UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PLANT HARVEST AGE AND PLANT PARTS ON PHYTOCHEMICAL COMPOUNDS OF Andrographis paniculata (Burm. F.) Nees

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By

NOR ELLIZA BINTI TAJIDIN

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

January 2017

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DEDICATION

Dedicated to my beloved husband, parents, siblings, in laws, supervisors and fellow friends for their endless love, support, understandings, sacrifices, motivation, advice and encouragement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF PLANT HARVEST AGE AND PLANT PARTS ON PYTOCHEMICAL COMPOUNDS OF *Andrographis paniculata* (Burm. F.) Nees

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January 2017

Chairperson : Siti Hajar Ahmad, PhD
Faculty : Agriculture

Andrographis paniculata (Burm. F.) Nees (hempedu bumi) is harvested from the wild or cultivated area, causing variations in quality and consistency of the plant extracts and active compounds. Variations in its bioactive compounds, andrographolide (ANDRO), neoandrographolide (NAG), 14-deoxy-11, 12-didehydroandrographolide (DDAG), especially due to harvest age and plant parts used affect medicinal properties of the plant. The objective of this study was to determine optimum harvest age and suitable plant parts for quality and consistency of phytochemical compounds in *A. paniculata*. In Experiment 1, seedlings were raised in a nursery, field transplanted at 8 weeks after sowing (WAS), and harvested at 14, 16, 18, 20 and 22 WAS. Plant growth, phytochemical compounds, and heavy metal contaminations were determined. In Experiment 2, optimum harvest age of 19 WAS that produced highest bioactive compounds were selected for further phytochemical compound analysis in different plant parts (leaves, stems, and aerials) of young and mature development stages. In Experiment 3, verification in metabolites, bioactive compounds in young and mature leaves, and also young leaves harvested at 14 to 22 WAS were conducted using proton magnetic resonance (^1H NMR) combined with multivariate analysis. The maximum increment rate in height and number of leaves and branches were at 16 and 22 WAS, respectively. Specific leaf area decreased by 41% as plant harvest age increased. Maximum ANDRO, NAG, and DDAG contents were obtained at 19, 18.5 and 18 WAS, respectively. Plant fresh and dry weights did not correlate with bioactive compounds. Total phenolic content increased by 17.6% from 14 to 19.06 WAS (36 to 42.36 mg GAE/100 g DW) and decreased by 3% until 22 WAS. Total flavonoid content showed a cubic relationship ($r^2 = 0.97$) with harvest age. Antioxidant activities, except for reducing power, were highest at seed formation stage. The chlorophyll content started to increase by 47.7% from 14 (3.54 mg/g) to 20 (5.23 mg/g) WAS, remaining constant until seed formation. Heavy metal contents were below the permissible limits of WHO. In Experiment 2, the leaves had a highest extraction yield, followed by aerial parts and stems. ANDRO was 56.2% in young compared to mature plants. ANDRO of mature leaves (1.18 mg/g) and mature aerial parts (1.03 mg/g) were 87.1% and 62.7% higher, respectively, compared to mature stems (0.63 mg/g). The NAG content of mature stage was 2.9 times higher than young stage. Mature leaves have highest NAG compared to young aerial and young stems. DDAG in mature stems were lower than other plant parts. Total flavonoid content, hesperetin and DPPH free radical scavenging activity was highest at young stage while rutin, FRAP and reducing power were highest at a mature stage. No significant differences were recorded in contents of total phenolics, naringin, kaempferol, and glycosaponin, and scavenging activity of hydrogen peroxide in young and mature plants. Seven metabolites, ANDRO, NAG, DAG, glucose, sucrose, choline, and alanine, were identified. Principle component analysis (PCA) indicated clear discrimination between young and mature leaves. This discrimination was influenced by the presence of glucose, sucrose, NAG and choline in mature leaves compared to ANDRO and DAG in young leaves. Analysis using PC 1 and PC 2 of young leaves harvested from 14 to 22 WAS showed trajectory changes in metabolites. A loading plot confirmed differences in metabolic patterns of the groups. From regression analysis ANDRO, NAG and DAG were maximum at pre-flowering and early flowering stages while glucose and choline increased with harvest age. Thus, *A. paniculata* young leaves should be harvested at pre-flowering stage (19 WAS) to obtain maximum yield and phytochemical properties with minimal heavy metal contents.

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

KESAN UMUR TUAIAN TANAMAN DAN BAHAGIAN TUMBUHAN TERHADAP KOMPAUN FITOKIMIA BAGI *Andrographis paniculata* (Burm. F.) Nees

Oleh

NOR ELLIZA BINTI TAJIDIN

Januari 2017

Pengerusi : Siti Hajar Binti Ahmad, PhD
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Kualiti kandungan kimia di dalam ekstraks tumbuhan *Andrographis paniculata* (Burm. f.) Nees yang dituai dari kawasan hutan berbeza dengan yang ditanam. Faktor utama yang mempengaruhi variasi kandungan andrograpfolida (ANDRO), neoandrograpfolida (NAG), and 14-deoxy-11, 12-didehydroandrograpfolida (DDAG) adalah umur tanaman dan bahagian tanaman yang berbeza. Objektif utama kajian ini ialah untuk menentukan umur tuaian tanaman dan bahagian tanaman yang sesuai untuk memastikan kestabilan kualiti kompoun kimia di dalam *A. paniculata*. Bagi Experimen 1, anak pokok dibesarkan di dalam nurseri sehingga berusia 8 minggu selepas disemai (MSS), kemudian dipindahkan ke ladang seterusnya dituai pada 14, 16, 18, 20 and 22 MSS. Pertumbuhan pokok, kompoun fitokimia dan kandungan logam berat ditentukan pada setiap peringkat umur tanaman. Umur tanaman pada 19 MSS dipilih untuk Experimen 2, kerana mengandungi kompaun fitokimia yang tinggi. Tanaman dibahagikan kepada bahagian muda dan matang, setiap bahagian terdapat daun, batang dan aerial. Experimen 3, varisi diantara daun muda dan daun matang, serta daun muda yang dituai pada 14 hingga 22 MSS kemudiannya dianalisa menggunakan resonan proton magnetik (^{1}H NMR) dan analisis multivariat. Kadar ketinggian pokok adalah maksimum pada minggu ke 16, manakala bilangan daun dan batang maksimum pada 22 MSS. Ketinggian pokok berkorelasi dengan bilangan daun (r = 0.97) dan batang (r = 0.94). Keluasan spesifik daun menurun sebanyak 41% dengan peningkatan umur tuaian tanaman. Kandungan ANDRO, NAG dan DDAG berada pada tahap maksimum apabila tanaman dituai pada 19, 18.5 dan 18 MSS. Analisa korelasi juga menunjukkan bahawa berat basah dan kering tidak mempunyai hubungan yang signifikan dengan ANDRO, NAG dan DDAG. Kandungan fenolik meningkat sebanyak 17.6% apabila tanaman dituai dari minggu 14 hingga minggu 19.06 (36 dan 42.36 mg GAE/100 g DW), kemudiaannya kandungan fenolik menurun sebanyak 3% apabila tanaman dituai pada 22 MSS. Kandungan flavonoid menunjukkan hubungan kubik (r^2 = 0.97) dengan umur tuaian tanaman. Aktiviti antioksidan adalah ditahap maksimum pada peringkat pembentukan benih, kecuali

bagi kuasa penurunan. Dari minggu 14 kandungan klorofil (3.54 mg/g) meningkat sebanyak 47.7% dibandingkan dengan kandungan klorofil (5.23 mg/g) pada tanaman dituai minggu 20, kandungan klorofil berada pada tahap yang sama apabila penuaian dilakukan pada peringkat berbunga dan berbuah. Kesemua kandungan logam berat berada dibawah tahap yang dibenarkan oleh WHO. Untuk eksperimen kedua, hasil ekstrak daripada daun adalah tinggi diikuti oleh bahagian aerial dan batang. Kandungan ANDRO meningkat sebanyak 56.2% pada bahagian muda berbanding dengan bahagian matang. ANDRO pada bahagian daun (1.18 mg/g) dan aerial yang matang (1.03 mg/g) adalah meningkat sebanyak 87.1% dan 62.7% berbanding dengan bahagian batang matang (0.63 mg/g). NAG pada bahagian matang adalah 2.9 kali ganda lebih tinggi berbanding bahagian muda. DDAG pada batang matang adalah rendah berbanding bahagian tanaman yang lain. Jumlah kandungan flavonoid, hesperetin dan DPPH tinggi pada bahagian muda, manakala rutin, FRAP dan kuasa penurunan adalah maksimum apabila tumbuhan dituai pada peringkat pertumbuhan matang. Tiada perbezaan yang signifikan dicatatkan bagi kandungan fenolik, naringin, kaempferol, aktiviti pemerangkapan hidrogen peroksida dan glikosaponin pada peringkat pertumbuhan muda dan matang. Terdapat tujuh metabolit yang berjaya dikenalpasti iaitu ANDRO, NAG, DAG, glukosa, sukrosa, kholin and alanin. Prinsip analisis komponen (PCA) menunjukkan diskriminasi yang nyata diantara daun muda dan matang. Diskriminasi ini telah dipengaruhi oleh kandungan glukosa, sukrosa, NAG dan kholin yang terdapat pada daun matang. Sementara itu, daun muda pula didominasi oleh kandungan ANDRO dan DAG. Daun muda menunjukkan perubahan trajektori pada PC 1 dan PC 2 terhadap kandungan metabolit apabila dituai pada minggu ke 14 hingga minggu ke 22 MSS. Perbezaan pada corak kumpulan metabolit juga turut disahkan melalui plot loading. Analisis regrasi menunjukkan kandungan ANDRO, NAG dan DAG adalah optimum pada peringkat pra-berbunga dan berbunga manakala kandungan glukosa dan kholin meningkat seiring dengan umur tuaian. Oleh yang demikian, daun muda hendaklah dituai pada peringkat pra-pembungaan (19 MSS) bagi mendapatkan kadar pertumbuhan, hasil tanaman dan fitokimia yang tinggi serta kandungan logam berat yang minimum.

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I certify that a Thesis Examination Committee has met on 17 January 2017 to conduct the final examination of Nor Elliza binti Tajidin on her thesis entitled "Effects of Plant Harvest Age and Plant Parts on Phytochemical Compounds of *Andrographis paniculata* (Burm.F.) 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree Doctor of Philosophy. The members of Supervisory Committee were as follows:

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µm	Micrometer
°C	Degree Celsius
g	Gram
mL	Millilitre
h	Hour
mg	Milligram
min	Minute
%	Percentage
mm	Millimetre
RP-HPLC	Reversed phase - high performance liquid chromatography
hPa	Hectopascals
µL	Microliter
rpm	Revolutions per minute
N	Normality
ppm	Parts per million
nm	Nanometer
w/v	Weight per volume
mM	Millimolar
M	Molarity
µg/mL	Microgram per millilitre
µg/g	Microgram per gram
w/w	Weight per weight
PP	Plant part
PS	Plant stage
SE	Standard error

DW	Dry weight
mg/g	Milligram per gram
ANDRO	Andrographolide
NAG	Neoandrographolide
DDAG	14-deoxy-11, 12-didehydroandrographolide
DAG	14-deoxyandrographolide
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
H_2O_2	Hydrogen peroxide
ROS	Reactive oxygen species
^1H NMR	Proton nuclear magnetic resonance
PCA	Principle component analysis

CHAPTER 1

INTRODUCTION

The use of herbal plants as alternative medicines has tremendously increased in the past decade and become a public interest. It is estimated that nearly four billion people around the world rely on herbal products as alternatives to modern medicines for primary source of healthcare (Ekor, 2013). The annual worldwide market income generated from this herbal industry is approximately US$60 billion (Wachtel-Galor and Benzie, 2011; Tilburt and Kaptchuk, 2008). There are several factors that influence public interest in herbal remedies: (i) efficiency or effectiveness of medicinal herbs, (ii) preference of consumers for natural remedies (iii) low cost and minimal side effect compared with modern medicines (iv) easily available and (v) for treating self-diagnosed disorders or symptoms (Ekor, 2013; Bandaranayake, 2006).

There are 18 herbal plants that have been selected under the National Key Economic Area (NKEA) research grant schemes, and one of them is the *Andrographis paniculata* (Burm. f.) Nees. (MOA, 2012). *A. paniculata* is an herbaceous plant that belongs to the Acanthaceae family (Mishra et al., 2014), widely found and cultivated in tropical and subtropical Asia and India (Benoy et al., 2012). *A. paniculata* has a wide range of medicinal and pharmacological applications. Traditionally, *A. paniculata* extract from the aerial parts are used to get rid of body heat, dispel toxins from the body, prevent common cold and sinusitis (Joselin and Jeeva, 2014), fever, and as well as an antidote against snake and insect poisons (Samy et al., 2008). Most of the *A. paniculata* products available in the market were highly consumed as stomachic, hepatoprotective, dyspepsia, anthelmintic and bitter tonic (Kataky and Handique, 2010).

According to Valdiani et al. (2012), the price of superior quality dried *A. paniculata* leaves were US$5 per kg, while the purified bioactive compounds such as andrographolide (ANDRO) and its derivatives, could be sold for US$100,000.00/kg (Valdiani et al. 2012). The consumption of aerial *A. paniculata* in India is around 250 tonnes/year (Pholphana et al., 2013; Sharma et al., 2008). There is a large market demand for raw plant materials and extracts of *A. paniculata*. The production of *A. paniculata* in Malaysia is only 1 tonne/year (DOA, 2014) and Malaysia is importing 70% of herbal products, including *A. paniculata*, to meet the local demand (Mohd Noor and Sukir, 2000). Conversely, the production of wild growing *A. paniculata* in India is estimated to be 5000 tonnes/year (Kumar and Kumar, 2013).

Several researches on *A. paniculata*, that focused on the reproductive systems and genetic variations (Chia, 2009; Abdalla, 2005; Biffa, 2003), crossability and genetic analysis (Valdiani, 2012), and salt stress (Talei, 2012), have been conducted in Malaysia. According to Biffa (2003), about 26 accessions of *A. paniculata* have been collected around the Peninsular of Malaysia from April to June, 2002. Among the 26 accessions, only five accessions (11179S, 11269P, 11265P, 11261P and 11284P) had been shortlisted as promising (Abdalla, 2005; Biffa, 2003). Accessions 11265P was selected and named as 'Harapan' due to the beneficial morphological traits (Biffa, 2003) and phytochemical constituents (Abdalla, 2005) with the potential to be commercialized in Malaysia.

The selection of a potential plant accession, variety or cultivar has been based on growth performance, yield and quality of the plant material. However, environmental effects, pest infestation, diseases, weeds (Chanli, 2012), and agronomic practices, especially plant harvest age and plant parts used (Chen et al., 2012) need to be studied. Plant harvest age and plant parts are important factors that influence the phytochemical properties of a herbal plant (Raya et al., 2015; Couto et al., 2013). Since *A. paniculata* is categorized under an angiosperm plant, most of the studies have stated that changes in phenological stage affect the growth characteristics and phytochemical contents of the plants (Ahl and Sabra, 2016; Ben Farhat et al., 2015; Chauhan et al., 2013; Chen et al., 2012; Chouaieb et al., 2012). According to Fenner (1998), the phenology of growth and reproduction in plants are influenced and closely related to the abiotic and biotic factors. Several recommendations, mostly from India, China and Thailand, where the geography, climate and weather condition are different from Malaysia, have been reported on the suitable harvesting age or stage of *A. paniculata*. (Pholphana et al., 2013; Kumar and Kumar, 2013; Parashar et al., 2011; Singh et al., 2011; Pandey and Mandal, 2010). There is a lack of information on the optimum plant harvest age of Harapan (11265P).

Plant parts are a major factor that influences the phytochemical properties of a plant extract (Couto et al., 2013; Pholphana et al., 2013; Gajbhiye and Khristi, 2010; Zhoa et al., 2007). A study conducted on *Clinacanthus nutans* reported that plant parts and vegetative stage did influence the bioactive compounds of the plant (Raya et al., 2015). Panawala et al. (2016) also claimed that plant parts and development stage also affected the overall quantity of phytochemical properties of *Pimenta dioica* (L.) Merr. The aerial plant parts (leaves and stems) of *A. paniculata* are normally used for the extraction of bioactive compounds and phytochemicals, and each compound possessed different potency in pharmacological activities (Thisoda et al., 2006). Normally, farmers harvest the whole aerial parts in order to obtain a higher biomass yield, which is dominated by the weight of the stems. Since the contribution of stems to the final herbage is substantial and amount of bioactive compounds present in stems could change the final concentration of ANDRO, NAG and DDAG, it becomes vital to study the distribution of this bioactive compound in the different plant parts in order to control the quality of the final crude extract.

Nowadays, metabolomic is gaining much attention among scientists since it is a holistic, non-biased method and could provide comprehensive, qualitative and quantitative overview of the metabolites present in the plant (Shuib et al. 2011). By using ^{1}H NMR and multivariate analysis, the results obtained could provide information about metabolic fingerprints, detect chemical variations and provide a possible explanation of discrimination between the metabolomes in the plant extracts (Mediani et al., 2015; Khoo et al., 2015). However, there is no information available on the variation in the metabolic discrimination of young and mature plant parts of *A.*

paniculata at different harvest age using advanced tools, such as the ^{1}H NMR and multivariate analysis.

Since Harapan is a recommended accession to be commercialized in Malaysia, therefore studies on plant harvest age and plant parts should be carried out in order to achieve optimum growth and high phytochemical contents for production of superior quality plant extract that could meet international quality standards. Thus, the main objective of this study was to determine optimum plant harvest age and suitable plant parts for superior quality and consistency of phytochemical compounds in *A. paniculata*. The specific objectives were as follows:

1. To determine the effects of plant harvest age on growth characteristics, phytochemical compounds, and heavy metal contents of *A. paniculata*.
2. To compare the phytochemical properties of different plant parts of *A. paniculata* when harvested at young and mature development stages.
3. To discriminate young versus mature leaves and explore the changes in the metabolites profile of young leaves harvested at different harvest age/stage.

REFERENCES

Abdalla, J. A. 2005. Genetic Variation and Anticancer Activity of *Andrographis paniculata* Germplasm from Malaysia, Master Thesis, Universiti Putra Malaysia, Malaysia.

Abu-Darwish, M.S., Al-Fraihat, A.H., Al-Dalain, S.Y., Afifi, F.U. and Al-Tabbal, J.A. 2011. Determination of Essential Oils and Heavy Metals Accumulation in *Salvia officinalis* Cultivated in Three Intra-raw Spacing in Ash-Shoubak, Jordan. *International Journal of Agriculture and Biology* 13 (6): 981–985.

Ahl, H.A.H.S. and Ali S.S. 2016. Growth, Herb and Essential Oil of *Marrubium vulgare* as Affected by Phenological Stages and Planting Dates. *Journal of Chemical and Pharmaceutical Research* 8 (5): 863–72.

Ainsworth, E.A. and Bush, D.R. 2011. Carbohydrate Export from the Leaf: A Highly Regulated Process and Target to Enhance Photosynthesis and Productivity. *Plant Physiology* 155 (1): 64–69.

Akbarirad, H., Gohari Ardabili, A., Kazemeini, S. M. and Mousavi Khaneghah, A. 2016. An Overview on Some of Important Sources of Natural Antioxidants. *International Food Research Journal* 23 (3): 928–33.

Akinyele, I.O. and Shokunbi, O.S. 2015. Comparative Analysis of Dry Ashing and Wet Digestion Methods for the Determination of Trace and Heavy Metals in Food Samples. *Food Chemistry* 173: 682-684.

Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. 2013. Review on in Vivo and in Vitro Methods Evaluation of Antioxidant Activity. *Saudi Pharmaceutical Journal* 21 (2): 143–152.

Alimpić, A., Mariana, O., Matevski, V., Marin, P.D. and Sonja, D.L. 2014. Antioxidant Activity and Total Phenolic and Flavonoid Contents of *Salvia amplexicaulis* Lam. Extracts. *Archives of Biological Sciences* 66 (1): 307–316.

Amoghein, R.S., Tobeh, A. and Jamaati-e-Somarin, S. 2012. Effect of Plant Density on Phenology and Oil Yield of Safflower Herb under Irrigated and Rainfed Planting Systems. *Journal of Medicinal Plants Research* 6 (12): 2493–2503.

Anju, D., Jugnu, G., Kavita, S., Arun, N. and Sandeep, D. 2012. A Review on Medicinal Prospectives of *A. paniculata* Nees. *Journal of Pharmaceutical and Scientific Innovation* 1 (1): 1–4.

Anonymous. 2005. Cereal Growth Stages-The Link to Cereal Management [Electronic version]. Barton ACT, Australia: Grains Research and Development Corporation.

Ariffin, Z., Mamat, W. Z. W., Hussain, Y. and Yaacob. M. 2006. Manual Teknologi Penanaman Hempedu Bumi, Serdang: Mardi.

Ashok, K., Amit, A., Sujatha, M., Murali, B. and Anand, M.S. 2002. Effect of Aging on Andrographolide Content in *A. paniculata*. *Journal of Natural Remedi Es* 2 (2): 179–81.

Awang, Y., Shaharom, A.S., Mohamad, R.B. and Selamat, A. 2010. Growth Dynamics of *Celosia cristata* Grown in Cocopeat, Burnt Rice Hull and Kenaf Core Fiber Mixtures. *American Journal of Agricultural and Biological Sciences* 5 (1): 70–76.

Bandaranayake, W. M. 2006. Quality Control, Screening, Toxicity and Regulation of Herbal Drugs. In *Modern Phytomedicine: Turning Medicinal Plants into Drugs*, ed. I. Ahmad, F. Aqil, and M. Owais, pp. 25–53. KGaA, Weinheim, Germany: Wiley-VCH Verlag GmbH and Co.

Bandaranayake, W.M. 2002. Bioactivities, Bioactive Compounds and Chemical Constituents of Mangrove Plants. *Wetlands Ecology and Management* 10 (6): 421–452.

Barceló, A.R. 1998. Hydrogen Peroxide Production is a General Property of the Lignifying Xylem from Vascular Plants. *Annals of Botany* 82 (1): 97–103.

Barter, L.M. and Klug, D.R. 2005. A Unified Picture of Energy and Electron Transfer in Primary Photosynthesis. *Chemical Physics* 319 (1): 308–315.

Ben Farhat, M.B., Chaouch-Hamada, R., Sotomayor, J.A., Landoulsi, A. and Jordán, M.J. 2015. Antioxidant Properties and Evaluation of Phytochemical Composition of *Salvia verbenaca* L. Extracts at Different Developmental Stages. *Plant Foods for Human Nutrition* 70 (1): 15–20.

Bennett, T. and Leyser, O. 2006. Something on the Side: Axillary Meristems and Plant Development. *Plant Molecular Biology* 60(6): 843–854.

Benoy, G.K., Animesh, D.K., Aninda, M., Priyanka, D.K. and Sandip, H. 2012. An Overview on *A. paniculata* (Burm. f.) Nees. *International Journal of Research in Ayurveda and Pharmacy* 3 (6): 752–60.

Bhan, M.K., Dhar, A.K., Khan, S., Lattoo, S.K., Gupta, K.K. and Choudhary, D.K. 2006. Screening and Optimization of *A. paniculata* (Burm. f.) Nees. for Total Andrographolide Content, Yield and its Components. *Scientia Horticulturae* 107 (4): 386–391.

Biesiada, A. and Tomczak, A. 2012. Biotic and Abiotic Factors Affecting the Content of the Chosen Antioxidant Compounds in Vegetables. *Vegetable Crops Research Bulletin* 76: 55–78.

Biffa, M. A. 2003. Conservation and Diversity of Hampedu Bumi (*Andrographis paniculata* Nees) Germplasm in Malaysia, Master Thesis, Universiti Putra Malaysia, Malaysia.

Bingol, K., Bruschweiler-Li, L., Da-Wei, L. and Brüschweiler, R. 2014. Customized Metabolomics Database for the Analysis of NMR $^{1}H–^{1}H$ TOCSY and $^{13}C–^{1}H$

HSQC-TOCSY Spectra of Complex Mixtures. *Analytical Chemistry* 86 (11): 5494–5501.

Bixenmann, R.J., Coley, P.D. and Kursar, T.A. 2013. Developmental Changes in Direct and Indirect Defenses in the Young Leaves of the Neotropical Tree Genus *Inga* (Fabaceae). *Biotropica* 45 (2): 175–184.

Borzak, C.L., Potts, B.M., Davies, N.W. and O'Reilly-Wapstra, J.M. 2014. Population Divergence in the Ontogenetic Trajectories of Foliar Terpenes of a *Eucalyptus* Species. *Annals of Botany* 115(1):159-170.

Bouayed, J. and Bohn. T. 2010. Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. *Oxidative Medicine and Cellular Longevity* 3 (4): 228–237.

Brenes-Arguedas, T., Horton, M.W., Coley, P.D., Lokvam, J., Waddell, R.A., Meizoso-O'Meara, B.E. and Kursar, T.A. 2006. Contrasting Mechanisms of Secondary Metabolite Accumulation during Leaf Development in Two Tropical Tree Species with Different Leaf Expansion Strategies. *Oecologia* 149 (1): 91–100.

Bystrická, J., Vollmannová, A., Margitanová, E. and Čičová, I. 2010. Dynamics of Polyphenolics Formation in Different Plant Parts and Different Growth Phases of Selected Buckwheat Cultivars. *Acta Agriculturae Slovenica* 95 (3): 225-229.

Cai, Y., Luo, Q., Sun, M. and Corke, H. 2004. Antioxidant Activity and Phenolic Compounds of 112 Traditional Chinese Medicinal Plants Associated with Anticancer. *Life Sciences* 74 (17): 2157–2184.

Calabrese, C., Berman, S.H., Babish, J.G., Ma, X., Shinto, L., Dorr, M., Wells, K., Wenner, C.A. and Standish, L.J. 2000. A Phase I Trial of Andrographolide in HIV Positive Patients and Normal Volunteers. *Phytotherapy Research* 14 (5): 333–338.

Campos-Vega, R. and Oomah, B.D. 2013. Chemistry and Classification of Phytochemicals. In *Handbook of Plant Food Phytochemicals*, ed. B.K. Tiwari, N. P. Brunton and C. S. Brennan, pp. 5–48. John Wiley and Sons Ltd: Oxford.

Chanli, H.U. 2012. Factors Affecting Phytochemical Composition and Antioxidant Activity of Ontario Vegetable Crops, Master Thesis. The University of Guelph, Ontario, Canada.

Chao, W.W. and Lin, B.F. 2012. Hepatoprotective Diterpenoids Isolated from *A. paniculata*. *Chinese Medicine* 3 (3): 136–143.

Chauhan, N.K., Singh, S., Haider, S.Z. and Lohani, H. 2013. Influence of Phenological Stages on Yield and Quality of Oregano (*Origanum vulgare* L.) Under the Agroclimatic Condition of Doon Valley (Uttarakhand). *Indian Journal of Pharmaceutical Sciences* 75 (4): 489–493.

Chear, N.J.Y, Khaw, K.Y., Murugaiyah, V. and Lai, C.S. 2016. Cholinesterase Inhibitory Activity and Chemical Constituents of *Stenochlaena palustris* Fronds at Two Different Stages of Maturity. *Journal of Food and Drug Analysis* 24 (2): 358–366.

Chen, Y., Zhu, Z., Guo, Q., Zhang, L. and Zhang, X. 2012. Variation in Concentrations of Major Bioactive Compounds in *Prunella vulgaris* L. Related to Plant Parts and Phenological Stages. *Biological Research* 45 (2): 171–175.

Cheung, H.Y., Cheung, C.S. and Kong, C.K. 2001. Determination of Bioactive Diterpenoids from *A. paniculata* by Micellar Electrokinetic Chromatography. *Journal of Chromatography A* 930 (1): 171-176.

Chia, S. H. 2009. Reproductive System and Genetic Diversity of Hempedu Bumi (*A. paniculata*) Germplasm in Peninsular Malaysia, Master Thesis, Universiti Putra Malaysia, Malaysia.

Chouaieb, H., Ayadi, I., Zouari, S., Fakhfakh, N., Zaidi, S. and Zouari, N. 2012. Effect of Phenological Stage and Geographical Location on Antioxidant Activities of Tunisian Horehound: *Marrubium vulgare* L. (Lamiaceae). *Journal of Biologically Active Products from Nature* 2 (4): 232–38.

Chua, L.S., Yap, K.C. and Jaganath, I.B. 2013. Comparison of Total Phenolic Content, Scavenging Activity and HPLC-ESI-MS/MS Profiles of Both Young and Mature Leaves and Stems of *A. paniculata*. *Natural Product Communications* 8 (12): 1725–1729.

Çirak, C., Radusiene, J., Ivanauskas, L., Jakstas, V. and Çamaş, N. 2014. Phenological Changes in the Chemical Content of Wild and Greenhouse-Grown *Hypericum pruinatum*: Flavonoids. *Turkish Journal of Agriculture and Forestry* 38 (3): 362–370.

Coşge Şenkal, B, Kiralan, M. and Yaman, C. 2014. The Effect of Different Harvest Stages on Chemical Composition and Antioxidant Capacity of Essential Oil from *Artemisia annua* L. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* 21: 71–77.

Couto, A.G., Kunzler, M.L., Spaniol, B., Magalhães, P.M., Ortega, G.G. and Petrovick, P.R. 2013. Chemical and Technological Evaluation of the *Phyllanthus niruri* Aerial Parts as a Function of Cultivation and Harvesting Conditions. *Brazilian Journal of Pharmacognosy* 23 (1): 36–43.

Croteau, R., Kutchan, T.M. and Lewis, N.G. 2000. Natural Products (Secondary Metabolites).” In *Biochemistry Molecular Biology of Plants*, ed. B. Buchanan, W. Gruissem, and R. Jones, pp. 1250–1318. Rockville, Maryland, USA: American Society of Plant Biologists, John Wiley and Son.

Dai, J. and. Mumper, R.J. 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules* 15 (10): 7313–52.

Dall’Acqua, S., Cervellati, R., Loi, M.C. and Innocenti, G. 2008. Evaluation of in Vitro Antioxidant Properties of Some Traditional Sardinian Medicinal Plants:

Investigation of the High Antioxidant Capacity of *Rubus umifolius*. *Food Chemistry* 106(2) :745-749.

de Costa, F., Yendo, A.C.A., Fleck, J.D., Gosmann, G. and Fett-Neto, A.G. 2013. Accumulation of a Bioactive Triterpene Saponin Fraction of *Quillaja brasiliensis* Leaves is Associated with Abiotic and Biotic Stresses. *Plant Physiology and Biochemistry* 66: 56–62.

Digel, B., Tavakol, E., Verderio, G., Tondelli, A., Xu, X., Cattivelli, L., Rossini, L. and von Korff, M. 2016. Photoperiod-H1 (Ppd-H1) Controls Leaf Size. *Plant Physiology* 172 (1): 405–15.

de León, P.I. and Montesano, M. 2013. Activation of Defense Mechanisms against Pathogens in Mosses and Flowering Plants. *International Journal of Molecular Sciences* 14 (2): 3178–3200.

Deveci, M., and Uzun, E. 2011. Determination of Phenolic Compounds and Chlorophyll Content of Spinach (*Spinacia oleracea* L.) at Different Growth Stages. *Asian Journal of Chemistry* 23 (8): 3739–3743.

Dghaim, R., Al Khatib, S., Rasool, H. and Ali Khan, M. 2015. Determination of Heavy Metals Concentration in Traditional Herbs Commonly Consumed in the United Arab Emirates. *Journal of Environmental and Public Health* 2015: 1–6.

DOA. 2014. *Herbs and Spices Statistic Malaysia* [Electronic version], pp. 5-6 Putrajaya: Department of Agriculture.

Doughari, J.H., Human, I.S., Bennade, S. and Ndakidemi, P.A. 2009. Phytochemicals as Chemotherapeutic Agents and Antioxidants: Possible Solution to the Control of Antibiotic Resistant Verocytotoxin Producing Bacteria. *Journal of Medicinal Plants Research* 3 (11): 839–848.

Doughari, J.H. 2012. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In *A Global Perspective of Their Role in Nutrition and Health*, ed. R. Venketeshwer, pp.1–32. INTECH Open Access Publisher.

Dragana Jakovljević, Z., Milan Stanković, S. and Marina Topuzović, D. 2013. Seasonal Variability of *Chelidonium majus* L. Secondary Metabolites Content and Antioxidant Activity. *EXCLI Journal* 12: 260–268.

Duda, S.C., Mărghitaş, L.A., Dezmirean, D., Duda, M., Mărgăoan, R. and Bobiş, O. 2015. Changes in Major Bioactive Compounds with Antioxidant Activity of *Agastache foeniculum*, *Lavandula angustifolia*, *Melissa officinalis* and *Nepeta cataria*: Effect of Harvest Time and Plant Species. *Industrial Crops and Products* 77: 499–507.

Dunn, W.B. and Ellis, D.I. 2005. Metabolomics: Current Analytical Platforms and Methodologies. *Trends in Analytical Chemistry* 24 (4): 285–294.

Ekor, M. 2013. The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. *Frontiers in Pharmacology* 4 (177): 1-10.

El Senousy, A.S., Farag, M.A., Al-Mahdy, D.A. and Wessjohann, L.A. 2014. Developmental Changes in Leaf Phenolics Composition from Three Artichoke cvs. (*Cynara scolymus*) as Determined via UHPLC–MS and Chemometrics. *Phytochemistry* 108: 67–76.

Elger, A., Lemoine, D.G., Fenner, M. and Hanley, M.E. 2009. Plant Ontogeny and Chemical Defence: Older Seedlings Are Better Defended. *Oikos* 118 (5): 767–773.

Ellinger, J.J., Chylla, R.A., Ulrich, E.L. and Markley, J.L. 2013. Databases and Software for NMR-Based Metabolomics. *Current Metabolomics* 1 (1): 1-22.

Embuscado, M.E. 2015. Spices and Herbs: Natural Sources of Antioxidants - A Mini Review. *Journal of Functional Foods* 18: 811-819.

England, J.R. and Attiwill, P.M. 2005. Changes in Leaf Morphology and Anatomy with Tree Age and Height in the Broadleaved Evergreen Species, *Eucalyptus regnans* F. Muell. *Trees* 20 (1): 79–90.

Ezeabara, C.A., Okeke, C.U., Aziagba, B.O., Ilodibia, C.V. and Emeka, A.N. 2014. Determination of Saponin Content of Various Parts of Six Citrus Species. *International Research Journal of Pure and Applied Chemistry* 4 (1): 137–143.

Falster, D.S. and Westoby, M. 2003. Leaf Size and Angle Vary Widely across Species: What Consequences for Light Interception?. *New Phytologist* 158 (3): 509–525.

Farias, K.S., Santos, T.S.N., Paiva, M.R.A.B., Almeida, S.M.L., Guedes, P.T., Vianna, A.C.A., Favaro, S.P., Bueno, N.R. and Castilho, R.O. 2013. Antioxidant Properties of Species from the Brazilian Cerrado by Different Assays. *Revista Brasileira de Plantas Medicinais* 15 (4): 520–528.

Fasola, T.R., Ayodele, A.E., Odetola, A.A. and Umotok, N.E. 2010. Foliar Epidermal Morphology and Anti-Diabetic Property of *A. paniculata* (Burm. F.) Wall Ex. Nees. *Ethnobotanical Leaflets* 14: 593–598.

Fenner, M. 1998. The Phenology of Growth and Reproduction in Plants. *Perspectives in Plant Ecology, Evolution and Systematics* 1 (1): 78–91.

Fenwick, G.R., Price, K.R., Okubo, K. and Tsukamoto. C. 1991. Saponins. In *Saponins in Toxic Substances in Crop Plants*, ed. F.J.P.D. Mello, C.M. Duffus, and J.H. Duffus, pp. 285–327. Cambridge: The Royal Society of Chemistry.

Fernando, I.D.N.S., Abeysinghe, D.C. and Dharmadasa, R.M. 2013. Determination of Phenolic Contents and Antioxidant Capacity of Different Parts of *Withania somnifera* (L.) Dunal. from Three Different Growth Stages. In *Proceedings of 12th Agricultural Research Symposium*, 160–164.

Gajbhiye, N.A. and Khristi, S. 2010. Distribution Pattern of Andrographolide and Total Lactones in Different Parts of Kalmegh Plant. *Indian Journal of Horticulture* 67: 591–593.

Gao, Z., Huang, K., Yang, X. and Xu, H. 1999. Free Radical Scavenging and Antioxidant Activities of Flavonoids Extracted from the Radix of *Scutellaria baicalensis* Georgi. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1472 (3): 643–650.

Gaur, A. and Adholeya, A. 2004. Prospects of Arbuscular Mycorrhizal Fungi in Phytoremediation of Heavy Metal Contaminated Soils. *Current Science* 86 (4): 528–534.

Ghasemzadeh, A., and Ghasemzadeh, N. 2011. Flavonoids and Phenolic Acids: Role and Biochemical Activity in Plants and Human. *Journal of Medicinal Plants Research* 5 (31): 6697–6703.

Ghasemzadeh, A., Jaafar, H.Z.E., Ashkani, S., Rahmat, A., Juraimi, A. S., Puteh, A. and Mohamed, M.T.M. 2016. Variation in Secondary Metabolite Production as well as Antioxidant and Antibacterial Activities of *Zingiber zerumbet* (L.) at Different Stages of Growth. *BMC Complementary and Alternative Medicine* 16 (1): 1–10

Ghasemzadeh, A., Nasiri, A., Jaafar, H.Z.E., Baghdadi, A. and Ahmad, I. 2014. Changes in Phytochemical Synthesis, Chalcone Synthase Activity and Pharmaceutical Qualities of Sabah Snake Grass (*Clinacanthus nutans* L.) in Relation to Plant Age. *Molecules* 19 (11): 17632–17648.

Gholivand, M. B. and Piryaei, M. 2012. The Antioxidant Activity, Total Phenolics and Total Flavonoids Content of *Bryonia dioica* Jacq. *Biologija* 58 (3): 99–105.

Gill, S.S. and Tuteja, N. 2010. Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. *Plant Physiology and Biochemistry* 48 (12): 909–930.

Goss, J.A. and Gray, P.P. 2013. *Physiology of Plants and Their* Cells [Electronic version], pp. 48-66. Pergamon Biological Sciences Series.

Gülçin, İ. 2006. Antioxidant Activity of Caffeic Acid (3,4-Dihydroxycinnamic Acid). *Toxicology* 217 (2): 213–220.

Haarry-Asobara, J.L., and Okon, E. 2014. Comparative Study of the Phytochemical Properties of *Jatropha curcas* and *Azadirachta indica* Plant Extracts. *Journal of Poisonous and Medicinal Plants Research* 2 (2): 20–24.

Hall, R., Beale, M., Fiehn, O., Hardy, N., Sumner, L. and Bino, R. 2002. Plant Metabolomics: The Missing Link in Functional Genomics Strategies. *The Plant Cell* 14 (7): 1437–40.

Hazrati, S., Sarvestani, Z.T. and Ramezani, S. 2011. Effect of Different Harvest Dates on Growth Characteristics and Aloin Content of *Aloe barbadensis* Miller. *Advances in Environmental Biology* 5 (2): 439–442.

Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. 2002. Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationships. *The Journal of Nutritional Biochemistry* 13 (10): 572–584.

Heyman, H.M, and Meyer, J.J.M. 2012. NMR-Based Metabolomics as a Quality Control Tool for Herbal Products. *South African Journal of Botany* 82: 21–32.

Hörtensteiner, S. and Kräutler, B. 2011. Chlorophyll Breakdown in Higher Plants. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1807 (8): 977–988.

Hossain, M.S., Urbi, Z., Sule, A.B. and Rahman, H.K.M. 2014. *A. paniculata* (Burm. f.) Wall. Ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. *Scientific World Journal* 2014:1-28.

Hue, S.M., Boyce, A.N. and Somasundram, C. 2011. Influence of Growth Stage and Variety on the Pigment Levels in *Ipomoea batatas* (Sweet Potato) Leaves. *African Journal of Agricultural Research* 6 (10): 2379–2385.

Huijser, P. and Schmid, M. 2011. The Control of Developmental Phase Transitions in Plants. *Development* 138 (19): 4117–4129.

Hüner, N., Dahal, K., Kurepin, L.V., Savitch, L., Singh, J., Ivanov, A.G., Kane, K. and Sarhan, F. 2014. Potential for Increased Photosynthetic Performance and Crop Productivity in Response to Climate Change: Role of CBFs and Gibberellic Acid. *Frontiers in Chemistry* 2 (18): 1-14.

Hunt, R., Causton, D.R., Shipley, B. and Askew, A.P. 2002. A Modern Tool for Classical Plant Growth Analysis. *Annals of Botany* 90 (4): 485–488.

Hunt, R., Thomas, B., Murphy, D.J. and Murray, D. 2003. Growth Analysis, Individual Plants. *Encyclopedia of Applied Plant Sciences* 2: 579–588.

Ignat, I., Volf, I. and Popa, V.I. 2011. A Critical Review of Methods for Characterisation of Polyphenolic Compounds in Fruits and Vegetables. *Food Chemistry* 126 (4): 1821–1835.

Irshad, M., Zafaryab, M., Singh, M. and Rizvi, M.M. 2012. Comparative Analysis of the Antioxidant Activity of *Cassia fistula* Extracts. *International Journal of Medicinal Chemistry* 2012:1-6.

Isayenkov, S.V. 2014. Plant Vacuoles: Physiological Roles and Mechanisms of Vacuolar Sorting and Vesicular Trafficking. *Cytology and Genetics* 48 (2): 127–137.

Isha, A., Yusof, N.A. and Ismail, I.S. 2016. Metabolomics Profilling of *A. paniculata* Leaf Extracts with Anti-Inflammatory Activity by ^{1}H NMR Spectroscopy. In *Proceedings of the Fundamental Science: Basis for Technology Advancement, Serdang, Malaysia, August 9-10, 2016, UPM: Serdang.*

Islam, M., Saha, S., Akand, H. and Rahim, A. 2011. Effect of Spacing on The Growth And Yield of Sweet Pepper (*Capsicum annuum* L.). *Journal of Central European Agriculture* 12 (2): 328–335.

Ivanov, A.G., Ignatova, N.S. and Christov, A.M. 1990. Comparative Ultrastructural and Fluorescence Studies of Grapevine (*Vitis vinifera* L.) Chloroplasts Isolated from Stem and Leaf Tissues. *Plant Science* 67: 253–257.

Jaafar, H.Z.E., Karimi, E., Ibrahim, M.H. and Ghasemzadeh, A. 2013. Phytochemical Screening and Antioxidant Activity Assessment of the Leaf Stem and Root of (*Labisia paucifolia*). *Australian Journal of Crop Science* 7 (2): 276–280.

Jarukamjorn, K. and Nemoto, N. 2008. Pharmacological Aspects of *A. paniculata* on Health and its Major Diterpenoid Constituent Andrographolide. *Journal of Health Science* 54 (4): 370–381.

Jide-Ojo, C. 2013. Extracts of *Jatropha curcas* L. Exhibit Significant Insecticidal and Grain Protectant Effects against Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Stored Products and Postharvest Research* 4 (3): 44–50.

Johnson, I.T. 2013. Phytochemicals and Health. In *Handbook of Plant Food Phytochemicals*, ed. B.K. Tiwari, B.P. Nigel, and B.S. Charles, pp. 49–67. Oxford: John Wiley and Sons Ltd.

Joselin, J. and Jeeva, S. 2014. Medicinal and Aromatic Plants *A. paniculata* : A Review of Its Traditional Uses, Phytochemistry and Pharmacology. *Medicinal and Aromatic Plants* 3(4): 169-184.

Jung, M.C. 2008. Heavy Metal Concentrations in Soils and Factors Affecting Metal Uptake by Plants in the Vicinity of a Korean Cu-W Mine. *Sensors* 8 (4): 2413–2423.

Kabera, J.N., Semana, E., Mussa, A.R. and He, X. 2014. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. *Journal of Pharmacy and Pharmacology* 2: 377–392.

Kamdem, R.E., Sang, S. and Ho, C. 2002. Mechanism of the Superoxide Scavenging Activity of Neoandrographolide−A Natural Product from *A. paniculata* Nees. *Journal of Agricultural and Food Chemistry* 50 (16): 4662–4665.

Kancheva, R., Borisova, D. and Georgiev, G. 2014. Chlorophyll Assessment and Stress Detection From Vegetation Optical Properties. *Ecological Engineering and Environment Protection* 1: 34–43.

Karavin, N. 2013. Effects of Leaf and Plant Age on Specific Leaf Area in Deciduous Tree Species *Quercus cerris* L. Var. Cerris. *Bangladesh Journal of Botany* 42 (2): 301–306.

Karimi, E., Jaafar, H.Z.E and Ahmad, S. 2011. Phytochemical Analysis and Antimicrobial Activities of Methanolic Extracts of Leaf, Stem and Root from

Different Varieties of *Labisa pumila* Benth. *Molecules* 16 (6): 4438–4450.

Kasote, D.M., Katyare, S.S., Hegde, M.V. and Bae, H. 2015. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. *International Journal of Biological Sciences* 11 (8): 982–991.

Kataky, A. and Handique, P.J. 2010. A Brief Overview on *A. paniculata* (Burm. f.) Nees., a High Valued Medicinal Plant: Boon over Synthetic Drugs. *Asian Journal of Science and Technology* 6: 113–118.

Khoo, L.W., Mediani, A., Zolkeflee, N.K.Z., Leong, S.W., Ismail, I.S., Khatib, A., Shaari, K. and Abas, F. 2015. Phytochemical Diversity of *Clinacanthus nutans* Extracts and Their Bioactivity Correlations Elucidated by NMR Based Metabolomics. *Phytochemistry Letters* 14: 123–133.

Khorasani Esmaeili, A., Mat Taha, R., Mohajer, S., and Banisalam, B. 2015. Antioxidant Activity and Total Phenolic and Flavonoid Content of Various Solvent Extracts from in Vivo and in Vitro Grown *Trifolium pratense* L. (Red Clover). *BioMed Research International* 2015: 1-11.

Khozirah, S. 2015. Discovering Future Cures from Phytochemistry to Metabolomics. Serdang, Malaysia: UPM Press.

Kim, H.K.; Choi, Y.H. and Verpoorte, R. 2010. NMR-Based Metabolomic Analysis of Plants. *Nature Protocols* 5: 536–549.

Kim, T.G., Hwi, K.K. and Hung, C.S. 2005. Morphological and Biochemical Changes of Andrographolide-Induced Cell Death in Human Prostatic Adenocarcinoma PC-3 Cells. *In Vivo* 19 (3): 551–557.

Kondo, N. and Kawashima, M. 2000. Enhancement of the Tolerance to Oxidative Stress in Cucumber (*Cucumis sativus* L.) Seedlings by UV-B Irradiation: Possible Involvement of Phenolic Compounds and Antioxidative Enzymes. *Journal of Plant Research* 113 (3): 311–317.

Kuczynska, P., Jemiola-Rzeminska, M. and Strzalk, K. 2015. Photosynthetic Pigments in Diatoms. *Marine Drugs* 13 (9): 5847–5881.

Kumar, A., Dora, J., Singh, A. and Tripathi, R. 2012. A Review on King of Bitter (Kalmegh). *International Journal of Research in Pharmacy and Chemistry* 2 (1): 116–124.

Kumar, S. and Kumar, A. 2013. Spatial and Harvesting Influence on Growth, Yield, Quality and Economic Potential of Kalmegh (*A. paniculata* Wall Ex. Nees.). *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 114 (1): 69–76.

Kumar, S. and Pandey, A.K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal* 2013: 1–16.

Küpper, H., Mijovilovich, A., Meyer-Klaucke, W., and Kroneck, P.M.H. 2004. Tissue- and Age-Dependent Differences in the Complexation of Cadmium and Zinc in the Cadmium/zinc Hyperaccumulator *Thlaspi Caerulescens* (Ganges Ecotype) Revealed by X-Ray Absorption Spectroscopy. *Plant Physiology* 134 (2): 748–757.

Kurzawa, M., Filipiak-Szok, A., Kłodzińska, E. and Szłyk, E. 2015. Determination of Phytochemicals, Antioxidant Activity and Total Phenolic Content in *A. paniculata* Using Chromatographic Methods. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 995-996: 101–106.

Lavinsky, A.O., Magalhães, P.C., Ávila, R.G., Diniz, M.M. and de Souza, T.C. 2015. Partitioning between Primary and Secondary Metabolism of Carbon Allocated to Roots in Four Maize Genotypes under Water Deficit and Its Effects on Productivity. *The Crop Journal* 3 (5): 379–386.

Lawal, U., Mediani, A., Maulidiani, H., Shaari, K., Ismail, I.S., Khatib, A. and Abas, F. 2015. Metabolite Profiling of *Ipomoea aquatica* at Different Growth Stages in Correlation to the Antioxidant and α-Glucosidase Inhibitory Activities Elucidated by ^{1}H NMR-Based Metabolomics. *Scientia Horticulturae* 192: 400–408.

Li, S., Fang, Y., Ning, H. and Wu, Y. 2012. Heavy Metals in Chinese Therapeutic Foods and Herbs. *Journal of the Chemical Society of Pakistan* 34 (5): 1091–1095.

Li, S., Han, Q., Qiao, C., Song, J., Cheng, C.L. and Xu, H. 2008. Chemical Markers for the Quality Control of Herbal Medicines: An Overview. *Chinese Medicine* 3 (7): 1–16.

Li, W., Xu, X., Zhang, H., Ma, C., Fong, H., van Breemen, R. and Fitzloff, J. 2007. Secondary Metabolites from *A. paniculata*. *Chemical and Pharmaceutical Bulletin* 55 (3): 455–458.

Li, Y., Johnson, D.A., Su, Y., Cui, J. and Zhang, T. 2005. Specific Leaf Area and Leaf Dry Matter Content of Plants Growing in Sand Dunes. *Botanical Bulletin-Academia Sinica Taipei* 46: 127–34.

Lin, J.T., Chen, S.L., Liu, S.C. and Yang, D.J. 2009. Effect of Harvest Time on Saponins in Yam (*Dioscorea pseudojaponica* Yamamoto). *Journal of Food and Drug Analysis* 17 (2): 116–122.

Liu, J., Zeng, D., Lee, D.K., Fan, Z. and Zhong, L. 2008. Leaf Traits and Their Interrelationship of 23 Plant Species in Southeast of Keerqin Sandy Lands, China. *Frontiers of Biology in China* 3 (3): 332–337.

Liu, N.Q., Cao, M., Frédérich, M., Choi, Y.H., Verpoorte, R. and van der Kooy, F. 2010. Metabolomic Investigation of the Ethnopharmacological Use of *Artemisia afra* with NMR Spectroscopy and Multivariate Data Analysis. *Journal of Ethnopharmacology* 128 (1): 230–235.

Liu, W. T., Ni, J.C. and Zhou, Q.X. 2013. Uptake of Heavy Metals by Trees: Prospects for Phytoremediation. *Materials Science Forum* 743-744: 768–81.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. 2010. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. *Pharmacognosy Reviews* 4 (8): 118–126.

Lodish, H., Berk, A. and Zipursky, S.L. 2000. Photosynthetic Stages and Light-Absorbing Pigments. In *Molecular Cell Biology*, ed. H. Lodish, A. Berk, L.S. Zipursky, P. Matsudaira, J. Darnell, and D. Baltimore, New York: Freeman and Co.

Lomlim, L., Jirayupong, N. and Plubrukarn, A. 2003. Heat-Accelerated Degradation of Solid-State Andrographolide. *Chemical and Pharmaceutical Bulletin* 51 (1): 24–26.

Luís, Â., Gil, N., Amaral, M.E., Domingues, F. and Duarte, A. P. 2012. *Ailanthus altissima* (Miller) Swingle: A Source of Bioactive Compounds with Antioxidant Activity. *BioResources* 7 (2): 2105–2120.

Ma, X., Li, H., Dong, J. and Qian, W. 2011. Determination of Hydrogen Peroxide Scavenging Activity of Phenolic Acids by Employing Gold Nanoshells Precursor Composites as Nanoprobes. *Food Chemistry* 126 (2): 698–704.

Malar, J., Chairman, K., Singh, A.R.J., Vanmathi, J.S., Balasubramanian, A. and Vasanthi, K. 2014. Antioxidative Activity of Different Parts of the Plant *Lepidium sativum* Linn. *Biotechnology Reports* 3:95–98.

Malaysian Standard 2541. 2013. Phytopharmaceutical Aspect of Water and Water-Ethanol Extracts from *Orthosiphon stamineus* (Misai Kucing) Leaves-Specification. Kuala Lumpur: SIRIM.

Máthé, Á. and Máthé, I. 2008. Quality Assurance of Cultivated and Gathered Medicinal Plants. *Acta Horticulturae* 765: 67–76.

Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A. and Nishi, K. 1994. Cell Differentiation-Inducing Diterpenes from *A. paniculata* Nees. *Chemical and Pharmaceutical Bulletin* 42 (6): 1216–1225.

Maulidiani, Abas, F., Khatib, A., Shitan, M., Shaari, K. and Lajis, N.H. 2013. Comparison of Partial Least Squares and Artificial Neural Network for the Prediction of Antioxidant Activity in Extract of Pegaga (*Centella*) Varieties from ^{1}H Nuclear Magnetic Resonance Spectroscopy. *Food Research International* 54 (1): 852–60.

Mediani, A. 2012. Influence of Growth Stages and Harvesting Season and Drying Methods on Phytochemicals Content and Antioxidant Activities of *Cosmos caudatus* Kunth Leaves, Master Thesis, Universiti Putra Malaysia.

Mediani, A., Abas, F., Khatib, A., Tan, C.P., Ismail, I.S., Shaari, K., Ismail, A. and Lajis, N.H. 2015. Phytochemical and Biological Features of *Phyllanthus niruri* and *Phyllanthus urinaria* Harvested at Different Growth Stages Revealed by ^{1}H NMR-Based Metabolomics. *Industrial Crops and Products* 77: 602–613.

Mediani, A., Abas, F., Ping, T.C., Khatib, A. and Lajis, N.H. 2012. Influence of Growth Stage and Season on the Antioxidant Constituents of *Cosmos caudatus*. *Plant Foods for Human Nutrition* 67 (4): 344–350.

Michalak, A. 2006. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Plant Cell* 15 (4): 523–530.

Milenković, S.M., Zvezdanović, J.B., Andelković, T.D. and Marković, D.Z. 2012. The Identification of Chlorophyll and Its Derivatives in the Pigment Mixtures: HPLC-Chromatography, Visible and Mass Spectroscopy Studies. *Advanced Technologies* 1 (1): 16–24.

Mishra, N., Agrawal, S., Jadhav, S.K. and Kumar, A. 2014. Traditional Applications and Phytochemical Investigation of *A. paniculata* from Four Districts of Chhattisgarh. *Advances in Bioresearch* 5 (2): 172–182.

Mishra, S.K., Sangwan, N.S. and Sangwan, R.S. 2007. *A. paniculata* (Kalmegh): A Review. *Plant Review* 1 (2): 283–298.

Mishra, S., and Jain, A. 2014. Effect of INM on Vegetative Growth, Flowering and Fruiting of *A. paniculata*. 2 (3): 93–96.

Misra, P., Pal, N.L., Guru, P.Y., Katiyar, J.C., Srivastava, V. and Tandon, J.S. 1992. Antimalarial Activity of *A. paniculata* (Kalmegh) against *Plasmodium berghei* NK 65 in *Mastomys natalensis*. *Pharmaceutical Biology* 30 (4): 263–274.

MOA. 2012. National Key Economic Area. Retrieved 3 June 2016 from http://www.moa.gov.my/web/guest/epp-1.

Mohd Noor, M.S., and Sukir, S. 2000. Problems and Obstacles in Herbs and Local Medicinal Plants Processing Industry in Malaysia. In *Proceedings of the 16th National Seminar on Natural Products: Interdisciplinary Approaches in Natural Products Research, 183–188. Serdang, Selangor*.

Mohd Rani, M.Y., Mohamed Zabawi, A.G., Tunku Mahmud, T.Y., Mohd Syukri, M.A., Mahamud, C.H., Tosiah, S., Ghani, A., Hasbullah, M., Norlia, Y. and Lo, N.P. 2002. Intercropping Misai Kucing, Hempedu Bumi, Kacip Fatima and Mengkudu with Rubber: A Case Study in Agroforestry. In *Proceedings of the 13th Malaysian Society of Plant Physiology Conference: Towards Sustainable Development in Agroforestry: New Paradigm for Plant Physiologists Malacca (Malaysia), 10-12 Sept 2002*, 18–27. Serdang.

Mohtashami, S., Babalar, M. and Mirjalili, M.H. 2013. Phenological Variation in Medicinal Traits of *Dracocephalum moldavica* L. (Lamiaceae) under Different Growing Conditions. *Journal of Herbs, Spices and Medicinal Plants* 19 (4): 377–390.

Mojica, L., Rui, L. and de Mejia, E.G. 2012. *Hibiscus sabdariffa* L.: Phytochemical

Composition and Nutraceutical Properties. In *Hispanic Foods: Chemistry and Bioactive Compounds*, ed. M.H. Tunick and E.G. de Mejía, pp. 17–279. Washington, D.C.: American Chemical Society.

Moses, T., Papadopoulou, K.K. and Osbourn, A. 2014. Metabolic and Functional Diversity of Saponins, Biosynthetic Intermediates and Semi-Synthetic Derivatives. *Critical Reviews in Biochemistry and Molecular Biology* 49 (6): 439–462.

Mullineaux, P.M., Karpinski, S. and Baker, N.R. 2006. Spatial Dependence for Hydrogen Peroxide-Directed Signaling in Light-Stressed Plants. *Plant Physiology* 141 (2): 346–350.

Naeem, M., Nasir Khan, M., Masroor A Khan, M. and Moinuddin. 2013. Adverse Effects of Abiotic Stresses on Medicinal and Aromatic Plants and Their Alleviation by Calcium. In *Plant Acclimation to Environmental Stress*, ed. N. Tuteja and S.S. Gill, pp. 101–146. New York, NY: Springer New York.

Naghdi, B.H., Soroshzadeh, A., Rezazadeh, S., Sharifi, M., Ghalavand, A. and Rezai, A. 2005. Evaluation of Phytochemical and Production Potential of Borage (*Borago officinalis* L.) During the Growth Cycle. *Journal of Medicinal Plants* 7 (4): 1–7.

Naghiloo, S., Movafeghi, A., Delazar, A., Nazemiyeh, H., Asnaashari, S. and Dadpour, M.R. 2012. Ontogenetic Variation of Total Phenolics and Antioxidant Activity in Roots, Leaves and Flowers of *Astragalus compactus* Lam. (Fabaceae). *BioImpacts* 2 (2): 105–109.

Naser, H.M., Sultana, S., Mahmud, N.U., Gomes, R. and Noor, S. 2012. Heavy Metal Levels in Vegetables with Growth Stage and Plant Species Variations. *Bangladesh Journal of Agricultural Research* 36 (4): 563–574.

Nasr, S.B., Aazza, S., Mnif, W. and Miguel, M. 2014. Phenol Content and Antioxidant Activity of Different Young and Adult Plant Parts of Tobacco from Tunisia, Dried at 40 and 70 ^{o}C. *Journal of Applied Pharmaceutical Science* 4 (8): 23–31.

Ndamba, J., Lemmich, E. and Mølgaard, P. 1994. Investigation of the Diurnal, Ontogenetic and Seasonal Variation in the Molluscicidal Saponin Content of *Phytolacca dodecandra* Aqueous Berry Extracts. *Phytochemistry* 35 (1): 95–99.

Niranjan, A., Tewari, S. K. and Lehri, A. 2010. Biological Activities of Kalmegh (*A. paniculata* Nees.) and Its Active Principles-A Review. *Indian Journal of Natural Products and Resources* 1 (2): 125–135.

Nor Qhairul Izzreen, M.N. and Mohd Fadzelly, A.B. 2013. Phytochemicals and Antioxidant Properties of Different Parts of *Camellia sinensis* Leaves from Sabah Tea Plantation in Sabah, Malaysia. *International Food Research Journal* 20 (1): 307–312.

Noriham, A., Dian-Nashiela, F., Hafifi, B.K., Nooraain, H. and Azizah, A.H. 2015. Influences of Maturity Stages and Extraction Solvents on Antioxidant Activity of

Cosmos caudatus Leaves. *International Journal of Research Studies in Biosciences* 3 (12): 1–10.

Nouvellon, Y., Laclau, J.P., Epron, D., Kinana, A., Mabiala, A., Roupsard, O., Bonnefond, J.M., Le Maire, G., Marsden, C., Bontemps, J.D. and Saint-Andre, L. 2010. Within-Stand and Seasonal Variations of Specific Leaf Area in a Clonal *Eucalyptus* Plantation in the Republic of Congo. *Forest Ecology and Management* 259 (9): 1796–1807.

Oh, M., Carey, E.E. and Rajashekar, C.B. 2010. Regulated Water Deficits Improve Phytochemical Concentration in Lettuce. *Jornal of American Society of Horticultural Science* 135 (3): 223–229.

Ojha, S., Bharti, S., Golechha, M., Sharma, A.K., Rani, N., Kumari, S. and Arya, D.S. 2012. *A. paniculata* Extract Protect against Isoproterenol-Induced Myocardial Injury by Mitigating Cardiac Dysfunction and Oxidative Injury in Rats. *Acta Poloniae Pharmaceutica-Drug Research* 69 (2): 269–278.

Okhuarobo, A., Falodun, J.E., Erharuyi, O., Imieje, V., Falodun, A. and Langer, P. 2014. Harnessing the Medicinal Properties of *A. paniculata* for Diseases and Beyond: A Review of its Phytochemistry and Pharmacology. *Asian Pacific Journal of Tropical Disease* 4 (3): 213–222.

Oladele, O. O., and Aborisade, A. T. 2009. Influence of Different Drying Methods and Storage on the Quality of Indian Spinach (*Basella rubra* L.). *American Journal of Food Technology* 4 (2): 66–70.

Orroño, D.I. and Lavado, R.S. 2009. Heavy Metal Accumulation in *Pelargonium hortorum*: Effects on Growth and Development. *Phyton (Buenos Aires)* 78: 75–82.

Otake, T., Mori, H., Morimoto, M., Ueba, N., Sutardjo, S., Kusumoto, I.T., Hattori, M. and Namba, T. 1995. Screening of Indonesian Plant Extracts for Anti-Human Immunodeficiency Virus—type 1 (HIV-1) Activity. *Phytotherapy Research* 9 (1): 6–10.

Panawala, P.B.C., Abeysinghe, D.C. and Dharmadasa, R.M. 2016. Phytochemical Distribution and Bioactivity of Different Parts and Leaf Positions of *Pimenta dioica* L. Merr (Myrtaceae). *World Journal of Agricultural Research* 4 (5): 143–146.

Pandey, A.K. and Mandal, A.K. 2010. Variation Morphological Characteristics and Andrographolide Content in *A. paniculata* of Central India. *Iranica Journal of Energy and Environment* 1 (2): 165–169.

Parashar, R., Upadhyay, A., Singh, J., Diwedi, S.K. and Khan, N.A. 2011. Morpho-Physiological Evaluation of *A. paniculata* at Different Growth Stages. *World Journal of Agricultural Sciences* 7 (2): 124–127.

Parasher, R., Upadhyay, A., Khan, N.A. and Dwivedi, S.K. 2011. Biochemical

Estimation and Quantitative Determination of Medicinally Important Andrographolide in *A. paniculata* at Different Growth Stages. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 10 (7): 2479–2486.

Pehlivan, E., Özkan, A.M., Dinç, S. and Parlayici, Ş. 2009. Adsorption of Cu^{2+} and Pb^{2+} Ion on Dolomite Powder. *Journal of Hazardous Materials* 167 (1): 1044–1049.

Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G. and Gardea-Torresdey, J. 2009. The Biochemistry of Environmental Heavy Metal Uptake by Plants: Implications for the Food Chain.*The International Journal of Biochemistry and Cell Biology* 41 (8–9): 1665–1677.

Phansawan, B. and Pongsabangpho, S. 2014. Determination of Gallic Acid and Rutin in Extracts *Cassia alata* and *A. paniculata*. *ScienceAsia* 40 (6): 414-419.

Pholphana, N., Rangkadilok, N., Saehun, J., Ritruechai, S. and Satayavivad, J. 2013. Changes in the Contents of Four Active Diterpenoids at Different Growth Stages in *A. paniculata* (Burm. f.) Nees. (Chuanxinlian). *Chinese Medicine* 8 (1) : 1- 13.

Pholphana, N., Rangkadilok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M. and Satayavivad, J. 2004. Determination and Variation of Three Active Diterpenoids in *A. paniculata* (Burm. f.) Nees. *Phytochemical Analysis* 15 (6): 365–371.

Pilarski, J., Tokarz, K. and Kocurek, M. 2007. Comparison of Photosynthetic Pigment Contents in Stems and Leaves of Fruit Trees: Cherry, Sweet Cherry, Common Plum, and Walnut Tree. *Folia Horticulturae* 19 (1): 53–65.

Podolak, I., Galanty, A. and Sobolewska, D. 2010. Saponins as Cytotoxic Agents : A Review. *Phytochemistry Reviews* 9: 425–474.

Poljsak, B., Šuput, D. and Milisav, I. 2013. Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants. *Oxidative Medicine and Cellular Longevity* 2013: 1-11.

Pons, T.L., Jordi, W. and Kuiper, D. 2001. Acclimation of Plants to Light Gradients in Leaf Canopies: Evidence for a Possible Role for Cytokinins Transported in the Transpiration Stream. *Journal of Experimental Botany* 52 (360): 1563–1574.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. and Villar, R. 2009. Causes and Consequences of Variation in Leaf Mass per Area (LMA): A Meta-Analysis. *New Phytologist* 182 (3):565–588.

Prakash, D., Gupta, C. and Sharma, G. 2012. Importance of Phytochemicals in Nutraceuticals. *Journal of Chinese Medicine Research and Development* 1 (3): 70–78.

Prathanturarug, S., Soonthornchareonnon, N., Chuakul, W. and Saralamp, P. 2007. Variation in Growth and Diterpene Lactones among Field-Cultivated *A. paniculata*. *Journal of Natural Medicines* 61 (2): 159–163.

Prisca, A. 2013. Growth, Physiology and Quality of *Andrographis paniculata* in Response to Organic Fertilizer Rate, Bachelor Thesis, Universiti Putra Malaysia, Malaysia.

Quansah, E, and Karikari, T.K. 2016. Potential Role of Metabolomics in the Improvement of Research on Traditional African Medicine. *Phytochemistry Letters* 17: 270–277.

Radhika, P. and Lakshmi, K.R. 2010. Antimicrobial Activity of the Chloroform Extracts of the Root and the Stem of *A. paniculata* Nees. *Research Journal of Microbiology* 1: 37–39.

Rafat, A., Philip, K. and Muniandy, S. 2010. Antioxidant Potential and Content of Phenolic Compounds in Ethanolic Extracts of Selected Parts of *A. paniculata*. *Journal of Medicinal Plants Research* 4 (3): 197–202.

Rajput, Z.I., Hu, S., Xiao, C.W. and Arijo, A.G. 2007. Adjuvant Effects of Saponins on Animal Immune Responses. *Journal of Zhejiang University*. *Science B* 8 (3): 153–161.

Ramakrishna, A. and Ravishankar, G.A. 2011. Influence of Abiotic Stress Signals on Secondary Metabolites in Plants. *Plant Signaling and Behavior* 6 (11): 1720–1731.

Rasheed, N.M.A., Nagaiah, K., Goud, P.R. and Sharma, V.U.M. 2012. Chemical Marker Compounds and Their Essential Role in Quality Control of Herbal Medicines. *Annals of Phytomedicine* 1 (1): 1–8.

Rastogi, R.P., Mehrotra, B.N. and Pastogi, R.P. 2001. *Compendium of Indian Medicinal Plants*, Volume 3, New Delhi: Council of Scientific and Industrial Research.

Raya, K.B., Ahmad, S.H., Sanusi, F.F., Munirah, M., Tajidin, N.E. and Parvez, A. 2015. Changes in Phytochemical Contents in Different Parts of *Clinacanthus nutans* (Burm. F.) Lindau due to Storage Duration. *Bragantia* 74 (4): 445–452.

Rehman, A., Farhan, Iqbal, T., Ayaz, S. and Rehman, H.U. 2013. Investigations of Heavy Metals in Different Medicinal Plants. *Journal of Applied Pharmaceutical Science* 3 (8): 72–74.

Riahi, L., Chograni, H., Elferchichi, M., Zaouali, Y., Zoghlami, N. and Mliki, A. 2013. Variations in Tunisian Wormwood Essential Oil Profiles and Phenolic Contents between Leaves and Flowers and Their Effects on Antioxidant Activities. *Industrial Crops and Products* 46: 290–296.

Rice-Evans, C., Miller, N. and Paganga, G. 1997. Antioxidant Properties of Phenolic Compounds. *Trends in Plant Science* 2 (4): 152–159.

Rolland, F., Moore, B. and Sheen, J. 2002. Sugar sensing and signalling in plants.

Plant Cell (14):185–205.

Sabu, K.K., Padmesh, P. and Seeni, S. 2001. Intraspecific Variation in Active Principle Content and Isozymes of *A. paniculata* (Kalmegh): A Traditional Hepatoprotective Medicinal Herb of India. *Journal of Medicinal and Aromatic Plant Sciences* 23 (4): 637–47.

Salisbury, F.B. and Ross, C.W. 1992. *Plant Physiology*. Belmont, California: Wadsworth Publishing Company.

Samy, R.P., Thwin, M.M., Gopalakrishnakone, P. and Ignacimuthu, S. 2008. Ethnobotanical Survey of Folk Plants for the Treatment of Snakebites in Southern Part of Tamilnadu, India. *Journal of Ethnopharmacology* 115 (2): 302–312.

Şanli, A., Karadoğan, T., Tosun, B., Tonguç, M. and Erbaş, S. 2016. Growth Stage and Drying Methods Affect Essential Oil Content and Composition of Pickling Herb (*Echinophora tenuifolia* subsp. *sibthorpiana* Tutin). *SDÜ Fen Bilimleri Enstitüsü Dergisi* 20 (1): 43-49.

Santos, M.S., Jezler, C.N., de Oliveira, A.R., Oliveira, R.A., Mielke, M.S. and Costa, L.C. 2012. Harvest Time and Plant Age on the Content and Chemical Composition of the Essential Oil of *Alpinia zerumbet*. *Horticultura Brasileira* 30 (3): 385–390.

Sarkar, M.A., Salim, M., Islam, N. and Rahman, M.M. 2007. Effect of Sowing Date and Time of Harvesting on the Yield and Yield Contributing Characters of Sesame (*Sesamum indicum* L.) Seed. *International Journal of Sustainable Crop Production* 2: 31–35.

Scheepens, J.F., Frei, E.S. and Stöcklin, J. 2010. Genotypic and Environmental Variation in Specific Leaf Area in a Widespread Alpine Plant after Transplantation to Different Altitudes. *Oecologia* 164 (1): 141–150.

Schoefs, B. 2002. Chlorophyll and Carotenoid Analysis in Food Products. Properties of the Pigments and Methods of Analysis. *Trends in Food Science and Technology* 13 (11): 361–371.

Sellami, I.H., Maamouri, E., Chahed, T., Wannes, W.A., Kchouk, M.E. and Marzouk, B. 2009. Effect of Growth Stage on the Content and Composition of the Essential Oil and Phenolic Fraction of Sweet Marjoram (*Origanum majorana* L.). *Industrial Crops and Products* 30 (3): 395–402.

Sen, S., De, B., Devanna, N. and Chakraborty, R. 2013. Total Phenolic, Total Flavonoid Content, and Antioxidant Capacity of the Leaves of *Meyna spinosa* Roxb., an Indian Medicinal Plant. *Chinese Journal of Natural Medicines* 11 (2): 149–157.

Shaban, N.S., Abdou, K.A. and Hassan, N.E.Y. 2016. Impact of Toxic Heavy Metals and Pesticide Residues in Herbal Products. *Beni-Suef University Journal of Basic and Applied Sciences* 5 (1): 102–106.

Sharma, A., Shanker, C., Tyagi, L.K., Singh, M. and Rao, C.V. 2008. Herbal Medicine for Market Potential in India: An Overview. *Academic Journal of Plant Sciences* 1 (2): 26–36.

Sharma, A., Lal, K. and Handa, S.S. 1992. Standardization of the Indian Crude Drug Kalmegh by High Pressure Liquid Chromatographic Determination of Andrographolide. *Phytochemical Analysis* 3 (3): 129–131.

Sharma, B.K. and Jain, A.K. 2015. Studies on Some Aspects of Reproductive Biology of *A. paniculata* (Acanthaceae). *The International Journal of Plant Reproductive Biology* 7 (2): 1–6.

Sharma, M. and Sharma, R 2013. Identification, Purification and Quantification of Andrographolide from *A. paniculata* (Burm. f.) Nees. by HPTLC at Different Stages of Life Cycle of Crop. *Journal of Current Chemical and Pharmaceutical Sciences* 3 (1): 23–32.

Sharma, M., Sharma, A. and Tyagi, S. 2012. Quantitative HPLC Analysis of Andrographolide in *A. paniculata* at Two Different Stages of Life Cycle of Plant. *Acta Chimica and Pharmaceutica Indica* 2 (1): 1–7.

Sharma, S.N., Sinha, R.K., Sharma, D.K. and Jha, Z. 2009. Assessment of Intra-Specific Variability at Morphological, Molecular and Biochemical Level of *A. paniculata* (Kalmegh). *Current Science* 96 (3): 402–408.

Shen, Y.H., Li, R.T., Xiao, W.L., Xu, G., Lin, Z.W., Zhao, Q.S. and Sun, H.D. 2006. Ent-Labdane Diterpenoids from *A. paniculata*. *Journal of Natural Products* 69 (3): 319–322.

Shuib, N.H., Shaari, K., Khatib, A., Kneer, R., Zareen, S., Raof, S.M., Lajis, N.H. and Neto, V. 2011. Discrimination of Young and Mature Leaves of *Melicope Ptelefolia* Using ^{1}H NMR and Multivariate Data Analysis. *Food Chemistry* 126 (2): 640–645.

Shukri, M.A., Razali, M., Khozirah, S., Norlia, Y., Mohamed Senawi, M.T. and Abdul Ghani, I. 2005. Determination of Andrographolide and Neoandrographolide Levels in Hempedu Bumi (*A. paniculata* Nees.) Grown under Rubber Ecosystem. *Journal of Tropical Agriculture and Food Science* 33 (2): 303–309.

Silva, E.M., Souza, J.N.S., Rogez, H., Rees, J.F. and Larondelle, Y. 2007. Antioxidant Activities and Polyphenolic Contents of Fifteen Selected Plant Species from the Amazonian Region. *Food Chemistry* 101 (3): 1012–1018.

Singh, M., Singh, A., Tripathi, R.S., Verma, R.K., Gupta, M.M., Mishra, H.O., Singh, H.P. and Singh, A.K. 2011. Growth Behavior, Biomass and Diterpenoid Lactones Production in Kalmegh (*A. paniculata* Nees.) Strains at Different Population Densities. *Agricultural Journal* 6 (3): 115–118.

Siriwardane, A.S., Dharmadasa, R.M. and Samarasinghe, K. 2013. Distribution of Withaferin A, an Anticancer Potential Agent, in Different Parts of Two Varieties

of *Withania somnifera* (L.) Dunal. Grown in Sri Lanka. *Pakistan Journal of Biological Sciences* 16 (3):141–144.
Slesak, I., Libik, M., Karpinska, B., Karpinski, S. and Miszalski, Z. 2007. The Role of Hydrogen Peroxide in Regulation of Plant Metabolism and Cellular Signalling in Response to Environmental Stresses. *Acta Biochimica Polonica* 54 (1): 39–50.

Song, Y.X., Liu, S.P., Jin, Z., Qin, J.F. and Jiang, Z.Y. 2013. Qualitative and Quantitative Analysis of *A. paniculata* by Rapid Resolution Liquid Chromatography/time-of-Flight Mass Spectrometry. Molecules 18 (10): 12192–21207.

Sparg, S.G., Light, M.E. and Van Staden, J. 2004. Biological Activities and Distribution of Plant Saponins. *Journal of Ethnopharmacology* 94: 219–243.

Srivastava, N., Chauhan, A.S. and Sharma, B. 2012. Isolation and Characterization of Some Phytochemicals from Indian Traditional Plants. *Biotechnology Research International* 2012: 1–8.

Sroka, Z. and Cisowski, W. 2003. Hydrogen Peroxide Scavenging, Antioxidant and Anti-Radical Activity of Some Phenolic Acids. *Food and Chemical Toxicology* 41 (6): 753–758.

Stankovic, M.S., Niciforovic, N., Mihailovic, V., Topuzovic, M. and Solujic, S. 2012. Antioxidant Activity, Total Phenolic Content and Flavonoid Concentrations of Different Plant Parts of *Teucrium polium* L. subsp. Polium. *Acta Societatis Botanicorum Poloniae* 81 (2): 117–122.

Straumite, E., Kruma, Z. and Galoburda, R. 2015. Pigments in Mint Leaves and Stems. *Agronomy Research* 13 (4): 1104–1111.

Street, R.A. 2012. Heavy Metals in Medicinal Plant Products-An African Perspective. *South African Journal of Botany* 82: 67–74.

Sudhakaran, M.V. 2012. Botanical Pharmacognosy of *A. paniculata* (Burm. f.) Wall. Ex. Nees. *Pharmacognosy Journal* 4 (32): 1–10.
Suwanbareerak, K. and Chaichantipyuth, C. 1991. Num-Lai-Pung-Porn, *A. paniculata*. *Journal of Medicinal Plant Thailand Society* 7: 3–9.

Szakiel, A., Pączkowski, C. and Henry, M. 2011. Influence of Environmental Abiotic Factors on the Content of Saponins in Plants. *Phytochemistry Reviews* 10 (4): 471–491.

Taiz, L. 1992. The Plant Vacuole. *Journal of Experimental Biology* 172 (1): 113–122.

Taiz, L. and Zeiger, E. 2006. *Plant Physiology*, 5th ed. Belmont, California: Sinauer Associates, Inc.

Talei, Daryush. 2012. Morphological, Phytochemical and Proteomic Responses of *Andrographis paniculata* Nees. to Salt Stress, Ph. D Thesis, Universiti Putra Malaysia, Malaysia.

Talei, D., Valdiani, A., Abdullah, M.P. and Hassan, S.A. 2012. A Rapid and Effective Method for Dormancy Breakage and Germination of King of Bitters (*A. paniculata* Nees.) Seeds. *Maydica*. 57: 98-105.

Tangahu, B.V., Sheikh Abdullah, S.R., Basri, H., Idris, M., Anuar, N. and Mukhlisin, M. 2011. A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *International Journal of Chemical Engineering* 2011: 1–31.

Tessmer, O.L., Jiao, Y., Cruz, J.A., Kramer, D.M. and Chen, J. 2013. Functional Approach to High-Throughput Plant Growth Analysis. *BMC Systems Biology* 7 (6): 1–13.

Thakur, A.K., Chatterjee, S.S. and Kumar, V. 2015. Adaptogenic Potential of Andrographolide: An Active Principle of the King of Bitters (*A. paniculata*). *Journal of Traditional and Complementary Medicine* 5 (1): 42–50.

Thi, N.D. and Hwang, E. S. 2014. Bioactive Compound Contents and Antioxidant Activity in Aronia (*Aronia melanocarpa*) Leaves Collected at Different Growth Stages. *Preventive Nutrition and Food Science* 19 (3): 204–212.

Thisoda, P., Rangkadilok, N., Pholphana, N., Worasuttayangkurn, L., Ruchirawat, S. and Satayavivad, J. 2006. Inhibitory Effect of *Andrographis paniculata* Extract and Its Active Diterpenoids on Platelet Aggregation. *European Journal of Pharmacology* 553 (1): 39–45.

Tilburt, J.C. and Kaptchuk, T.J. 2008. Herbal Medicine Research and Global Health: An Ethical Analysis. *Bulletin of the World Health Organization* 86 (8): 594–599.

Tipakorn, N., 2002. Effects of *A. paniculata* (Burm. f.) Nees. on Performance, Mortality And Coccidiosis in Broiler Chickens, PhD Thesis, Georg-August-Universität Göttingen, Germany.

Tiwari, R. and Rana, C.S. 2015. Plant Secondary Metabolites: A Review. *International Journal of Engineering Research and General Science* 3 (5): 661–670.

Tupe, R.S., Kemse, N.G. and Khaire, A.A. 2013. Evaluation of Antioxidant Potentials and Total Phenolic Contents of Selected Indian Herbs Powder Extracts. *International Food Research Journal* 20 (3): 1053–1063.

Upadhya, V., Pai, S.R., and Hegde, H.V. 2015. Effect of Method and Time of Extraction on Total Phenolic Content in Comparison with Antioxidant Activities in Different Parts of *Achyranthes aspera*. *Journal of King Saud University - Science* 27 (3): 204–208.

Valdiani, Alireza. 2012. Crossability and Genetic Analyses of *Andrographis paniculata* Nees. Populations Using Phenotypic and Molecular Markers, Ph. D Thesis, Universiti Putra Malaysia, Malaysia.

Valdiani, A., Kadir, M.A., Tan, S.G., Talei, D., Abdullah, M.P. and Nikzad, S. 2012. Nain-E Havandi *A. paniculata* Present Yesterday, Absent Today: A Plenary Review on Underutilized Herb of Iran's Pharmaceutical Plants. *Molecular*

Biology Reports 39 (5):5409–5424.

Verpoorte, R., Choi, Y.H. and Kim, H.K. 2007. NMR-Based Metabolomics at Work in Phytochemistry. *Phytochemistry Reviews* 6 (1): 3–14.

Vicente, R., Morcuende, R. and Babiano, J. 2011. Differences in Rubisco and Chlorophyll Content among Tissues and Growth Stages in Two Tomato (*Lycopersicon esculentum* Mill.) Varieties. *Agronomy Research* 9 (2): 501–507.

Wachtel-Galor, S., and Benzie, I.F.F. 2011. Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In *Herbal Medicine: Biomolecular and Clinical Aspects*, ed. I.F.F. Benzie and S. Wachtel-Galor, 2nd ed., pp. 1–10. Boca Raton, Florida: CRC Press/Taylor and Francis.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S. and Sharma, H.C. 2012. Mechanisms of Plant Defense against Insect Herbivores. *Plant Signaling and Behavior* 7 (10):1306–1320.

Wasman, S.Q., Mahmood, A.A., Chua, L.S., Alshawsh, M.A. and Hamdan, S. 2011. Antioxidant and Gastroprotective Activities of *A. paniculata* (Hempedu Bumi) in Sprague Dawley Rats. *Indian Journal of Experimental Biology* 49 (10): 767–772.

Webber, III C.L., and Bledsoe, V.K. 2002. Kenaf Yield Components and Plant Composition. In *Trends in New Crops and New Uses*, ed. J. Janick and A. Whipkey, pp. 348–57. Alexandria, VA: ASHS Press.

WHO. 2007. *World Health Organization (WHO) Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*. Geneva, Switzerland: WHO Press.

Wiart, C., Kumar, K., Yusof, M.Y., Hamimah, H., Fauzi, Z.M. and Sulaiman, M. 2005. Antiviral Properties of Ent-Labdene Diterpenes of *A. paniculata* Nees., Inhibitors of Herpes Simplex Virus Type 1. *Phytotherapy Research* 19 (12): 1069–1070.

Wijarat, P., Keeratinijakal, V., Toojinda, T., Vanavichit, A. and Tragoonrung, S. 2011. Genetic Diversity and inbreeder Specie of *A. paniculata* (Burm. f.) Nees. by Randomly Amplified Polymorphic Deoxyribonucleic Acid (RAPD) and Floral Architecture Analysis. *Journal of Plant Breeding and Crop Science* 3(12): 327-334.

Wingler, A., von Schaewen, A., Leegood, R.C., Lea, P.J. and Quick, W.P. 1998. Regulation of Leaf Senescence by Cytokinin, Sugars, and Light : Effects on NADH-Dependent Hydroxypyruvate Reductase. *Plant Physiology* 116 (1): 329–335.

Wink, M. 1993. The Plant Vacuole: A Multifunctional Compartment. *Journal of Experimental Botany* 231-246.

Witzell, J., Gref, R. and Näsholm, T. 2003. Plant-Part Specific and Temporal Variation in Phenolic Compounds of Boreal Bilberry (*Vaccinium myrtillus*) Plants.

Biochemical Systematics and Ecology 31 (2): 115–127.

Wong, K.J. 2016. Optimization of Seed and Seedling Production in Hempedu Bumi (*Andrographis paniculata* (Burm. F.) Wall. Ex Ness), Master Thesis, Universiti Putra Malaysia, Malaysia.

Woodruff, D.R., Bond, B.J. and Meinzer, F.C. 2004. Does Turgor Limit Growth in Tall Trees? *Plant, Cell and Environment* 27 (2): 229–236.

Wu, T.S., Chern, H.J., Damu, A.G., Kuo, P.C., Su, C.R., Lee, E.J. and Teng, C.M. 2008. Flavonoids and Ent-Labdane Diterpenoids from *A. paniculata* and Their Antiplatelet Aggregatory and Vasorelaxing Effects. *Journal of Asian Natural Products Research* 10 (1): 17–24.

Xiao, S., Chen, S.Y., Zhao, L.Q. and Wang, G. 2006. Density Effects on Plant Height Growth and Inequality in Sunflower Populations. *Journal of Integrative Plant Biology* 48 (5): 513–519.

Xu, Y. 2009. Adaptive Immune Response-Modifying and Antimicrobial Properties of *A. paniculata* and Andrographolide, PhD Thesis, The University of Southern Queensland, Autralia.

Yamasaki, T., Kudoh, T., Kamimura, Y. and Katoh, S. 1996. A Vertical Gradient of the Chloroplast Abundance among Leaves of *Chenopodium album*. *Plant and Cell Physiology* 37: 43–48.

Yan, E.R., Milla, R., Aarssen, L.W. and Wang, X.H. 2012. Functional Relationships of Leafing Intensity to Plant Height, Growth Form and Leaf Habit. *Acta Oecologica* 41: 20–29.

Yan, S.K., Liu, R.H., Jin, H.Z., Liu, X.R., Ye, J., Shan, L. and Zhang, W.D. 2015. 'Omics' in Pharmaceutical Research: Overview, Applications, Challenges, and Future Perspectives. *Chinese Journal of Natural Medicines* 13 (1): 3–21.

Yang, M., Shen, Q., Li, L.Q., Huang, Y.Q. and Cheung, H.Y. 2015. Phytochemical Profiles, Antioxidant Activities of Functional Herb *Abrus cantoniensis* and *Abrus mollis*. *Food Chemistry* 177: 304–312.

Yoshimatsu, K., Iida, O., Kitazawa, T., Sekine, T., Kojoma, M., Makino, Y. and Kiuchi, F. 2004. Growth Characteristics of *Cannabis sativa* L. Cultivated in a Phytotron and in the Field. *Bulletin of National Institute of Health Sciences* 20 (122): 16–20.

Yusof, N.A., Isha, A., Ismail, I.S., Khatib, A., Shaari, K., Abas, F. and Rukayadi, Y. 2015. Infrared-Metabolomics Approach in Detecting Changes in *A. paniculata* Metabolites due to Different Harvesting Ages and Times. *Journal of the Science of Food and Agriculture* 95 (12): 2533–2543.

Yusron, M. 2008. Dukungan Teknologi Budidaya Untuk Pengembangan Sambiloto (*A. paniculata* Nees.). *Perkembangan Teknologi Tanaman Rempah Dan Obat* 20 (2): 63–74.

Zainol, M., Khairi, M., Abdul Hamid, A., Abu Bakar, F. and Pak Dek, M.S. 2009. Effect of Different Drying Methods on the Degradation of Selected Flavonoids in *Centella asiatica*. *International Food Research Journal* 16 (4): 531–537.

Zamani, N., Mianabadi, M. and Abdolzadeh, A. 2011. Changes in Antioxidant Activity of *Thymus transcaspicus* (Klokov) during Growth and Developmental Stages. *Journal of Cell and Molecular Research* 3 (1): 12–18.

Zeinalov, Y. 2005. Mechanisms of Photosynthetic Oxygen Evolution and Fundamental Hypotheses of Photosynthesis. In *Handbook of Photosynthesis*, ed. M. Pessarakli, 2nd ed. Boca Raton, Florida: CRC Press.

Zhang, Q.F., Zhang, Z.R., and Cheung, H.Y. 2009. Antioxidant Activity of *Rhizoma Smilacis glabrae* Extracts and Its Key Constituent-Astilbin. *Food Chemistry* 115 (1): 297–303.

Zhang, Y.J., Gan, R.Y., Li, S., Zhou, Y., Li, A.N., Xu, D.P. and Li, H.B. 2015. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules 20 (12): 21138–21156.

Zhao, C., Li, C. and Zu, Y. 2007. Different Solanesol Contents in Leaves of Several Tobacco Varieties and Their Changes during Seedling Growth. *Plant Physiology Communications* 43 (2): 298–308.

Zhoa, C., Zu, Y., Li, C. and Tian, C. 2007. Distribution of Solanesol in Nicotiana Tabacum. *Journal of Forestry Research* 18 (1): 69–72.

Zofia L., Kmiecik, Ã.W. and Korus, A. 2006. Content of Vitamin C, Carotenoids, Chlorophylls and Polyphenols in Green Parts of Dill (*Anethum Graveolens* L.) Depending on Plant Height. *Journal of Food Composition and Analysis* 19 (2):134–140.