

UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF DRYING TECHNIQUES AND STORAGE ON RAW
MATERIAL**

**SAFETY AND STABILITY OF PHYTOCHEMICALS OF HEMPEDU BUMI
(*Andrographis paniculata* (Burm.f.) Wall. Ex Nees)**

NOR SHARIAH BINTI SALLEH

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By

NOR SHARIAH BINTI SALLEH



Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**EFFECTS OF DRYING TECHNIQUES AND STORAGE ON RAW MATERIAL SAFETY AND STABILITY OF PHYTOCHEMICALS OF HEMPEDU BUMI
(*Andrographis paniculata* (Burm.f.) Wall. Ex Nees)**

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August 2017

Chairman : Mahmud Tengku Muda Mohamed, PhD
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Controlling the quality of finished herbal products with regard to safety aspect begins with proper production, harvesting and postharvest handling systems. However, there is very limited information that gives an overview on the safety and quality of hempedu bumi or *Andrographis paniculata* focusing on drying techniques and storage duration. Therefore, the objective of this study was to ascertain the effects of drying and storage on safety and stability of phytochemicals of *A. paniculata*.

In terms of safety aspects, heavy metal contaminations showed that Al, As, Cr, Cu, Hg, Ni, Pb and Zn were detected in soil and only Al, Cr, Cu, Ni and Zn found in tissues. Sample collected from Jabatan Pertanian, Serdang, Selangor had less concentrations of Al, Zn, Cr and Cu than Taman Pertanian Universiti (TPU), Universiti Putra Malaysia, Serdang and Kg. Bukit Pulau, Melaka. On the other hand, microbial contaminations depended on the drying technique used and however, increased during storage. Total count of fungi, yeast and bacteria also specific bacteria, *Escherichia coli* and *Staphylococcus aureus* were found less in vacuum oven and freeze drying throughout storage. Fortunately, heavy metal and microbial contaminations of dried *A. paniculata* were within the permissible limits prescribed by WHO.

The quality of *A. paniculata* leaf colour was greatly affected by drying techniques. The leaf colour of freeze-dried samples was much better than other drying techniques, with bright and greenish colour leaves. In addition, the chemical markers of andrographolide (AG) and neoandrographolide (NAG) content of freeze-dried samples were found to be more stable during storage compared to 14-deoxy-11, 12-didehydroandrographolide (DDAG). Microbial contaminations had a significant negative correlation with AG content, which

indicating that the presence of microorganism may led to the decreased in AG. Apart of this, freeze-dried samples also showed highest content of TPC, TFC and antioxidant activities (DPPH and FRAP) throughout the storage duration. However, sun drying gave the highest content of DDAG and TFC throughout storage period as compared to vacuum and conventional oven drying techniques. This study also showed high correlation between TPC and TFC which demonstrated that antioxidant activities were contributed by phenolic and flavonoid compounds.

Therefore, sun and freeze dryings were selected for future optimization of their quality using proton nuclear magnetic resonance ($^1\text{H-NMR}$). Eight compounds were identified in both techniques. Result obtained in PCA revealed that freeze-dried samples had higher amounts of AG and NAG, and also with relatively high amount of chlorogenic acid and alanine. AG, NAG and alanine compounds in freeze-dried samples were optimum within 6 to 8 weeks of storage. Chlorogenic acid, a compound related to antioxidant activities was found increased with storage duration. However, sun-dried samples were rich in DAG and glucose compounds, where, the estimated optimum amount of these compounds was found within 5 to 6 weeks of storage. Conversely, the level of choline in sun-dried samples increased with storage period. Thus, freeze drying is considered to be an appropriate technique for drying *A. paniculata* commercially, because this technique can stabilize most of the phytochemicals, at the same time safe for consumption.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**TAJUK TESIS PENGARUH TEKNIK PENGERINGAN DAN PENYIMPANAN
TERHADAP KESELAMATAN BAHAN MENTAH DAN KESTABILAN
FITOKIMIA HEMPEDU BUMI (*Andrographis paniculata* (Burm.f.) Wall. Ex
Nees)**

Oleh

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Jaminan kualiti barang herba terutama dari segi aspek keselamatan bermula di peringkat penghasilan produk, penuaian dan pengendalian lepas tuai dengan cara yang betul. Walau bagaimanapun, maklumat yang ada masih sedikit berkaitan dengan keselamatan dan kualiti hempedu bumi atau *Andrographis paniculata* dengan fokus kepada teknik pengeringan dan tempoh penyimpanan. Oleh itu, tujuan kajian ini adalah untuk menguji kesan pengeringan dan penyimpanan terhadap keselamatan dan kestabilan sebatian fitokimia *A. paniculata*.

Dari segi aspek keselamatan, pencemaran logam berat menunjukkan bahawa Al, As, Cr, Cu, Hg, Ni, Pb dan Zn telah berjaya dikenalpasti pada sampel tanah dan hanya Al, Cr, Cu, Ni dan Zn dijumpai di dalam sampel tisu. Sampel yang diambil dari Jabatan Pertanian, Serdang didapati kurang kandungan Al, Zn, Cr dan Cu berbanding TPU, Universiti Putra Malaysia, Serdang dan Kg. Bukit Pulau, Melaka. Selain itu, pencemaran mikrob dilihat bergantung kepada teknik pengeringan dan meningkat semasa tempoh penyimpanan. Jumlah kandungan kulat, yis dan bakteria dan juga bakteria spesifik seperti *Escherichia coli* dan *Staphylococcus aureus* didapati rendah kandungannya di dalam pengeringan ketuhar bervakum dan pengeringan sejuk beku sepanjang tempoh penyimpanan. Jumlah kiraan kulat, yis dan bakteria serta spesifik bakteria *Escherichia coli* dan *Staphylococcus aureus* didapati kurang dalam pengeringan ketuhar bervakum dan pengeringan sejuk beku semasa penyimpanan. Selain itu, pencemaran logam berat dan mikrob pada sampel kering *A. paniculata* masih di dalam had yang dibenarkan oleh WHO.

Kualiti warna daun *A. paniculata* sangat dipengaruhi oleh teknik pengeringan. Warna daun untuk pengeringan sejuk beku adalah lebih baik berbanding teknik pengeringan lain, yang mana menunjukkan warna daun yang cerah dan kehijauan. Tambahan pula, kandungan AG dan NAG daripada pengeringan sejuk beku didapati lebih stabil semasa penyimpanan berbanding DDAG. Pencemaran mikrob menunjukkan kolerasi negatif dengan kandungan AG, iaitu menunjukkan kehadiran mikroorganisma boleh menyebabkan pengurangan terhadap kandungan AG pada sampel kering *A. paniculata*.

Selain itu, pengeringan sejuk beku telah menunjukkan kandungan tertinggi untuk TPC, TFC dan aktiviti antioksidan (DPPH dan FRAP) sepanjang tempoh penyimpanan. Walau bagaimanapun, pengeringan matahari menunjukkan kandungan DDAG dan TFC yang tinggi sepanjang tempoh penyimpanan berbanding teknik pengeringan ketuhar bervakum dan ketuhar konvensional. Kajian ini juga menunjukkan korelasi yang tinggi di antara kandungan TPC dan TFC dengan aktiviti antioksidan dalam *A. paniculata*, membuktikan aktiviti antioksidan adalah berkait rapat dengan kandungan fenolik dan flavonoid.

Oleh itu, teknik pengeringan matahari dan sejuk beku telah dipilih untuk peroptimuman lanjut menggunakan proton-resonans magnetik nuklear ($^1\text{H-NMR}$). Lapan sebatian dikenalpasti daripada pengeringan matahari dan sejuk beku. Keputusan yang diperolehi melalui PCA telah menunjukkan bahawa sampel kering sejuk beku mempunyai jumlah AG dan NAG yang lebih tinggi dan juga jumlah asid klorogenik dan alanina yang agak tinggi. Sebatian AG, NAG dan alanina dalam sampel kering sejuk beku adalah optimum dalam tempoh penyimpanan antara 6 hingga 8 minggu. Asid klorogenik iaitu sebatian yang berkaitan dengan aktiviti antioksidan didapati meningkat dengan tempoh penyimpanan. Walau bagaimanapun, sampel kering matahari dikenalpasti tinggi dengan sebatian DAG dan glukosa, di mana jumlah optimum sebatian ini didapati dalam tempoh penyimpanan antara 5 hingga 6 minggu. Sebaliknya, tahap kolina pada sampel kering matahari meningkat apabila tempoh penyimpanan meningkat. Justeru, pengeringan sejuk beku dianggap sebagai teknik yang sesuai untuk pengeringan *A. paniculata* secara komersial, kerana teknik ini boleh menyebabkan sebatian fitokimia produk herba ini lebih stabil dan pada masa yang sama, selamat untuk digunakan.

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I certify that a Thesis Examination Committee has met on 2 August 2017 to conduct the final examination of Nor Shariah binti Salleh on her thesis entitled "Effects of Drying Techniques and Storage on Raw Material Safety and Stability of Phytochemicals of Hemedu Bumi (*Andrographis paniculata* (Burm.f.) Wall. ex Nees)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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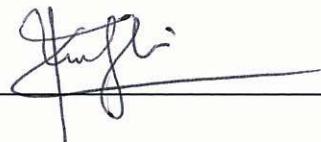
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LIST OF ABBREVIATIONS

a_w	Water activity
v/v	Volume per volume
w/v	Weight per volume
Q^2	Predictive ability
%	Percent
μl	Microliter
$^{1\text{H}}, ^{13\text{C}}$ -NMR	X-ray, Proton and Carbon-13 Nuclear Magnetic Resonance
$^1\text{H-NMR}$	^1H -Nuclear Magnetic Resonance
ABTS	2, 2'-azinobis (3- ethylbenzthiazoline)-6-sulphonic acid
AG	Andrographolide
Al	Aluminium
AlCl_3	Aluminum chloride
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
As	Arsenic
ASCII	American Standard Code for Information Interchange
BW	Body weight
C*	Chroma
Cd	Cadmium
CFS	Centre for Food Safety
CFU g^{-1}	Colony forming units per gram of sample
CH_2O_2	Formic acid
CH_3CN	Acetonitrile
$\text{CH}_3\text{OH-d}4$	Methanol-d4
cm	Centimeter
Co	Cobalt
Cr	Chromium
CRD	Completely randomized design
Cu	Copper
D	Drying
δ	Delta
D_2O	Deuterium oxide
DDAG	14-deoxy-11,12-didehydroandrographolide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
ESI-MS	Electrospray Ionisation Mass Spectrometry
FD	Freeze drying
FDA	Food and Drug Administration
$\text{FeCl}_3.6\text{H}_2\text{O}$	Iron (III) Chloride Hexahydrate
FRAP	Ferric reducing antioxidant power
g	Gram
h	Hour
h°	Hue angle
HCl	Hydrochloric acid
Hg	Mercury
HPLC	High Performance Liquid Chromatography

HSD	Tukey's Studentized Range
ICP-OES	Inductively coupled plasma optical emission spectrometry
K2S2O8	Potassium persulfate
kg	Kilogram
Kg.	Kampung
KH2PO4	Potassium dihydrogen phosphate
kPa	Kilopascal
L	Liter
L*	Lightness
LSD	Least significant difference
m	Meter
M	Molar
m2	Meter square
mbar	Millibars
MeOH	Methanol
mg	Milligram
mg g-1	Milligram per gram
mg kg-1	Milligram per kilogram
mg L-1	Milligram per liter
mg mL-1	Milligram per milliliter
mg N/ha	Milligram nitrogen per hectare
min	Minutes
mL	Mililiter
mL L-1	Mililiter per liter
mL/min	Mililiter per minutes
mm	Millimeter
mM	Millimolar
MSA	Mannitol Salt Agar
MVDA	Multivariate data analysis
NA	Nutrient agar
NAG	Neoandrographolide
NaOD	Sodium deuterium oxide
NaOH	Sodium hydroxide
ND	Not detected
Ni	Nickel
nm	Nanometer
NMR	Nuclear magnetic resonance-based
ns	No significant
O50	Oven drying at 50 °C
O70	Oven drying at 70 °C
°C	Degree celcius
Pb	Lead
PCA	Principal component analysis
PDA	Potato dextrose agar
PLS	Partial least-squares analysis
ppm	Parts per million
QE	Quercetin equivalent
R ²	R square
rpm	Revolutions per minute
S	Storage
SD	Sun drying

SE	Standard error
TE	Trolox equivalent
TE/g	Trolox equivalent per gram
TFC	Total flavonoid content
TNTC	Too numerous to count
ton/ha	Ton / hectare
TPC	Total phenolic content
TPTZ	6-triptyridyl-striazine
TPU	Taman Pertanian Universiti
TSP	3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt
UPM	Universiti Putra Malaysia
UV/VIS	Ultraviolet-visible
VD	vacuum oven drying
WHO	World Health Organization
Zn	Zinc
µm	Micrometer

CHAPTER 1

INTRODUCTION

Herbal remedies from medicinal plants have gained attention worldwide for having therapeutic effects which are the substitute medication of modern medicine. Together with the increasing demand for herbal products, quality and safety of medicinal plants and herbal products have become a major concern by the pharmaceutical industries, health authorities, and consumers (WHO, 2007). Thus, controlling the quality of finished herbal products should start with good field production, harvesting and postharvest handling practices.

In Malaysia, hempedu bumi or *Andrographis paniculata* was listed as a valuable herbs to be commercialized and was expected to generate the country's revenues. This plant has been effectively used as traditional medicines, in Asia as well as around the world, for centuries to treat various diseases. It is extensively used in the traditional Indian system of medicine, such as Ayurveda, Unani and Siddha, in home remedies for various diseases (Niranjan et al., 2010). Among many bioactive compounds in *A. paniculata*, andrographolide is considered to be the most active and valuable constituent which is responsible for the therapeutics activities (Parichatikanond et al., 2010).

Retention of bioactive compounds in harvested products is a major concern in the herbal industry. Bioactive compounds can be affected by postharvest handling, especially during drying process and storage. Drying treatments are important to decrease the moisture (Araújo and Bauab, 2012) in the herbs which would enable the extension of shelf life of the herbs. At the same time, drying will reduce the growth of microbes in medicinal plants but also can affect their phytochemical changes (Bernard et al., 2014). However, it can also give rise to other alterations that affect the stability of chemical markers.

Commonly, phytochemicals are unstable and vulnerable to degradation and decomposition. The stability of the phytochemicals is mostly influenced by various factors such as pH, oxygen, water activity, light, enzymes, concentration, structure, self-association, atmospheric composition, co-pigments, metallic ions, temperature and time of processing, the presence of antioxidants, and storage duration (Yang et al., 2013). Recently, various studies are reported in related to the factors that influence the stability of phytochemical in food products. The studies include the physical, chemical and/or biological changes that occur during processing or storage, such as loss of nutrients and loss of colour due to the degradation of pigments like carotenoids and anthocyanins (Nora et al., 2014). The author also reported that the DPPH scavenging activities in guabiju (*Myrcianthes pungens*) and red guava (*Psidium cattleyanum* Sabine) were reduced significantly through processing.

Changes in colour and essential oil contents of tarragon herb (*Artemisia dracunculus* L.) leaves at the storage depended on drying conditions and drying temperature in which 45 °C was suggested to maintain the quality nearest to the fresh material (Arabhosseini et al., 2007). However, storage temperature did not provide any significant impact on andrographolide which does not requires low temperature storage (Ibrahim and Chong, 2008). Nevertheless, Yang et al. (2013) have suggested that the loss phytochemicals could be reduced by minimizing the exposure to oxygen and light, as well as to select the appropriate temperature and pH during processing and storage of the food products.

The assessments of herbal quality are closely related to safety. Contamination can be defined as the undesired introduction of impurities of chemicals or foreign matters like heavy metals or microbiological nature into or onto a herbal products, at the production field, harvesting and postharvest handling including drying, packaging, storage and transport (WHO, 2007). Microbes are highly versatile and can enter the food chain at different steps, and adjust to the environment, then survive, growth and produce some toxic compounds in food (Havelaar et al., 2010).

Medicinal plants can be contaminated by enteric bacteria during growth in the farm and during harvest, pre-harvest and post-harvest activities as well as during transportation and further processing and handling (Havelaar et al., 2010). Ashiq et al. (2014) reported that various fungi contaminated medicinal plants during postharvest handling which could be held liable for damage and mycotoxin production. The contaminants found in medicinal herbs that caused a critical human health risk are pathogenic bacteria, such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Staphylococcus aureus* and other Gram positive and Gram negative strains of bacteria (Arias et al., 1999; Erich et al., 2001; Wolfgang et al., 2002; Adeleyea et al., 2003; Okunlola et al., 2007).

Many studies have shown that medicinal plants can accumulate heavy metals from contaminated soils and there is no exception with *A. paniculata*. Heavy metal uptake by *A. paniculata* is through metal transfer from sediments and water to food web (Mythili et al., 2011). All heavy metals can cause toxic effects to plants and, subsequently, to humans if consumed in high concentrations. There are three main mechanisms that need to be considered about heavy metal contaminations in herbal medicinal products: contamination during cultivation, cross contamination during processing and intentional introduction of heavy metals as a therapy (Rahimi et al., 2012).

Malaysia is well positioned to promote the growth and competitiveness of its herbal industry. Thus, all important factors related to maintenance of quality and safety of herbal products should be emphasized, especially in the area of postharvest handling practices, which is normally last to be considered. Until recently, the information on the effect of drying techniques and storage duration on the stability of phytochemicals of *A. paniculata* is still limited. A few reports of safety aspects have been studied for this plant (Mythili et al., 2011; Khandelwal

et al., 2013), yet did not cover heavy metals and microbial contamination during production or postharvest practices.

To fulfil the mission of improving the safety and stability of chemical markers of *A. paniculata*, it is pertinent to establish the scientific knowledge of its postharvest practices by focussing on drying techniques and storage. Therefore, the main objective of this study was to identify a suitable drying technique and storage duration so as to provide an optimum quality of *A. paniculata*, which ameliorate the stability of chemical markers and antioxidant properties.

The specific objectives were;

1. To identify heavy metals and microbial contaminants during production and drying of *A. paniculata*;
2. To determine the effect of drying techniques and storage durations on leaf colour and stability of three chemical markers (andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide);
3. To evaluate the effect of drying techniques and storage durations on total phenolic content, total flavonoid content and antioxidant properties of *A. paniculata*; and
4. To distinguish the metabolic variations in dried samples of *A. paniculata* and to discriminate the changes of the metabolite compositions of dried samples stored at different storage duration using ^1H -Nuclear Magnetic Resonance (^1H -NMR) based metabolomics coupled with multivariate data analysis (MVDA).

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