ASSESSMENT OF TOTAL PHENOLIC CONTENT AND FREE RADICAL-SCAVENGING CAPACITY OF *Leucaena leucocephala* EXTRACT

DOROTHY SARIM
HS2004-3173

DISSERTATION SUBMITTED AS A PARTIAL FULLFILLMENT OF REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOR

PLANT TECHNOLOGY PROGRAMME
SCIENCE AND TECHNOLOGY SCHOOL
UNIVERSITI MALAYSIA SABAH

APRIL 2007

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: ASSESSMENT OF TOTAL PHENOLIC CONTENT AND FREE-RADICAL-SCAVENGING CAPACITY OF Leucaena leucocephala EXTRACT

Ijazah: SARJANA MUDA DENGAN KEPUJIAN TEKNOLOGI TUMBUHAN

SESI PENGAJIAN: 2004 - 2007

Saya DOROTHY SARIM

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☐	TIDAK TERHAD	

Disahkan oleh

Dorothy
(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: KPG. TAMPARULI, TUARAN, KOTA KINABALU, SABAH

Nama Penyelia

Tarikh: 19/04/2007

Tarikh:

CATATAN: * Potong yang tidak berkenaan.
** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I declare that this writing is sole work of mine except for the quotation and summary which I have explained the source of each one of them.

12 March 2007

.....

DOROTHY SARIM

HS2004-3173

VERIFICATION

Signature

1. SUPERVISOR

(MR. RUZAIDI AZLI BIN MOHD. MOKHTAR)

RUZAIDI AZLI BIN MOHD MOKHTA

2. EXAMINER 1

(PROF. MADYA DR. MARKUS ATONG)

PROF. MADYA DR. MARKUS ATONG

3. DEAN

(SUPT/KS PROF. MADYA DR. SHARIFF OMANG)

ACKNOWLEDGEMENT

First of all, I would like to thank God for giving me opportunity to be able to finished my dissertation. I would also like to thank my supervisor, Mr. Ruzaidi Azli for his endless guidance. His guidance will be much appreciated.

Other than that, I would also like to express my gratitude to all the Biotechnology and Plant Technology laboratory assistants for their helps. And to all my friends to had helped me upon completing my dissertation, thank you very much.

ABSTRACT

Plants contain a variety of substances called the phytochemicals, for example phenolic compound and antioxidants. Interest in phenolic antioxidant in the last decade has increased remarkably because of their elevated capacity in scavenging free radical associated with various diseases. In the present study, aqueous, ethanol, and methanol extraction of different parts of *Leucaena leucocephala* (leaf, stem, and seed) were assessed for their total phenolic content and antioxidant activities. The total phenolic content was measured using Folin-Ciocalteu method while the free radical capacity was evaluated using DPPH (2,2'-diphenyl-1-pierythydrazyl) free-radical-scavenging assay. There are significant differences of total phenolic content in different parts of *Leucaena leucocephala*. The aqueous extraction of seed shows the highest result in total phenolic content (1.58 mg/ml) while the lowest were found in methanol extraction of stem (0.37mg/ml). The aqueous extraction was considerably better than the ethanol and methanol extraction in all three different part of *Leucaena leucocephala*. All different extraction for different parts of *Leucaena leucocephala* can act as radical-scavenger and there were significant differences of antioxidant activities in this different parts of *Leucaena leucocephala*. The highest scavenging effect was found in aqueous extraction of seed (87%) while the lowest scavenging effect were found in ethanol extraction of seed (55%). The results from this study can be used as future references to another study such as preventive effect of *Leucaena leucocephala* on diseases such as diabetes.

ABSTRAK

Tumbuhan mengandungi pelbagai jenis bahan yang di panggil bahan fitokimia sebagai contohnya ialah sebatian fenol dan antioksida. Akhir-akhir ini, minat terhadap antioksida fenol telah meningkat secara mendadak kerana kemampuannya dalam menyingkirkan radikal bebas yang boleh menyebabkan pelbagai penyakit. Dalam kajian ini, ekstrak akueus, etanol, dan methanol bagi bahagian-bahagian berbeza *Leucaena leucocephala* (daun, batang, dan biji) telah dikaji jumlah kandungan fenolik dan aktiviti antioksidanya. Jumlah kandungan fenol telah dikaji mengikut kaedah Folin-Ciocalteu manakala kemampuannya untuk menyingkirkan radikal bebas pula di kaji menggunakan kaedah *DPPH (2,2'-diphenyl-1-pierythydrazyl) radical-scavenging*. Terdapat perbezaan yang signifikan dalam kandungan fenol pada bahagian berbeza *Leucaena leucocephala*. Ekstrak akueus biji pada *Leucaena leucocephala* menunjukkan keputusan paling tinggi dalam jumlah sebatian fenol (1.58 mg/ml) manakala ekstrak metanol batang pula menunjukkan keputusan yang paling rendah (0.37mg/ml). Pengekstrakan akueus boleh dianggap lebih baik berbanding dengan pengekstrakan etanol dan metanol di dalam ketiga-tiga bahagian *Leucaena leucocephala*. Kesemua ekstrak yang berbeza untuk bahagian yang berlainan pada *Leucaena leucocephala* boleh bertindak sebagai agen penyingkir radikal bebas dan terdapat perbezaan yang signifikan untuk aktiviti antioksidan pada bahagian berlainan pada *Leucaena leucocephala* ini. Ekstrak akueus biji menunjukkan keputusan penyingkiran radikal bebas yang paling tinggi (87%) dan paling rendah dalam didapati dalam ekstrak etanol biji (55%). Keputusan yang diperolehi daripada kajian ini boleh dijadikan sebagai sumber rujukan oleh kajian lain seperti kesan pencegahan *Leucaena leucocephala* terhadap penyakit.

CONTENTS **PAGE**

TABLE LIST

.

FIGURE LIST

SYMBOL LIST

°C	Degree Celsius
°S	Degree South
°U	Degree North
g	Gram
ml	Milliliter
cm	centimeter
kg	Kilogram
µm	Micrometer
mg	Milligram
µl	Microliter
v/ v	Volume / volume
%	Percentage
<	Less Than
L	Liter
S.D.	Standard Deviation
ANOVA	Analysis Varian (*Analysis of variance*)
DPPH	2-2 diphenyl-1- pierythydrazyl

CHAPTER 1

INTRODUCTION

1.1 Introduction

Plants contain a variety of substances called phytochemicals which is the non-nutritive plant chemicals that have protective or disease preventive properties (Stratil *et al.*, 2006). These phytochemicals owe to naturally-occurring minor components present in plant. There are more than thousand known phytochemicals. It is well-known that plants produce these chemicals to protect itself but recent research demonstrates that these chemicals also can protect humans against diseases (Scalbert *et al.*, 2005). Example of plant phytochemicals that have pharmaceutical interest are phenolic compounds and antioxidants. The interest in phenolic antioxidant has increased remarkably in the last decade because of their elevated capacity in scavenging free-radical associated with various disease such as cancer, cardiovascular diseases, diabetes, and other pathologies.

From ancient times, plant medicine has been widely used by people of different ethnic origins in Malaysia. In several degenerative diseases such as diabetes and cardiovascular diseases, up until now there have been a lot of discoveries in plants and herbs that can control these diseases such as, *Retama raetam* (Maghrani *et al.*, 2003), *Occimum sanctum* Linnean (Suanarunsawat and Songsak, 2005), and *Annona squamosa*, (Gupta *et al.*, 2005). Such plants contain not only varied medical properties like antitumour and antiinflammatory but also have high total phenolic content and antioxidant activity.

Over the past 10 years, polyphenols have gained much more attention from researchers. The main reason for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet, and their role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Manach *et al.*, 2004). Manach *et al.* (2004) reported that polyphenols constitute the active substances found in many medicinal plants. Plants that contain high total phenolic content and antioxidant activity were believed can cure many of these age-related diseases.

Leucaena leucocephala is also known as petai belalang, is believed to be the cure for many diseases. It is believed that it can cure diseases such as cough, small wound, and stomach ache. *Leucaena leucocephala* has been used traditionally by Malaysian and Indonesian to threat or control diabetes mellitus. The seeds are being use traditionally to threat diarrhea. However, until now there are still no studies have been

done to determine the phenolic content and antioxidant activity of the different part of *Leucaena leucocephala*.

Leucaena leucocephala is in the family of Leguminosae/Fabaceae. *Leucaena leucocephala* is an erect, slender shrub or small tree, 1-5 meters high, with dense wood. It have bipinnate leaves with pale green to white colour, globose flowers in dense clusters and clustered, flat, dehiscent, dark brown pods containing flat, glossy brown seeds.

Leucaena leucocephala is common to very abundant in rural areas of large islands, where it has become naturalized in extensive stands. *Leucaena leucocephala* leaves and seeds from the young pods are protein-rich compound. Therefore, its leaves and seeds are used for direct human consumption in some areas of Central America, Mexico and Southeast Asia (Brewbaker and Sorensson, 1990). Seeds were eaten fresh or boiled. *Leucaena leucocephala* occasionally deliberately planted as fuelwood plantations or as shade in coffee plantations and also planted in home gardens.

Leucaena leucocephala is a very useful plant and are available abundantly not only in Malaysia but also in almost every part of the world. With the assessment of the total phenolic compound and antioxidant activity of this plant, it could provide future references for scientists to explore its use in the areas of plant medicine.

1.2 Objectives

To evaluate total phenolic content and the free radical-scavenging capacity of different parts of *Leucaena leucocephala* using it's aqueous, ethanolic, and methanolic extract.

1.3 Hypothesis

H_0: There are no significant differences of total phenolic compound and antioxidant activity in different parts of *Leucaena leucocephala.*

H_1: There are significant differences of total phenolic compound and antioxidant activity in different parts of *Leucaena leucocephala.*

and

H_0: There are no significant differences of total phenolic compound and antioxidant activity in different extraction of *Leucaena leucocephala.*

H_1: There are significant differences of total phenolic compound and antioxidant activity in different extraction of *Leucaena leucocephala.*

CHAPTER 2

LITERATURE REVIEW

2.1 *Leucaena leucocephala*

Figure 2.1.1: Examples of *Leucaena leucocephala* parts (Kewalramani *et al.,* 1987)

Leucaena leucocephala (Figure 2.1.1) is one of the fastest-growing leguminous trees. Its foliage is used as animal feed, and its leaves and seeds are used as human food in Central America, Indonesia, Malaysia, and Thailand (Sethi and Kulkarni, 1995). *Leucaena* foliage (leaflets plus stems) contain both nutrients and roughage, and make an almost complete ruminant feed, somewhat comparable to alfalfa forage (D'Mello and Thomas, 1977). Its amino acid pattern is comparable with that of soya bean and fish meal and other animal feed sources available in developing nations.

The mineral composition and mimosine content of the Leucaena plant vary considerably in different species (Kewalramani *et al.,* 1987) and even within the same species, that is, *Leucaena leucocephala* itself, for the various cultivars. Further variations in composition have also been observed in different parts of the plant and at different stages of growth. It is reported by Sethi and Kulkami (1995) that the nutritive value of *Leucaena leucocephala* has been studied. The aspects studied include crude protein, amino acid content of proteins, total nitrogen, free amino acids, and changes in free amino acid pools, total ash, ether extractives, carbohydrates, crude fibre, neutral detergent fibre, acid detergent fibre, hemicellulose, cellulose, lignin nitrogen-free extract, in vitro dry matter digestibility, digestible crude protein, total digestible nutrients, minerals, including calcium, phosphorus, sodium, potassium, magnesium copper, zinc, iron, cobalt, manganese, and iodine, carotene, xanthophylls, vitamin K, mimosine, tannins and other phenolic compounds, and organic solvent soluble mercury.

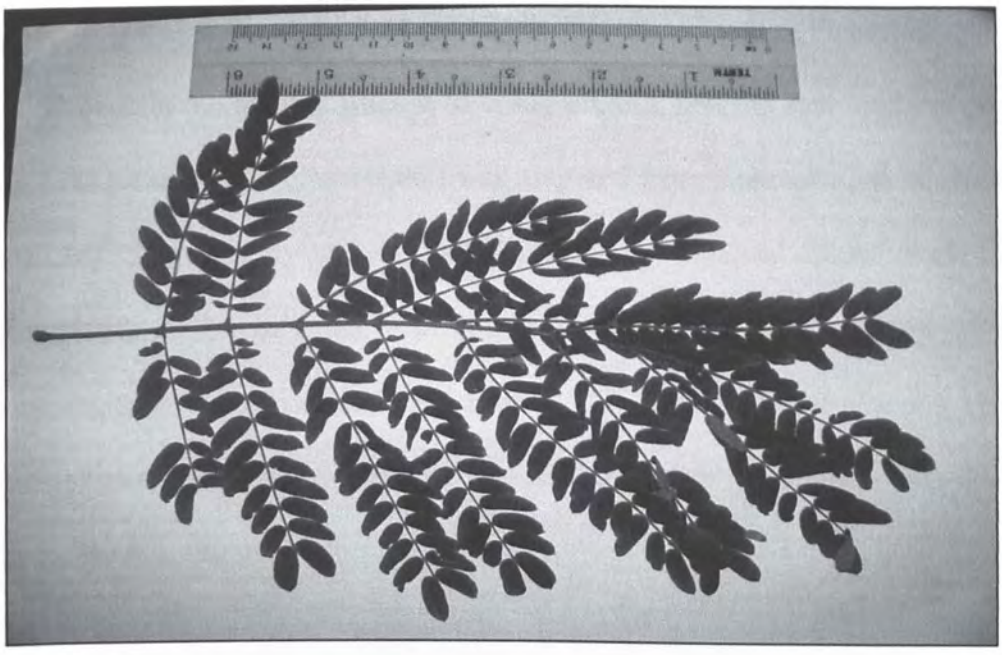

Figure 2.1.2: The leaves of *Leucaena leucocephala*

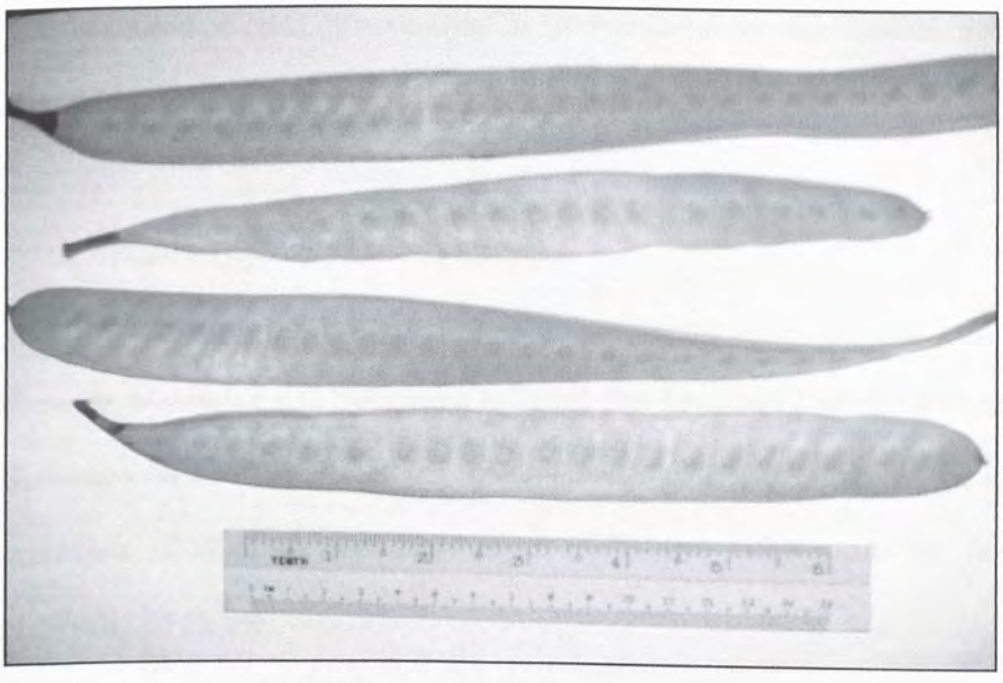

Figure 2.1.3: The seeds of *Leucaena leucocephala*

Among the various parts of the plant, immature leaves (Figure 2) and seeds (Figure 3) contain the highest amount of crude protein, and the stem and dry pods the lowest. Leaf protein concentrate (LPC) was prepared from *Leucaena leucocephala* leaf meal (LLM). The recovery of LPC was 7.6% and it contained 65.9% crude protein, compared with 29.2% in LLM. Ash content was 17.6%, and the levels of lysine, histidine, arginine, isoleucine, and leucine were 5.6%, 2.3%, 5.9%, 5.4%, and 11% on a dry matter basis, respectively. The LPC had higher in vivo digestibility than the LLM, 63.2% and 48.8%, respectively.

Leucaena leucocephala is spreading naturally and has been reported as a weed in more then 20 countries across all continents except Europe and Antarctica (Hughes, 2002). It is a weed of open, often coastal or riverine habitats, semi-natural, and other disturbed or ruderal sites and occasionally in agricultural land.

2.2 *Leucaena leucocephala* on diseases

Traditionally, Malaysian and Indonesian believed that *Leucaena leucocephala* can cure few diseases such as diabetes mellitus, small wounds, cough and diarrhea. Usually for the treatment of diabetes and diarrhea, the old and dried seeds of *Leucaena leucocephala* will be use.

Leucaena leucocephala leaves contain mimosine, a plant amino acids that can be toxic to animals and humans (Sethi and Kulkarni, 1995). This is the reason why *Leucaena leucocephala* leaves are not being used for any medicinal purposes up until now.

In some countries, this plant is treated as weeds (Hughes, 2002). The usage of *Leucaena leucocephala* in areas of plant medicine has been limited. Until now there were no scientific studies on *Leucaena leucocephala* to prove that it can cure any diseases.

2.3 Medicinal plants on disease

Plants have been used on the earth for food and medicine since ancient times. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs (Copper, 2004). There is an herb for every human affliction (Khan and Khanum, 2005). Herbal traditions have been passed down and refined with scientific understanding, providing information to assist in health maintenance. Today, the global movement towards a more natural lifestyle has brought about resurgence of interest in herbs.

Herbal-derived substances remain the basis for a large proportion of the commercial medications used today for the treatment of diseases such as heart disease, high blood pressure, pain, asthma and other illnesses (Saad *et al.,* 2005). One of the example is ephedra. Ephedra is an herb used in traditional Chinese medicine for more

than 2000 years to treat asthma and other respiratory problems. Nowadays ephedrine, which is the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems.

Other examples for medicinal plants use on diseases are in the areas of diabetes. There have been a lot of discoveries of plant that posess significant hypoglycaemic effect in diabetic model animals. Several plants such as *Retama raetam* (Maghrani *et al.,* 2003), diamed which is an herbal formulation composed of the aqueous extracts of three medicinal plants, *Azardirachta indica, Cassia auriculata* and *Momordica charantia* (Pari *et al.,* 2001), *Occimum sanctum Linnean* (Suanarunsawat and Songsak, 2005), *Annona squamosa*, (Gupta *et al.,* 2005) and *Blighia sapida* (Auerbach, 2001), have been proved to posses hypoglycaemic properties. It have been reported that these plants possess varied medicinal properties such as insecticidal, antiovulatory and antitumour. It has also been proved that soluble fibers, found in oats, beans, and fruits, helps to control diabetes (Bryne, 1952). Soluble fibers helps to stabilize blood sugar by slowing the absorption of carbohydrate (starches and sugars), thereby acting as the time-release mechanisms.

Herbs act on the blood, metabolism, and all cellular processes including the nervous system (Khan and Khanum, 2005). Thus, they are capable of bringing the body into harmony and health. Herbs are considered food for the body. They are valuable sources of natural medicine, vitamins, minerals and phytochemicals that have a remarkable history of curative effects.

2.4 Phenol in plants

Phenolic compounds, or polyphenols, constitute one of the most numerous and widely-distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known (Urquiaga and Leighton, 2000). Polyphenols are products of the secondary metabolism of plants with flavonoids and tannis as one of the example for pharmaceutical interest.

The expression "phenolic compounds" embraces a considerable range of substances that possess an aromatic ring bearing one or more hydroxyl substituents. Most of the major classes of plant polyphenol are listed in Table 2.4, according to the number of carbon atoms of the basic skeleton. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins (Harborne, 1980).

REFERENCES

Asami, D.K., Yun, J.H., Barrett, D.M., and Mitchell, A.E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Food Biochemistry* **26**: 453-465

Auerbach, P. S. 2001. *Wilderness Medicine*. 4th ed. Mosby

Baydar, N. G., Ozkan, G., Yasar, G. 2006. Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control Journal* **18**: 1131-1136

Beekwilder, J., Hall, R. D., and Vos, C. H. R. 2005. Identification and dietary relevance of antioxidants from raspberry. *Biofactors* **23**: 197-205

Blair, J. P. 1995. *Nutrition and Fitness*. Volume 4. 1st ed. MacMillan Health Encyclopedia.

Bravo, L. 1998 Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Review* **56**:317-333

Brewbaker, J. L., and Sorensson, C. T. 1990. *New Tree Crops from Interspecific Leucaena hybrids*. In: J. Janick, J., and Simon, J. E. Advances in New Crops. 1st ed. Timber Press

Bryne, K. D. 1952. Understanding and Managing Cholesterol: *A Guide for Wellness Professionals*. 1st ed. Illinois.

Cervato, G., Carabelli, M., Gervasio, S., Cittera, A., Cazzola, R., and Cestaro, B. 2000. Antioxidant properties of oregano (*Origanum vulgaare*) leaf extracts. *Journal of Food Biochemistry* **24**: 453-465

Chanwitheesuk, A. 2006. Screening and identification of antioxidants from local vegetables and Thai herbs. *Journal of Medicinal Food* **9**(2): 276-280.

Chung, K. T., Wong, T. Y., Wei, C. I, Huang, Y. W., and Lin, Y. 1998. Tannins and human health: a review. *Critical Revision Food Science Nutrition* **38**:421-464

Colin Hughes. 2002. This species has been nominated as among 100 of the "World's Worst" invaders. http//:

Cooper, E. L. 2004. Drug discovery, CAM and natural products. *Evidence Based Complement Alternative Medicine* **1**:215–217.

Crozier, A., Burns, J., Aziz A. A., Steward A. J., Rabiasz, H. S., Jenkins, G. I., Edwards, C. A., and Lean M. E. J. 2000. Antioxidant flavonols from fruits, vegetables and beverages: measurements and bioavailability. *Biology Res* **33**: 79-88

Danelisen, I., Lou, H., Sudha, T., and Singal, P. K. 2004. Antioxidant vitamins in cardiovascular disease. In: Nath, R., Khullar, M., and Singal, P. K. *Antioxidant and Cardiovascular Disease.* 1st ed. Narosa Publishing House.

D'Mello, J. P. F., and Thomas, D. 1997. Animal feed. In: Rushkin, F. R. *Leucaena*: promising forage and tree crops for the tropics: *National Academy of Sciences*: **30**(2)

Evans, C. A. R., Miller, N. J., and Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**:152-159

Gislene G. F., Nascimento, G. G. F., Locatelli, J., Freitas, P. C., and Giuliana L. Silva, G. L. 2000. Antibacterial activity of plant extract and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal Microbiology* **31**(4).

Gupta, R. K., Kesari, A. N., Watal, G., Murthy, P. S., Chandral, R., Maithall, K., and Thandal, V. 2005. Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* L. in experimental animals. *Current 1224 Science* **88**(8)

Hakimuglu, F., Kizil, G., Kanay, Z., Kizil, M., and Is, H., 2006. The effect of ethanol extract of *Hypericum lysimachioides* on lipid profile in hypercholesterolemic rabbits and its *in vitro* antioxidant activity. *Article in Press:* 10.

Harborne, J. B. 1980. *Plant Phenolics*. In: Bell, E. A., and Charlwood, B. V., *Encyclopedia of Plant Physiology*. Vol. 8. Springer-Verlag, New York.

Khan, I. A., and Khanum, A. 2005. *Hyderabad, Herbal Medicine for Human Diseases*. Vol. 2. Ukaaz Publication.

Kewalramani, N., Ramchandra, K. S., Upadhyay, U. S., and Gupta, V. K. 1987. Proximate composition, mimosine and mineral contents of *Leucaena* sp. and hybrids. *Indian Journal Animal Science* **57**:1117-1120.

Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R. 2005. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry Journal **100**: 1409–1418*

Maghrani, M., Lemhadri, A., Jouad, H., Michel, J. B., and Eddouks, M. 2003. Effect of the desert plant *Retama raetam* on glycaemia in normal and streptozotocin-induced diabetic rats. *Journal of Enthnopharmacology* **87**:21-25

Manach, C., Scalbert, A., Morand, C., Christian Rémésy, C., and Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* **79**(5):727-747

Matkowski, A., and Piotrowska, M. 2006. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Journal of Fitoterapia* 77: 346–353.

Pari, L., Ramakrishnan, R., and Venkateswaran, S. 2001. Antihyperglycaemic effect of diamed, a herbal formulation, in experimental diabetes rats. *Journal Pharmacies Pharmacology* **53**(8):1139-1143.

Prajapati, N. D., Purohit, S. S., Sharma, A. K., and Kumar, T. 2003. A *Handbook of Medical Plants: A complete Source Book.* 1st ed. Agrobios.

Rao, A. V., and Balachandran, B. 2004. *Role of Antioxidant Lycopene in Heart Disease.* In: Nath, R., Khullar, M., and Singal, P. K. *Antioxidant and Cardiovascular Disease.* 1st ed. Narosa Publishing House.

Rastogi, S. S., and Singh, R. B. 2001. Antioxidants, free radical stress and diabetes. In: Angel, A., Dhalla, N., Pierce, G., and Singal, P. *Diabetes and Cardiovascular Disease*. 1st ed. Kluwer Academic Plenum Publisher.

Saad, B., Azaizeh, H., and Said, O. 2005. Tradition and perspectives of Arab herbal medicine: A review. *Evidence Based Complement Alternative Medicine*. **2**(4): 475–479.

Scalbert, A., Johnson, I. T., and Saltmarsh, M. 2005. Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition,* Vol. **81**(1): 215-217

Sethi, P., and Kulkarni, P. R., 1995. *Leucaena leucocephala* A nutrition profile. *Food and Nutrition Profile* **16**(3).

Shi, J., Yu, J., Pohorly, J. J., Young, J. C., Bryan, M., and Wu, Y. 2003. *Optimization of* the extraction of polyphenols from grape seed meal by aqueous ethanol solution *Food, Agriculture and Environment Journal* **1(2)**: 42-47

Stratil, P., Klejdus, B., Kubaįn, V. 2006. Determination of phenolic compounds and their antioxidant activity in fruits and cereals. *Journal of Talanta 17: 1741-1751*

Suanarunsawat, T and Songsak, T. 2005. Anti-hyperglycaemic and anti-dyslipidaemic effect of dietary supplement of white *Ocimum Sanctum* Linnean before and after STZ-induced diabetes mellitus. *International Journal Diabetes and Metabolism* **13**: 18-23

Thomas, B., Murphy, D. J., and Murray, B. G. 2003. *Encyclopedia of Applied Plant Sciences*. 1st ed. Elsevier Academic Press.

Urquiaga, I., and Leighton, F. 2000. Plant polyphenol antioxidants and oxidative stress. *Biology Res* **33**(2).

Woodside, J. V., and Young, I. S. 2004. Preventation of cardiovascular disease: role of fruits, vegetables and antioxidant. In: Nath, R., Khullar, M., and Singal, P. K. *Antioxidant and Cardiovascular Disease*. 1st ed. Narosa Publishing House.