OPTIMIZATION OF EXTRACTION AND SPRAY DRYING PROCESSES OF TONGKAT ALI EXTRACTS USING RESPONSE SURFACE METHODOLOGY

WAN MASTURA BT WAN ZAMRI

UNIVERSITI TEKNOLOGI MALAYSIA

OPTIMIZATION OF EXTRACTION AND SPRAY DRYING PROCESSES OF TONGKAT ALI EXTRACTS USING RESPONSE SURFACE METHODOLOGY

WAN MASTURA BT WAN ZAMRI

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Engineering (Chemical)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

SEPTEMBER 2014

Dedicated to beloved Abah, Ummi, Abe, Maisarah, Manisah and Mawaddah.

ACKNOWLEDGEMENTS

I am grateful to Allah that I could finish my thesis. In preparing this thesis, I was in contact with many people, researchers and academicians, who have helped and contributed towards my understanding and thoughts. First and foremost, I would like to convey my gratitude to my supervisors, Prof. Ramlan bin Aziz and Assoc. Prof. Dr. Azila bt Abd. Aziz for their guidance, encouragement, criticisms and advices throughout this project.

I wish to express my deep appreciation to Dr. Harisun and Mrs. Noor Hafiza for their moral support, suggestions and time for endless discussions. I indebted to Mrs. Roshanida for helpful advice and suggestions towards the application of the statistical software and analysis. I also like to thank all the research officer, research assistant and technicians of Institute Bioproduct Development (IBD), especially to Mr. Ismail, Mrs. Fashya, Mrs. Muna and Mr. Syafiq for their guides and assistances.

A great appreciation for staff and technicians of Laboratory of Faculty of Chemical Engineering and Natural Sources, Universiti Malaysia Pahang for permission in using the spray dryer and their technical supports during the experiments. I am also grateful to Ministry of Agriculture and Agro-based Industry, Malaysia under NKEA 1: NKEA Herbal Research Grant Scheme for funding my master study.

My sincere thankfulness also extends to all my colleagues, my beloved father, mother, siblings and those whom directly and indirectly had helped in making this project a success. Thank you.

ABSTRACT

Tongkat Ali is a popular herb due to its various medicinal benefits. However, Tongkat Ali processing has a low yield and the quality and safety of spray dried Tongkat Ali had been an issue. Therefore, this study was done to optimize Tongkat Ali production process to obtain high quality and safe Tongkat Ali product. A Central Composite Design was employed to study the effects of solvent to raw material ratio, duration of extraction and particle size of raw material from Tongkat Ali extraction on total solid content and amount of eurycomanone. A Box Benhken Design was applied to determine the effects of inlet air temperature, feed temperature, air pressure and feed flow rate from Tongkat Ali spray drying process on the efficiency of spray drying. Effect of spray drying heat was investigated on four bioactive compounds of Tongkat Ali which were amount of eurycomanone, total polysaccharide, total protein, and total glycosaponins and its toxicity. The optimum condition for extraction process was achieved at 10:1 g/g of solvent to raw material ratio,1 hour of duration of extraction and 0.5-1.0 mm particle size which corresponded to 6.2290 mg/g solid content and 1.7139 % eurycomanone. Yield of extract at this condition was 6.8526 %. For spray drying process, the optimum condition was observed at 180 °C of inlet air temperature, 100 °C of feed temperature, 15.18 psi of air pressure and 5.44 ml/min of feed flowrate with 38.48 % efficiency of spray drying achieved.Temperature of the spray dryer was found to have no effect on the amount of eurycomanone, total polysaccharide, total glycosaponins and cytotoxicity except for total protein. Spray dried Tongkat Ali was safe to be used and no major difference was found in the cytotoxic test of both spray dried and freeze dried extracts. Response surface methodology was successful in identifying the optimal conditions of Tongkat Ali processing.

ABSTRAK

Tongkat Ali merupakan herbal terkenal kerana pelbagai kelebihannya dalam bidang perubatan. Walaubagaimanapun, pemprosesan Tongkat Ali mempunyai hasil yang rendah dan kualiti serta keselamatan Tongkat Ali hasil daripada pengeringan semburan menjadi isu. Olehitu, kajian ini telah dijalankan untuk mengoptimal pemprosesan produk Tongkat Ali bagi mendapatkan produk Tongkat Ali yang berkualiti tinggi dan selamat. Pusat rekabentuk komposit telah digunakan untuk mengkaji kesan nisbah pelarut kepada bahan mentah, tempoh pensarian dan partikel saiz bahan mentah daripada pensarian Tongkat Ali terhadap jumlah kandungan pepejal dan kuantiti *eurycomanone*. *Box Benhken Design* telah digunakan untuk mengkaji kesan suhu udara masuk, suhu masukan, tekanan udara dan kadar aliran masukan daripada pengeringan semburan Tongkat Ali terhadap kecekapan pengeringan semburan. Kesan pemanasan pengeringan semburan juga dikaji terhadap empat bioaktif kompaun dalam Tongkat Ali iaitu kuantiti *eurycomanone*, kuantiti polisakarida, kuantiti protein, dan kuantiti glikosaponin,dan juga toksisiti. Keadaan optimal untuk proses pensarian adalah pada 10:1 g/g nisbah pelarut kepada bahan mentah, 1 jam tempoh pensarian dan 0.5-1.0 mm partikel saiz bahan mentah bersamaan dengan 6.8526 mg/g jumlah kandungan pepejal dan 1.7139 % kuantiti *eurycomanone*. Hasil sarian pada keadaan ini adalah 6.8526 %. Untuk pengeringan semburan, keadaan optimal adalah pada 180 °C suhu udara masuk, 100 °C suhu masukan, 15.18 psi tekanan udara dan 5.44 ml/min kadar aliran masukan dengan 38.48 % kecekapan pengeringan semburan. Suhu daripada pengeringan semburan tidak member kesan kepada kuantiti *eurycomanone*, kuantiti polisakarida, dan kuantiti glikosaponin,dan sitotoksik kecuali kepada kuantiti protein. Tongkat Ali hasil daripada pengeringan semburan selamat digunakan dan tiada perbezaan ketara dalam ujian sitotoksik bagi kedua Tongkat Ali yang hasilkan daripada pengeringan semburan dan pengeringan beku. Kaedah tindakbalas permukaaan Berjaya dalam mengenalpasti keadaan optimal untuk pemprosesan Tongkat Ali.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
C	-	Concentration
CO_2	-	Carbon dioxide
D	-	Dilution factor
df	-	Degree of freedom
DMEM	-	Dulbecco's modified Eagle's medium
DMSO	-	Dimethyl sulfoxide
DOE	-	Design of experiment
F	-	Fisher
FBS	-	Fetal Bovine Serum
FV	-	Final volume
GC	-	Gas chromatography
GC-MS	-	Gas chromatography-mass spectrometry
HPLC	-	High Performance Liquid Chromatography
HPLC-MS	-	High Performance Liquid Chromatography- mass spectrometry
MS	-	Mean squares
MSA	-	Means of squares for factor A
MSE	-	Means of squares for error or residual
MTT	-	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
N	-	Normality
NMR	-	Nuclear Magnetic Resonance
O.D	-	Optical Density
P	-	Probability
RPMI	-	Roswell Park Memorial Institute

RSM	-	Response Surface Methodology
SS	-	Sum of squares
SSE	-	Sum of squares of residual error
SSR	-	Sums of squares of regression
SST	-	Sums of squares of total variation
TC_{50}	-	Concentration toxic in 50 % of the cell population
TLC	-	Thin Layer Chromatography
UV	-	Ultra Violet
UV-Vis	-	Ultra Violet Visible
V	-	Volume
W	-	Weight

LIST OF SYMBOLS

%	-	Percentage
β_i	-	Coefficients of the linear parameters
β_o	-	Constant term
°C	-	Degree of Celsius
µg	-	Microgram
µl	-	Microliter
cm	-	Centimeter
g	-	Gram
h	-	Hour
H_A	-	Alternative hypothesis
H_o	-	Null hypothesis
kg	-	Kilogram
m	-	Meter
mg	-	Milligram
min	-	Minutes
ml	-	Milliliter
mm	-	Millimeter
nm	-	Nanometer
ppm	-	Part per million
psi	-	Pounds per square inch
R^2	-	Coefficient of multiple determinations
rpm	-	Revolution per minute
x_i	-	Variables

α	-	Level of significance
ε	-	Residual
k	-	Number of variables

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Tongkat Ali (*Eurycoma longifolia*) is a popular herb used by natives Malaysian and Indonesian. There are various bioactive compounds can be found in this plant such as canthin-6-one alkaloids, β-carboline alkaloids, quassinoids, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans. The plant's phytochemicals contains several medicinal benefits, some of which are anti-pyretic, anti-malarial, energy boosting, anti-tumour, and aphrodisiac properties. Besides processing parameters, the geographical factors also influence the type of metabolite and concentration of the plant extract. Consistency of the phytochemical content must be ensured particularly for herbal medicine efficacy study (Chua *et al*., 2011). Tongkat Ali root has various benefits and is taken orally. The tap root is processed traditionally, by decocting the root and is drunk for the benefit (Kumaresan and Sarmidi, 2003). Nowadays, Tongkat Ali has become known globally due to its ability to treat erectile dysfunction and to improve sexual desire.

Due to Tongkat Ali's high market demand as health supplement, these phytochemical products have a high commercial value in local and global market. For instance, the prices of Tongkat Ali dried roots ranges between 20 and 25 US dollars/kg while the price of Tongkat Ali water extracts is about 26 US dollars per bottle of 60 capsules. Market demand of this plant has greatly increased as there are almost 200 products from Tongkat Ali available in health-food market specifically for its aphrodisiac properties. Tongkat Ali product are available are either in the form of capsules mixed with other aphrodisiac herbs, in raw crude powder form especially from roots, as additives mixed with coffee and ginseng, or as health products (Bhat and Karim, 2010).

Eurycomanone, a quassinoids found in Tongkat Ali extract has a potential to be developed as complementary for anti-cancer therapy due to its ability to inhibit cancer cells such as lung, liver, breast cancer cells, and, decrease tumorigenic and significant activities against Plasmodium falciparum strains (Chan *et al*., 1986; Kardono *et al*., 1991; Cheah and Azimahtol, 2004; Zakaria *et al*., 2009; Wong *et al*., 2012). Other compounds found in Tongkat Ali include total polysaccharide, total protein, and total glycosaponins also shows medicinal properties. Total polysaccharides have been reported to exhibit a variety of biological activity like anti-inflammation, anti-oxidation, anti-complement, anti-fatigue, anti-coagulation and enhancement of robiotic bacteria growth (Zheng *et al*., 2010). Polysaccharides also induce anti-tumor activity, anti-virus activity, anti-bacterial activity, immune activating activity, and anti-diabetic activity (Nakamura *et al*., 2009; Xi *et al*., 2010).

Total protein are essential to human’s health because they are a major sources of energy and contain essential amino-acids such as lysine, tryptophan, methionine, leucine, isoleucine, and valine which the body cannot synthesize. Total glycosaponins have a wide spectrum of activities such as anti-bacterial and anti-fungal agents, inhibiting cancer cell growth, and lowering blood cholesterol (Sezgin and Artik, 2010). Glycosaponins also found to be having molluscicidal properties, anti-microbial activity, anti-inflammatory activity, anti-dermatophytic activity, anthelmintic properties, and antitussives properties (Chen *et al*., 2010). Since eurycomanone, total polysaccharides, total protein and total glycosaponins have great benefits on human’s health, processing techniques of Tongkat Ali that could achieve high concentration while retaining the biological activities of the compounds are very important.

The production of Tongkat Ali water extract is mainly carried out through various traditional methods, such as boiling or soaking which often lead to high losses and low product yield. Hence more efforts are needed to develop the various stages of phytochemical processing, be it in planting and harvesting, raw material preparation, processing or as value added production (Athimulam *et al*., 2006). It is desirable to have Tongkat Ali in powder form to then put into a capsule due to its bitter taste.

The ability of solvents to extract solutes present in the drug material is shown by the amount of dry residue (extracts) that can be obtained. Higher amount of extracts obtained from selected extraction conditions is required for spray drying process. The dry residue does not only affect yield of spray drying but also the properties of the final product. In addition, running spray dryer at the highest amount of dry residue is economically beneficial due the reduced amount of water to be evaporated (Fernandes *et al*., 2012). Therefore, maximum dry residue or total solid content of Tongkat Ali extraction was required in this study.

Spray drying is widely used in the production of Tongkat Ali extract in a powder form. Spray dried powders have good reconstitutional characteristics; lower water activity, good quality and are easier to be transported and stored which are important properties for powder products (Kha *et al*., 2010). The cost for spray drying extraction is cheaper compared to freeze drying (Gharsallaoui *et al*., 2007). Furthermore, freeze drying is a discontinuous process with low yield, energy, and time consuming. Spray drying on the other hand is relatively inexpensive, short time period process and allows continuous large capacity production. Compared to freeze drying method, spray drying can offer 6 times less expensive alternatives for every kg of water removed (Paez *et al*., 2012). A spray dried powder is characterized not only by its composition (protein, carbohydrates, fats, mineral and water) but also its physical properties such as bulk and particle density, hygroscopicity, insolubility index, and particle size. The physiochemical properties of spray dried powders depends on some process variables such as characteristics of the liquid feed (particle size, viscosity, flow rate), drying air (temperature, pressure), and the type of atomizer (Tonon *et al*., 2008).

1.2 Problem Statement

Batch solid liquid extraction and spray drying technology have been developed for Tongkat Ali processing. However, current extraction process based on batch solid liquid extraction has a low yield of 2-3 % w/w (Mohtar *et al*., 2007). Moreover, most studies only focuses on the yield extract and not much on eurycomanone yield. Thus, effects of extraction operating parameters need to be investigated in order to achieve the highest yield of extraction and eurycomanone at the optimum condition. Operating parameters that can affect the extract yield includes solvent used, solvent to raw material ratio, time of extraction, extraction temperature, raw material particles size and agitation speed of extraction vessel (Kumaresan and Sarmidi, 2003).

Another important process in the production of Tongkat Ali product is spray drying process. Tongkat Ali extracted is converted into powder form that is easier to handle and free of harmful bacteria and fungi. There is common conception that Tongkat Ali powder produced by freeze dryer is more quality as compared to those produced using spray drying method (Chan and Chew, 2003). However, freeze drying is a very expensive method and require higher costs. Spray drying powders however may have some problems on their properties such as hygroscopicity, stickiness, and solubility which lead to low efficiency of spray drying process (Tonon *et al*., 2008). Hence, the effect of processing parameters of spray drying process needs to be studied to attain highest process yield.

Quality of phytochemicals and safety of Tongkat Ali extract from spray drying process has also been an issued by majority due to the use of elevated temperature during the process. Degradation of phytochemicals will diminish its biological activity and might produce harmful byproducts. Therefore, the effects of spray drying heat on the spray dried Tongkat Ali extracts were observed by investigating the quality of the compounds in the extract and their safety is examined by cell toxicity study.

1.3 Objective of the Research

In this research the effects of processing parameters during Tongkat Ali extraction and spray drying process were investigated. The objective of this research is to optimize Tongkat Ali production process to obtain high quality and safe Tongkat Ali product.

1.4 Scope of the Research

In order to achieve the objectives of the research, the experimental works were divided into three major scopes. The scopes of the research are:

i) To optimize Tongkat Ali extraction process on total solid content and amount of eurycomanone. The optimization of Tongkat Ali extraction was carried out at three different parameters which are solvent to raw material ratio, duration of extraction and particle sizes of raw material.

ii) To optimize Tongkat Ali spray drying process on efficiency of spray drying. The optimization of Tongkat Ali spray drying was carried out at four parameters which are air inlet temperature, feed temperature, air pressure and feed flow rate.

iii) To investigate the effects of spray drying heat on eurycomanone, total polysaccharide, total protein, total glycosaponins, and toxicity of Tongkat Ali. Cultured colon cancer cell line; HT-29 was used as a model in cytotoxicity study.

REFERENCES

Ahmad, A., Mohamad, M., Ali, M. W., and Ripin, A. (2013). Effect of Extraction Process Parameters on the Yield of Bioactive Compounds from the Roots of *Eurycoma Longifolia*. *Jurnal Teknologi*. 60:51-57.

Amaro, M. I., Tajber, L., Corrigan, O. I. and Healy, A. M. (2011). Optimisation of Spray Drying Process Conditions for Sugar Nanoporous Microparticles (Npmps) Intended for Inhalation. *International Journal of Pharmaceutics*. 421:99-109.

American Type Culture Collection, ATCC (2012). *Thawing, Propagating and Cryopreserving Protocol, NCI-PBCF-HTB38 (HT-29) Colon Adenocarcinoma*. Version 1.6. Manassas, United State.

Ang, H. H. and Lee, K. L. (2005). Analysis of Lead in Tongkat Ali Hitam Herbal Preparations in Malaysia. *Toxicological and Environmental Chemistry*. 87:521-528.

Ang, H. H., Lee, E. L. and Cheang, H. S. (2004). Determination of Mercury by Cold Vapor Atomic Absorption Spectrophotometer in Tongkat Ali Preparations obtained in Malaysia. *International Journal of Toxicology*. 23:65-71.

Ang, H. H., Ngai, T. H. and Tan, T. H. (2003). Effect of *Eurycoma longifolia Jack* on Sexual Qualities in Middle Aged Male Rats. *Pyhtomedicine*. 10:590-593.

Ang, H. H. and Sim, M. K. (1997). *Eurycoma Longifolia Jack* Enhances Libido in Sexually Experienced Male Rats. *Journal of Experimental Animal Science*. 46:287–290.

Asep, E. K., Jinap, S. Tan, T. J., Russly, A. R., Harcharan, S. Nazimah, S. A. H. (2008). The Effects of Particle Size, Fermentation and Roasting of Cocoa Nibs on Supercritical Fluid Extraction of Cocoa Butter. *Journal of Food Engineering*.85:450-458.

Ashwani, K. and Ashish, B. (2012). Comparative, Qualitative and Quantitative Chemotypic Characterization among North Indian Tribulus Terrestris. *International Research Journal of Pharmacy*. 3(6):212-218.

Athimulam, A., Kumaresan, S., Foo, D. C. Y., Sarmidi, M. R. and Aziz, R. A. (2006). Modelling and Optimization of *Eurycoma Longifolia* Water Extract Production. *Food and Bioproduct Processing.* 84 (C2):139-149.

Azmir, J., Zaidul I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N.A.N., and Omar, A. K. M. (2013). Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *Journal of Food Engineering*. 117:426-436.

Berhow, M. A., Cantrell, C. L., Duval, S, M., Dobbins, T. A., Maynes, J. and Vaughn, S. F. (2002). Analysis and Quantitative Determination of Group B Saponins in Processed Soybean Products. *Phytochemical Analysis*. 13:343-348.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. and Escaleira, L. A (2008). Response Surface Methodology (RSM) as a Tool for Optimization in Analytical Chemistry. *Talanta.* 76: 965–977.

Bhat, R. and Karim, A. A. (2010). Tongkat Ali (*Eurycoma longifolia Jack*): A Review on its Ethnobotany and Pharmacological Importance. *Fisioterapia*. 88: 669-679.

Bodeker, G., Salleh, H., Hashim, R. S., Jaenicke, C., Gruenwald, J. and Abidin, Z. Z. (2009). *Health and Beauty from the Rainforest*, *Malaysian Traditions of Ramuan*. (pp. 88). Malaysia: Biotropics Malaysian Berhad.

Brummer, Y. and Cui, S. W. (2005). Understanding Carbohydrate Analysis. Food Carbohydrates: Chemistry, Physical Properties and Applications. (pp.68-99). United Kingdom: Taylor and Francis Group, LLC.

Cacace, J. E and Mazza, G. (2003). Mass Transfer Process during Extraction of Phenolic Compounds from Milled Berries. *Journal of Food Engineering*. 59:379-389.

Chan, H. K. and Chew, N. Y. K. (2003). Novel alternative methods for the delivery of drugs for the treatment of asthma. *Advanced Drug Delivery Reviews*. 55, 793–805.

Chan, K. L, Choo, C. Y., Abdullah, N. R., and Ismail, Z. (2004). Antiplasmodial Studies of *Eurycoma Longifolia Jack* using the Lactate Dehydrogenase Assay of *Plasmodium Falciparum*. *Journal of Ethnopharmacology*. 92:223–227.

Chan, K. L. and Choo, C. Y. (2002). The toxicity of some quassinoids from *Eurycoma longifolia. Planta Medica*. 68, 662-664.

Chan, K. L. O'neill, M. J., Phillipson, J. D., Warhurst, D. C. (1986). Plants as Sources of Antimalarial Drugs, Part 31-Eurycoma longifolia. *Planta Medica*. 52:105-107.

Cheah, S. C. and Azimahtol, H. L. P. (2004). Eurycomanone Exert Antiproliferative Activity via Apoptosis upon MCF-7 Cells. *Proceeding Symposium Biologi Kebangsaan Malaysia ke-7*. 73-77.

Chegini, R. G. and Ghobadian, B. (2005). Effect of Spray-Drying Conditions on Physical Properties of Orange Juice Powder. *Drying Technology* 23: 657-668.

Chegini, R. G. and Ghobadian, B. (2007). Spray Dryer Parameters for Fruit Juice Drying. *World Journal of Agricultural Science*. 3: 230-236.

Chen, Y. F., Yang, C. H., Chang, M. S., Ciou, Y. P. and Huang, Y. C. (2010). Foam Properties and Detergent Abilities of the Saponins from *Camellia oleifera*. *International Journal of Molecular Sciences*. 11:4417-4425.

Chen, Y., Roan, H., Lii, C, Huang, Y. and Wang, T. (2011). Relationship between Antioxidant and Antiglycation Ability of Saponins, Polyphenols, and Polysaccharides in Chinese Herbal Medicines used to Treat Diabetes. *Journal of Medicinal Plants Research*. 5(11):2322-2331.

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M. and Ho, C. W. (2011). Effect of Ethanol Concentration Extraction Time and Extraction Temperature on the Recovery of Phenolic Compounds and Antioxidant Capacity of *Centella asiatica* Extracts. *International Food Research Journal*. 18:571-578.

Chua, L. S., Amin, N. A. M., Neo, J. C. H., Lee, T. H., Lee, C. T., Sarmidi, M. R., and Aziz, R. A. (2011). LC–MS/MS-based Metabolites of *Eurycoma longifolia* (Tongkat Ali) in Malaysia (Perak and Pahang). *Journal of Chromatography B.* 879:3909– 3919.

Cornell, J. A. (1984). *How to Apply Response Surface Methodology*. Volume 8. American Society for Quality Control. Statistics Division.

Couto, R. O., Edemilson, C. C., Luiza, T. C., Ezequiane, M. S. O. and Frederico, S. M. (2012). Spray-dried Rosemary Extracts: Physicochemical and Antioxidant Properties. *Food Chemistry*. 131:99-105.

Dent, M., Dragovic-Uzelac, V., Penic, M., Brncic, M., Bosiljkov, T., and Levaj., B. (2013). The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts. *Food Technology and Biotechnology*. 51(1):84-91.

Devore, J. L. and Farnum, N. R. (1999). Applied Statistic for Engineers and Scientist. Pacific Grove: Duxbury Press.

Diankov, S., Karsheva, M. and Hinkov, I. (2011). Extraction of Natural Antioxidants from Lemon Peels. Kinetics and Antioxidant Capacity. *Journal of the University of Chemical Technology and Metallurgy.* 46(3):315-319.

Dulekgurgen, E. (2004). Proteins (Lowry) Protocol. UIUC.

Effendy, N. M., Mohamed, N., Muhammad, N.,Mohamad, I. N and Shuid, A. N. (2012). Eurycoma Longifolia: Medicinal Plant in the Prevention and Treatment of Male Osteoporosis Due to Androgen Deficiency. *Evidence-Based Complementary and Alternative Medicine.* 60:1-9.

Ehrman, T. (1994). Standard Test Method for Moisture, Total Solids, and Total Dissolved Solids in Biomass Slurry and Liquid Process Samples. *Chemical Analysis and Testing Task, Laboratory Analytical Procedure, LAP-012*.

Farouk, A. E. and Benafri, A. (2007). Antibacterial activity of *Eurycoma longifolia Jack*. A Malaysian Medicinal Plant. *Saudi Medical Journal*. 28:1422–1424.

Fernandes, L. P. Candido, R. C. and Oliveira, W. P. (2012). Spray Drying Microencapsulation of *Lippia Sidoides* Extracts in Carbohydrate Blends. *Food and Bioproducts Processing*. 90:425-432.

Fournier, E. (2001). *Colorimetric Quantification of Carbohydrates*. Current Protocols in Food Analytical Chemistry. John Wiley and Sons, Inc.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voiley, A. and Saurel R. (2007). Applications of Spray Drying in Microencapsulation of Food Ingredients: An Overview. *Food Research International*. 40:1107-1121.

Goel, N., Sirohi, S. K. and Dwivedi, J. (2012). Estimation of Total Saponins and Evaluate Their Effect on in Vitro Methanogenesis and Rumen Fermentation

Pattern in Wheat Straw Based Diet. *Journal of Advanced Veterinary Research*. 2:120-126.

Gohel, M. C., Parikh, R. K., Nagori, S. A., Bariya, S. H., Gandhi, A. V., Shroff, M. S., Patel, P. K., Gandhi, C. S., Patel, V. P., Bhagat, N. Y., Poptani, S. D., Kharadi, S. R., Pandya, R. B. and Patel, T. C. (2009). Spray Drying: A Review. Retrieved September 20, 2013, from http://www.pharmainfo.net.

Haaland, P. D. (1989). "Experimental Design in Biotechnology". (pp:259). Marcel Dekker, New York.

Handa, S. S., Khanuja S. P. S., Longo, G. and Rakesh, D. D. (2008). Extraction Technologies for Medicinal. International Centre for Science and High Technology. Trieste.

Herzi, N., Bouajila, J., Camy, S., Romdhane, M. and Condoret, J. S. (2013). Comparison of Different Methods for Extraction from Tetraclinis articulate: Yield, Chemical Composition and Antioxidant Activity. *Food Chemistry*. 141:3537-3545.

Hiai, S., Oura, H. and Nakajima, T. (1976). Color Reaction of Some Sapogenins and Saponins with Vanillin and Sulfuric Acid. *Plant Medica*. 29:116-122.

Husen, R., Hawariah, A., Pihie, L., Nallappan, M. (2004). Screening for Antihyperglycaemic Activity in Several Local Herbs of Malaysia. *Journal of Ethnopharmacolog.* 95:205–208.

Ismail, Z., Ismail, N. and Lassa, J. (1999). Malaysian Herbal Monograph. Kuala Lumpur. Malaysian Monograph Committee.

Jiwajinda, S., Santisopasri, V., Murakami, A., Kawanaka, M., Kawanaka, H., and Gasquet, M. (2002). In Vitro Anti-Tumor Promoting and Anti-Parasitic Activities of the Quassinoids from Eurycoma Longifolia, a Medicinal Plant in Southeast Asia. *Journal of Ethnopharmacology*. 82:55–58.

Joshi, D. D. (2012). Herbal Drugs and Fingerprints: Evidence Based Herbal Drugs. India: Springer.

Juntachote, T., Berghofer, E., Bauer, F. and Siebenhand, S. (2006). The Application of Response Surface Methodology to the Production of Phenolic Extracts of Lemon Grass, Galangal, Holy Basil And Rosemary. *International Journal of Food Science and Technology*. 41: 121–133.

Kardono, L. B. S., Angerhofer, C. K., Tsauri, S., Padmawinata, K., Pezzuto, J. M. and Kinghorn, D. (1991). Cytotoxic and Antimalarial Constituents of the Roots of Eurycoma Longifolia. *Journal of Natural Product*. 54(5):1360-1367.

Kareru, P. G., Keriko, J. M., Gachanja, A. N. and Kenji, G. M. (2007). Direct Detection of Triterpenoid Saponins in Medicinal Plants. *African Journal of Traditional, Complementary and Alternative Medicines*. 5(1):56-60.

Kaur, I. Kumaresan, S., and Sarmidi, M. R. (2003). A Study into the Effect of Laboratory Scale Processing Parameters and Scale Up on *Eurycoma Longifolia* Water Extract Yield. *Proceedings of the Symposium of Malaysia Chemical Engineers*, 29-30 Disember 2003. Copthorne Hotel, Penang: Institute of Malaysian Chemical Engineers. 294-299.

Kha, T. C., Nguyen, M. H. and Roach, P. D. (2010). Effects of Spray Drying Conditions on the Physicochemical and Antioxidant Properties of the Gac (*Momordica cochinchinensis*) Fruit Aril Powder. *Journal of Food Engineering*. 98:385-392.

Kim, J. H., Park, S. Y., Lim, H. K., Park, A. Y., Kim, J. S., Kang, S. S., Youm, J. R. and Han, S. B. (2007). Quantitative Evaluation of *Radix Astragali* through the Simultaneous Determination of Bioactive Isoflavonoids and Saponins by HPLC/UV and LC-ESI-MS/MS. *Bul. Korean Chem. Soc*. 28 (7): 1187-1194.

Kordono, L. B. S., Angerhofer, C. K., Tsauri, S., Padmawinata, K., Pezzuto, L. M. and Kinghom, A. D. J. (1991). Cytotoxic and Antimalarial Constituents of the Roots of *Eurycoma longifolia. Journal Natural Product*. 54:1360-1367.

Krishnaiah D., Sarbatly, R. and Nithyanandam, R. (2012). Microencapsulation of *Morinda Citrifolia* L. Extarct by Spray Drying. *Chemical Engineering Research and Design*. 90:622-632.

Krishnaiah, D., Sarbatly R. and Nithyanandam, R. (2011). Optimization of Spray Drying for Drying *Morinda citrifolia* L. Fruit Extract. *Journal of Applied Sciences*. 11 (13):2276-2283.

Kumaresan, S. and Sarmidi, M. R. (2003). A Preliminary Study into the Effect on Eurycoma longifolia Water Extract Yield. *Proceedings of International Conference on Chemical and Bioprocess Engineering*, 27th-28th August 2003, Universiti Malaysia Sabah, Kota Kinabalu, Sabah.

Kuo, P., Damu., A. G., Lee, K. and Wu, T. (2004). Cytotoxic and Antimalarial Constituents from the Roots of *Eurycoma longifolia*. *Bioorganoc & Medicinal Chemistry*. 12:537-544.

Kurozawa, L. E., Park, K. J. and Hubinger, M. D. (2009). A Effect of Carrier Agents on the Physicochemical Properties of a Spray Dried Chicken Meat Protein Hydrolysate. *Journal of Food Enginnering*. 12:326-333.

Linus, H. W., Eijkelboom, C. and Hagendoorn, M. J. M. (1995). Relation between Primary and Secondary Metabolism in Plant Cell Suspensions. *Plant Cell, Tissue and Organ Culture.* 43:111-116.

Liu, X., Qiu, Z., Wang, L., and Chen, Y. (2011). Quality Evaluation of Panax Notoginseng Extract Dried by Different Drying Methods. *Food and Bioproducts Processing*.89:10-14.

Malaysian Standard (2010). *MS No. 07U001R0*, Phytopharmaceutical Aspect of Freeze Dried Water Extract from Tongkat Ali Roots – Specification, Department of Standards Malaysia. 1-13.

Marsden, J. O. and House, C. I. (2006). *The Chemistry of Gold Extraction*. (2nd ed.). Society for Mining, Metallurgy, and Exploration, Inc: United States of America.

Masters, K. (2002). Spray Drying Fundamentals. In Spray Drying in Practice. SprayDryConsult International ApS, Charlottenlund, Denmark. 19-125.

Mohamad, M., Ali, M. W. and Ahmad, A. (2010). Modelling for Extraction of Major Phytochemical Components from *Eurycoma longifolia*. *Journal of Applied Science.* 10: 2572-2577.

Mohd Ajib Mohtar (2005). *Modelling and Optimization of Eurycoma longofolia Extraction Utilising a Recirculating Flow Extractor (RFE).* Bachelor of Chemical Engineering, Universiti Teknologi Malaysia.

Mohtar, M. A., Kumaresan, S., Sarmidi, M. R. and Aziz, R. A. (2007). Modelling and Optimisation of *Eurycoma longifolia* Extraction Utilising a Recirculating Flow Extractor. *Journal of Applied Sciences.* 7 (15):2168-2173.

Mustafa, A. and Turner, C. (2011). Pressurized Liquid Extraction as a Green Approach in Food and Herbal Plants Extraction: A Review. *Analytica Chimica Acta*. 703:8-18.

Nakamura, T., Nishi, H. and Kakehi, K. (2009). Investigation on the Evaluation Method of Fungi-Polysaccharide Marker Substance for the Identification by Gel Permeation Chromatography. *Chromatography*. 30(1): 25-35.

National Coordinating Committee on Food and Nutrition (NCCFN). (2005). Recommended Nutrient Intakes for Malaysia: Protein. Ministry of Health Malaysia, Putrajaya. Digibook Sdn Bhd, Petaling Jaya. Pages: 52-65.

Negi, J. S., Singh, P., Pant, G. J. N and Rawat, M. S. M. (2011). High-Performance Liquid Chromatography Analysis of Plant Saponins: An update 2005-2010. *Pharmacognosy Review*. 5 (10): 155-158.

Nurhanan, M. Y., Azimahtol, H. L. P., Mohd-Ilham, A. and Mohd-Shukri, M. A. (2005). Cytotoxic Effects of the Root Extracts of *Eurycoma longifolia Jack. Phytotherapy Research*. 19:994-996.

Nurkhasanah, M and Azimahtol, H. L. P. (2008). Eurycomanone Induces Apoptosis through the Up-Regulation of p53 in Human Cervical Carcinoma Cells. *Journal of Cancer Molecules*. 4(4):109-115.

Obon, J. M., Castellar, M. R., Alacid, M. and Fernandez-Lopez, J. A. (2009). Production of a Red–Purple Food Colorant From *Opuntia Stricta* Fruits by Spray Drying and its Application in Food Model Systems. *Journal of Food Engineering*. 90: 471-479.

Olson, B. J. S. C. and Markwell, J. (2007). *Assays for Determination of Protein Concentration*. (pp:1-29). Current Protocols in Proteins Science. John Wiley and Sons, Inc.

Paez, R., Lavari, L., Vinderola, G., Audero, G., Cuatrin, A., Zaritzky, N. and Reinheimer, J. (2012). Effect of Heat Treatment and Spray Dying on Lactobacilli Viability and Resistance to Simulated Gastrointestinal Digestion. *Food Research International*. 48:748-754.

Patel, R. P., Patel, M. P. and Suthar, A. M. (2009). Spray Drying Technology: an Overview. *Indian Journal of Science and Technology*. 2: 44-47.

Paudel, A., Worku, Z. A., Meeus, J., Guns, S. and Mooter, G. V. (2012). Manufacturing of Solid Dispersions of Poorly Water Soluble Drugs by Spray Drying: Formulation and Process Considerations. *International Journal of Pharmaceutics*. 453(1):253-284.

Phisut, N. (2012). Spray Drying Technique of Fruit Juice Powder: Some Factors Influencing the Properties of Product. *International Food Research Journal*. 19 (4): 1297-1306.

Pisecky, J. (2004). Spray Drying in the Cheese Industry. *IDF Symposium on Cheese, International Dairy Federation*, Denmark.

Razak, M. F. A and Aidoo, K. E. (2011). Toxicity Studies of *Eurycoma longifolia* (*Jack*)-based Remedial Products. *Asian Journal of Pharmaceutical and Clinical Research*. 4(3):23-27.

Sambandan, T.G., Saad, J.M., Rha, C.K., Aminudin, N. & Abdul-Kadir, A. (2006). US Patent No. 7 132 117 B2, *Bioactive Fraction of Eurycoma longifolia*.

Satayavivad, J., Soonthornchareonnon, N., Somanabandhu, A. and Thebtaranonth, Y. (1998). Toxicological and antimalarial activity of eurycomalactone and *Eurycoma longifolia* Jack extracts in mice. *Thai J Phytopharm*. 5: 14–27.

Sezgin, A. E. C. and Artik, N. (2010). Determination of Saponin Content in Turkish Tahini Halvah by Using HPLC. *Advance Journal of Food and Science Technology*. 2(2):109-115.

Sim, C. C., Kumaresan, S. and Sarmidi M.R. (2004). Mass Transfer Coefficients of *Eurycoma longifolia* Batch Extraction Process, *Proceedings of the 18th Symposium of Malaysian Chemical Engineers*. Pages 362 to 367.

Sivakumar Kumaresan (2008). *A Process Emgineering Approach to the Standardization of Eurycoma in Eurycoma longifolia Water Extract*. Doctor of Philosophy, Universiti Teknologi Malaysia.

Stacey, G. N., Doyle, A. and Ferro, M. (2001). *Cell Culture Methods for in vitro Toxicology*. Vol 17(4&5). (pp:102). Kluwer Academic Publisher, The Netherlands.

Stat-Ease, Inc. (2002). "Design-Expert 6 user's guide".Version 6.0.8. Design Expert Software.

Tai, C. J., Wang, C. K., Tai, C. J., Lin, Y. F., Lin, C. S., Jian, J. Y., Chang, Y. J. and Chang, C. C. (2013). Aqueous Extract of Solanum nigrum Leaves Induces Autophagy and Enhances Cytotoxicity of Cisplatin, Doxorubicin, Docetaxel, and 5-Fluorouracil in Human Colorectal Carcinoma Cells. *Evidence-Based Complementary and Alternative Medicine*. 9:1-12.

Tee, L. H., Chuah, L. A., Pin, K. Y., Rashih, A. A. and Yusof, Y. A. A. (2012). Optimization of Spray Drying Process Parameters of *Piper betle* L. (Sirih) Leaves Extract Coated with Maltodextrin. *Journal of Chemical and Pharmaceutical Research*. 4(3):1833-1841.

Tee, T. T. and Azimahtol, H. L. P. (2005). Induction of Apoptosis by *Eurycoma longifiolia Jack* Extract. UKM. *Anticancer Research*. 25:2205-2214.

Tonon, R. V., Brabet C. and Hubinger, M. D. (2010). Anthocyanin Stability and Antioxidant Activity of Spray-Dried Açai (*Euterpe oleracea Mart*.) Juice Produced with Different Carrier Agents. *Food Research Interna*tional. 43: 907-914.

Tonon, R. V., Brabet, C. and Hubinger, M. D. (2008). Influence of Process Conditions on the Physicochemical Properties of Acai (*Euterpe oleraceae Mart*.) Powder Produced by Spray Drying. *Journal of Engineering*. 88: 411-418.

Tzannis S. T. and Prestrelski S. J. (1999). Activity-stability Considerations of Trypsinogen During Spray Drying: Effects of Sucrose. *Journal Pharmaceutical Sciences.* 88(3):351-9.

Voung., Q. V., Hirun, S., Roach, P. D., Bowyer, M. C., Phillips, P. A., Scarlett, C. J. (2013). Effect of Extraction Conditions on Total Phenolic Compounds and Antioxidant Activities of Carica Papaya Leaf Aqueous Extracts. *Journal of Herbal Medicine*. 3:103:111.

Wang, A., Lu, Y., Zhu, R., Li, S. and Ma, X. (2009). Effect of Process Parameters on the Performance of Spray Dried Hydroxyapatite Microspheres. *Powder Technology*. 191:1-6.

Wondesen Workneh (2011). *Extraction and Characterization of Essential Oil from Margosa Seed*. Degree of Masters. Addis Ababa University.

Wong, P.-F., Cheong, W.-F., Shu, M.-H., Teh, C.-H., Chan, K.-L. and Bakar, S. A. (2012). Eurycomanone Suppresses Expression of Lung Cancer Cell Tumor Markers, Prohibitin, Annexin 1 and Endoplasmic Reticulum Protein 28. *Phytomedicine*. 19:138– 144.

Xi, X., Wei, X. Wang, Y., Chu, Q. and Xiao, J. (2010). Determination of Tea Polysaccharides in Camellia Sinensis by a Modified Phenol-Sulfurica Acid Method. *Archives of Biological Science Belgrade*. 62(2): 669-676.

Xiu-zhen, N., Bing-qing, W., Yuan, Z., Ning-ning, W., Xu, Z., Shan-shan, L., Gui-hua, T., Yi-fa, Z. and Ji-ming, Z. (2010). Total Fractionation and Analysis of Polysaccharides from Leaves of Panax Ginseng C. A. Mayer. *Chemical Research Chinese Universities*. 26 (2):230-234.

Yaakob, H., Malek, R. A., Misson M., Jalil, M. F. A., Sarmidi, M. R. and Aziz, R. (2011). Optimization of Isoloflavone Production from Fermented Soybean using Response Surface Methodology. *Food Sci. Biotechnol*. 20(6): 1525-1531.

Yunus, M. A. C., Yaw, C. L. and Idham, Z. (2011). Effects of Variables on the Production of Red-fleshed Pitaya Powder using Response Surface Methodology. *Jurnal Teknologi*. 56:15-29.

Zakaria, Y., Rahmat, A., Pihie, A. H., Abdullah, N. R. and Houghton, P. J. (2009). Eurycomanone Induce Apoptosis in HepG2 Cells Up-regulation of p53. *Cancer Cell International*. 9:16.

Zheng, M., Shen, J., Yang, K. Qian, S. and Feng, S. (2010). Rapid Determination of Polysaccharides in BianTi Soft Extract by Spectophotometry Coupled with Gas Chromatography-mass Spectrometry. *Pharmacognosy Magazine*. 6(22): 106-110.