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PHYSIOLOGICAL RESPONSES AND PHYTOCHEMICAL COMPOSITION OF GYNURA PROCUMBENS (LOUR.) MERR. AFFECTED BY SHADE AND PLANT DENSITY

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By

OMAR ALI AHMED

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

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DEDICATION

To the spirit of my late father. To my precious mother, my wife and children (Ahmed, Maryam, Abdulrahman and Ali), my brothers and sisters who stood behind me and were supporting me all the time.

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

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September 2020

Chairman: **Martini Mohammad Yusoff, PhD**
Faculty: **Agriculture**

Gynura procumbens (Lour.) Merr., locally known as *Sambung Nyawa*, has been documented to possess high phytochemicals. Light intensity and plant density are known parameters that affect composition and quantity of phytochemicals in plants. The present 3-part study examined the effects of these parameters on growth and development of *G. procumbens* aimed at producing higher biomass yield with consistently high secondary metabolite contents. The first experiment was conducted to determine the effects of four levels of shades (0, 30, 50 and 70%) on growth, physiological attributes, biomass yield and phytochemical contents using nested design with four replications. Results showed significant effects of shade levels on plants grown under 30% shade recording high total leaf fresh weight (TLFW), total fresh weight (TFW), total leaf dry weight (TLDW) and total dry weight (TDW), with increased number of branches and higher crop growth rate. Control treatment (0% of shade) revealed the lowest fresh and dry biomass yield in TLFW, TFW, TLDW and TDW corresponding to low net photosynthesis rate, total chlorophyll content, leaf area and number of branches. Total phenol, flavonoid contents, C/N ratio and antioxidant activities decreased with increase in shade levels. The highest phenol and flavonoid yields per plant were recorded from 30% shade producing high biomass yield, while high phytochemical contents and antioxidant activities were recorded from control plants. The second experiment was conducted to evaluate the effects of different shade levels (0 and 30% shade) and plant density (9, 15 and 25 plants m^{-2}) on shoot-root ratio (SRR) and its relationship with growth, physiology and phytochemical composition. Increasing shade level to 30% significantly affected shoot-root ratio (SRR), total phenolic content (TPC) and total flavonoid content (TFC). Increasing plant density from 9 to 25 plants m^{-2} resulted in significant decrease in SRR, whereas, TPC and TFC increased. Under stressed conditions in control and high plant density, size of above-ground parts was significantly reduced compared to below-ground parts which resulted in low SRR with high phytochemicals. In the third experiment, the effects of plant density and shade levels were evaluated on growth, physiological attributes, biomass yield and phytochemical contents using split-plot design with four replications. Results showed higher total leaf dry weight (TLDW) and total shoot dry weight (TShDW) from 30% shade with 9 plants m^{-2} density as reflected in high net photosynthesis rate, total chlorophyll content, leaf area and number of branches. Higher dry weight per square meter, TLDW m^{-2} and TShDW m^{-2} were observed from 25 plants m^{-2} density which were associated with higher leaf area index. High total phenol, total flavonoid and antioxidant activities were also detected due to high C:N ratio and low protein content. The highest yield in phytochemical was recorded from 25 plants m^{-2} density, implying that this density was the best approach for *G. procumbens* to balance the trade-off between biomass and quantity of secondary metabolites in achieving high photochemical contents with high biomass yield per unit area. In conclusion, the selection of appropriate light intensity and plant density improves both biomass yield and phytochemical composition of *G. procumbens*.

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

TINDAK BALAS FISIOLOGI DAN KOMPOSISI FITOKIMIA *Gynura procumbens* (Lour.) MERR. DIPENGARUH NAUNGAN DAN KEPADATAN TANAMAN

Oleh

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Gynura procumbens (Lour.) Merr., dengan nama tempatan *Sambung Nyawa,* telah didokumen sebagai mengandungi fitokimia yang tinggi. Keamatan cahaya dan kepadatan tanaman adalah parameter yang mempengaruhi komposisi dan kuantiti fitokimia tumbuhan. Kajian 3-bahagian ini meneliti kesan parameter tersebut ke atas pertumbuhan dan perkembangan *G. procumbens* bertujuan untuk menghasilkan biojisim dengan kandungan metabolit sekunder yang tinggi dan konsisten. Kajian pertama dijalankan untuk menentukan kesan empat paras naungan (0, 30, 50 dan 70%) ke atas pertumbuhan, sifat fisiologi, hasil biojisim dan kandungan fitokimia dalam reka bentuk *nested* dalam empat replikasi. Keputusan menunjukkan kesan ketara paras naungan ke atas tanaman di bawah naungan 30% dengan mencatatkan jumlah berat daun segar (TLFW), jumlah berat segar (TFW), jumlah berat kering daun (TLDW) dan jumlah berat kering (TDW) berserta penambahan bilangan dahan dan kadar tumbesaran tanaman yang tinggi. Rawatan kawalan (0% naungan) menghasilkan biojisim segar dan kering yang terendah bagi TLFW, TFW, TLDW dan TDW seiring dengan kadar fotosintesis bersih, jumlah kandungan klrofil, keluasan daun dan bilangan dahan yang rendah. Jumlah kandungan fenol dan flavonoid, nisbah C/N dan aktiviti antioksidan didapati mengurang dengan peningkatan paras naungan. Nilai tertinggi fenol dan flavonoid bagi setiap tanaman mencatatkan biojisim tertinggi daripada naungan 30%, sementara kandungan fitokimia dan aktiviti antioksidan yang tinggi dicatatkan daripada kawalan. Eksperimen kedua dijalankan untuk menilai kesan paras naungan (0 dan 30% naungan) dan kepadatan tanaman (9, 15 dan 25 pokok m^{-2}) ke atas nisbah pucuk-akar (SRR) dan hubungannya dengan pertumbuhan, fisiologi dan komposisi fitokimia. Peningkatan naungan ke 30% memberi kesan yang ketara ke atas nisbah pucuk-akar (SRR), jumlah kandungan fenol (TPC) dan jumlah kandungan flavonoid (TFC). Peningkatan kepadatan tanaman daripada 9 ke 25 tanaman m^{-2} menyebabkan pengurangan SRR, sementara TPC dan TFC meningkat dengan ketaranya. Dalam keadaan tekanan tanpa naungan dan kepadatan tanaman yang tinggi, saiz tanaman di bahagian atas tanah menyusut dengan ketara berbanding bahagian bawah menyebabkan SRR rendah, tetapi tinggi dalam fitokimia. Dalam eksperimen ketiga, kesan kepadatan tanaman dan paras naungan telah

dinilai dari segi pertumbuhan, sifat fisiologi, hasil biojisim dan kandungan fitokimia dengan menggunakan rekabentuk 'split-plot' dalam empat replikasi. Keputusan menunjukkan jumlah berat kering daun (TLDW) dan jumlah berat kering pucuk (TShDW) yang tinggi daripada paras naungan 30% dengan kepadatan 9 tanaman m^{-2} sepertimana yang dilihat dalam kadar fotosintesis bersih, jumlah kandungan klorofil, keluasan daun dan bilangan dahan. Berat kering per meter persegi, TLDW m^{-2} dan TShDW m^{-2} dicatatkan daripada kepadatan 25 tanaman m^{-2} yang berkaitan dengan indeks keluasan daun yang tinggi. Jumlah fenol, flavonoid dan aktiviti antioksidan yang tinggi juga direkodkan yang disebabkan oleh nisbah C: N yang tinggi dan kandungan protin yang rendah. Hasil tertinggi fitokimia yang telah direkodkan daripada kepadatan 25 tanaman m^{-2} memberi implikasi bahawa kepadatan ini adalah pendekatan yang terbaik bagi *G. procumbens* untuk mengimbangkan pertukaran antara biojisim dan kuantiti metabolit sekunder dalam menghasilkan kandungan fitokimia yang tinggi dengan hasil biojisim per meter persegi yang tinggi. Pada kesimpulannya, penentuan intensity cahaya yang sesuai dan kepadatan tanaman boleh memperbaiki kedua-dua penghasilan biojisim dan komposisi fitokimia *G. procumbens*.

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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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CHAPTER 1

INTRODUCTION

1.1 Background

Presently, medicinal plants play an important role in the provision of remedies for human ailments of much of the world's population, with undoubted increase in demand both herbal medicinal products and beneficial plant raw materials being traded. Medicinal plants have been truly valuable sources of herbal products and stand as important sources of food and medicines especially in developing countries (Chen et al., 2016). It has been recorded that more than 80% of population in developing countries are dependent on herbal drugs for their primary healthcare, and more than 25% of prescribed medicines in developed countries are resultant from wild plant species (Dubey et al., 2004; Hamilton, 2004; Jasemi et al., 2016). Recently, there exists a global trend of increasing acceptance for many popular and effective plant species in Europe, North America, and Asia, with growth in demand of between 8 and 15% per year due to increasing knowledge in herbal medicines because of fewer or no side-effects compared to conventional medicines (Grunwald & Buttel, 1996). In this context, world trade volume of medicinal plants has been recorded to be more than US$43 billion and has been predicted to reach US$5 trillion by the year 2050 (Mashayekhan et al., 2016; Niyaki et al., 2011).

Gynura procumbens is one of the well-known medicinal herbs found in Southeast Asia. It has long been used as a vegetable and medicinal plant (Yam et al., 2009). As traditional medicine, the leaves were used for treatment of diseases caused by oxidative stresses, such as inflammation, cancer, diabetes and hypertension as well as other general diseases (Perry & Metzger, 1980).

In Malaysia, fresh leaves are usually consumed raw as salads and also used as one of ingredients in a number of dishes (Hew & Gam, 2011). In Thailand, the leaves are commonly consumed raw or boiled and eaten with chili paste or used in curries, as well as garnishing or ingredient in soups and entrees. Presently, people in the Tropics are consuming an increasing amount of G. procumbens leaves, believing that this vegetable can cure several illnesses and diseases (Akowuah et al., 2002; Yusoff et al., 2019).

Literature has it that the leaves or leaf extracts of G. procumbens have numerous beneficial health properties including anti-herpes simplex virus (Nawawi et al., 1999), antihyperglycemic (Akowuah et al., 2002; Li et al., 2009), antihyperlipidemic (Zhang & Tan, 2000), anti-inflammatory (Iskander et al., 2002), anti-carcinogenic (Agustina et al., 2006), blood hypertension reduction capabilities (Hoe et al., 2007; Kim et al., 2006), anti-proliferative on human mesangial cells (Lee et al., 2007), antioxidative (Puangpronpitag et al., 2010; Yam et al., 2009; Yam et al., 2008), and antiulcerogenic (Mahmood et al., 2010). On the other hand, leaves of G. procumbens do not have any toxic effects (Rohin et al., 2018; Yam et al., 2009).

Undoubtedly, advances in plant biochemistry and herbal medicines bring forth the need for experimental evidence for the benefits derived from them. Over the past decades, hundreds of phytochemicals have been identified in plants (Khaled-Khodja et al., 2014). The most extensively studied group of plant secondary metabolites has been polyphenols including flavonoids, phenolic acids, stilbenes, lignans and others (Sung et al., 2016). In this regard, several studies have reported the presence of secondary metabolites with health benefits when consuming G. procumbens mainly related to a number of bioactive compounds such as saponins, flavonoids and terpenoids (Akowuah et al., 2002; Kaewseejan et al., 2015).

The quality of medicinal plants is mainly determined by their superior genotypes and high biomass yield with consistent secondary metabolite contents (Kozai et al., 2005). Generally, environmental factors have remarkable effects on secondary metabolite biosynthesis (Seigler, 1996). Due to the unpredictability of other abiotic factors, high quality medicinal plants can only be produced under controlled environments (Zobayed et al., 2005). Agronomic practices have been reported to be the key factor in influencing quantitative and qualitative characteristics of herbal medicines (Chikezie et al., 2015). The major advantages of growing plants under controlled environments include optimization of plant biomass and consistency, and sustenance of quality of bioactive compounds. Environmental factors such as light intensity can significantly improve growth and alter metabolite concentrations (Ma et al., 2010).

Light intensity which affects many physiological processes in plants is one of the most important environmental factors affecting plant's survival, growth, reproduction and distribution (Keller & Lüttge, 2005; Kumar et al., 2011). Phenol and flavonoid biosyntheses are enhanced by light. Flavonoid formation is definitely light-dependent, and its biosynthetic rate is related to light intensity (Ghasemzadeh et al., 2010). However, different plants respond differently to light intensity which results in differences in their production of secondary metabolites (Nasiri, 2016).

Plant density is also one of the important agronomic practices for optimal use of the environmental resources, as it has a major role in the regulation of plant competition within the plant canopy (Singh et al., 2006). Optimal plant population enhances the capacity of the canopy to capture environmental resources such as light, water, and nutrients (Rossini et al., 2011).

Due to various reported health benefits of G. procumbens, commercialization of the species appears to be a promising market. In this regard, optimization of plant productivity would be a promoting key to encourage investment. As earlier mentioned, agricultural practices have significant effects on medicinal plants yield and productivity. Despite their importance as yield-limiting factors in herbal crops, documented information on their effects on physiological responses, biomass yield and chemical compositions of G. procumbens under local conditions is scarcely available. This lack of information is the major hindrance in exploiting this economically important herb. Thus, further research on the issues is crucial and justified to be conducted.

1.2 Objectives of Study

1.2.1 General Objective

The general objective of the present study was to optimize cultivation practices for G. procumbens for high biomass and phytochemical contents under Malaysian conditions. This is an initiative to meet the ever-growing demand for G. procumbens as a natural source of food, natural antioxidants and alternative remedy for treatments of an assortment of health issues.

1.2.2 Specific Objectives of Study

The present study had the following specific objectives:

a) To determine growth, physiological responses, biomass yield and phytochemical contents of *G. procumbens* as affected by different shade levels.
b) To evaluate the effects of plant density on growth, physiological responses and secondary metabolites of *G. procumbens* at two different shade levels.
c) To investigate and determine the relationship between shoot and root systems on phytochemical contents of *G. procumbens* under different shade levels and planting density.

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LIST OF PUBLICATIONS

Ahmed, O. A., Yusoff, M. M., Misran, A., & Wahab, P. E. M. Growth and Physiological Responses to Shade and Nitrogen Fertilizer Levels on Gynura Procumbens.

Yusoff, M. M., Misran, A., Ahmed, O. A., Wan Majid, W. H. D., Wahab, P. E. M., & Ahmad, N. F. (2019). Gynura procumbens: Agronomic Practices and Future Prospects in Malaysia. *Pertanika Journal of Tropical Agricultural Science*, *42*(2).