UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION, ANTIOXIDANT PROPERTIES AND HYPOCHOLESTEROLEMIC EFFECTS OF DIFFERENTLY TREATED ROSELLE (*Hibiscus sabdariffa* L.) SEEDS

EMMY HAINIDA BINTI KHAIRUL IKRAM

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By

EMMY HAINIDA BINTI KHAIRUL IKRAM

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Chairman : Amin Ismail, PhD

Faculty : Medicine and Health Sciences

Roselle (*Hibiscus sabdariffa L.*) seeds, grown in Malaysia, were investigated. Effects of drying and boiling-drying of the seeds on the proximate analysis, minerals, amino acids content, vitamins, polyphenols and antioxidant activity were studied. Effect of dried roselle seeds (DRS), on lipid profiles of rats induced hypercholesterolemia was also conducted. Protein, lipids and dietary fiber were found to be high in all the seeds studied. Raw (RRS), dried (DRS) and boiled seeds (BRS) contained 57.3%, 9.9% and 9.8% moisture; 35.4%, 33.5% and 30.6% protein; 27.2%, 22.1% and 29.6% lipids; 2.3%, 13.0% and 4.0% available carbohydrate; 25.5%, 18.3%, and 19.2% total dietary fibers and 7.4%, 7.5% and 6.6% ash, respectively. There was a significant difference ($p < 0.05$) in protein, lipid and carbohydrate contents between treated and raw seeds. The predominant inorganic elements in the seeds were potassium (99 – 109 mg/100 g), magnesium (26 – 28 mg/100 g) and calcium (24 – 31 mg/100 g). The total dietary fiber of the seeds was considerable with the best ratio between soluble and insoluble ranging from 1.2 to

3.3. The study detected 17 essential and non-essential amino acids. The seeds were rich in lysine (14 – 15 g/100 g), arginine (30 – 35 g/100 g), leucine (15.4-18.6 g/100 g), phenylalanine (11 – 12 g/100 g) and glutamic acid (21 – 24 g/100 g). Sulphur containing amino acids, cystine (4.04 - 5.32 g/100 g protein) and methionine (2.50-3.96 g/100 g protein) were the limiting amino acids of the seeds powder. Vitamin A content in DRS was (99.97 ± 2.99 µg retinol), followed by BRS (87.73 ± 0.49 µg retinol) and RRS (69.38 ± 0.36 µg retinol). BRS was found to contain the highest vitamin E value (0.19 ± 0.05 mg/g sample), followed by DRS (0.19 ± 3.20 mg/g sample) and RRS (0.07 ± 0.78 mg/g sample). Phenolic content for RRS, DRS and BRS were 105.6 ± 0.49, 39.6 ± 0.12 and 28.5 ± 0.61 mg/g sample, expressed as gallic acid equivalents (GAE), respectively. The total ferulic acids in RRS, BRS and DRS were 17.99 ± 0.29 mg/g, 1.96 ± 0.60 mg/g and 1.25 ± 0.62 mg/g samples, respectively. Methanolic extracts of DRS and BRS exhibited high antioxidant activity compared with ethanolic and n-butanolic extracts. β-carotene bleaching assay resulted in higher antioxidant activity in DRS compared to BRS powder in methanolic, ethanolic and n-butanolic extracts. In contrast, scavenging activitiy of BRS was found to be significantly higher than DRS. Significant differences ($p < 0.05$) were found among the following three extracts with values in the descending order of 80% methanol > absolute ethanol > n-butanol. In *in-vivo* study to determine the effect on lipid profile, 20 males Sprague-Dawley rats were randomly divided into 4 groups and fed with four different diets, respectively. The rats were fed with normal, hypercholesterol, hypercholesterol + 5% (w/w) DRS and hypercholesterol + 15% (w/w) DRS diets, respectively. Treatments were given for a total of 6 weeks.

Results indicated that the addition of 5% and 15% DRS had significantly lowered ($p<0.05$) the total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels. However, there were no significant differences in triglyceride level. Furthermore, there was no significant increase in high density lipoprotein cholesterol (HDL-C) levels, with addition of DRS. In conclusion, findings indicated that the nutritional values of treated roselle seeds for human consumption are still considerable when compared to raw seeds. Nutritional benefits of raw and treated roselle seeds revealed that these seeds are promising with a high source of proteins, dietary fiber, essential amino acids and lipids. The present findings also indicated that the addition of 5% and 15% DRS showed to have a potential hypocholesterolemic effects.

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

KOMPOSISI KIMIA, KANDUNGANANTIOKSIDAN DAN KESAN HIPOKOLESTEROLEMIK KE ATAS BIJI ROSELLE (*Hibiscus sabdariffa* L.) YANG DIBERI RAWATAN BERBEZA

Oleh

EMMY HAINIDA BINTI KHAIRUL IKRAM

Julai 2006

Pengerusi : Amin Ismail, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kajian ke atas biji buah roselle (*Hibiscus sabdariffa L.*) yang terdapat di Malaysia telah dijalankan. Kesan pengeringan dan rebusan keatas analisis proksimat, mineral, kandungan asid amino, vitamin, polifenol dan aktiviti antioksidan dikaji. Kajian kesan serbuk biji roselle kering (DRS), ke atas profil lipid tikus diaruh hiperkolesterolemia juga telah dijalankan. Kandungan protein, lipid dan fiber diet didapati tinggi dalam semua serbuk biji yang dikaji. Biji mentah (RRS), kering (DRS) and rebus (BRS) mengandungi 57.3%, 9.9% dan 9.8% kandungan air; 35.4%, 33.5% dan 30.6% protein; 27.2%, 22.1% dan 29.6% lipid; 2.3%, 13.0% dan 4.0% karbohidrat perlu; 25.5%, 18.3%, dan 19.2% fiber diet serta 7.4%, 7.5% dan 6.6% abu. Terdapat perbezaan yang signifikan ($p < 0.05$) dalam kandungan protein, lipid dan karbohidrat dalam biji yang diberi rawatan haba berbanding biji mentah. Elemen pre-dominan bukan organik yang terdapat dalam biji roselle adalah natrium (99 – 109 mg/100 g), magnesium (26 – 28 mg/100 g) dan kalsium (24 – 31 mg/100 g). Jumlah kandungan fiber dalam biji roselle boleh dianggap baik dengan nisbah 1.2

hingga 3.3 diantara fiber larut dan tak larut. Kajian ini telah mengesan 17 asid amino perlu dan tak perlu dalam biji roselle yang dikaji. Biji roselle kaya dengan lisina (14 – 15 g/100 g), arginin (30 – 35 g/100 g), leusina (15.4-18.6 g/100 g), phenylalanin (11 – 12 g/100 g) dan asid glutamik (21 – 24 g/100 g). Kandungan asid amino yang paling rendah adalah jenis asid amino sulfur iaitu, sistina (4.04 - 5.32 g/100 g protein) dan metionina (2.50-3.96 g/100 g protein). Kandugan vitamin A dikesan dalam DRS (99.97 ± 2.99 µg/g retinol), diikuti dengan BRS (87.73 ± 0.49 µg/g retinol) dan RRS (69.38 ± 0.36 µg/g retinol). Kandungan vitamin E adalah paling tinggi dalam BRS (0.19 ± 0.05 mg/g sampel), diikuti dengan DRS (0.19 ± 3.20 mg/g sampel) dan RRS (0.07 ± 0.78 mg/g sampel). Kandungan fenolik bagi RRS, DRS and BRS adalah 105.6 ± 0.49, 39.6 ± 0.12 dan 28.5 ± 0.61 mg/g sampel. Manakala, jumlah kandungan asid ferulik untuk ujian kandungan polifenol dalam RRS, BRS and DRS adalah 17.99 mg/g, 1.96 mg/g dan 1.25 mg/g sampel. Aktiviti antioksidan ekstrak metanol bagi DRS dan BRS adalah tinggi berbanding ekstrak etanol dan n-butanol. Aktiviti antioksidan bagi ekstrak metanol, etanol dan n-butanol DRS menggunakan kaedah pelunturan β-carotene adalah tinggi berbanding BRS. Sebaliknya, dalam kaedah pemusnahan radikal bebas, aktiviti antioksidan BRS didapati tinggi secara signifikan berbanding DRS. Terdapat perbezaan yang signifikan ($p < 0.05$) diantara ketiga-tiga ekstrak dengan nilai tertinggi diikuti terendah iaitu 80% metanol > etanol absolut > n-butanol. Dalam kajian *in-vivo*, 20 tikus Sprague-Dawley jantan telah dibahagikan secara rawak kepada 4 kumpulan dan diberi 4 diet berbeza. Kumpulan tikus-tikus ini telah diberi diet normal, diet hiperkolesterol, diet hiperkolesterol + 5% (w/w) DRS dan diet hiperkolesterol + 15%

(w/w) DRS mengikut kumpulan masing-masing. Rawatan diet telah diberikan selama 6 minggu. Keputusan menunjukkan bahawa tambahan 5% dan 15% DRS telah menurunkan secara signifikan ($p<0.05$) kandungan jumlah kolesterol darah (TC) dan nilai kolesterol lipoprotein berketumpatan rendah (LDL-C). Walau bagaimanapun, tiada perbezaan yang signifikan didapati bagi kandungan plasma trigliserida. Tiada peningkatan signifikan juga didapati dalam kolesterol lipoprotein berketumpatan tinggi (HDL-C) setelah diberi rawatan diet DRS. Kesimpulannya, kajian ini mendapati kandungan nutrisi bagi biji roselle yang dirawat dengan haba masih baik jika dibandingkan dengan biji mentah. Kebaikan nutrisi biji roselle sama ada yang mentah dan dirawat menjanjikan sumber protein, fiber diet, asid amino dan lipid yang baik. Kajian juga mendapati potensi kesan hipokolesterolemik dalam diet tambahan 5% dan 15% DRS.

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I certify that an Examination Committee has met on 4 July 2006 to conduct the final examination of Emmy Hainida bt. Khairul Ikram on her Master Science thesis entitled "Chemical Composition, Antioxidant Properties and Hypocholesterolemic Effects of Differently Treated Roselle (*Hibiscus sabdariffa* L.) Seeds" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

ROKIAH MOHD YUSOF, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairperson)

AZIZAH ABDUL HAMID, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ASMAH RAHMAT, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

AYUB MOHD YATIM, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Independent Examiner)

_____

HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

AMIN ISMAIL, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

NORHAIZAN MOHD ESA, PhD
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

NORMAH HASHIM, MSc
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis 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 other institutions.

_____

EMMY HAINIDA KHAIRUL IKRAM

Date:

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LIST OF ABBREVIATIONS

BHT	Butylated hydroxytoluene
BRS	Boiled roselle seeds
C	Celsius
CV	Coefficient variation
CVD	Cardiovascular disease
DPPH	2, 2-diphenyl-1-piccrylhydrazyl
DRS	Dried roselle seeds
EAA	Essential amino acid
FAO	Food and Agriculture Organization
g	Gram
GAE	Gallic acid equivalent
HDL-C	High density lipoprotein cholesterol
HPLC	High Performance Liquid Chromatography
hr	Hour
IDF	Insoluble dietary fiber
kg	Kilogram
l	Liter
LDL-C	Low density lipoprotein cholesterol
M	Molarity
mg	Milligram
min	Minute

ml	Milliliter
NCCFN	National Coordinating Committee on Food and Nutrition
NEAA	Non-essential amino acid
PEM	Protein Energy Malnutrition
RRS	Raw roselle seeds
SDF	Soluble dietary fiber
TAC	Total available carbohydrate
TC	Total cholesterol
TDF	Total dietary fiber
TG	Triglyceride
WHO	World Health Organization
µl	Microliter

CHAPTER I

INTRODUCTION

Background

In Malaysia, the ever rising number of mortality due to heart diseases, cardiovascular diseases (CVD), cancer and also protein-energy malnutrition in children require great attention. Although the numbers of death are not critical compared to other developed countries, prevention is much more important than treatment. It has been clearly identified that along with lifestyle, dietary factors are associated with greater risk of cancer, CVD and other diseases. Thus, it is important for nutrition scientist to explore more beneficial candidate from food source especially within the natural based materials like plants and animals to exploit useful products for the better health of the populations.

One of the most common flower plants grown worldwide is hibiscus. There are more than 300 species of hibiscus around the world. One of them is roselle (*Hibiscus sabdariffa*, Linn), which is a member of a plant family, Malvaceae. The origin of roselle is not fully known, but it is believed to be native to tropical Africa. It is known by different synonyms and vernacular names. It also known as roselle (Tsai *et al.*, 2002; Chenowarin *et al.*, 1999; Abu-Tarboush *et al.*, 1997), karkade (Abu-Tarboush *et al.*, 1997) and mesta (Rao, 1996). It was introduced to West Indies, and cultivated mainly as an ornamental plant.

Nowadays, this crop is cultivated extensively for its pleasant red coloured calyces which were mainly used for food application such as making jams, jellies and beverages. For non-food applications, the flower and fleshy fruit are used in pharmaceutical to relieve symptoms of bronchitis and coughs. In addition, its calyx is used for the treatment of hypertension, diarhea, Ceylon mouth and many other diseases (Faraji *et al.*, 1999; Chewonarin *et al.*, 1999). Moreover, the kernels and seeds are roasted and consumed as such, though slightly bitter (Morton, 1987).

Roselle was first introduced into Malaysia three centuries ago. Its farm was located in Pahang, Melaka, Perak, Johor and Terengganu. Most of the roselle was being planted commercially by smallholders in Terengganu on the bris soils. The planting has later expanded to some parts of Johor and Perak in Peninsular Malaysia. A roselle plantation which is operated by Gulf and Pacific Estates Company covers an area of 10 km^2 in Terengganu (Mohd, 1998). Recently in Malaysia, the Malaysian Ministry of Agriculture and Agro-Based Industry has proposed to make roselle drink as our national drink (Utusan Malaysia, 2004). In addition, the proposed RM6 billion ($1.6 billion) Roselle Valley project, when completed in 2013 (News Strait Times, 2003) are expected to make Malaysia a key producer in the world's beverage, herbal and food supplement markets. The agro-technology project was started in 2004 and will be completed in three phases over a 10-year period. Regrettably, this mega project will lead into innumerable amounts of discarded and unexploited velvety capsules containing the seeds. At present, the seed of the roselle plant are merely discarded by our local manufacturers.

The importance and health benefits of grains and beans consumption in the prevention of chronic diseases have been documented. Although nutritional guidelines put grains and grains product at the base of food guide pyramid to emphasize their importance for optimal health, nevertheless, little attention has been paid to grains, beans and also seeds consumption compared to fruits and vegetables. Seeds are one of the cheap sources of nutritious food. Researchers have confirmed its nutritional usefulness and many different interpretations and view have been discussed about its nutritional benefits (Salleh,1992; Barampama and Simard, 1993). Chau *et al.* (1998) reported that protein concentrates from legume seeds (*Phaseolus angularis, Phaseolus calcaratus* and *Dolichos lablab*) had a very pronounced hypocholesterolemic effect compared to casein. Furthermore, Dabai *et al.* (1996) had showed that seeds of different legumes species contribute different hypocholesterolemic effects. However, limited research has been embarked on the exploitation and utilization of seeds as a potential candidate for human alternative food source.

Previous studies had significantly shown that roselle seeds contained high amounts of protein, dietary fiber, and minerals such as phosphorus, magnesium and calcium (Rao, 1996). Abu-Tarboush *et al.* (1997) found that roselle seed flour contained high amounts of protein, oil and carbohydrate. In other countries, the seeds have been exploited as a substitute for coffee for human consumption and are considered an excellent feed for chicken (Morton, 1987). The seeds are also seen to be used for their oil in China and eaten in West Africa (Robert, 1996). Hence, it would be

advantageous if we could fully utilize the roselle calyces and seed, and minimize the waste production from roselle processing. Utilization of the seeds may diversify into health care product, functional foods, nutraceuticals and food supplement products. In addition, such utilization may also provide income for small and medium industries.

Statement of Problems

The burden of chronic diseases is rapidly increasing worldwide. Malnutrition and non-communicable diseases such as cancers and heart disease account for 60% of the global total, divided more or less equal between the developing and developed world (WHO, 2000). Currently, one-third of all global deaths (15.3 million) is caused by cardiovascular disease (CVD), accounting for 86% worldwide (WHO, 2003). In Malaysia, it is estimated about 30% of all medically reported deaths were attributed to CVD (MOH, 2001).

Studies on dietary interventions such as increase intake of antioxidants, or replacing animal protein with plant proteins have shown to have positive effects in reducing the risk of chronic diseases (Robert *et al*., 1999; Chau *et al*., 2004). Dietary sources from new crop especially cheaply available crops should be investigated and explored to minimize morbidity and mortality of the populations, especially the percentage of mortality due to malnutrition and chronic diseases.