GROWTH PERFORMANCE AND PHYTOCHEMICALS OF Vernonia amygdalina Delile AS AFFECTED BY LIGHT INTENSITY, GROWING MEDIA, HARVEST TIME AND STORAGE DURATION

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FP 2019 33

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By

NURSUHAILI A. BAKAR

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

January 2019

DEDICATION

Specially dedicated to my beloved late father, Mr. A. Bakar Sulong, my mother, Mrs. Lijah Yusof and my siblings, Mrs. Nurhima A. Bakar, Mrs. Norhanisah A. Bakar, Mr. Mohd Amin A. Bakar and Mr. Mohd Aimran A. Bakar, for their endless love, sacrifices, support, understandings, motivation, advice and encouragement.

Abstract of thesis presented to the senate of universiti putra malaysia in fulfillment of the requirement for the degree of Master of Science

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Chair : Martini Mohammad Yusoff, PhD
Faculty : Agriculture

Vernonia amygdalina Del., locally known as pokok bismillah, is a potential herb with high medicinal value for the cure of many types of human ailments. It is found grown in a variety of habitats, causing wide variation in the growth performance and phytochemical content. In this regard, a field experiment and two laboratory experiments were conducted at Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia. The objectives of the study were to evaluate the effects of light intensity and suitable growing media on growth, physiology and biomass yield of *V. amygdalina*. The treatments for the field experiment comprised of three levels of light intensity (30%, 50% and 100% that equivalent to open field) and four types of growing media (soil, cocopeat, empty fruit bunch (EFB) and burnt paddy husk (BPH). Seedlings were raised in a nursery and transferred to the field at 4 weeks after propagated. Plants were harvested at 18 weeks after transplanted (WAT) to the field. Results indicated that *V. amygdalina* grown under 50% light intensity in EFB growing medium had the highest biomass yield (490 kg ha^{-1}) with a greater plant height (182 cm), number of branches (3 branches), stem diameter (22 mm), root surface area (3631 cm) and photosynthesis rate (22.6 µmol CO_2 $m^{-2}s^{-1}$) among the treatments imposed.

Experiment 2 was carried out to determine the suitable harvest time for optimum phytochemical contents in *V. amygdalina* leaves. Leaves samples from plants grown at the optimum requirement of the light intensity and growing media on *V. amygdalina* (50% light intensity, EFB) obtained from Experiment 1 were used in this study. The experiment comprised of six treatments of different harvest time (3, 6, 9, 12, 15 and 18 WAT). Results revealed that total phenolic content obtained were 91 and 84 mg GAE/g DW in leaf samples of plants harvested at 9 and 18 WAT, respectively. Similar pattern was found on total flavonoid content where it showed an increment from 3 to 9 WAT, then decreased sharply at week

12 but rising again until 18 WAT. Antioxidant activity (DPPH radical scavenging activity) was high from plants harvested at 9, 15 and 18 WAT. As harvest time increased the total chlorophyll content and plant nutrients content tended to increase. Heavy metals content were below the permissible limits of World Health Organization (WHO).

Experiment 3 was carried out to quantify the phytochemical contents of dried *V. amygdalina* leaves at different storage durations. The experiment consisted of seven treatments of different storage durations (0, 3, 6, 9, 12, 15 and 18 weeks). The results showed the 12-week storage duration of dried leaves of *V. amygdalina* achieved high stability of phytochemicals of total phenolic and flavonoid contents. There were decreases of 44% and 58% of total phenolic and flavonoid contents for 18-week storage duration, respectively. The nutrient content also decreased until 18 weeks of storage. However, *V. amygdalina* could be as a great source of nitrogen, potassium and magnesium since the contents were above the recommended daily allowance (RDA) set by WHO. Therefore, *V. amygdalina* is suitable to be harvested at 18 WAT in order to get the optimum phytochemicals content with minimum heavy metal contamination as well as high in biomass yield. Heavy metal contents and microbial contamination were at tolerable levels as they were below the permissible limits prescribed by WHO. Thus, *V. amygdalina* dried leaves were considered safe to be consumed until 18 weeks of storage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PRESTASI PERTUMBUHAN DAN KANDUNGAN FITOKIMIA BAGI *Vernonia amygdalina* Delile YANG DIPENGARUHI OLEH INTENSITI CAHAYA, MEDIA TANAMAN, MASA TUAIAN DAN TEMPOH PENYIMPANAN

Oleh

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Vernonia amygdalina Del., juga dikenali sebagai pokok bismillah, ialah sejenis herba yang berpotensi dengan nilai perubatan yang tinggi untuk merawat pelbagai penyakit manusia. Ianya ditemui hidup dalam pelbagai habitat yang menyebabkan variasi yang luas dalam pertumbuhan dan kandungan fitokimianya. Justeru, satu eksperimen lapangan dan dua eksperimen di makmal telah dijalankan di Jabatan Sains Tanaman, Fakulti Pertanian, Universiti Putra Malaysia. Eksperimen pertama dilaksanakan bagi menilai kesan intensiti cahaya dan kesesuaian media tanaman ke atas pertumbuhan, fisiologi, dan hasil biomas *V. amygdalina*. Rawatan untuk eksperimen di lapangan adalah terdiri daripada tiga tahap intensiti cahaya (30%, 50% dan 100% (bersamaan dengan lapangan terbuka)) dan empat jenis media tanaman (tanah, habuk kelapa, tandan buah kosong (EFB) dan sekam padi bakar (BPH). Anak pokok dibesarkan di nurseri dan dipindahkan ke lapangan 4 minggu selepas disemai. Pokok-pokok dituai pada minggu ke 18 selepas ditanam di lapangan (MST). Keputusan kajian menunjukkan *V. amygdalina* yang ditanam di bawah 50% intensiti cahaya dan menggunakan media tanaman EFB menunjukkan hasil biomas tertinggi (490 kg hektar^{-1}) dengan ketinggian pokok yang lebih tinggi (182 cm), bilangan cabang (3 cabang), diameter batang (22 mm), luas permukaan akar (3631 cm) dan kadar fotosintesis (22.6 μmolm^{-2}s^{-1}).

Eksperimen 2 dijalankan bagi menentukan masa tuaian yang sesuai bagi mendapatkan kandungan fitokimia yang optimum dalam daun *V. amygdalina.* Sampel daun daripada pokok yang ditanam dengan keperluan optimum intensiti cahaya dan media tanaman ke atas *V. amygdalina* (50% intensiti cahaya, EFB) diperolehi daripada daripada Eksperimen 1 telah digunakan dalam kajian ini. Eksperimen ini terdiri daripada enam rawatan masa tuaian (3, 6, 9, 12, 15 dan 18 MST). Keputusan kajian mendapati kandungan jumlah kandungan fenolik yang diperolehi ialah 91 dan 84 mg GAE/g berat kering dalam sampel daun

daripada pokok yang dituai pada 9 dan 18 MST. Pola yang sama didapati ke atas jumlah kandungan flavonoid di mana ia menunjukkan peningkatan bermula pada tempoh 3 hingga 9 MST, kemudian turun mendadak pada 12 MST dan meningkat semula sehingga 18 MST. Aktiviti antioksida adalah tinggi pada pokok yang dituai pada 9, 15 dan 18 MST. Semakin meningkat masa tuaian, kandungan jumlah klorofil dan nutrien yang diambil oleh pokok juga meningkat. Kandungan logam berat berada di bawah tahap yang dibenarkan oleh Pertubuhan Kesihatan Sedunia (WHO).

Eksperimen 3 dilaksanakan bagi menentukan kuantiti kandungan fitokimia dalam daun kering *V. amygdalina* pada tempoh penyimpanan yang berbeza. Eksperimen ini mengandungi tujuh rawatan tempoh penyimpanan (0, 3, 6, 9, 12, 15 dan 18 minggu). Keputusan kajian menunjukkan tempoh penyimpanan selama 12 minggu bagi daun kering *V. amygdalina* mencapai kestabilan fitokimia yang tinggi bagi kandungan jumlah fenolik dan flavonoid. Terdapat penurunan sebanyak 44% dan 58% bagi kandungan jumlah fenolik dan flavonoid sehingga tempoh penyimpanan selama 18 minggu. Kandungan nutrien juga menurun sehingga minggu ke 18 penyimpanan. Walau bagaimanapun, *V. amygdalina* boleh dijadikan sebagai sumber yang terbaik bagi nitrogen, kalium dan magnesium memandangkan kandungannya adalah melebihi peruntukan harian yang ditetapkan oleh WHO. Justeru itu, *V. amygdalina* adalah sesuai dituai pada minggu ke 18 MST untuk mendapatkan kandungan fitokimia yang optimum dengan kontaminasi logam berat yang minimum sebagaimana hasil biomas. Kandungan logam berat dan kontaminasi mikrob berada di bawah paras maksima yang dibenarkan oleh WHO. Oleh itu, daun kering *V. amygdalina* adalah selamat untuk digunakan sehingga tempoh penyimpanan selama 18 minggu.

ACKNOWLEDGEMENTS

In the name of Allah SWT, The most Beneficent, The most Merciful, in whom I always trust, for the wisdom he bestowed upon me, the patience, endurance and strength to finish my study.

First, I would like to express my special gratitude to my supervisor, Dr. Martini Mohammad Yusoff for her generous guidance, advices, support, patience and finding of her time for me in her busy schedule in completion of this thesis. It is a great honour to work under her supervision. “Dr., words can never be enough to thank your kindness”.

Sincere gratitude and thanks to my co-supervisors, Prof. Dr. Mahmud Tengku Muda Mohamed and Dr. Azizah Misran for imparting their knowledge and expertise, invaluable support and also guidance during this study. I am also highly indebted to my Ex co-supervisor, Assoc. Prof. Dr. Siti Aishah Hassan for giving the precious advices and very constructive criticisms regarding of my research.

It is a genuine pleasure to express my deepest gratitude towards my supportive family, especially my beloved mother, who served as my greatest inspiration and sources of my strength to pursue this undertaking.

I acknowledge with great pleasure to all of the staff of the Department of Crop Science and Department of Land Management, who always was ready to lend me a hand during my field and laboratory work. Without them, the success of this work would have not been achieved. I also gratefully acknowledge gratitude to the Ministry of Higher Education Malaysia for the financial support of this project provided under the Fundamental Research Grant Scheme (FRGS) Grant.

Last but not least, I would like to mention here that I am greatly indebted to my colleagues and people who has willingly helped me out with their abilities during my study but whose name does not find a place in this acknowledgement. I express my profound appreciation and sincere respect to all of them, thank you very much.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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cm	Centimetre
^{o}C	Degree celsius
π	Pi
β	Beta
g	Gram
h	Hour
µ	Micro
kg	Kilogram
L	Litre
MJ	Megajoule
m	Metre
min	Minute
mg	Miligram
mL	Millilitre
mm	Millimetre
nm	Nanometer
r	Radius
s	Second
v/v	Volume per volume
w	Weight
%	Percentage
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
Al	Aluminium
$AlCl_3$	Aluminium chloride
AOAC	Association of Official Agricultural Chemists
BPH	Burnt paddy husk
CO_2	Carbon dioxide
Ca	Calcium
CFU	Colony forming units
Chl	Chlorophyll
Cu	Copper
DPPH	1, 1-diphenyl-2-picrylhydrazyl
DW	Dry weight
EFB	Empty fruit bunch
EPP	Entry point project
ETP	The economic transformation programme
FAO	Food and Agricultural Organization
Fe	Iron
Fi	The fraction of incident PAR
FRAP	Ferric reducing antioxidant power

GAE	Gallic acid equivalent
GNI	Gross national income
Gs	Stomatal conductance
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
K	Potassium
LAI	Leaf area index
LDPE	Low-density polyethylene
LSD	Least significant difference
Mg	Magnesium
NKEA	National Key Economic Area
M	Molar
MPL	Maximum permissible limits
N	North
N	Nitrogen
NA	Nutrient agar
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
P	Phosphorus
PAR	Photosynthetically active radiation
Pb	Lead
PDA	Potato dextrose agar
Pn rate	Net photosynthesis rate
PSII	Photosystem II
QE	Quercetin
RDA	Recommended daily allowance
ROS	Reactive oxygen species
RuBP	Ribulose-1,5-bisphosphate
TFC	Total flavonoid content
TPC	Total phenolic content
S	South
SE	Standard error
SLA	Spesific leaf area
T	Transpiration rate
UV	Ultraviolet
WAT	Weeks after transplanted
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

Malaysia has been ranked 4th in Asia as the most biodiverse country due to the richness of the nation's various plant species, many of which have high medicinal values (Mohammad Azmin et al., 2016). A herbal plant is a plant species which has high medicinal value commonly utilized in herbal medicine. In herbal medicine, almost all plant parts such as leaves, barks, roots, seeds, flowers and fruits are valuable for therapeutic purposes (Craig, 1999). Due to the potential of many species of herbs, the goverment under the Agriculture National Key Economic Area (NKEA) in The Economic Transformation Programme (ETP) has included the herbal industry as one of the Entry Point Project (EPPs) marked as a new source of economic growth and sustainability in Malaysia. The initial phase of this EPP was targeted at commercializing five types of herbs, namely Kacip Fatimah, Tongkat Ali, Hempedu Bumi, Misai Kucing and Dukung Anak (Ministry of Agriculture and Agro-based Industry, 2011). This initiative supports the country to become a potential hub for high-value herbal products in order to generate high returns for gross national income (GNI) by year 2020 (Malaysia Investment Development Authority, 2016).

One of the potential herbal plants is *Vernonia amygdalina* from the Family Asteraceae, native to Africa and widely distributed in Asia and commonly found growing wild in Malaysia and Singapore (Wong et al., 2013). It is known as "*Bitter Leaf*" or "*Daun Bismillah*" which contains phytochemical compounds that are useful for pharmacological purposes (Ramachendrin, 2015). The leaves are green in colour with bitter taste and have their own characteristic aroma (Udochukwu et al., 2015). This herbal plant has been confirmed to show anti-cancer, anti-malarial, antibacterial, antiparasitic, antihelmitic, antithrombotic and antidiabetic properties (Audu et al., 2012). Traditionally, the leaves of *V. amygdalina* are consumed as vegetable or liquid extract (tonics) to cure fever, hiccups, anemia, kidney and stomach disorders and to relieve toothache and gingivitis as it acts as antimicrobial agent (Imaga & Bamigbetan, 2013).

Vernonia amygdalina is commonly consumed in Cameroon which has a high market demand for its products. The price of dried leaves has been sold at USD 7.5 by Afriproduct per kg in 2010. Besides, there were also health products that have been commercialized, namely Diabetes 5 and EdoTide Plus (Yeap et al., 2010). According to Atangwho et al. (2013), this herb was only recently found to grow wild in Malaysia and it is traditionally used in the control of hypertension and diabetes mellitus, but no commercial product has been developed. However, many studies on its nutritional properties, pharmacological, medicinal and antioxidant value have been reported in recent years (Kadiri & Olawoye, 2016).

Since the consumption of herbal products has been on the increase nowadays, this means that the supply of raw materials of this plant needs to be increased and be easily obtained by entrepreneurs and manufacturers. Production of high yielding *V. amygdalina* with higher phytochemicals content requires optimum agronomic as well as effective postharvest handling practices. Both of these factors are crucial since it can influence the growth, yield of herbal plants and quality of medicinal products (Fonseca et al., 2006).

Vernonia amygdalina has been found to grow in nature close to rivers and forest margins, and is classified as a drought tolerant plant although adequate water supply is more suitable for its growth (Oyeyemi et al., 2017). Presently, information on the commercial cultivation of this plant is rather scarce and the herbal plant has not been well studied. In consideration of abundance of phytochemicals in this herb for pharmacological purposes, determination of suitable crop agronomy practices for better plant growth and yield of phytochemical content is therefore important. The agronomic practices are related to crop management such as choice of growing media in relation to abiotic factors such as light, water supply, temperature and others.

Light is one of the abiotic factors that has long been known to influence not only plant performance, but also the production of both primary and secondary metabolites (Hemm et al., 2004; Liu et al., 2002). Photosynthetically active radiation (PAR) in quantum flux density in active wavebands of 400-700 nm are the determinants of plant growth and crop yield (Biscoe & Gallanger, 1977; Williams et al., 1965). Normal plant growth needs optimal light irradiance because excessively high and low irradiances would result in photo-inhibition and light deficiency, respectively (Nur Faezah et al., 2015). In addition, the incident quantum flux intercepted by a plant canopy determines the photosynthetic rate, hence its biomass yield (Hipps et al., 1983).

Growing media is one of the planting components needed to cultivate *V. amygdalina* as this herb has yet to be grown commercially by the farmers. Even though this herb is said to thrive easily in any types of soil, better growth on humus-rich soil has been reported by Oyeyemi et al. (2017). Recently, organic matter is gaining interest as a growing media for sustainable agricultural practices in order to reuse the agricultural waste, ensure better environmental safety issues and promote soil conservation (Yadav et al., 2013). Agricultural organic wastes have been reported as a great source of natural organic fertilizer with their biological properties in plant physiology, such as improving plant yield and ensuring sufficient nutrient uptake by the roots (Zandonadi et al., 2013). Growing media, apart from enhancing soil biological, physical and chemical properties also aids in increasing the production of phytochemicals in the plants. Soils amended with agricultural wastes as a source of organic nutrients have the ability to improve the nutritional value and phytochemical content in crops (Theunissen et al., 2010).

Other than the requirement of optimum light and growing media, harvesting time also influences the phytochemical compounds in herbs (Raya et al., 2015). The production of phytochemicals in medicinal plants was found to vary at different harvesting times since it is also influenced by hormonal and enzyme balance, carbohydrates, nutrients and water content in the plants (Brasileiro et al., 2015).

Plants are good sources of natural phytochemicals of antioxidants and secondary metabolites (Ghasemzadeh et al., 2010). There are an abundance of bioactive compounds in *V. amygdalina* such as sesquiterpene lactones, phenol, flavonoids, alkaloids, tannins, cyanogenic glycosides, saponins, steroid, phytate, anthraquinone and oxalate which have been reported (Udochukwu et al., 2015; Yeap et al., 2010). It is well known that the antioxidant activity of medicinal plants and herbs are contributed more by the phenolic and flavonoid compounds (Ferreira et al., 2007; Rodrigo et al., 2006).

In addition, *V. amygdalina* is prompt to immediate physiological and physical deterioration when harvested (Ejike & Ndukwu, 2017). Thus, appropriate postharvest handling during storage of dried herbs as well as storage duration are the most fundamental factors in maintaining plant metabolic activity instead of using synthetic antioxidants to preserve the medicinal plants before processing the plant raw materials into herbal products (Mediani et al., 2014). However, the environmental factors such as light, temperature and also microbial contamination could affect the capacity of dried materials to maintain their quality and shelf life during storage (Masand et al., 2014).

As *V. amygdalina* is a new potential herb in pharmacology, understanding its underlying process in agronomic practices in the manipulation of light intensity and growing media is desirable for developing package technology for the commercial cultivation of this herb, including the suitable preference of plant harvesting time and storage duration in order to obtain desirable quality of phytochemical compounds. The specific objectives of the study on *V. amygdalina* were as follows:

1. To evaluate the effects of optimum light intensity and suitable growing media on physiological parameters, growth and yield of *V. amygdalina*.
2. To determine the suitable harvesting time for optimum phytochemical content of *V. amygdalina.*
3. To quantify the phytochemical content of *V. amygdalina* at different storage duration.

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