UNIVERSITI PUTRA MALAYSIA

GROWTH AND PHYTOCHEMICAL COMPOSITION OF Andrographis paniculata (Burm.f.) Wall. ex Nees IN RELATION TO DIFFERENT LIGHT INTENSITIES, PHOTOPERIOD AND PRUNING

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FP 2018 41

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By

NUR FAEZAH BINTI OMAR

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

January 2018

DEDICATION

This thesis is dedicated to my beloved husband, children, parents, supervisors and fellow friends for their everlasting love, support, understandings, sacrifices, motivation and encouragement.

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

GROWTH AND PHYTOCHEMICAL COMPOSITION OF *Andrographis paniculata* (Burm.f.) Wall. ex Nees IN RELATION TO DIFFERENT LIGHT INTENSITIES, PHOTOPERIOD AND PRUNING

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January 2018

Chair: Associate Professor Siti Aishah Hassan, PhD
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Vegetative stage of *Andrographis paniculata* when grown under Malaysia conditions is found to be short as compared to those grown in India. Both quality and quantity of light will affect plant development. In addition, pruning is one of the way to extend vegetative stage and delay flower formation. Therefore, this study was conducted to determine the effect of different light intensities, photoperiod and pruning frequency on growth and phytochemical composition of *A. paniculata*. Four weeks old seedlings were arranged in a split plot design with three replications where the main plots were shade levels while the sub plots were pruning frequencies. Plant samples were taken and analyzed for their morphological, physiological, and phytochemical changes. The A_{max} of *A. paniculata* was achieved at light intensity of 1000 µ mol $m^{-2}s^{-1}$. A significant interaction effect was found on N, P and Mn content. Higher andrographolide content obtained from plant with 50% shade and single pruning. Conversely, total phenolic and total flavonoid content decreased under shade levels for all pruned and non-pruned plants. Optimum shoot dry weight was obtained in single pruned plant at 50% shade levels, thus shade levels of 50% and single pruning have been chosen for photoperiod study. Photoperiodism of *A. paniculata* was further studied under four different long day photoperiods (12, 13, 14 and 15 h). The maximum growth rate of plant height (6.96 cm/week) was obtained from plant exposed to 13 h light. Flower development was influenced by long day treatments with the plant exposed to 13 h light prolonged the days to flower by 2.1 days compared to 12 h. The lowest percentage of flowering was detected under 13 h. Besides, leaf dry weight of 13 h photoperiod improved by 26% as compared to 15 h. The result of 13 h photoperiod were further tested in determination of suitable plant age for initiation of light treatment. The initiation of 13 h light length were tested from 3rd, 5th and 7th week after transplanting. The total leaf area of 3rd WAT (week after transplanting) had 10.5% greater as compared to normal photoperiod, 7.2% higher as compared to 5th WAT and 7.3% higher as compared to 7th WAT. The flowering percentage was 29.6% higher from plant without light treatment as compared to 3rd WAT. Leaf dry weight recorded from 3rd WAT was greater by 10.6 %, 6.9% and 7.2% as compared to normal photoperiod, 5th WAT and 7th WAT. Total

plant fresh weight and dry weight were improved with initiation of light at 3rd WAT. Total phenol and total flavonoid content were improved with light initiation at 3rd WAT. Micrographs of SEM showed that development of floral meristem involved at least three stages which consisted of vegetative stage, transition stage and development of flower parts. The flower initiation of plant exposed to light at 3rd and 5th WAT was delayed compared to control and 7th WAT. In conclusion, growth and phytochemical composition of *A. paniculata* can be altered by growing the crop under suitable light intensities, photoperiod induction and undergone pruning.

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

PERTUMBUHAN DAN KOMPOUN FITOKIMIA BAGI *Andrographis paniculata* (Burm.f.) Wall. ex Nees YANG DIPERNGARUHI OLEH KEAMATAN CAHAYA, FOTOPERIOD DAN KEKERAPAN PEMANGKASAN

Oleh

NUR FAEZAH BINTI OMAR

January 2018

Pengerusi: Professor Madya Siti Aishah Hassan, PhD
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Peringkat vegetatif *Andrographis paniculata* apabila ditanam di bawah kondisi Malaysia di dapati pendek berbanding dengan yang ditanam di India. Kedua-dua kualiti dan kuantiti cahaya dijangka memberi kesan terhadap perkembangan tumbuhan. Di samping itu, pemangkasan adalah salah satu cara untuk memanjangkan peringkat vegetatif dan melambatkan pembentukan bunga. Oleh itu, kajian ini dijalankan untuk menentukan kesan tahap teduhan yang berbeza, fotoperiod dan cara pemangkasan terhadap pertumbuhan dan komposisi fitokimia *A. paniculata*. Anak benih berusia empat minggu disusun dengan rekabentuk 'split-plot' dengan tiga replikasi di mana plot utama adalah tahap teduhan manakala sub-plot adalah frekuensi pemangkasan. Sampel tumbuhan diambil dan dianalisa bagi perubahan morfologi, fisiologi, dan fitokimia. A_{max} diperolehi pada keamatan cahaya 1000 μ mol $m^{-2}s^{-1}$. Kesan interaksi yang signifikan di dapati pada kandungan N, P dan Mn. Kandungan andrographolida yang tinggi diperolehi daripada tumbuhan dengan teduhan 50% dan mengalami pemangkasan sekali. Sebaliknya, jumlah kandungan fenolik dan kandungan flavonoid menurun dengan tahap teduhan untuk semua tumbuhan yang dipangkas dan tidak dipangkas. Berat kering optimum telah diperolehi daripada tumbuhan dengan tahap teduhan 50% dan dipangkas sekali, oleh yang demikian, tahap teduhan 50% dan pemangkasan sekali telah dipilih untuk kajian fotoperiod. Fotoperiodisme *A. paniculata* kemudiannya diuji di bawah empat fotoperiod yang berlainan panjang siangnya (12, 13, 14 and 15 j). Perkembangan bunga dipengaruhi oleh rawatan siang yang panjang di mana tanaman yang terdedah kepada 13 h cahaya memanjangkan hari untuk berbunga sebanyak 2.1 hari berbanding 12 h.Kadar pertumbuhan yang maksimum bagi tinggi pokok (6.96 cm / minggu) diperoleh daripada tumbuhan yang terdedah kepada 13 j cahaya. Tanaman yang terdedah kepada 13 j cahaya memerlukan sebanyak 2.1 hari untuk berbunga berbanding 12 j. Peratusan berbunga yang terendah dikesan pada fotoperiod 13 j. Disamping itu, berat kering daun bagi 13 j fotoperiod dikenal pasti meningkat sebanyak 26% berbanding dengan 15 j. Keputusan bagi 13 h fotoperiod seterusnya diuji untuk penentuan waktu yang sesuai memulakan rawatan

cahaya. 13 h rawatan cahaya telah diuji terhadap tanaman yang dipindahkan pada minggu ke-3, ke-5 dan ke-7. Jumlah luas daun bagi minggu ke-3 selepas pemindahan mempunyai 10.5% lebih besar berbanding dengan normal fotoperiod, 7.2% lebih tinggi berbanding minggu ke-5 dan 7.3% lebih tinggi berbanding minggu ke-7. Peratusan berbunga adalah lebih tinggi sebanyak 29.6% bagi tanaman tanpa rawatan cahaya berbanding dengan minggu ke-3. Berat kering daun yang direkodkan oleh minggu ke-3 adalah lebih tinggi sebanyak 10.6%, 6.9% dan 7.2% berbanding dengan fotoperiod normal, minggu ke-5 dan minggu ke-7. Jumlah berat basah dan berat kering meningkat dengan permulaan rawatan cahaya pada minggu ke-3. Jumlah kandungan fenol dan flavonoid telah meningkat dengan permulaan cahaya pada minggu ke-3. Mikrograf SEM menunjukkan perkembangan meristem bunga melibatkan sekurang-kurangnya tiga peringkat yang terdiri daripada peringkat vegetatif, peringkat peralihan dan perkembangan bahagian bunga. Pembentukan bunga bagi tumbuhan yand didedahkan pada cahaya pada minggu ke-3 and ke-5 adalah lambat berbanding kawalan dan minggu ke-7. Kesimpulannya, pertumbuhan dan komposisi fitokimia bagi *A. paniculata* boleh diubah dengan menanam pokok di bawah keamatan cahaya yang sesuai, induksi fotoperiod dan mengalami pemangkasan.

ACKNOWLEDGEMENT

Praise be to Allah SWT and peace and blessings be upon Prophet Muhammad SAW. First and foremost, I wish to express my sincere appreciation to Assoc. Prof. Dr. Siti Aishah Hassan, the chairman of my supervisory committee, for her enlightening, suggestions and guidance throughout this study. I am also thankful to Prof. Dato' Dr. Mohd Fauzi Hj. Ramlan, Dr. Puteri Edaroyati Megat Wahab and Prof. Madya Dr. Mohd. Puad Abdullah, the members of my supervisory committee for their invaluable advice, guidance and support to complete this study.

I am enormously grateful to the love of my life husband, son and daughter for their unfailing love and support throughout this journey. I am deeply indebted to my dearest parents, parent in law, sisters and brother, and friends for their understanding, encouragement and moral support throughout my study.

I also would like to acknowledge Mr. Yusof Yasin, Mr. Mazlan Bangi, Mr. Khoiri Kandar and Mr. Wahidin who always lend a hand during my laboratory works. Besides, I would like to thanks the staffs of Field 2, UPM, for providing me the experimental sites and assisting me during physical works. I owe deepest gratitude to all my friends for all those advices, guidance and comments. Last but not least, I would like to extend my greatest appreciation to Ministry of Higher Education Malaysia for giving me financial support through MyBrain program (MyPhD).

I certify that a Thesis Examination Committee has met on 5th January 2018 to conduct the final examination of Nur Faezah binti Omar on her thesis entitled "Growth and Phytochemical Composition of *Andrographis paniculata* (Burm.f.) Wall. ex Nees in Relation to Different Light Intensities, Photoperiod and Pruning" 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 fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
℃	Degree Celsius
h	Hour
min	Minute
LSD	least significant different
*	Significant at $P \leq 0.05$
**	Significant at $P \leq 0.01$
ns	non-significant
r	coefficient correlation
r2	coefficient of determination
g	gram
µg/g	Microgram per gram
µl	Microliter
ml	Milliliter
mm	Millimeter
cm	Centimeter
nm	Nanometer
rpm	Revolutions per minute
FW	Fresh weight
DW	Dry weight
WAT	Week after transplanting
RP-HPLC	Reversed phase-high performance liquid chromatography
AG	Andrographolide
NAG	Neoandrographolide

CHAPTER 1

INTRODUCTION

Herbs have been used as sources of food, medicine, beauty enhancers and fragrances. Herbs and natural products are valuable sources of therapeutic properties and their values for healing is well recognised. At this time, herbal medicines are currently in demand and their popularity is raising worldwide as consumers conscious that natural health medicine has the benefits with minor or no side effects. Perhaps, this has contributed to greater demand for organic herbs mainly to the health awareness consumers and environmental friendly technology awareness for alternative medicinal production (Siti Aishah et al., 2017). In recent years, approaches of using non-traditional to heal diseases has been revitalised worldwide (Ekor, 2013).

Due to impressively increased of the usage of herbal plants as alternative medicines in the past few decades, it is become a public interest with the approximately 4 billion people around the world depend on herbal products as alternatives to modern medicines (Ekor, 2013). Nowadays, health care system in Malaysia is rising trends of users which is varying from using synthetic allophatic drug to herbal cures as treatments (Ghasemzadeh et al., 2014). Medicinal herbs have moved from margin to mainstream and extensive consideration has been compensated to utilize eco-friendly and bio-friendly plant based products for preventing and curing of various human ailments (Ghasemzadeh and Ghasemzadeh, 2011). The estimation of 80% of world's population were trusted in traditional medicine, mainly plant based drugs for their crucial health precaution (Dubey et al., 2004).

In Malaysia, the herbal industry is still new although aromatic plant have been used by native as traditional since ancient years (Ganesan, 2011). Recently, herbal industry in Malaysia has been spotted as one of the agriculture Entry Points Projects (EPPs) under the National Key Economic Areas (NKEAs) in the Economic Transformation Programmed (ETP). Due to extensive acquaintance of herbs for curing of various illnesses and the recognition of biomedical values, the Malaysia Economic Transformation Program over NKEA Agriculture sector has recognised *Andrographis paniculata* as one of the great importance herbal crop that need to be commercially exploited for economic growth in the herbal industry. In addition, the Discovery Team have listed some of herbs need to be emphasized in order to analyse the significance sequence from crop to the market including dukung anak, kacip fatimah, tongkat ali, and misai kucing (MOA, 2012).

The potential used for treatment of many diseases led to higher demand for hempedu bumi (Valdiani et al., 2012). Recently, it was conveyed that the price of dried aerial part of *A. paniculata* were US$5 per kg whereas purified bioactive compounds mainly andrographolide components were almost US$100,000 per kg (Valdiani et al., 2012). *Andrographis paniculata* or locally known as hempedu bumi, is one of herbal plant that has been confirmed to possess numerous pharmacological activities comprising anticancer and immunostimulatory, anti-malarial as well as anti-inflammatory (Mishra

et al., 2014). Both leaves and stems of the plants are normally used for the extraction of active phytochemicals and each compounds have different strength in pharmacological activities (Mishra et al., 2014). On the other hand, information to optimize the chemical constituents of this medicinal herb by proper manipulation of the environment which generally associated with cultivation as well as cultural practices is still lacking.

Pruning is a significant cultural practice that has been proven to boost the plant quality and productivity. Previous report showed that heavy pruning resulting in vigorous leafy growth and fewer fruit. A research report by Yilmaz et al. (2004) pointed out pruning on tea plant lead to increase in a number of branch and prior to this, it revitalizes the tea bushes causing in a large number of tender leaves. The importance of pruning is to divert stored carbohydrate to production of new emerging shoots. However, the time of pruning depend on agronomic management, cultivars and the climatic condition of the areas (Pal et al., 2014). Until recently, there are many studies have been carried out to determine the effect of pruning on growth and secondary metabolites of various herbs, but little is known regarding its effect on hempedu bumi.

Plant growth and the biosynthesis of primary and secondary metabolites are controlled by light (Hemm et al., 2004). Both quality and quantity of light will affect plant development. The quantity of light comprises the light intensity and the period of acceptance of light whereas, light quality involves the wavelength of light and light received by the plant (Acquaah, 2002). According to Ghasemzadeh et al. (2010) light intensity influenced the concentration of secondary metabolites of plants. Normally, *A. paniculata* will grow well in shaded areas such as undergrowth in forest (Saravanan et al., 2008). A study by Siti Aishah et al., (2014) reveals that *A. paniculata* grown under 60% shade had significantly higher shoot dry weight compared to under full sunlight. Besides the light intensities and cultural practices, photoperiod is one of the important factor in controlling developmental processes of plants because it is directly influence the vegetative to flowering stage. In addition, optimum photoperiod is a key factor for obtaining higher yield with the minimum amount of growth is essential for plants to respond to photoperiodic cycles. Normally, the leaves for *A. paniculata* will be harvested after three months of planting once it has already achieved the minimum amount of growth to distinguish the photoperiod at this phase (Animesh et al., 2012). It is advised as short day plant due to its well adapted to India, tropical and subtropical Asia, also south-east Asia (Chang, 1986). However there is no literature to indicate the classification of *A. paniculata* based on Malaysia condition.

According to Tajidin (2017), the vegetative stage of this plant is found to be shorter when grown under Malaysia conditions as compared to those grown in India, thus probably lower the yield. According to Sharma and Sharma (2013) the vegetative stage of this plant started after transplanting process until 110 days thereafter while Bhan et al. (2006) mentioned that the vegetative stage of *A. paniculata* is at 70 days after transplanting. In addition, India is of the higher producer for aerial part of *A. paniculata* which around 250 tonnes per year (Pholphana et al., 2013), meanwhile in Malaysia the production of this herb is only 1 tone per year (DOA, 2014). According to Mohd Noor and Sukir (2000), Malaysia is importing 70% of herbal products including *A. paniculata* to meet the local market demand. Due to high demand, *A. paniculata* is commercially cultivated. However, documented information on suitable light intensities and photoperiod on

growth, physiology and phytochemical compound for organic production under tropical condition especially in Malaysia is still lacking. Furthermore, there is no standardized farming practice in Malaysia for 'hempedu bumi' to be produced commercially. With no standardized farming practices in place, it is difficult to attain stability in output and quality, with supply often not being enough to cope with market demand (Ganesan, 2011). There is a necessity to study the impact of different light intensities, photoperiod and pruning frequencies on growth and phytochemical content in *A. paniculata* as a means of improving the productivity and quality of this valuable herbs. Hence, the study was carried out with these objectives:

1. To evaluate the effect of different light intensities and pruning frequency on growth performance, flowering production and phytochemical compounds of *A. paniculata* accession Harapan (11265).
2. To identify photoperiodism of *A. paniculata* by determing the critical day length of flowering as influenced by different day length treatments.
3. To determine the suitable plant age for light induction of *A. paniculata* accession Harapan (11265) to delay flowering.

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LIST OF PUBLICATIONS

i. Publications in journal

Nur Faezah, O, Siti Aishah, H, Puteri Edaroyati, M.W, Ramlan, M.F, Puad, M.A. 2015. Growth and dry matter partitioning of *Andrographis paniculata* to different light intensities and pruning. Global Advanced Research Journal of Agricultural Science, 4(12): 851-857.

Nur Faezah, O, Siti Aishah, H, Puteri Edaroyati, M.W, Ramlan, M.F, Puad, M.A. 2016. Growth and phytochemical responses of *Andrographis paniculata* as influenced by different shade levels and pruning. Journal of Tropical Plant Physiology, 8: 61-69.

Siti Aishah H., Nur Faezah O., Rosenani A.B., Thohirah A., Siti Hajar A., Puteri Edaroyati M.W. and Nurulhasanah I. 2017. Organic fertilizer and biochar application improved andrographolide content and antioxidant activity of *Andrographis paniculata.* Asian Academic Research Journal of Multidisciplinary, 4(3): 9-16.

ii. Publications in proceeding of conferences and seminars

Nur Faezah, O, Siti Aishah, H, Puteri Edaroyati, M.W, Ramlan, M.F, Puad, M.A. 2015. Changes in physiological characters of *Andrographis paniculata* under different shade levels and pruning. 3rd International Symposium on Applied Engineering and Sciences 2015, Serdang (Oral presenter)

Nur Faezah, O, Siti Aishah, H, Puteri Edaroyati, M.W, Ramlan, M.F, Puad, M.A. 2016. Growth and phytochemical responses of *Andrographis paniculata* as influenced by different shade levels and prunings. MSPP Conference 2015, Perak (Poster presenter)

Nur Faezah, O, Siti Aishah, H, Puteri Edaroyati, M.W, Ramlan, M.F, Puad, M.A. 2016. Determination of andrographolide, a biologically active ingredient in *Andrographis paniculata* under different shade levels and pruning frequency. International Agriculture Congress 2016, Putrajaya (Best poster presenter -1st place)

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