



UNIVERSITI PUTRA MALAYSIA

***GROWTH RESPONSES AND SECONDARY METABOLITES
PROPERTIES OF THREE ACCESSIONS OF *Justicia Gendarussa* Burm
F. AFFECTED BY PLANT AGE, WATER STRESS AND CHICKEN
MANURE***

MOHD HAFIZAD BIN RAHMAT

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By

MOHD HAFIZAD BIN RAHMAT

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

December 2020

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DEDICATION

I dedicate this thesis to ALLAH for the guidance, strength and power of mind to complete this thesis.

I also would like to dedicate to my parents and my family, whose unconditional love, support, and encouragement have enriched my soul and inspired me to pursue and complete this research.



Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

GROWTH RESPONSES AND SECONDARY METABOLITES PROPERTIES OF THREE ACCESSIONS OF *Justicia Gendarussa* Burm F. AFFECTED BY PLANT AGE, WATER STRESS AND CHICKEN MANURE

By

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December 2020

Chair : Hawa ZE Jaafar, PhD
Faculty : Agriculture

Justicia gendarussa Burm F., (family: Acanthaceae) have been used to treat diseases in the traditional medicine. Many pharmacological studies have demonstrated that this plant is beneficial for human health and has the potential for commercialization due to its therapeutic properties. Morphological and secondary metabolites changes in plants can occur naturally during the growth periods as to be influenced by of environmental factors. Therefore, this study aimed to evaluate the effects of accessions, plant age, water stress, and chicken manure on the growth of *J. gendarussa* based on biomass, morphological characters, and secondary metabolites compounds (phenolic (TPC) and flavonoid (TFC))

Two glasshouse experiments were carried out at glasshouse complex at Field 2, Faculty of Agriculture, Universiti Putra Malaysia. The first experiment aimed to examine the effects of plant age on growth, gas exchange attributes, and secondary metabolites of three *J. gendarussa* accessions ('Black stem'; JGB, 'Green stem'; JGG, and, 'Variegata'; JGV). The treatments were laid in a randomized complete block design (RCBD) and blocked five times. The second experiment was design to evaluate the effect of water stress (100%, 75%, 50%, and 25% of evapotranspiration replacement, ET-R) and chicken manure application (0 kg N ha⁻¹ and 90 kg N ha⁻¹) on growth, gas exchange attributes as well as secondary metabolites of *J. gendarussa*. The treatments were laid out in RCBD with three blocks. The recorded parameters were measured within three regular intervals of every four weeks at 4, 8, and 12 WAT and were statistically analysis.

Among three accessions evaluated at three different plant ages, JGG exhibited the highest dry weight at each observation weeks (3.9 g to 11.5 g). This JG accession also produced highest growth (for example 42% to 162% higher in leaves number than other accessions) at 12 WAT. For the secondary metabolites evaluation had shown that

JGB exhibited a highest TPC and TFC at 8 WAT of plant ages (0.75 mg GAE g⁻¹ DW), and TFC (0.39 mg QE g⁻¹ DW). As plant became older (12 WAT), JGG exhibited a highest dry weight and total secondary metabolite production (TPC: 4.2 mg GAE; TFC: 2.60 mg QE). Therefore, JGG was then chosen to be used in the second experiment. The combined treatments of water stress and chicken manure did not affected growth as well as secondary metabolite production in JGG. However, water stress had significantly ($p \leq 0.01$) affected most of the growth, and physiological attributes of JGG which reduced to 3.5% to 7% as the water stress severity increased compared to control (4.4g). In contrast, secondary metabolites accumulations were increased under the water stress, especially at level of w_{50} , which demonstrated the highest total production in JGG at 12 WAT. The highest secondary metabolites production was recorded in JGG exposed to water stress at 50% ET-R (TPC: 0.9 mg GAE g⁻¹ DW dan TFC: 0.37 mg QE g⁻¹ DW). The chicken manure application at 90 kg N ha⁻¹ (n_{90}) had significant improved 2.7% of total dry weigh JGG. However, the chicken manure application had no significant ($p < 0.05$) effect on the secondary metabolites' accumulation and total production.

This study suggests that accessions, plant age, water stress, and chicken manure had significantly affected the growth and secondary metabolites of *J. gendarussa*. JGG harvest at age of 12 WAT and subjected to 50% ET-R of water stress level have shown to yield different amount of secondary metabolites production (TPC and TFC). Additional chicken manure application improved growth attributes of JGG. Thus, cultivation of JG based on the suggestion made from this study may increase its potential in bio business, which can further aid the socio-economic farming community in Malaysia.

Keywords: *Justicia gendarussa* Burm F., secondary metabolites, plant age, water stress, chicken manure

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

**RESPON PERTUMBUHAN DAN KANDUNGAN METABOLIT SEKUNDER
TIGA ASESI *Justicia gendarussa* Burm F. DIPENGARUHI OLEH UMUR
TANAMAN, KETEGASAN AIR DAN TINJA AYAM.**

Oleh

MOHD HAFIZAD BIN RAHMAT

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Justicia gendarussa Burm F., (keluarga: Acanthaceae) telah digunakan dalam perubatan tradisional untuk merawat penyakit. Banyak kajian farmakologi menunjukkan bahawa tanaman ini bermanfaat untuk kesihatan manusia dan berpotensi untuk dikomersialkan kerana sifat terapeutiknya. Perubahan morfologi dan kandungan metabolit sekunder pada tanaman boleh berlaku secara semula jadi semasa tempoh pertumbuhan akibat dipengaruhi oleh faktor persekitaran. Sehubungan dengan itu, kajian ini dilakukan bagi tujuan menilai kesan asesi, umur tanaman, ketegasan air, dan tinja ayam terhadap pertumbuhan *J.gendarussa* berdasarkan parameter biomas, morfologi, dan kandungan metabolit sekunder (fenolik (TPC) dan flavonoid (TFC)).

Dua eksperimen dijalankan di kompleks rumah kaca di Lapangan 2, Fakulti Pertanian, Universiti Putra Malaysia. Eksperimen pertama adalah mengkaji kesan umur tanaman terhadap pertumbuhan, atribut pertukaran gas, dan kandungan metabolit sekunder dari tiga jenis asesi *J. gendarussa* ('Batang hitam', 'Batang hijau', dan 'Variegata'). Reka bentuk adalah blok lengkap secara rawak (RCBD) dan di blok sebanyak lima kali. Eksperimen kedua dijalankan untuk mengkaji kesan ketegasan air (100%, 75%, 50%, dan 25% daripada kadar penggantian evapotranspirasi) dan pembajaan tinja ayam (0 kg N ha⁻¹ dan 90 kg N ha⁻¹) pada pertumbuhan, atribut pertukaran gas serta kandungan metabolit sekunder *J. gendarussa*. Rekabentuk RCBD di blok sebanyak tiga kali digunakan dalam eksperimen kedua ini. Parameter yang direkodkan secara tetap bagi setiap empat minggu pada 4, 8, dan 12 WAT dan data dianalisis menggunakan perisian statistik.

Di antara tiga asesi yang dinilai pada tiga usia tanaman yang berbeza, JGG merekodkan berat kering tertinggi pada setiap minggu pemerhatian (3.9 g hingga 11.5 g). Asesi JG ini juga menunjukkan pertumbuhan tertinggi (contohnya 42% hingga 162% lebih tinggi pada jumlah daun daripada aksesori lain) pada 12 WAT. Manakala penilaian

kandungan metabolit sekunder menunjukkan bahawa JGB mengandungi TPC dan TFC tertinggi pada 8 WAT usia tanaman ($0.75 \text{ mg GAE g}^{-1} \text{ DW}$), dan TFC ($0.39 \text{ mg QE g}^{-1} \text{ DW}$). Ketika tanaman menjadi lebih tua (12 WAT), JGG menghasilkan berat kering tertinggi dengan jumlah kandungan metabolit sekunder (TPC: 4.2 mg GAE ; TFC: 2.60 mg QE). Oleh yang demikian, aksesori JGG dipilih untuk digunakan dalam eksperimen kedua. Kombinasi rawatan ketegasan air dan tinja ayam tidak mempengaruhi pertumbuhan serta kandungan metabolit sekunder JGG. Walau bagaimanapun, ketegasan air secara signifikan ($p \leq 0.01$) mempengaruhi sebahagian besar pertumbuhan, dan parameter fisiologi JGG yang berkurang menjadi 3.5% hingga 7% setelah ketegasan air ditingkatkan berbanding dengan kawalan (4.4 g). Sebaliknya, penghasilan kandungan metabolit sekunder meningkat di bawah ketegasan air, terutama pada tahap w_{50} , yang menunjukkan jumlah tertinggi pada 12 WAT. Penghasilan metabolit sekunder tertinggi dicatatkan pada JGG yang terdedah kepada ketegasan air pada 50% ET-R (TPC: $0.9 \text{ mg GAE g}^{-1} \text{ DW}$; TFC: $0.37 \text{ mg QE g}^{-1} \text{ DW}$). Pembajaan tinja ayam pada $90 \text{ kg N ha}^{-1} (n_{90})$ menghasilkan peningkatan 2.7% yang signifikan daripada jumlah berat kering JGG. Walau bagaimanapun, pembajaan tinja ayam tidak mempunyai pengaruh yang signifikan ($p < 0.05$) terhadap penghasilan metabolit sekunder.

Kajian ini menunjukkan bahawa asesi, umur tanaman, ketegasan air, dan tinja ayam telah mempengaruhi pertumbuhan dan kandungan metabolit sekunder *J.gendarussa*. Penuaian JGG pada usia 12 WAT dan mengalami 50% ET-R dari tahap ketegasan air telah terbukti menghasilkan kandungan metabolit sekunder yang berbeza (TPC dan TFC). Pembajaan tinja ayam meningkatkan pertumbuhan JGG. Kesimpulannya, penanaman JG mengikut syor dari kajian ini dapat meningkatkan potensinya dalam perniagaan bio, yang dapat membantu sosio-ekonomi untuk petani di Malaysia.

Kata kunci: *Justicia gendarussa* Burm F., kandungan metabolit sekunder, umur tanaman, ketegasan air, tinja ayam

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I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of (Mohd Hafizad Bin Rahmat) on his thesis entitled “Growth Responses And Secondary Metabolites Properties of Three Accessions of *Justicia Gendarussa* Burm F. Affected by Plant Age, Water Stress and Chicken Manure” 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 Master of Science (Plant Science).

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	vii
LIST OF TABLES	xiv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xxiii
CHAPTER	
1 INTRODUCTION	1
1.1 Problem statements	1
1.2 Glasshouse crop production in Malaysia	2
1.3 General objectives	2
1.4 Specific objectives	2
1.5 Hypothesis	3
2 LITERATURE REVIEW	4
2.1 Medicinal plants	4
2.2 Acanthaceae	5
2.3 Genus <i>Justicia</i>	6
2.3.1 <i>Justicia gendarussa</i> Burm F.	7
2.3.2 Taxonomy of <i>J. gendarussa</i>	7
2.3.3 Morphological characteristics	8
2.3.4 The uses and medicinal properties of <i>Justicia gendarussa</i>	9
2.4 Plant secondary metabolites	10
2.4.1 Phenolic	10
2.4.2 Flavonoid	11
2.4.3 Biosynthesis of phenolic and flavonoid in plants	12
2.5 Effects of water stress and nutrient on plant growth	14
2.6 Effect of plant genotype and plant age on secondary metabolites	15
2.7 Effects of water stress and nutrient on secondary metabolites	16
2.8 Chicken manure fertilizer	17
3 CHANGES IN GROWTH, PHYSIOLOGY AND SECONDARY METABOLITES OF THREE <i>Justicia gendarussa</i> ACCESIONS AT DIFFERENT PLANT AGES UNDER GLASSHOUSE CONDITION	19
3.1 Introduction	19
3.2 Materials and Methods	20
3.2.1 Study site	20
3.2.2 Plant material	20
3.2.3 Growing media preparation	21
3.2.4 Experimental layout	21

3.2.5	Watering program	22
3.2.6	Fertilizer program	22
3.2.7	Pest control	23
3.2.8	Weeding	23
3.2.9	Microclimate measurement in a glasshouse	23
3.2.10	Measurement of growth characteristics	23
3.2.10.1	Plant height	23
3.2.10.2	Basal stem diameter	24
3.2.10.3	Total number of leaves	24
3.2.10.4	Total leaf area	24
3.2.10.5	Specific leaf area	24
3.2.10.6	Total dry weight	24
3.2.10.7	Root to shoot ratio	25
3.2.11	Measurements of physiological traits	25
3.2.11.1	Leaf gas exchange	25
3.2.11.2	Chlorophyll concentration	25
3.2.12	Biochemical parameters	26
3.2.12.1	Extraction of leaf samples for TPC and TF	26
3.2.12.2	Determination of total phenolic content	26
3.2.12.3	Determination of total flavonoid content	27
3.2.12.4	Total secondary metabolite production	27
3.2.13	Statistical analysis	27
3.3	Results and discussions	28
3.3.1	Changes in growth characteristics	28
3.3.1.1	Total dry weight	28
3.3.1.2	Dry weight allocation	29
3.3.1.3	Root to shoot ratio	31
3.3.1.4	Plant height	32
3.3.1.5	Basal stem diameter	33
3.3.1.6	Total number of leaves	34
3.3.1.7	Total leaf area	34
3.3.1.8	Specific leaf area	35
3.3.2	Changes in physiological traits	36
3.3.2.1	Photosynthetic rate	36
3.3.2.2	Stomata conductance	37
3.3.2.3	Chlorophyll concentration	38
3.3.3	Changes in secondary metabolites	39
3.3.3.1	Total phenolic content	40
3.3.3.2	Total flavonoid content	40
3.3.3.3	Total production of secondary metabolites	41
3.3.4	Relationship between growth and secondary metabolites concentration	43
3.3.5	Effect of interaction between accession and plant age on growth and physiology traits of <i>Justicia gendarussa</i>	45
3.3.6	Effect of interaction between accession and plant age on secondary metabolites in <i>Justicia gendarussa</i> leaves	46
3.4	Conclusion	47

4	INFLUENCE OF WATER STRESS LEVEL AND CHICKEN MANURE APPLICATION ON GROWTH, PHYSIOLOGY, AND SECONDARY METABOLITES (TPC AND TFC) OF <i>Justicia gendarussa</i> ACCESSION; ‘GREEN STEM’	49
4.1	Introduction	49
4.2	Materials and Methods	50
4.2.1	Study site	50
4.2.2	Plant material	50
4.2.3	Growing media preparation	50
4.2.4	Experiment layout	51
4.2.4.1	Watering treatment	51
4.2.4.2	Chicken manure treatment	52
4.2.5	Watering program	53
4.2.6	Fertilizer program	53
4.2.7	Pest control	53
4.2.8	Weeding	53
4.2.9	Glasshouse microclimate	53
4.2.10	Measurement of growth characteristics	53
4.2.11	Measurements of physiological traits	54
4.2.11.1	Chlorophyll fluorescence	54
4.2.11.2	Leaf water potential	54
4.2.11.3	Relative water content	54
4.2.12	Biochemical parameters	55
4.2.13	Plant nutrient analysis	55
4.2.14	Statistical analysis	55
4.3	Results and discussions	55
4.3.1	Changes in growth characteristics	55
4.3.1.1	Total dry weight	56
4.3.1.2	Dry weight allocation	57
4.3.1.3	Root to shoot ratio	60
4.3.1.4	Plant height	61
4.3.1.5	Basal stem diameter	62
4.3.1.6	Total number of leaves	63
4.3.1.7	Total leaf area	64
4.3.1.8	Specific leaf area	66
4.3.2	Changes in physiological traits	67
4.3.2.1	Water relation attributes	67
4.3.2.2	Photosynthetic rate	70
4.3.2.3	Stomata conductance	71
4.3.2.4	Chlorophyll concentration	72
4.3.2.5	Chlorophyll fluorescence	74
4.3.3	Changes in secondary metabolites	75
4.3.3.1	Total phenolic content	75
4.3.3.2	Total flavonoid content	76
4.3.3.3	Total production of secondary metabolites	78
4.3.4	Changes in nutrients content	80
4.3.5	Relationship between growth and secondary metabolites concentration	83
4.3.6	Effect of water stress and chicken manure application on growth and physiology	85

4.3.7	Effect water stress and chicken manure application on secondary metabolites	87
4.3.8	Effect of water stress and chicken manure application on nutrients content	88
4.4	Conclusion	88
5	SUMMARY, CONCLUSION AND FUTURE RECOMMENDATIONS FOR FUTURE RESEARCH	90
5.1	Summary	90
5.2	Conclusion	90
5.3	Recommendation for future research	91
	REFERENCES	92
	APPENDICES	110
	BIODATA OF STUDENT	128
	PUBLICATION	129

LIST OF TABLES

Table	Page
2.1 Example of some genera of Acanthaceae found around the world	6
2.2 Nutrient content in chicken manure	18
3.1 Effect of accession and plant age on leaves, stem and root dry weight of <i>Justicia gendarussa</i>	30
3.2 Pearson's correlation for growth and secondary metabolites parameters; n=45.	44
4.1 The quantity of water supplied based on water stress level treatments to each individual polybag containing <i>Justicia gendarussa</i> 'green stem' plants.	52
4.2 The quantity of chicken manure application of each polybag application	52
4.3 Effect of water stress or chicken manure application rate on leaves, stem and root dry weight of <i>Justicia gendarussa</i>	59
4.4 Total nitrogen content (N), phosphorus content (P), and potassium content (K) in <i>Justicia gendarussa</i> 'green stem' leaves as affected by water stress levels and chicken manure application harvested calculated at three different harvest age (4 WAT, 8WAT, 12 WAT); n=24.	82
4.5 Pearson's correlation coefficient between growth characteristic and secondary metabolites of JGG measured in the study; n=24	84
A 1 Mean square for the effects of accession and plant age at time of harvest, and their interactions on total weight, leaf dry weight, stem dry weight and root dry weight.	110
A 2 Mean square for the effects of accession and plant age at time of harvest, and their interactions on root to shoot ratio, plant height, and basal stem diameter.	110
A 3 Mean square for the effects of accession and plant age at time of harvest, and their interactions on total number of leaves, total leaf area, and specific leaf area.	111
A 4 Mean square for the effects of accession and plant age at time of harvest, and their interactions on photosynthetic rate, stomata conductance, and chlorophyll concentration.	111

A 5	Mean square for the effects of accession and plant age at time of harvest, and their interactions on total phenolic content, total flavonoid content, total production of phenolic content and total production of flavonoid content.	112
B 1	Mean square for the effects of water stress level and chicken manure application, and their interactions on total dry weight at 4, 8, and 12 WAT	113
B 2	Mean square for the effects of water stress level and chicken manure application, and their interactions on leaf dry weight at 4, 8, and 12 WAT	113
B 3	Mean square for the effects of water stress level and chicken manure application, and their interactions on stem dry weight at 4, 8, and 12 WAT	114
B 4	Mean square for the effects of water stress level and chicken manure application, and their interactions on root dry weight at 4, 8, and 12 WAT	114
B 5	Mean square for the effects of water stress level and chicken manure application, and their interactions on root to shoot ratio at 4, 8, and 12 WAT	115
B 6	Mean square for the effects of water stress level and chicken manure application, and their interactions on plant height at 4, 8, and 12 WAT	115
B 7	Mean square for the effects of water stress level and chicken manure application, and their interactions on basal stem diameter at 4, 8, and 12 WAT	116
B 8	Mean square for the effects of water stress level and chicken manure application, and their interactions on total number of leaves at 4, 8, and 12 WAT	116
B 9	Mean square for the effects of water stress level and chicken manure application, and their interactions on total leaf area at 4, 8, and 12 WAT	117
B 10	Mean square for the effects of water stress level and chicken manure application, and their interactions on specific leaf area at 4, 8, and 12 WAT	117
B 11	Mean square for the effects of water stress level and chicken manure application, and their interactions on relative water content at 4, 8, and 12 WAT	118

B 12	Mean square for the effects of water stress level and chicken manure application, and their interactions on leaf water potential at 4, 8, and 12 WAT	118
B 13	Mean square for the effects of water stress level and chicken manure application, and their interactions on photosynthetic rate at 4, 8, and 12 WAT	119
B 14	Mean square for the effects of water stress level and chicken manure application, and their interactions on stomata conductance at 4, 8, and 12 WAT	119
B 15	Mean square for the effects of water stress level and chicken manure application, and their interactions on total chlorophyll concentration at 4, 8, and 12 WAT	120
B 16	Mean square for the effects of water stress level and chicken manure application, and their interactions on chlorophyll fluorescence at 4, 8, and 12 WAT	120
B 17	Mean square for the effects of water stress level and chicken manure application, and their interactions on total phenolic content at 4, 8, and 12 WAT	121
B 18	Mean square for the effects of water stress level and chicken manure application, and their interactions on total flavonoid content at 4, 8, and 12 WAT	121
B 19	Mean square for the effects of water stress level and chicken manure application, and their interactions on total production of phenolic content at 4, 8, and 12 WAT	122
B 20	Mean square for the effects of water stress level and chicken manure application, and their interactions on total production of flavonoid content at 4, 8, and 12 WAT	122
B 21	Mean square for the effects of water stress level and chicken manure application, and their interactions on total nitrogen at 4, 8, and 12 WAT	123
B 22	Mean square for the effects of water stress level and chicken manure application, and their interactions on total phosphorus at 4, 8, and 12 WAT	123
B 23	Mean square for the effects of water stress level and chicken manure application, and their interactions on total potassium at 4, 8, and 12 WAT	124
E 1	Characteristics of Serdang series soil	127
E 2	Table N calculation of chicken manure fertilizer	127

LIST OF FIGURES

Figure		Page
2.1	Taxonomy hierarchy of <i>Justicia gendarussa</i>	7
2.2	<i>Justicia gendarussa</i> Burm F. Morphology; (1) flowering twigs, (2) flower, (3) stamens and pistil (Padua et al., 1999).	8
2.3	The generic structure of flavonoids	11
2.4	Structures of main classes of dietary flavonoids	12
2.5	Principle biosynthetic pathways leading to synthesis of secondary metabolites (Ramawat et al., 2009).	13
3.1	Three different <i>Justicia gendarussa</i> Burm F. Accessions used in the experiment namely: <i>J. gendarussa</i> 'Black stem' (a; SK2939/15), <i>J. Gendarussa</i> 'Green stem' (b; SK2940/15), and <i>J. gendarussa</i> 'Variegata' (c; SK2941/15). Each accession is differentiated by either the colour of the leaf petiole or midrib, or the colour the leaf lamina.	21
3.2	Experimental layout of combined treatments in a randomized complete block design (RCBD). Abbreviation: a1='Black stem'; a='Green stem'; a3='Variegata'; h=plant age; h ₁ = plant age at 4 WAT; h ₂ =plant age at 8 WAT; h ₃ =plant age at 12 WAT.	22
3.3	Total weight of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45.	28
3.4	Effect of accession on root to shoot ratio of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	31
3.5	Effect of plant age on root to shoot ratio of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	31
3.6	Effect of accession on plant height of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	32
3.7	Effect of plant age on plant height of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	32
3.8	Effect of (a) accession and (b) plant age on basal stem diameter of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates	

	mean with significant different using DMRT ($p \leq 0.05$). Total n=45	33
3.9	Effect of plant age on basal stem diameter of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	33
3.10	Total number of leaves of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45	34
3.11	Total leaf area of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45.	35
3.12	Specific leaf area of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45	35
3.13	Effect of accession on photosynthesis of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	36
3.14	Effect of plant age on photosynthesis of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	37
3.15	Effect of accession on stomata conductance of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	38
3.16	Effect of plant age on stomata conductance of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	38
3.17	Effect of accession on chlorophyll concentration of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	39
3.18	Effect of plant age on chlorophyll concentration of <i>Justicia gendarussa</i> . Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=45.	39
3.19	Total phenolic content of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=15	40
3.20	Total flavonoid content of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45	41

3.21	Total phenolic content production of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45.	42
3.22	Total flavonoid content production of <i>Justicia gendarussa</i> as affected by the interaction between accession and different plant ages. Bars represent standard error means. Total n=45.	42
4.1	Plants arrangement according to eight combined treatments on a randomized complete block design (RCBD) placed perpendicular to the sunlight movement to reduce errors. Abbreviation, n0=chicken manure application at 0 kg N ha ⁻¹ , n90=chicken manure application at 90 kg N ha ⁻¹ , w25= severe water stressed, w50=high water stressed, w75=moderate water stressed, w100=well-watered	51
4.2	Effect of water stress on total weight of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	56
4.3	Effect of chicken manure on total weight of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	57
4.4	Effect of water stress on root to shoot ratio of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	60
4.5	Effect of chicken manure on root to shoot ratio of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	60
4.6	Effect of water stress on plant height of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	61
4.7	Effect of chicken manure on plant height of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	61
4.8	Effect of water stress on basal stem diameter of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	62

4.9	Effect of chicken manure on basal stem diameter of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	63
4.10	Effect of water stress on number of leaves of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	64
4.11	Effect of chicken manure on number of leaves of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$	64
4.12	Effect of water stress on total leaf area of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	65
4.13	Effect of chicken manure on total leaf area of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	65
4.14	Effect of water stress on specific leaf area of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	66
4.15	Effect of chicken manure on specific leaf area of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$	67
4.16	Effect of water stress on relative water content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	68
4.17	Effect of chicken manure on relative water content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	68
4.18	Effect of water stress on leaf water potential of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	69

4.19	Effect of chicken manure on leaf water potential of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	69
4.20	Effect of water stress on photosynthetic rate of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	70
4.21	Effect of chicken manure on photosynthetic rate of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	71
4.22	Effect of water stress on stomata conductance of <i>Justicia gendarussa</i> . Each Harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	72
4.23	Effect of chicken manure on stomata conductance of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	72
4.24	Effect of water stress on chlorophyll concentration of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	73
4.25	Effect of chicken manure on chlorophyll concentration of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	73
4.26	Effect of water stress on chlorophyll fluorescence of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	74
4.27	Effect of chicken manure on chlorophyll fluorescence of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24.	75
4.28	Effect of water stress on total phenolic content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total n=24	76
4.29	Effect of chicken manure on total phenolic content of <i>Justicia</i>	

	<i>gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	76
4.30	Effect of water stress on total flavonoid content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	77
4.31	Effect of chicken manure on total flavonoid content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$	77
4.32	Effect of water stress on total production of phenolic content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	78
4.33	Effect of chicken manure on total production of phenolic content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph in each harvest indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	79
4.34	Effect of water stress on total production of flavonoid content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	79
4.35	Effect of water stress on total production of flavonoid content of <i>Justicia gendarussa</i> . Each harvest was analysed independently. Different alphabet in the bar graph indicates mean with significant different using DMRT ($p \leq 0.05$). Total $n=24$.	80
C.1	Calibration curve of chlorophyll concentrations against SPAD values for <i>Justicia gendarussa</i> ; 'Black stem' (JGB), 'Green stem' (JGG), Variegata (JGV); $n=10$.	125
D.1	The calibration graph for the quantification of phenolic in the sample using different concentration of Gallic acid measured at 760 nm; $n=5$.	126
D.2	The calibration graph for the quantification of flavonoid in the sample using different concentration of Quercetin measured at 593 nm; $n=5$.	126

LIST OF ABBREVIATIONS

%	percentage
$^{\circ}\text{c}$	degree Celsius
ANOVA	analysis of variance
c.v	coefficient variation
chl	chlorophyll
cm	centimetre
cm^2	centimetre square
DMRT	Duncan Multiple Range Test
DW	dry weight
ET-R	evapotranspiration replacement
fv/fm	PSII photochemical efficiency system
fw	fresh weight
g	gram
GAE	Gallic acid equivalent
ha	hectare
IRGA	infra-red gas analyser
JG	<i>Justicia gendarussa</i> Burm F.
JGB	'black stem'
JGG	'green stem'
JGV	'variegata'
kg N ha^{-1}	kilogram Nitrogen per hectare
M	molar
mg	milligram
ml	millilitre

n	number of samples
N	nitrogen
NOL	total number of leaves
n.s	non-significant
pH	measurement of acidity/alkalinity
QE	quercetin equivalent
r ²	coefficient of determination or r square
RCBD	randomized complete block design
RWC	relative water content
SEM	standard error of difference between means
SPAD	soil plant analysis development
TFC	total flavonoid content
TLA	total leaf area
TPC	total phenolic content
uv	ultra violet ray
v/v	volume per volume
WAT	weeks after treatment
w ₁₀₀	100% ET-R –well watered
w ₇₅	75% ET-R –moderate water stressed
w ₅₀	50% ET-R -high water stressed
w ₂₅	25% ET-R-severe water stressed
μmol m ⁻² s ⁻¹	micro mole per meter square per second

CHAPTER 1

INTRODUCTION

Medicinal plants have played a vital role in the traditional medicinal systems throughout the world to be used as a treatment against diseases. In Malaysia the increase in the use of herbal medicine has resulted in the expansion of the production from 2,176 hectares in 2014, to 2,312 ha increasing the yield of local medicinal plant to 11,649 tonnes (MOA, 2016). By 2020, the market value of the medicinal plants' industry in Malaysia is also projected to reach RM29 billion, with the annual growth rate of 15% (BERNAMA, 2013). Thus, the growing market of medicinal plants industry offers an opportunity as wealth creation, especially to Malaysian farmers to compete in the global markets.

One of the potential medicinal plants to be planted is *Justicia gendarussa*; a perennial plant from a family of Acanthaceae and for usually grows in a small area as ornamental plants and domestic uses. There are a number of accessions of *J. gendarussa* commonly found and available in Malaysia including 'Black Stem', 'Green stem', and 'Variegata'. In general, the fresh leaves of *J. gendarussa* have been used traditionally, pounded and applied topically, to treat inflammation, headache, flatulence (Ong et al., 2011), and stomach ache (Kulip, 2003).

1.1 Problem statements

To our knowledge, there is so far no comprehensive information on these agronomy practices of large-scale cultivation of *Justicia gendarussa* under glasshouse condition in Malaysia. As consequence, it has contributed to the production of low quantity and quality herbal products. Despite the long-term and widespread use of *J. gendarussa*, little information on secondary metabolites production is known especially for *J. gendarussa* accessions; 'Green stem' and 'Variegata' and each accession may contain different type of beneficial secondary metabolite compounds. In many cases, each plant genotype may differ in its responses to the agronomic practice. Hence, it is important to identify the best accessions containing high beneficial secondary metabolite compounds.

The other problem associated with *Justicia gendarussa* is that there are lack of knowledge and information about agronomic practice of specific medicinal herbs such as in *J. gendarussa*. A numbers of studies have shown that secondary metabolites compounds in plants can be induced according to different factors besides genotype, plant age, water availability, nutrient supply, etc. (Jaafar et al., 2012, Ibrahim et. al, 2013, Ghasemzadeh et. al, 2014). With regard to *J.gendarussa*, there is little published data on secondary metabolites compounds of accessions and its interaction with the environment. Therefore, this study was proposed to examine the cultivation of potential *J. gendarussa* accessions under glasshouse condition within the vegetative growth

periods with two important agronomic practices (water and organic nutrition) being determined.

1.2 Glasshouse crop production in Malaysia

In the tropics such Malaysia, agriculture sectors in particularly conventional open growing system always subjected to adverse climatic variation including temperature, rainfall, flooding and etc. (Alam et al., 2011). Controlled environment agriculture system plays an important role as an alternative to conventional open growing system in order to sustain agriculture productivity. Controlled environment system including glasshouse are designed to adapt the plant microclimate for optimizing plant growth and quality (Niu & Masabi, 2018).

Plant productions of some medicinal herb in controlled environmental system structures such as glasshouse have many advantages compared to open field (Ibrahim & Jaafar, 2011b). This production system warrants higher yield under better microclimatic condition with good water and nutritional control. Moreover, this production system not only make the planning and scheduling of production can be possible, but also could reduce or eliminated the contamination (diseases, insects, metals and other harmful factors) (Kozai, Kubota et al., 2000). Therefore, cultivation under a controlled environment can be considered an alternative way to ensure the safety and efficiency of the medicinal plant and its beneficial secondary metabolites.

1.3 General objectives

The main objective of this study was to evaluate the growth, physiology, and secondary metabolites production of three *Justicia gendarussa* accessions cultivated in soil medium under glasshouse conditions. The plant was evaluated at different plant age and was exposed to water stress and chicken manure application. For herbal plant growers, this study could help them to identify the best accessions of *J. gendarussa* that contains pharmacological active ingredient, and provide the growing information (plant age, water management, chicken manure application,) for large-scale cultivation. In economic perspective, producing this potential herb can diversify growers' income, increase their profits, and contribute to the enhancement of the development of national herbal industry.

1.4 Specific objectives

Two experiments were carried out with specific objectives:

1. To examine the changes in growth and physiological characteristics of three different *J. gendarussa* accessions leaves collected at three plant ages;

2. To determine the secondary metabolites (total phenolic content TPC and total flavonoid content, TFC) production of three different *J. gendarussa* accessions leaves collected at three plant ages;
3. To determine the interaction effects of water stress and chicken manure rates on growth, physiology, and production of secondary metabolites compounds (TPC and TFC) in *J. gendarussa*; and
4. To calculate the total productions of secondary metabolites compounds (TPC and TFC) of *J. gendarussa* extract as a result of water stress imposition and chicken manure rates.

1.5 Hypothesis

It was hypothesized that secondary metabolites concentrations of *J. gendarussa* accessions may vary at different age as determine by harvest period, and according to water stress and chicken manure application rate. This secondary metabolite compound would increase through introduction of water stress and low chicken manure application.

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