH) was higher

(70.53%) in *I. batatas* shoots than that of *A. occidantale* shoots extract (32.26%). The phenolic content of these two herbs and the free radical scavenging activities showed no positive relationship. Among the seven standards used, the main flavonoid found in both *I. batatas* and *A. occidentale* shoots was quercetin. The wound healing activity of ethanolic extract of *I. batatas* (sweet potato) and *A. occidentale* (cashew) shoots were investigated using excision wound models on *Sprague-Dawley* rats. Animals were randomly divided into four groups of eight rats and treated orally with (i) sweet potato shoots extract (250 mg/kg/day), (ii) cashew shoots extract (250 mg/kg/day), (iii) the negative control treated with distilled water only and (iv) the positive control treated with *Gelam* honey (250 mg/kg/day). Healing was assessed by the rate of wound contraction, antioxidant activity and tissue granulation. On day 14, the sweet potato and cashew shoots extract exhibited 96.0% and 90.5% reduction in the wounds area, respectively. The negative control group (distilled water) exhibited 86.8% and positive control group (honey) exhibited 96.7% wound reduction. The results also indicated that *I. batatas* and *A. occidentale* shoots extract possesses potent antioxidant activity by inhibiting blood lipid peroxidation significantly ($P < 0.05$) and caused increase in the superoxide dismutase (SOD), glutathione peroxidase and catalase activities. Histopathological examination of granulation tissues revealed increase collagen deposition in the treatments groups as compared to negative control group. The results suggest that *I. batatas* has antioxidant properties, which may be responsible and favorable for faster wound healing, and this plant extract may be useful to accelerate healing almost equivalent to honey.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

CIRI-CIRI ANTIOKSIDAN DAN PENYEMBUHAN LUKA EKSTRAK PUCUK GAJUS DAN UBI KELEDEK KE ATAS TIKUS

Oleh

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September 2009

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Pucuk *Ipomoea batatas* (ubi keledek) dan *Anacardium occidentale* (gajus) adalah sayuran ruji yang dikenalpasti mempunyai kebaikan kesihatan. Kajian ini bertujuan menyiasat kandungan polifenol, aktiviti antioksidan dan ciri-ciri penyembuhan luka oleh ekstrak etanol pucuk *Ipomoea batatas* dan *Anacardium occidentale.* Jumlah kandungan fenolik kedua-dua jenis herba ini dinilai menggunakan ujian Folin-Ciocalteau dan aktiviti antioksidan dinilai menggunakan ciri penguraian radikal bebas melawan radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH). Pengenalpastian flavonoid telah dilakukan dengan menggunakan kromatografi cecair prestasi tingsi (HPLC). Keputusan ujian menunjukkan ekstrak etanol pucuk *A. occidantale* mempunyai jumlah kandungan polifenol (304.75 mg/GAE/g) yang lebih tinggi berbanding ekstrak pucuk *I. batatas* (86.5 mg/GAE/g). Walaubagaimanapun, aktiviti penguraian radikal bebas (DPPH) adalah lebih tinggi (70.53%) di dalam ekstrak pucuk *I. batatas* berbanding ekstrak pucuk *A. occidantale* (32.26%). Kandungan

fenolik dan aktiviti penguraian radikal bebas kedua-dua herba ini menunjukkan tiada hubungan positif diantara satu sama lain. Diantara tujuh piawaian yang telah digunakan, kandungan flavonoid utama yang dijumpai di dalam pucuk *I. batatas* dan *A. occidantale* adalah kuercetin. Aktiviti penyembuhan luka oleh ekstrak etanol pucuk *I. batatas* (ubi keledek) dan *A. occidantale* (gajus) dilakukan menggunakan model torehan luka ke atas tikus Sprague Drawley. Haiwan ini dibahagikan secara rawak kepada empat kumpulan yang terdiri daripada lapan ekor tikus dan diberikan makanan secara oral iaitu (i) ekstrak pucuk ubi keledek (250 mg/kg/hari), (ii) ekstrak pucuk gajus (250 mg/kg/hari), (iii) minuman air suling sahaja sebagai kawalan negatif dan (iv) kawalan positif diberikan madu Gelam (250 mg/kg/hari). Penyembuhan dinilai melalui kadar pengecutan luka, aktiviti antioksidan dan granulasi tisu. Pada hari ke-14, ekstrak pucuk ubi keledek dan gajus tersebut menunjukkan 96.0% dan 90.5% pengecutan terhadap kawasan luka masing-masing. Kumpulan kawalan negatif (air suling) menunjukkan 86.8 % dan kumpulan kawalan positif (madu) menunjukkan 96.7% pengecutan luka. Keputusan ini juga menunjukkan bahawa ekstrak pucuk *I. batatas* dan *A. occidantale* mempunyai keupayaan antioksidan untuk membantut peroksidaan lipid darah ($P<0.05$) dan menyebabkan kenaikan aktiviti superoxide dismutase (SOD), glutathione peroxidase dan catalase. Ujian histopatologi terhadap tisu granulasi menunjukkan kenaikan mendakan kolagen di dalam kumpulan rawatan berbanding kumpulan kawalan negative. Keputusan menunjukkan bahawa *I. batatas* mempunyai ciri antioksidan yang bertanggungjawab dalam penyembuhan luka dan ekstrak pokok ini mampu mempercepatkan penyembuhan luka setanding dengan madu.

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This thesis submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that this project is based on my original work, except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or any other institutions.

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Mehdi Javadi

Date: 28th September 2009

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LIST OF ABBREVIATIONS

µL	microliter
/	Per
°C	degree Celsius
αVβ3	fibrinogen/fibrin receptor
BA	Beniazuma
BHD	Butylated Hydroxytoluene
bFGF	basic Fibroblast Growth Factor
CA	Caffeic acid
CAT	Catalase
CAJ	Cashew Apple Juice
ChA	Chlorogenic acid
CNSL	Cashew Nut Shell Liquid
C*0*A	*P*-coumaric acid
CQA	Caffeoylquinic acid
Cu	Copper
DM	dry matter
DPPH	radical scavenging activity
DTNB	5-5´ dithiobis 2-nitrobenzoic acid
3,5-diCQA	3,5-di-o-caffeoylquinic acid
4,5-diCQA	4,5-di-o-caffeoylquinic acid
3,4,5-triCQA	3,4,5-tri-o-caffeoylquinic acid
EDTA	Ethylenediaminetetraacetic acid
EEAO	ethanolic extract of *Anacardium occidentale*

FGF	Fibroblast Growth Factor
Fe	Iron
g	gram
GAE	Gallic Acid Equivalents
GPx	Glutathione peroxidase
GSH	Glutathione
H_2SO_4	Sulphuric acid
HPLC	High Performance Liquid Chromatography
KS	Koganesengan
LC/MS	Liquid Chromatography/Mass Spectrophotometery
LDL	Low Density Lipoprotein
LPS	Lipopoly Saccharide
MDA	Malondialdehyde
NaOH	Sodium Hydroxide
PDGF	Platelet derived growth factor
QA	Quinic acid
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
SAR	Structure Activity Relationships
SE	Standard Error
SD	Standard Deviation
SOD	Superoxide dismutase
TAC	Total Antioxidant Capacity

TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TIC	Total Ion chromatography
TGF	Transforming Growth Factor
TGF-α	Transforming growth factor-α
TGF-β	Transforming growth factor- β
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
Zn	Zinc

CHAPTER I

INTRODUCTION

1.1 Introduction

The Malaysian population particularly the Malays is renowned for consuming traditional vegetables and herbs, raw as salad or cooked with their main meal. These vegetables are consumed mainly for their scent and taste as well as an appetite inducer. One of the commonly consumed vegetables is the shoot of *Anacardium occidentale*. This herb has been used to treat various ailments including malaria and yellow fever as well as diarrhea (Razali, 2008). The leaves of *A. occidentale* have antibacterial activity against the gram-negative bacteria *Escherichia coli*, while the stem barks have been reported to inhibit prostaglandin production from cells (Ibewuike *et al.*, 1997). Recently, the methanol extract of the stem bark of *A. occidentale* was reported to possess anti-inflammatory effects in an *in vivo* model (Olajide *et al.*, 2004).

The consumption of sweet potato (*Ipomoea batatas*) greens (like leaves and shoots) fresh or cooked as a vegetable in many parts of the world indicates that they are adequate as edible vegetables like other traditional leafy vegetables. They are rich in vitamin B, ß-carotene, iron, calcium, zinc and protein, and the crop is more tolerant of diseases, pests and high moisture than many other leafy vegetables grown in the tropics (Ishiguro *et al.*, 2004; Yoshimoto *et al.*, 2003). Previous experimentation

exposed that sweet potato leaves are an excellent source of antioxidative polyphenolics compared to other commercial vegetables (Islam *et al.,* 2002a). Sweet potato leaves contains high concentrations of polyphenolics, when compared with the major commercial vegetables such as spinach, broccoli, cabbage, lettuce. *I. batatas* leaf is a physiologically functional food that offers protection from diseases related to oxidation, such as cancer, hepatotoxicity, allergies, aging, human immunodeficiency virus, and cardiovascular problems. Consequently, sweet potato leaves used as a vegetable, a tea, in noodles, breads, confectioneries, and as a nutritional supplement can become a food source for beneficial polyphenolic compounds (Islam *et al.*, 2006).

Wounds are physical injuries that result in an opening or break of the skin. Accurate healing of wounds is essential for the renovation of disrupted anatomical stability and disturbed functional status of the skin. Healing is a complex and involved process initiated in response to an injury that restores the function and integrity of damaged tissues. The phases of normal wound healing include hemostasis, inflammation, proliferation, and remodeling (Nayak *et al.,* 2007). Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. A number of factors affect the process of wound healing that are as diverse as age to nutritional status and neuropathy following wounding (Reddy *et al.,* 2008). The process of wound healing is promoted by several natural products, plant products, which are composed of active principles like triterpenes, alkaloids, flavonoids and biomolecules (Panchatcharam *et al.,* 2006). Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to support wound repair.

Recently, investigators and consumers are interested in seeking natural antioxidant components in the diet, which may help to reduce oxidative damage, Oxidative stress, caused by Reactive Oxygen Species (ROS) or free radicals, has been shown to be related with the progression of many diseases including cancer, heart disease, depression, and others (Wang *et al.*, 2008). In order to protect tissues and organs from oxidative damages and free radicals, the body possesses both enzymatic and non-enzymatic systems (Schäfer and Werner, 2008). The main enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Lipid peroxidation is an autocatalytic process which is a common significance of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging. Polyunsaturated fatty acids of the membrane are peroxidized by free radical-mediated reactions. Malondialdehyde (MDA) is one of the end-products in the lipid peroxidation process (Inal, 2001).

The present study was taken up to determine polyphenols and radical scavenging activity of cashew and sweet potato shoots and also investigate the efficacy of these properties on healing process of dermal wounds in normal rats when administered orally. Recently, research on phytochemicals focused on the search for antioxidants, hypoglycemic agents, and anticancer agents from vegetable, fruit, tea, spice and medical herbs. The goal of this study is to make use of the potential of antioxidant activity and reactive oxygen species (ROS) scavenging during wound healing process. Overproduction of ROS results in oxidative stress thereby causing cytotoxicity and delayed wound healing (Shetty *et al.*, 2007). The potential of each sample for scavenging the ROS and also effect on wounds should be different. Hence this study wants to find out these effects and investigate it.

1.2 Objectives

The objectives of this study are:

1. To identify the phenolic compounds and evaluate antioxidant activity in ethanolic extract of cashew and sweet potato shoots.

2. To investigate the wound healing properties of cashew and sweet potato shoots ethanolic extract.

CHAPTER II

LITERATURE REVIEW

2.1 Sweet potato

Sweet potato (*Ipomoea batatas*) is a dicotyledonous plant (Figure 2.1), which belongs to the *Convolvulaceae* family (Table 2.1). Scientists believe that the sweet potato was domesticated more than 5000 years ago. The origin of sweet potato is thought to be Central or South America but it is now grown worldwide in tropical and subtropical regions. Sweet potato plants produce underground storage roots, which are typically ready to harvest 3-4 months after planting. Based on the production volume, sweet potato ranks as seventh and fifth most important food crops in the world and developing countries, respectively (CIP, 2006). Since the 16th century, sweet potatoes have been a traditional food in Asia primarily in China. Sweet potatoes were one of the primary food commodities in China due to the well adaptability of the crop to the environmental conditions in that country. Compared to other crops, the storage root of sweet potato is able to grow at an accelerating rate in various environmental conditions, thus multiplying in quantity in a short period of time. For that reason, sweet potato spread throughout Asia, Africa, and Latin America during the 17th and 18th centuries. Since then, sweet potato has become one of the most grown crops in Asia, Africa, and America and so did the demand for sweet potato among consumers. Globally, sweet potato is ranked as the seventh most important food commodity after wheat, rice, maize, potato, barley and cassava.