PHOTOSYNTHETIC ACTIVITY AND YIELD OF PEGAGA (*Hydrocotyle bonariensis*) GROWN UNDER NATURAL AND ARTIFICIAL LIGHT

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PHOTOSYNTHETIC ACTIVITY AND YIELD OF PEGAGA (*Hydrocotyle bonariensis*) GROWN UNDER NATURAL AND ARTIFICIAL LIGHT

ABSTRACT

Large leaf pennywort (*Hydrocotyle bonariensis*) also known as Pegaga in Malaysia, is an herb belonging to the family Araliaceae and commonly used as a culinary leafy vegetable and in preparation of traditional medicines to treat various symptoms of ophthalmic diseases and eczema. The plant is also used as an emetic, diuretic and laxative. With the increasing demand for *H. bonariensis* by the herbal industry, sourcing plant materials from natural habitats is no longer an economically viable or environmentally sustainable proposition. *H. bonariensis* is highly sensitive to direct sunlight exposure and water scarcity, which makes successful farming dependent on weather conditions and cultivation practices. Thus, indoor cultivation with optimized lighting system, pertinent water and nutritional supply will be ideal for large-scale production of *H. bonariensis.* Light-emitting diodes (LEDs) have emerged as one of the promising artificial lighting systems for the plant growth. LED offer technical advantages over traditional lighting sources such as high flexibility for customizing the light spectrum, lower thermal radiation emission, lower energy requirement and high safety performance. In this study, we investigated the influence of LED lighting on growth and photosynthesis of *H. bonariensis*. Three experimental LED lighting systems, a) Blue and Red (B+R), b) Blue, Red and Green (B+R+G), and c) Blue, Red and Ultraviolet (B+R+U) were compared with natural daylight (NL) for *H. bonariensis* growth and photosynthesis (20 biological replicates x 3 experimental replicates = 60 plants). Results showed plants grown under the B+R lighting system for a period of 50 days to have higher fresh and dry biomass (fresh biomass; 1180.04 ± 0.085 mg, dry biomass; 190.86 ± 0.002 mg) compared to

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B+R+G (fresh biomass; 665.84 ± 0.030 mg, dry biomass; 156.56 ± 0.004 mg), B+R+U (fresh biomass; 403.00 ± 0.002 mg, dry biomass; 120.50 ± 0.011 mg) and NL (fresh biomass; 436.00 ± 0.003 mg, dry biomass; 110.84 ± 0.007 mg). Furthermore, photosynthetic pigment content (Chl-a; 17.91 ± 0.14 µg/ml, Chl-b; 8.33 ± 0.19 µg/ml, carotenoids; 3.72 ± 0.38 µg/ml) in plants grown under the B+R lighting system was found to be much higher compared to that of plants grown under the other LED lighting combinations and under NL. The higher photosynthetic pigment and biomass followed by large leaf area (5.61 ± 0.04 cm^2), greater plant height (5.87 ± 0.638 cm), and higher number of leaves (17.50 ± 0.761) for plants grown under the B+R LED lighting system indicates that B+R lighting is the most suitable of the systems tested for efficient *H. bonariensis* farming.

Keywords: *Hydrocotyle bonariensis*, indoor cultivation, light-emitting diodes (LEDs), photosynthesis, plant growth.

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AKTIVITI FOTOSINTESIS DAN PENGHASILAN PEGAGA (*Hydrocotyle bonariensis*) YANG DITANAM DI BAWAH PENCAHAYAAN SEMULAJADI DAN PENCAHAYAAN BUATAN

ABSTRAK

Large leaf pennywort (*Hydrocotyle bonariensis*) yang juga dikenali sebagai Pegaga di Malaysia, adalah herba milik keluarga Araliaceae. Daun daripada tumbuhan ini lazimnya digunakan dalam masakan dan sebagai persediaan ubat-ubatan tradisional untuk merawat pelbagai gejala penyakit mata dan eksim. Tumbuhan ini juga digunakan sebagai emetik, diuretik dan julap. Dengan peningkatan permintaan untuk *H. bonariensis* dalam industri berasaskan herba, perolehan bekalan tumbuhan dari alam semulajadi tidak lagi ekonomik dan mapan. *H. bonariensis* sangat sensitif apabila terdedah secara langsung kepada cahaya matahari dan kekurangan air. Justeru, pertanian yang berjaya bergantung pada keadaan cuaca dan amalan penanaman. Oleh itu, penanaman dalam bilik menggunakan sistem lampu yang optimum, di samping bekalan air dan bekalan nutrisi yang sesuai adalah langkah yang ideal untuk mengusahakan penanaman *H. bonariensis* dalam skala besar. Diod pemancar cahaya (LED) merupakan salah satu sistem pencahayaan buatan yang berpotensi untuk pertumbuhan tanaman. LED menawarkan kelebihan teknikal berbanding sumber pencahayaan tradisional dari segi fleksibiliti yang tinggi untuk menyesuaikan spektrum cahaya, pelepasan radiasi haba yang lebih rendah, keperluan tenaga yang lebih rendah dan prestasi keselamatan yang tinggi. Dalam kajian ini, kami menyiasat pengaruh pencahayaan LED pada pertumbuhan dan fotosintesis *H. bonariensis*. Tiga sistem lampu LED telah dieksperimentasikan, a) Biru dan Merah (B+R), b) Biru, Merah dan Hijau (B+R+G), dan c) Biru, Merah dan Ultraviolet (B+R+U), dan ketiga-tiga sistem telah dibandingkan dengan cahaya semulajadi (NL) untuk

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pertumbuhan *H. bonariensis* dan fotosintesis (20 replika biologi x 3 percubaan eksperimen = 60 tumbuhan). Keputusan menunjukkan tumbuh-tumbuhan yang ditanam di bawah sistem pencahayaan B+R untuk tempoh 50 hari telah mencatatkan biomas segar dan kering yang lebih tinggi (biomas segar; 1180.04 ± 0.085 mg, biomas kering; 190.86 ± 0.002 mg) berbanding dengan B+R+G (biomas segar; 665.84 ± 0.030 mg, biomas kering; 156.56 ± 0.004 mg), B+R+U (biomas segar; 403.00 ± 0.002 mg, biomas kering; 120.50 ± 0.011 mg) dan NL (biomas segar; 436.00 ± 0.003 mg biomas kering; 110.84 ± 0.007 mg). Tambahan pula, kandungan pigmen fotosintetik (Ch-a, 17.91 ± 0.14 µg/ml, Ch-b, 8.33 ± 0.19 µg/ml, karotenoid; 3.72 ± 0.38 µg/ml) dalam tumbuhan yang ditanam di bawah sistem pencahayaan B+R lebih tinggi berbanding dengan tumbuhan yang ditanam di bawah kombinasi pencahayaan LED yang lain dan di bawah NL. Pigmen fotosintesis dan biomas yang lebih tinggi, diikuti dengan keluasan daun yang besar (5.61 ± 0.04 cm^2), tangkai yang panjang (5.87 ± 0.638 cm), dan jumlah daun yang lebih tinggi (17.50 ± 0.761) yang terdapat pada tumbuh-tumbuhan yang ditanam di bawah sistem lampu LED B+R menunjukkan bahawa sistem ini adalah paling sesuai untuk pertanian *H. bonariensis* yang lebih efisien.

Kata kunci: *Hydrocotyle bonariensis*, penanaman dalam bilik, diod pemancar cahaya (LEDs), fotosintesis, pertumbuhan tumbuhan.

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LIST OF SYMBOLS AND ABBREVIATIONS

µg	:	Microgram
µmol $m^{-2}s^{-1}$	:	Micromole per Second and Square Meter
˚C	:	Degrees Celsius
A_{665}	:	Absorbance at A_{665}
A_{649}	:	Absorbance at A_{649}
A_{480}	:	Absorbance at A_{480}
ATP	:	Adenosine triphosphate
B+R	:	Blue and Red
B+R+G	:	Blue, Red and Green
B+R+U	:	Blue, Red and Ultraviolet
Ca	:	Calcium
Chl-a	:	Chlorophyll a
Chl-b	:	Chlorophyll b
cm^2	:	Square Centimetre
cm	:	Centimetre
Cu	:	Copper
DLI	:	Daily Light Integral
DMSO	:	Dimethyl sulphoxide
ETP	:	Economic Transformation Programme
EPP	:	Entry Point Projects
Fe	:	Iron
GBIF	:	Global Biodiversity Information Facility
H. bonariensis	:	*Hydrocotyle bonariensis*

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HID	:	High-Intensity Discharge
LED	:	Light-Emitting Diodes
ml	:	Millilitre
Mg	:	Magnesium
mg	:	Milligram
NADPH	:	Nicotinamide Adenine Dinucleotide Phosphate
NKEA	:	National Key Economic Area
NL	:	Natural Daylight
PBF	:	Plant Biotech Facility
PSI	:	Photosystem-I
PS II	:	Photosystem-II
ROS	:	Reactive Oxygen Species
UV	:	Ultraviolet
Zn	:	Zinc

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CHAPTER 1: INTRODUCTION

Plants and herbs have been an integral part of traditional healthcare for centuries around the world. Indigenous peoples have been using an array of traditional herbs to heal a myriad of maladies (reviewed in Li et al., 2017). With the advancement in a civilization, direct use of plant products has been largely superseded with modern drugs which are based on individual active compounds with explicit modes of action derived from several plant products or analogues of these products (reviewed in Zahidin et al., 2017). Despite advances, modern drugs may fall short in providing efficacious remedies for some diseases with complicated roots, such as cancer, diabetes, neurological and autoimmune diseases, thus it has been suggested that the plant kingdom serves as an important resource for facilitating continuous new drug discovery (reviewed in Li et al., 2017). Traditional herbal medicines are developed by trial and error over thousands of years. Hence, ancient wisdom concentrated into traditional herbal medicines is a valuable resource to be explored.

Pennywort herb species, comprising more than 100 species, have been used as herbal medicines for centuries. The species of pennyworts have been known by an array of common names in different regions of the world such as pegaga (in Malaysia), Fo-titieng (in Chinese), indischer Wassernabel (in German), tsubo-kusa (in Japanese), hydrocotyle asiatique (in French), idrocotile (in Italian), hierba de clavo (in Spanish), daunkaki kuda (in Indonesia), gotu kola (in Sinhala), Brahamamanduki (in Hindi) and Mandukaparni (in Sanskrit) (Singh et al., 2010; Sudhakaran et al., 2017). Pennywort herb species include Asiatic pennywort (*Centella asiatica*), large leaf marsh pennywort (*Hydrocotyle bonariensis*), and lawn marsh pennywort (*Hydrocotyle sibthorpioides*) (Hashim, 2011;

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Maulidiani et al., 2012) are abundantly grown in Asia. Among them, *H. bonariensis* is the most abundantly grown in Malaysia (Maulidiani et al., 2012).

H. bonariensis, also known as large leaf marsh pennywort, has been used as food and a traditional therapeutic for centuries (Maulidiani et al., 2012). The chemically active compounds in *H. bonariensis* are terpenoids, flavonoids, alkaloids, tannin, saponin and sulfated polysaccharides (Ajani et al., 2009; Dantas-Santos et al., 2012; Tabopda et al., 2012). The most active compounds are the bonarisriosides, a class of terpenoids compounds, accumulated mainly in the leaf and effective in treating colon cancer cell lines leaf (Tabopda et al., 2012). The species *H. bonariensis* has been known by an array of common names in different regions of the world such as pegaga (in Malaysia) and Karo (in Nigeria). *H. bonariensis* prefers to grow in swampy areas in temperate and tropical regions and wetlands (Florinsiah et al., 2013; Masoumian et al., 2011a). This plant also can be found in Africa and America and inhabits mostly beach dunes, moist, open sandy areas, wet ditches and edges of ponds (reviewed in Ajani et al., 2009). The Indigenous people of the United States of America use preparation of this herb used as emetics, diuretics and laxatives (Evans, 1992); the herb is also used in treating various symptoms of ophthalmic diseases among some local populations in Western Nigeria (Edeoga et al., 2005), and for the treatment of tuberculosis, relieving the pain of rheumatism and arthritis, to increase brain capacity and for longevity (Masoumian et al., 2011a). In addition to medicinal usage, *H. bonariensis* is also popular as a culinary vegetable in several parts of Asia. In Malaysia, *H. bonariensis* is consumed by people of Malay, Chinese and Indian ethnicity in Malaysia as "ulam" (fresh green salad) or juice (Reihani et al., 2012), whereas in Taiwan, *H. bonariensis* is used in the preparation of a popular folk drink known as Pai-Tsao-Tsa (Huang et al., 2008).

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Recent policies in Malaysia have aimed towards capitalizing on its mega biodiversity and thus, the Malaysian government has listed high-value herbal-derived products as one of Entry Point Projects (EPPs) within the Agriculture National Key Area (NKEA) under the Economic Transformation Programme (ETP) (PEMANDU, 2010). The primary aims of the EPP are to develop products that can penetrate the global high-end market segment, which will be possible if the R&D outputs are validated by clinical studies and the products contain standardized extracts (PEMANDU, 2010). To achieve the objectives, the initial phase of this EPP was launched aiming to support upstream and downstream activities of high-value local product development containing ten traditional plant species including pegaga (ETP Annual Report, 2013).

With the increasing demand for pennyworth by the herbal industry, sourcing plant materials from natural habitats is no longer an economically viable proposition (reviewed by Hashim, 2011; Lokanathan et al., 2016). Furthermore, pegaga is highly sensitive to direct sunlight exposure and water scarcity, which makes it unsuitable for cultivation in open agricultural areas (James et al., 2009; reviewed by Hashim, 2011). Hence, steady large-scale enclosed cultivation is necessary to meet the exponentially increasing demand of the industry, while protecting natural habitats from overexploitation. Furthermore, *H. bonariensis* recorded seeding survival rates less than 1% and it has been reported that the reproduction strategies are primarily via vegetative clonal growth (Joesting et al., 2012).

Based on the above, it is proposed that an indoor facility with an optimized lightening system with pertinent water and nutritional supply will be an ideal option for large-scale production of *H. bonariensis*. This is because the light environment influences critical developmental and phytochemical pathways in plants. Photoreceptors, a specialized pigment protein in plants function to absorb solar radiation in order to signal photomorphogenic responses to help plants adapt to changes within their light

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environment (Kong & Okajima, 2016). Thus this project was designed to test the hypothesis that different combination of LED light wavelengths has different effects on the photosynthetic activity, growth rates, and biomass accumulation in *H. bonariensis*.

The main objective of this project was to evaluate the impact of selected sets of LED light source on the chlorophyll content and biomass accumulation in *H. bonariensis* with an aim to provide practical knowledge for optimised growth of this plant to support the Malaysian herbal industry.

The specific objectives of this project were:

1. To determine the photosynthetic activity of *H. bonariensis* under three different LED light spectra and under natural daylight by measuring chlorophyll and carotenoid content.
2. To establish the growth rates of *H. bonariensis* under three different LED light spectra and under natural daylight.
3. To compare biomass accumulation of *H. bonariensis* under three different LED light spectra and under natural daylight.

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CHAPTER 2: LITERATURE REVIEW

2.1 *Hydrocotyle bonariensis*

Hydrocotyle bonariensis is a large leaf marsh pennywort species (Figure 2.1). The plant has been used as a food and as a traditional therapeutic for centuries (Maulidiani et al., 2012). This herb is used traditionally to treat ophthalmic diseases (Edeoga et al., 2005), tuberculosis, and for relieving the pain of rheumatism and arthritis (Masoumian et al., 2011a).

Figure 2.1: Study plant: *Hydrocotyle bonariensis*.

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2.2 Taxonomic classification of *H. bonariensis*

Hydrocotyle bonariensis is a member of a family Araliaceae. The details of the classification are described in Box 2.1.

Box 2.1: Classification of *H. bonariensis*. (Retrieved from the Integrated Taxonomic Information System on-line database).

Kingdom	: Plantae – plantes, Planta, Vegetal, plants
Subkingdom	: Viridiplantae – green plants
Infrakingdom	: Streptophyta – land plants
Superdivision	: Embryophyta
Division	: Tracheophyta – vascular plants, tracheophytes
Subdivision	: Spermatophytina – spermatophytes, seed plants, phanérogames
Class	: Magnoliopsida
Superorder	: Asteranae
Order	: Apiales
Family	: Araliaceae
Genus	: *Hydrocotyle*
Species	: *Hydrocotyle bonariensis* – largeleaf pennywort

2.3 Origin and geographical distribution of *H. bonariensis*

H. bonariensis colonises a wide range of habitats around the world. It is distributed near beach dunes, moist, open sandy areas, wet ditches and at the edges of ponds (reviewed in Ajani et al., 2009). This plant is native to tropical and subtropical regions of Northern America, Southern America and Africa (USDA, Agricultural Research Service, National Plant Germplasm System, 2019). This plant also found in Asian countries such as China, Indo-China, India, Sri Lanka, Indonesia as well as in Malaysia (Goh, 2007).

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However, there is no clear scientific evidence on how this plant was introduced to each country (Goh, 2007). The native occurrence of this species has been recorded and presented by the Global Biodiversity Information Facility (GBIF) in their online database. (Figure 2.2).

Figure 2.2: Occurrence of *H. bonariensis* throughout the world. Source: Global Biodiversity Information Facility (GBIF). (https://www.gbif.org/species/3034611).

2.4 Botanical description

J. P. Tournefort was the first to introduce the genus *Hydrocotyle (*Hylander, 1945*)*. The genus 'Hydrocotyle' is derived from a Greek words 'hydro' meaning water and 'kotyle' meaning dish or plate, referring to the aquatic habitat and dish or plate-shaped leaf of the species (reviewed in Bandara et al., 2011). The genus was later validated and expanded by C. Linnaeus in 1753 (Konstantinova & Yembaturova, 2010). The name of the species *H. bonariensis* was derived from a Latin word Bonariensis meaning 'from Buenos Aires, Argentina' as they are a native species of Argentina that grows in the province of Buenos Aires on the Rioplatense coast. *H. bonariensis* is a semiaquatic perennial herb with long stolons (runners), from which roots, leaves and inflorescences are produced at nodes (Figure 2.3A). The nodes can act as physiologically independent

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units and can be propagated vegetatively (Evans & Whitney, 1992). The leaves are circular to widely elliptical, with palmate venation, arranged in small clusters of 1 to 5, of glossy leaflets, 1.2 to 4 cm in diameter and attached by a petiole of 2.0 to 37.5 cm long (Figure 2.3B). Inflorescences are produced opposite the leaf of a node (Figure 2.3C). The inflorescence is umbelliferous and about 5.08–7.62 cm long with clusters of white fragrant flowers. The hermaphroditic flower contains 5 calyx, petals, stamens and 2 separate carpels with inferior ovary surmounted by a style and a stigma (Figure 2.3C). From the flowers arise green schizocarp fruit which turn bright yellow when mature (Figure 2.3D-E).

2.5 Nutrient composition and bioactive compounds

Although *H. bonariensis* has been used traditionally as food for centuries, little is known about the macronutrient composition (Table 2.1). The primary micronutrients found in *H. bonariensis* have been reported as Ca, Mg, Fe, Zn and Cu (Table 2.1). The phenolic compound tannin was also reported in *H. bonariensis* (Ajani et al., 2009).

The major bioactive compounds reported in *H. bonariensis* are terpenoids, flavonoids, alkaloids, tannin, saponin and sulfated polysaccharides (Ajani et al., 2009; Dantas-Santos et al., 2012; Maulidiani et al., 2014; Tabopda et al., 2012;). Amongst the terpenoids, bonarienosides is the only chemical constituent identified in *H. bonariensis* that has been demonstrated to have a cytotoxic effect on human colon cancer lines HCT 116 and HT-29 (Tabopda et al., 2012) (Table 2.2). In addition, there is only one *in vivo* study reported on the effect of *H. bonariensis* extracts on the remediation of cataract onset in albino rats (Ajani et al., 2009) (Table 2.2). Sulfated polysaccharides are well known for their pharmacological activities such as anticoagulant, antioxidant, antiproliferative,

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antitumoral, anticomplementary, anti-inflammatory, and antiviral properties (Dantas-Santos et al., 2012).

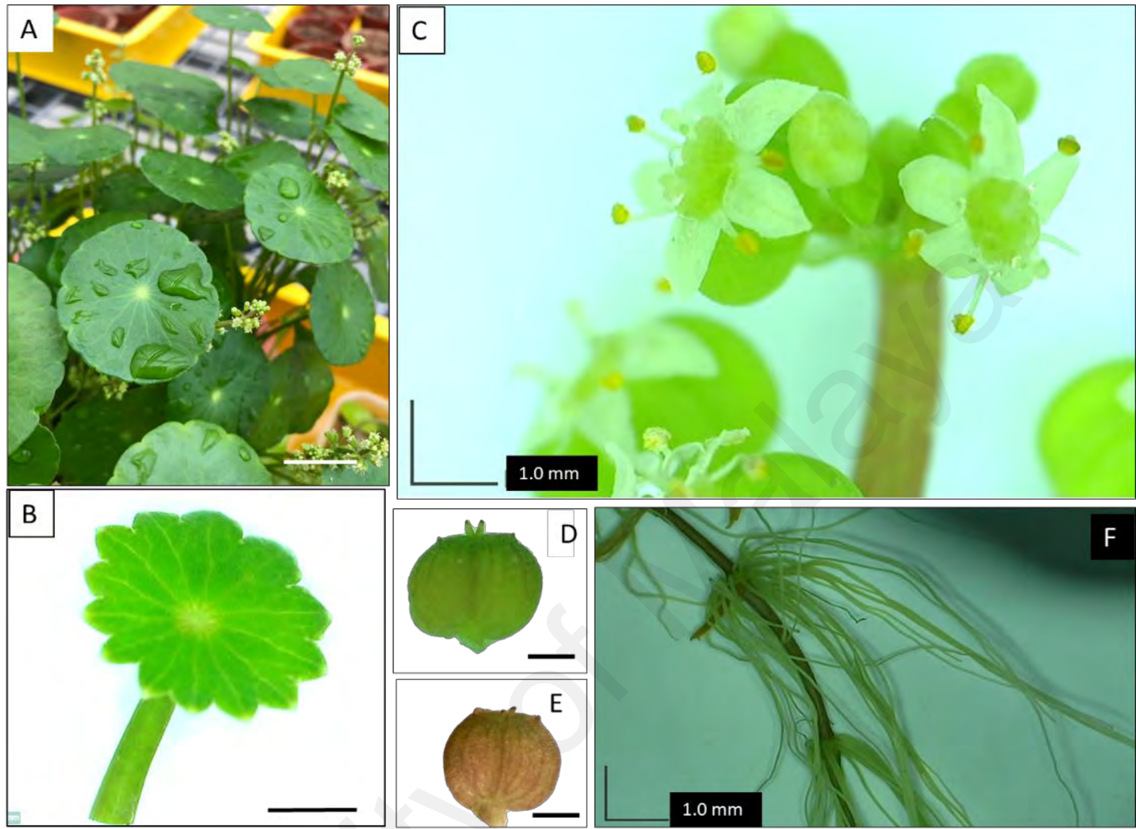

Figure 2.3: Botanical description of *Hydrocotyle bonariensis.* (A) *H. bonariensis* plant with inflorescence in the pot(40x, bar=40mm) ; (B) Microscopic view of *H. bonariensis* leaf (40x, bar=30mm); (C) Compound umbel inflorescence of *H. bonariensis* (40x, bar=1mm); (D) The indehiscent schizocarp of *H. bonariensis* (40x, bar=1mm); (E) Mature schizocarp *H. bonariensis* (40x, bar=1mm); (F) Root of the *H. bonariensis* (40x, bar=1mm).

Flavonoids are yellow pigments present in plants and also collectively known as vitamin P and citrin (Imohiosen et al., 2014). Flavonoids act as an antioxidant agent in the human body by scavenging or chelating free-radicals (Schmitt-Schillig et al., 2005; Chandrika et al., 2015). The description of the known phytochemicals isolated from *H. bonariensis* from previous studies is summarized in Table 2.3.

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Several strategies have been implemented to enhance the levels of beneficial phytochemicals in crop plants. Methods tested range from the exogenous application of chemical inducer (reviewed in Alothman et al., 2009) to genetic manipulation of metabolic pathway-associated genes (Singh, 2016). Masoumian et al. (2011b) reported accelerated flavonoid production of *in vitro* leaf-callus tissues *H. bonariensis* by supplementing precursors of flavonoids such as phenylalanine, proline, glutamine and naringenin at different concentrations. The study found that the callus that produced the highest flavonoid content was grown on the medium containing, either 3mg/l phenylalanine (11.4 mg/g dry weight), 4mg/l proline (10.7 mg/g dry weight), 1mg/l of glutamine (10.5 mg/g dry weight) or 4mg/l naringenin (10.1 mg/g dry weight).

Table 2.1: Phytonutrient content of *H. bonariensis*.

Phytonutrients of *H. bonariensis*	Concentration	References
Ca	60%	Monyn et al., 2016
Mg	35%	
Fe	3%	
Zn	1%	
Cu	1%	

Table 2.2: Functional studies on *H. bonariensis*.

Type of studies	Functional studies	References
In vivo	*Hydrocotyle bonariensis* protects against galactose-induced cataract, and that administration of the extract after cataract onset reduced cataract progression	Ajani et al., 2009
In vitro	Two compounds showed weak cytotoxicity with IC_{50} 24.1 and 24.0, 83.0 and 83.6 µM against HT-29 and HCT 116, respectively.	Tabopda et al., 2012

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Table 2.3: Phytochemical content reported for *H. bonariensis*.

Group	Chemical structure	Molecular formula/ (Molecular weight)	Chemical Name (Common name)	Reference (PubChem CID)
Saponins		$C_{54}H_{86}O_{23}$ (1103.26g/mol)	3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-l-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}-21-*O*-(2-methylbutyroyl)-22-*O*-acetyl-R_1-barrigenol (Common name: bonarienoside A)	Tabopda et al., 2012 (PubChem CID: 56951544)
		$C_{54}H_{86}O_{23}$ (1103.26g/mol)	3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-l-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}-21-*O*-(2-methylbutyroyl)-28-*O*-acetyl-R_1-barrigenol (Common name: bonarienoside B)	Tabopda et al., 2012 (PubChem CID: 56951656)

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	$C_{49}H_{78}O_{22}$ (1019.14g/mol)	3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-l-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}-21-*O*-acetyl-R_1-barrigenol (Common name: bonarienoside C)	Tabopda et al., 2012 (PubChem CID: 56951657)
	$C_{47}H_{76}O_{21}$ (977.10 g/mol)	3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-l-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}-R_1-barrigenol (Common name: bonarienoside D)	Tabopda et al., 2012 (PubChem CID: 56949673)
	$C_{52}H_{84}O_{21}$ (1045.22 g/mol)	3-*O*-{β-D-glucopyranosyl-(1 → 2)-[α-l-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranosyl}-22-*O*-(2-methylbutyroyl)-A_1-barrigenol (Common name: bonarienoside E)	Tabopda et al., 2012 (PubChem CID: 56949674)

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		$C_{52}H_{84}O_{21}$ (1045.22g/mol)	21-*O*-[2-methylbutanoyl]-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene 3-*O*-[α-L-arabinopyranosyl(1→3)]β-D-glucopyranosyl (1→2)-beta-D-glucuronopyranoside (Common name: saniculoside-R1)	Tabopda et al., 2012, Schöpke et al., 1998 (PubChem CID: 9491771)
Flavonols		$C_{27}H_{30}O_{16}$ (610.52 g/mol)	Quercetin-rutinoside	Maulidiani et al., 2014 (PubChem CID: 124221768)

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	$C_{26}H_{28}O_{16}$ (596.49 g/mol)	Quercetin-3-*O*-pentosyl-7-*O*-hexoside	Maulidiani et al., 2014 (PubChem CID: 133053374)
	$C_{27}H_{30}O_{15}$ (594.52 g/mol)	Kaempferol-3-*O*-glucoside 7-*O*-rhamnoside	Maulidiani et al., 2014 (PubChem CID: 14035324)

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	$C_{21}H_{20}O_{12}$ (466.39 g/mol)	Quercetin-3-*O*-glucoside	Maulidiani et al., 2014 (PubChem CID: 5280804
	$C_{35}H_{60}O_6$ (576.86 g/mol)	3-*O*-β-D-glucopyranosyl-sitosterol	Ajani et al., 2017 (PubChem CID: 5742590)
	$C_{15}H_{10}O_7$ (302.24 g/mol)	Quercetin	Ajani et al., 2017 (PubChem CID: 5280343)

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Tripertenes		$C_{41}H_{68}O_{15}$ (800.98 g/mol)	3β,15α,16α,21β,22*x*,28-hexahydroxy-Δ^{12}-oleanane-3-*O*-[α-L-arabinopyranosyl-(1→ 6)]-β-D-glucopyranoside (Common name: Ranuncoside I)	Maulidiani et al., 2014, Greca et al., 1994 (PubChem CID: 101672524)
		$C_{47}H_{78}O_{20}$ (963.12 g/mol	)3β,15α,16*x*,21β,22α,28-hexahydroxy-Δ^{12}-oleanane-3-*O*-[α-L-arabinopyranosyl-(1→ 6)]-β-D-glucopyranosyl-(1→ 2)- β- D-glucopyranoside (Common name: Ranuncoside II)	Maulidiani et al., 2014, Greca et al., 1994 (PubChem CID: 101673080)

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	$C_{54}H_{86}O_{22}$ (1087.26 g/mol)	3β,15α,16α,21β,22α,28-hexahydroxy-Δ^{12}-oleanane-21-*O*-tigloyl-22acetyl-3-*O*-[α-L-arabinopyranosyl-(1→ 6)][β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside (Common name: Ranuncoside IV)	Maulidiani et al., 2014, Greca et al., 1994 (PubChem CID: 101672525)
	$C_{54}H_{88}O_{22}$ (1089.28 g/mol)	3β,15α,16α,21β,22α,28-hexahydroxy-Δ^{12}-oleanane-21-*O*-[2-methylbutyroyl)-28-*O*-acetyl-3-*O*-[α-L-arabinopyranosyl-(1→ 6)][β-D-glucopyranosyl-(1 → 2)]- β-D-glucopyranoside (Common name: Ranuncoside V)	Maulidiani et al., 2014, Greca et al., 1994 (PubChem CID: 101672526)

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2.6 Effect of environmental factors on *H. bonariensis*

H. bonariensis commonly inhabits both coastal sand dunes and inland coastal areas. These environments differ in many aspects: in coastal sand dune, the plants are exposed to high growing season air and sand temperatures, high incident sunlight, salt spray, and periodic saltwater inundation. In contrast, plants grown in inland coastal areas are exposed to more variable temperature and incident sunlight due to increased canopy cover. The ability of *H. bonariensis* to thrive in two different environments is directly linked to the phenotypic plasticity characteristic of the plant (reviewed in Chiarello et al., 2016). Phenotypic plasticity is defined as the ability of an organism to adjust its morphology and/or physiology in response to variations in abiotic factors.

Based on the research reported by Chiarello et al. (2016), *H. bonariensis* grown in soil from inland coastal areas recorded greater leaf area, petiole fresh weight, petiole thickness, petiole length, and abaxial stomata density compared to the plants grown in sand dune soil. This indicates that soil characteristics and chemistry play a pivotal role for the plant to thrive and reproduce successfully. It was reported that soil from inland coastal areas consists of loamy sand, composed of 81.9% sand, 11.8% silt, and 6.3% clay, and was more acidic with higher organic matter, potassium, nitrate and ammonium content compared to sand dune soil. Meanwhile, sand dune soil consists of 100% sand and had lower organic matter, potassium, nitrate, and ammonium but higher phosphorus content compared to inland coastal soil. Based on the soil composition, greater organic matter content and the resulting greater potential water holding capacity and nutrient availability in inland coastal soil may have led to greater photosynthesis, carbon gain, and growth of *H. bonariensis*.

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In addition, *H. bonariensis* that inhabits coastal sand dunes have been exposed to a harsh environment for plant growth and reproduction (Joesting et al., 2011). The sand dune environment is exposed with high incident sunlight in combination with abiotic stress factors including sand burial and abrasion, salt spray, high wind, periodic inundation by saltwater, substrate temperatures over 50 ˚C, and maximum growing season air temperatures up to 40 ˚C. These factors may contribute to risk of reduced photosynthetic efficiency during the growing season. In order to sustain in this environment, the plant should possess certain adaption for their survival. Leaf inclination is the strategy adopted by *H. bonariensis* to reduce midday leaf-level sunlight incidence, leaf temperature, and photoinhibition in the sand dune environment (reviewed in Joesting et al., 2016). Leaf inclination reduces the total daily amount of sunlight incidence and altered the diurnal distribution pattern of sunlight incidence on leaf surfaces compared to horizontal leaf surfaces (Joesting et al., 2016).

2.7 Photosynthesis and light

2.7.1 Photosynthesis

Photosynthesis by plants is a process which converts water (H_2O) and carbon dioxide (CO_2) into oxygen and complex organic molecules such as carbohydrates. In plants, photosynthesis occurs in two separate reactions which are called as the ‘light’ and ‘dark’ reactions. In the light reaction, water is split using light into oxygen, protons and electrons, and in the dark reaction, the protons and electrons are used to reduce CO_2 to carbohydrates (reviewed in Sujatha, 2015). The chemical equations are represented as below:

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Light reaction: $2H_2O + light \rightarrow O_2 + 4H^+ + 4e^-$

Dark reaction: $CO_2 + 4H^+ + 4e^- \rightarrow CH_2O + H_2O$

Overall: $H_2O + light + CO_2 \rightarrow CH_2O + O_2$

2.7.2 Photosynthetic pigments

Photosynthesis in plants begins with the absorption of light by pigment molecules located in the thylakoid membrane. The main photosynthetic pigments are chlorophyll and carotenoids (Johnson, 2016). Five types of chlorophyll molecules have been identified in photosynthetic organisms which include chlorophyll a, b, c, d, and f. These five molecules have similar chemical structures, which are characterized by a chlorin ring with a central magnesium ion and have small variations in five-membered ring structures or side chains (Croft & Chen, 2017). Chlorophyll a (Figure 2.4) is the most abundant chlorophyll located in the reaction centers and in the light-harvesting complexes which act as a primary donor of an electron (Croce & van Amerongen, 2014). Meanwhile, chlorophyll b (Figure 2.4) is mostly present in higher plants as a light-harvesting accessory pigment. The remaining chlorophyll molecules (chlorophyll c, d, and f) are only present in algae and cyanobacteria (Croft & Chen, 2017).

Carotenoids are known as accessory pigments which assist in photosynthetic light harvest, and protect chlorophyll and the thylakoid membrane from the damage of absorbed energy by photo-oxidation (Johnson, 2016). Ritz et al. (2000) found that carotenoid pigments are crucial for harvesting energy from sunlight, particularly at those wavelengths in which chlorophyll molecules do not absorb strongly. Different types of carotenoids are present in higher plants. Commonly carotenoids are divided into carotenes and xanthophylls. The most common pigments present in leaves are b-carotene and five

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xanthophyll pigments which include lutein, zeaxanthin, violaxanthin, antheraxanthin, and neoxanthin (Young et al., 1997; Barzinji et al., 2015). Figure 2.5 shows the differences in chemical structure for some of the carotenoids found in higher plants.

The absorption spectra (Figure 2.6) has a characteristic for different chlorophylls and carotenoids as each pigment captures specific wavelengths of light more efficiently. The chlorophylls absorb blue (400–500 nm) and red (650–700 nm) spectral regions with maximum absorbance between 660 and 680 nm and maximum reflectance in green wavelengths (560 nm). The maximum reflectance of chlorophylls in green wavelengths (560 nm), accounts for the green colour of vegetation. Meanwhile, carotenoids absorb light only in the blue spectral region (400–500 nm) and so appear yellow/red (Croft & Chen, 2017). The presence of an alternating series of carbon single and double bonds which form a conjugated system π–electron system in these pigments are responsible for the light absorption (reviewed in Johnson, 2016).

Pigments and their relation to the rate of photosynthesis are highly influenced by environmental conditions. In *Adiantum* species, chlorophyll and carotenoid content varied with microclimatic conditions (Shaikh & Dongare, 2008). They found that the highest concentration of photosynthetic pigment was observed in plants that grow in moist and shady places while the lowest value was obtained from the plants which belong to dry and exposed areas. In addition, the ratio of chlorophyll-a and chlorophyll-b in terrestrial plants has been used as an indicator of response to light shade conditions (Maina & Wang, 2015). The small proportion of chlorophyll a to b is considered as a sensitive biomarker of pollution and environmental stress (reviewed in Sumanta et al., 2014).

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Figure 2.4: The chemical structures of the chlorophyll a and b present in the thylakoid membrane of higher plants. (Adapted from Johnson, 2016).

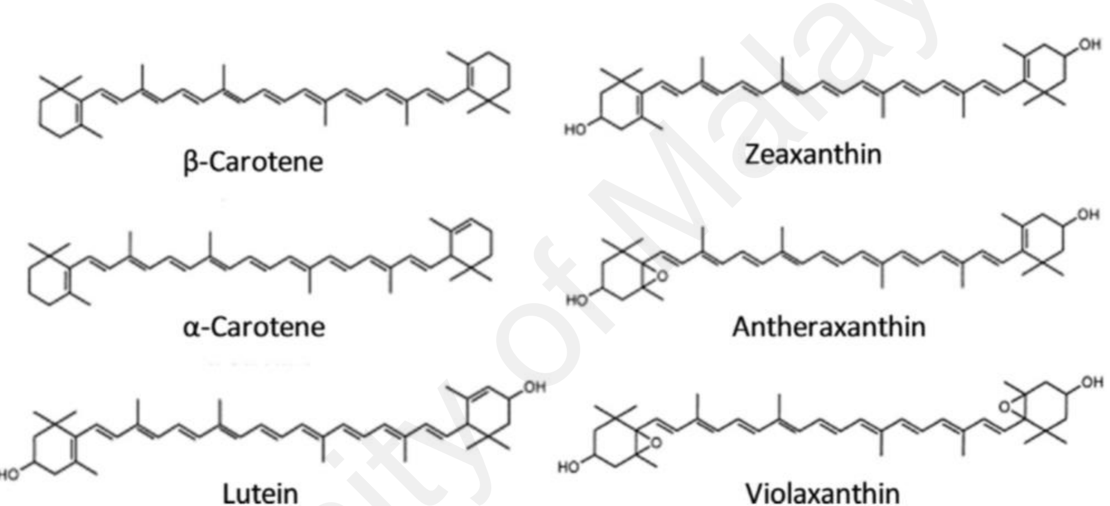

Figure 2.5: The chemical structures of several carotenoids present in higher plants. (Adapted from Oliver & Palou, 2000).

2.7.3 Light and plant growth and development

Light is an essential source of energy for plants for their whole life-span from germination to flower and seed production. Three principle characteristics that affect plant growth which is relevant to the plantation, greenhouse and nursery are quality, quantity, and duration of light. All three parameters have different effects on plant performance.

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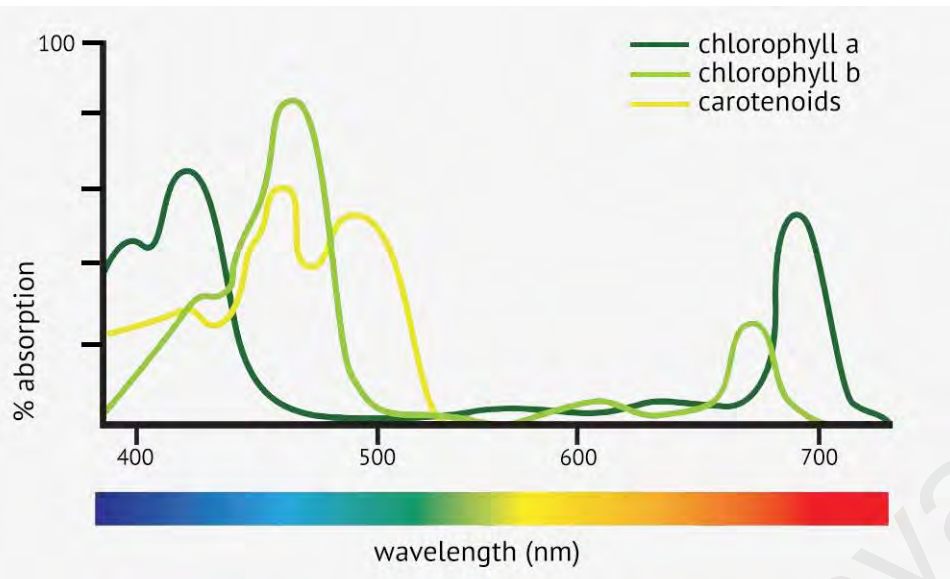

Figure 2.6: Absorption spectrum of photosynthetic pigments. (Adapted from www.philpoteducation.com).

Generally, with the increase of light intensity, the rate of the light-dependent reaction increases proportionately and therefore the rate of photosynthesis also increases (Nelson & Cox 2008). At high light intensity, more photons of light that fall on a leaf which will increase the number of ionized chlorophyll molecules. This results in the generation of more ATP and NADPH (reviewed in Amuenda et al., 2015). However, as light intensity is increased further, the rate of photosynthesis is eventually limited by some other factor (Rodrigues & Thomaz, 2010). The rate began to plateau and eventually, the rate of photosynthesis drops drastically as chlorophyll may be damaged at a very high light intensity (Gauslaa & Solhaug, 2000). Yoneda et al. (2017), showed that the higher the light intensity, the greater the leaf biomass in *Stevia rebaudiana*. Their results showed that high light intensities tend to improve morphological traits such as total leaf area and fresh weight, stem length, stem diameter, and leaf number per plant. However, they found that light intensity at 400 $mol^{-2}s^{-1}$ and above damages plant and causes brown colouration in leaves. This is due to the plant producing various reactive oxygen species (ROS) under

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excess light condition in the reaction centre protein of photosystem-II (PS II) and photosystem-I (PSI) in the thylakoid membrane (Melis, 1999; Li et al., 2009).

The duration of time exposed to light, which is also known as the photoperiod, mainly affects the flowering of the plants. The flowering time can be influenced by controlling the photoperiod. Increasing the photoperiod subsequently increased daily light integral (DLI). Therefore, plants are able to maintain high photosynthetic output. Yet, little attention has been paid to the manipulation of photoperiod to benefit vegetative plant growth despite an extensive literature available for manipulation of photoperiod to induce or inhibit flowering (Adams & Langton., 2004). Yoneda et al. (2017), showed leaf area was larger in *Stevia rebaudiana* for a 24 hour photoperiod treatment compared to an 8 hour photoperiod.

2.7.4 Artificial lighting sources

Artificial light can be defined as any light source that is produced by means of electrical energy. There are different types of artificial light which include incandescent lamps, fluorescent lamps, high-intensity discharge (HID) lamps and light emitting diodes (LED) (Landis et al., 2013). Artificial lights are available in a wide variety of shapes, sizes, colours of light emitted, and levels of brightness. The use of artificial lighting is crucial in agriculture and gardening, particularly in indoor cultivation.

Among artificial lighting systems, LED (light-emitting diode) technology has gained popularity as a sustainable and efficient light source for indoor farming (Singh et al., 2015). LEDs are solid-state, long-lasting and durable sources of narrow-band light that can be used in a variety of horticultural and photo-biological applications such as supplemental and photoperiod lighting for greenhouses (reviewed in Olle et al., 2013).

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LED lights allow the control of spectral composition and the adaptation of light intensity to be matched to the plant photoreceptors in order to furnish better growth and to influence plant morphology as well as different physiological processes such as flowering and photosynthetic efficiency (Yeh & Chung, 2009).

LED has advantages over other lighting options due to its high flexibility for customizing the spectrum, low thermal radiation emission and low energy requirement as well as a high safety performance (Singh et al., 2015). In addition, LED does not have any fragile glass envelope to break or no high touch temperature, also no hazardous materials such as mercury which are directly correlated with high safety performance.

Several leafy vegetables such as lettuce, spinach, cabbage, Chinese cabbage, and green onion showed improved photosynthetic activity and acceleration in biomass accumulation when grown under LED lighting systems (reviewed in Olle et al., 2013) (Table 2.4). The above studies showed that plant growth and development can be reprogrammed by manipulating the light spectrum with combinations of different wavelengths. As examples, a combination red (660-635 nm) and blue (460 nm) LED light resulted in a delay in plant flowering and an increase in biomass accumulation in mustard and basil (Tarakanov et al., 2012); use of a sole red LED spectrum (660 nm) produced an increase in anthocyanin content in cabbage (Mizuno et al., 2011) and an increase in fruit yield in tomato (Lu et al., 2012). To date, there are no reports of the effects of different LED light sources on the growth and quality of *H. bonariensis*.

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Table 2.4: Cultivation of plants under LED lighting from previous studies.

Wavelength range	Lighting condition	Plant (s)	Effects on growth and photosynthesis / Metabolic effects	Reference
Far red light (700-740 nm)	730 nm, 20 µmol m^{-2} s^{-1} in combination with 640 nm, 300 µmol m^{-2} $s-^{1}$	Red leaf lettuce *(Lactuca sativa)*	• Increased total biomass, leaf elongation. • Suppressed anthocyanin content and antioxidant potential.	Stutte et al., 2009
	734 nm, 160 µmol m^{-2} s^{-1} supplemental for cool white fluorescent lamps	Red leaf lettuce (*Lactuca sativa*)	• Decreased chlorophyll concentration by 14% as compared to white fluorescent lamps. The fresh weight, dry weight, stem length, leaf length and leaf width significantly increased by 28%, 15%, 14%, 44% and 15%, respectively, as compared to sole white fluorescent lamps. • Decreased anthocyanins and carotenoids concentration by 40% and 11% as compared to sole white fluorescent lamps.	Li and Kubota 2009
Red light (625-700 nm)	660 nm, 170 µmol m^{-2} s^{-1} in combination with blue 460 nm, 170 µmol m^{-2} s^{-1}	Indian mustard (*Brassica juncea* L.) and Basil (*Ocimum*	• Delayed or inhibited plant transition to flowering as compared to HPS or 460 nm+635 nm LED combination effects	Tarakanov et al., 2012

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	gratissimum L.)		
658 nm, 130 µmol m^{-2} s^{-1} supplemental for cool white fluorescent lamps	Baby leaf lettuce (*Lactuca sativa*)	• Phenolics concentration increased by 6% with a supplemental red light.	Li and Kubota 2009
640 nm, 253 µmol m^{-2} s^{-1} applied 7 days before harvesting (pretreatment with cool-white fluorescent and incandescent irradiance at 275 µmol m^{-2} s^{-1}	Kale plants (*Brassica oleracea*)	• Enhanced chlorophyll *a*, *b* accumulation. • Enhanced lutein accumulation.	Lefsrud et al., 2008
638 nm, 500 µmol m^{-2} s^{-1} supplemental for HPS, 130 µmol m^{-2} s^{-1} lighting and natural illumination in the greenhouse. 3 days of pre-harvest treatment.	Lettuce (*Lactuca sativa* (*Majorana hortensis*) Green onions (*Allium Cepa)*	• Reduction of nitrate concentration.	Samuolie et al., 2009
638 nm, 170 µmol m^{-2} s^{-1} supplemental for HPS, 130 µmol m^{-2} s^{-1} lighting and natural illumination in the greenhouse. 3 days of pre-harvest treatment.	Lettuce (*Lactuca sativa*): green leaf 'Lolo Bionda', 'Grand rapids', red leaf 'Lolo rosa'.	• Increased DPPH free radical scavenging activity. • Increased phenolic compound and α tocopherol content.	Žukauskas et al., 2011

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638 nm, 210 µmol m^{-2} s^{-1} in combination with HPS Lighting, 300 µmol m^{-2} s^{1} and natural illumination 3 days before harvesting in greenhouse	Green baby leaf lettuce (*Lactuca sativa* L.) 'Thumper' and 'Multibaby'	• Increased concentration of total phenolics (28.5%), tocopherols (33.5% in 'Multibaby'), sugars (52.0%) and antioxidant capacity (14,5%) but decreased concentration of ascorbic acid.	Samuolienė et al., 2012a
638-nm, 300 µmol m^{-2} s^{-1} in combination with HPS lighting, 90 µmol m^{-2} s^{-1} and natural illumination 3 days before harvesting in greenhouse	Red leaf 'Multired 4', green leaf 'Multigreen 3' and light green leaf 'Multiblond 2' lettuces (*Lactuca sativa* L.)	• Reduced content of nitrate in red (56,2%) and green (20,0%) leaf lettuce, but nitrate contents increased in light green leaf lettuce.	Samuolienė et al., 2011
638-nm LEDs (photoregulated flux) in combination with HPS lighting (90 µmol m^{-2} s^{-1}) and natural illumination 3 days before harvesting in greenhouse, total PPFD maintained at 300 µmol m^{-2}s^{-1}	White mustard (*Sinapsis alba* 'Yellow mustard'), Spinach (*Spinacia oleracea)* 'Giant d'hiver', Rocket (*Eruca sativa)* 'Rucola', Dill (*Anethum*	• Altered antioxidant activity, increased monosaccharide and decreased nitrate accumulation in dill and parsley. • Increase in vitamin C content in mustard, spinach, rocket, dill and green onion.	Bliznikas et al. 2012

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		graveolens) 'Mammouth', Parsley (*Petroselinum crispum)* 'Plain leaved', Green onions (*Allium cepa)* 'White lisbon'.		
Green light 490-550 nm	Green 510, 520 or 530 nm LEDs (PPFD 100, 200 and 300 µmol m^{-2} s^{-1})	Red leaf lettuce (*Lactuca sativa* L. cv Banchu Red Fire)	• High intensity (300 µmol m-2 s-1) green LED light was effective to promote lettuce growth (as compared to fluorescent light); 510 nm light had the greatest effect on plant growth.	Johkan et al. 2012
	Green 530 nm LEDs (30 µmol m^{-2} s^{-1}) supplemental for natural solar and HPS lamp (170 µmol m^{-2} s^{-1}) illumination in greenhouse	Baby leaf lettuce: red leaf "Multired 4", green leaf "Multigreen 3" and light green leaf "Multiblond 2"	• Reduction of nitrate concentration and increase in saccharide contents in all baby leaf lettuce varieties.	Samuolienė et al. 2012d

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	505, 535 nm LEDs (30 µmol m^{-2} s^{-1}) supplemental for HPS lighting (170 µmol m^{-2} s^{-1}) and natural illumination in the greenhouse	Red leaf 'Multired 4', green leaf 'Multigreen 3' and light green leaf 'Multiblond 2' baby leaf lettuce (*Lactuca sativa* L.)	• 535 nm green LEDs had greater positive effect on ascorbic acid, tocopherol contents and DPPH freeradical scavenging capacity when 505 nm LEDs had a greater effect on total phenol and anthocyanin contents.	Samuolienė et al. 2012b
Blue light 425- 490 nm	Sole 440 nm blue LEDs (10,6 µmol $m^{-2}s^{-1}$) applied 7 days before harvesting (pretreatment with cool-white fluorescent and incandescent irradiance at 275 µmol m^{-2} s^{-1})	Kale plants (*Brassica oleracea* L. cv Winterbor)	• Enhanced β- carotene contents	Lefsrud et al., 2008
	Blue (468nm) LEDs alone or in combination with red (665nm) LEDs. Total PPFD ~100 µmol $m^{-2}s^{-1}$	Red leaf lettuce seedlings (*Lactuaca sativa* L. cv. Banchu Red Fire)	• Stimulated biomass accumulation in the roots. • Resulted in compact lettuce seedling morphology. • Promoted the growth of lettuce after transplanting.	Johkan et al., 2010

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		• Greater polyphenol contents and total antioxidant status.	
Blue (440nm, 30 µmol m^{-2}s^{-1}) LEDs in combination with red (640nm, 270 µmol m^{-2}s^{-1})	Red leaf lettuce (*Lactuaca sativa* L. cv. Outredgeous)	• Leaf expansion. • An increased concentration of anthocyanins, higher antioxidant potentials.	Stutte et al., 2009
Blue LEDs (476 nm, 130 µmol m^{-2}s^{-1}) supplemented for cool white fluorescent lamps	Baby leaf lettuce (*Lactuaca sativa* L.) 'Red cross'	• Anthocyanins concentration increased by 31%. • Carotenoids concentration increased by 12%.	Li and Kubota, 2009
Blue 470nm LEDs, 50 µmol m^{-2}s^{-1}	Seedlings of cabbages (*Brassica olearacea* var. capitata L.) 'Kinshun' (green leaves) and 'Red Rookie' (Red leaves)	• Promoted petiole elongation in both cabbage varieties • Higher chlorophyll contents in green leaf cabbages	Mizuno et al., 2011
Blue 460nm LEDs alone and in combination with red 660nm light. Total PPFD of 80 µmol m^{-2}s^{-1}	Non- heading Chinese Cabbage (*Brassica campestris* L.)	• Higher chlorophyll concentration. • Blue LEDs benefit vegetative growth, while red LEDs and blue plus red LEDs support reproductive growth. • Concentration of vitamin C was the greatest under blue LEDs	Li et al., 2012

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UV-A light 315-380 nm	UV-A LEDs (373 nm, 18± 2 µmol $m^{-2}s^{-1}$) supplemental for cool white fluorescent lamps	Baby leaf lettuce (*Lactuaca sativa* L.) ‘Red cross’	• Anthocyanin concentration increased by 11%	Li and Kubota, 2009

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CHAPTER 3: METHODOLOGY

3.1 Lighting conditions and experimental setup

Plants were grown under three different LED lighting conditions (Figure 3.1); 1) Blue and Red; 2) Blue, Red and Green and 3) Blue, Red and Ultraviolet, with a standardised total fluence rate of 250 umol/m^{-2}/s^{-1} for a photoperiod of 16 hr at 25°C and a relative humidity of 70%. We have set 16 hr photoperiod as it was reported to be suitable for *H. bonariensis in vitro* growth (Masoumian et al., 2011). A set of control plants grown under natural daylight conditions (25°C and relative humidity 70%, 12hr photoperiod) under the same watering regimen. The distance between the LED lighting units and the surface of the rack was 60 cm. All the lighting treatments were conducted in Growth Room D, while the control plants were grown at greenhouse B of the Plant Biotech Facility (PBF), University of Malaya, Malaysia. Plants were grown for a period of 50 days and sampled at various times. Each experiment included 20 biological replicates and was repeated three times (n = 20 ×3 = 60).

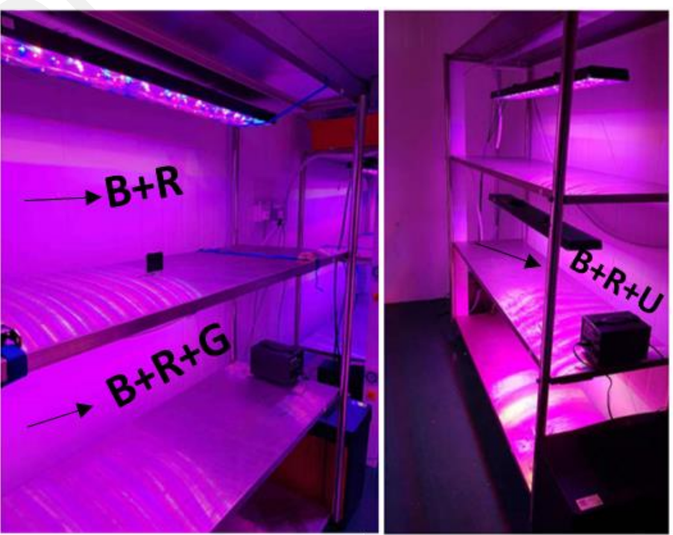

Figure 3.1: Installed lighting system: B+R+G, B+R+U and B+R.

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3.2 Plant materials and experimental setup

Hydrocotyle bonariensis plants were obtained from the Glorious Nursery, Petaling Jaya, Malaysia (Figure 3.2A). Ramets (runners with roots) of 0.5 cm in length were cut off from the stolon of the parent plants and potted separately (Figure 3.2B). Each pot of approximately 9.0 cm × 6.0 cm × 8.5cm size was filled with soil sterilised by autoclave (121° C, 30 minutes at 15 psi of pressure). Soil contained topsoil (Glorious Nursery, Petaling Jaya, Malaysia) and mushroom compost in 2:1 ratio. One ramet per pot was planted. The potted plants were watered on alternate days (Figure 3.2). Sufficient plants were established to allow for destructive sampling of biomass at each time point such that 20 plants could be measured in triplate for every assay.

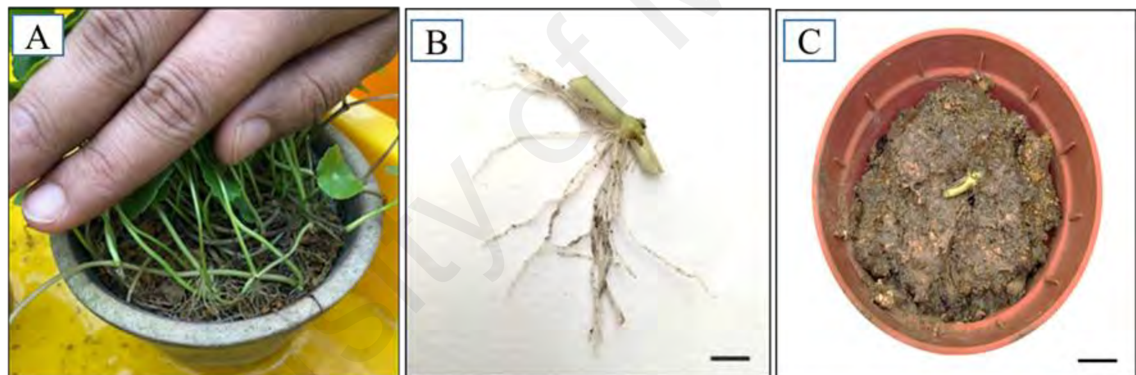

Figure 3.2: *Hydrocotyle bonariensis*. (A) Parent plant with ramets; (B) Planting material: Ramets (runners with root) (bar = 0.25 cm); (C) Plant at zero day (bar = 0.50cm).

3.3 Morphological parameter determination

Morphological parameters were recorded for all plantlets derived from each experimental condition as described below:

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3.3.1 Measurement of plant height

The plant height was measured using a metric ruler. The metric ruler it was placed so that it touched the surface of the soil and the reading was taken for the greatest height. All the readings were recorded in cm.

3.3.2 Number of leaves and measurement of leaf area

The number of leaves on each plant was recorded for each replicate at 20, 30, 40 and 50 days after the start of the experiment. For measurement of leaf area, a total of five leaves were selected from each lighting condition and control condition at 20, 30, 40, and 50 days after the start of the experiment. Each individual leaf was placed on a white sheet of paper together with a metric scale and photographed. The photographs were analysed to measure leaf area using Digimizer Image Analysis Software (Version 5.3.5, MedCalc, Belgium).

3.4 Measurement of aerial part biomass

Plants were harvested at 20, 30, 40, and 50 days after the start of the experiment. Root and aerial parts were excised separately and the fresh weight was recorded immediately by using an electronic balance (Shimadzu, Japan). Following that, dry weight was measured for the same tissues after wrapping in pre-weighed aluminum foil and drying in an oven at 75° C for 72 hr. Data was collected for 20 plants under each lighting condition and control.

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3.5 Determination of photosynthetic pigment

Photosynthetic activity was estimated by measuring chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid content according to the method of Sumanta et al., (2014). Approximately, 100 mg of fully expanded leaf from each plant was harvested and ground using a mortar and pestle, then 2 ml of dimethyl-sulphoxide (DMSO) solvent was gradually added to the mortar and grinding was continued until the mixture was homogenized and formed a green slurry. The green slurry was then transferred to a 2 ml centrifuge tube and the sample mixture was centrifuged for 10,000 rpm at 15min at 4^0C. Then, the supernatant was collected and diluted fivefold using DMSO and transferred to a plastic cuvette for optical density measurement using a spectrophotometer (Implen Gmbh, Germany). DMSO was used as a reference. The absorbance spectrum of the pigments was recorded at 665, 649, and 480 nm wavelength. The equation used for the quantification of chlorophyll-a, chlorophyll-b, and carotenoid was as follows:

$Chl\text{-}a = 12.47A_{665} - 3.62A_{649}$

$Chl\text{-}b = 25.06A_{649} - 6.5A_{665}$

Where, A_{665} = absorbance at A_{665}; A_{649}= absorbance at A_{649}

Carotenoids = $(1000A_{480} - 1.29Ca - 53.78Cb)/220$

Where, Ca = chlorophyll a; Cb= chlorophyll b

3.6 Statistical Analysis

All statistical analyses were performed using SPSS Version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Multiple comparisons were conducted with Tukey HSD test with $P \leq 0.05$ considered as significant.

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CHAPTER 4: RESULTS

The investigation of the effect of the light treatments1) Blue and Red (B+R), 2) Blue, Red and Green (B+R+G), 3) Blue, Red and Ultraviolet (B+R+U) on morphological characteristics of *Hydrocotyle bonariensis* showed differences in the plant growth. The plants showed variable growth pattern under different light treatment for a period of 20, 30, 40, and 50 days with plants grown under an LED lighting system showing improved growth compared to natural daylight irrespective of light wavelengths (Figure 4.1A-D).

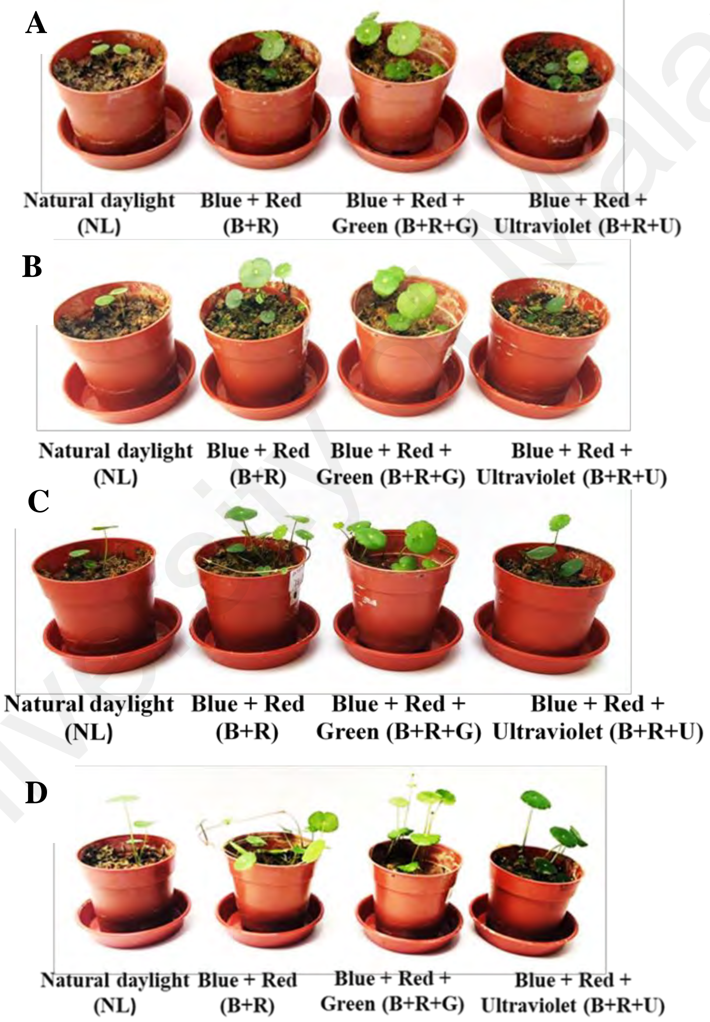

Figure 4.1: *H. bonariensis* grown under different lighting conditions. A: Image capture 20 days after the start of the experiment; B: Image capture 30 days after the start of the experiment; C: Image capture 40 days after the start of the experiment; D: Image capture 50 days after the start of the experiment**.**

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4.1 Effect of different lighting systems on plant height

The plants grown under different lighting conditions (B+R), (B+R+U), (B+R+G) and NL showed no significant difference in height after the first 10 days of growth (Figure 4.2). The difference in growth patterns was observed after 20, 30, 40, and 50 days of light treatments. A significant increase in the plant height was observed in all lighting systems compared to NL ($p < 0.05$). Among all lighting system tested, B+R showed greatest plant height (Figure 4.2). At 20 days of the treatment, the B+R showed plant height of 3.56 cm followed by B+R+U (2.85 cm), B+R+G (2.54 cm) and NL (1.50 cm). At 30 days of the treatment, the plant height of B+R significantly increased to 4.29 cm, followed by B+R+G (3.77 cm), B+R+U (3.38 cm) and NL (2.50 cm). At 40 days of the treatment, B+R showed plant height of 5.17 cm followed by B+R+G (4.31 cm), B+R+U (3.68 cm) and NL (2.88 cm). At 50 days of the treatment, the greatest plant height was observed for B+R (5.87 cm), followed by B+R+G (4.47 cm), B+R+U (3.70 cm) and NL (3.37 cm).

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4.2 Effect of different lighting systems on number of leaves

The plants grown under different lighting conditions (B+R), (B+R+U), (B+R+G) and NL showed no significant difference in the number of leaves at the first 10 days of growth (Figure 4.3). A difference in the number of leaves was observed after 20, 30, 40, and 50 days of light treatments. A significant increase in the number of leaves was observed in B+R compared to all lighting systems at 20, 30, 40, and 50 days of light treatment. Among all lighting system tested, B+R showed the highest number of leaves (Figure 4.3). At 20 days of the treatment, the B+R showed an average number of leaves

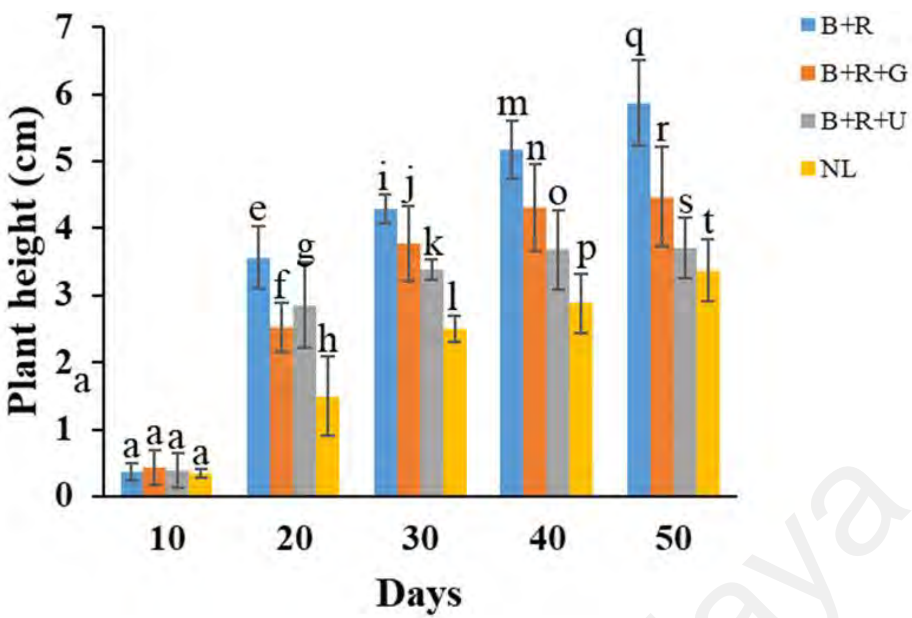

Figure 4.2: Height of *H. bonariensis* plants grown under different lighting conditions. NL: Natural light, B+R: Blue + Red B+R+U: Blue + Red + Ultraviolet, B+R+G: Blue + Red + Green. Each bar represent the mean from measurement of 60 plants and error bars represent the standard errors of the experiment. Different letters on the top of each bar indicate significant differences at $p \leq 0.05$.

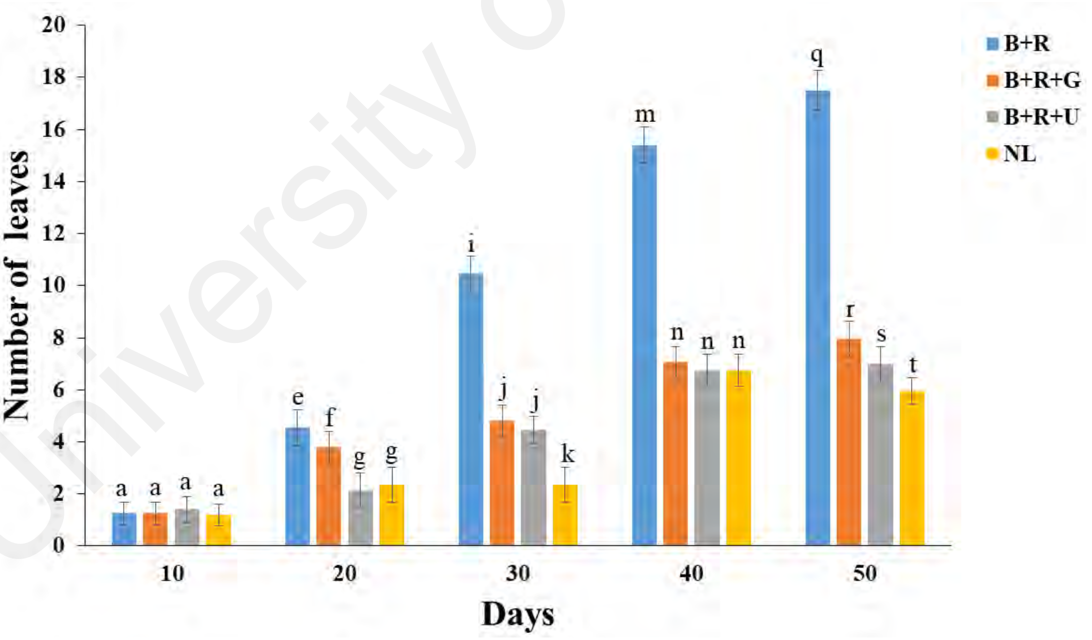

Figure 4.3: Number of leaves of *H. bonariensis* grown under different lighting conditions. NL: Natural light, B+R: Blue + Red B+R+U: Blue + Red + Ultraviolet, B+R+G: Blue + Red + Green. Each bar represent the mean from measurement of 60 plants and error bars represent the standard errors of the experiment. Different letters on the top of each bar indicate significant differences at $p \leq 0.05$.

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of 4.55, followed by B+R+G (3.80), NL (2.35), and B+R+U (2.15). At 30 days of the treatment, the number of leaves significantly increased to 10.45 in B+R, followed by B+R+G to 4.80, B+R+U to 4.45 and NL to 2.35. At 40 days of the treatment, the number of leaves significantly increased in the B+R treatment (15.40) compared to other lighting systems. However, the number of leaves at B+R+G, B+R+U and NL at 40 days of treatment did not show any significant difference. At 50 days of the treatment, B+R showed the highest number of leaves (17.50). The number of leaves demonstrated that B+R lighting system promotes higher leaves number compared to other lighting system tested.

4.3 Effect of different lighting systems on leaf area

The average leaf area (five biological replicates per treatment) measured using Digimizer Image Analysis Software (Version 5.3.5, MedCalc, Belgium) showed no significant differences in the plants grown under different lighting conditions (B+R), (B+R+U), (B+R+G) and NL up to 20 days of growth (Figure 4.4). However, the average leaf area after 30, 40, and 50 days of light treatments was observed to increase for all the lighting systems. The average leaf area was found to be significantly larger in B+R and B+R+G lighting systems compared to NL. Among all lighting systems tested, B+R showed the highest average leaf area (Figure 4.4). At 30 days of the treatment, the B+R showed leaf area of 2.82 cm^2 followed by B+R+G (2.44 cm^2), NL (2.16 cm^2) and B+R+U (1.37 cm^2). At 40 days of the treatment, the leaf area of B+R significantly increased to 5.34 cm^2, followed by B+R+G (4.44 cm^2), NL (3.93 cm^2) and B+R+U (3.42 cm^2). At 50 days of the treatment also, the highest leaf area was observed for B+R (5.61 cm^2),

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followed by B+R+G (4.98 cm^2), NL (4.39 cm^2) and B+R+U (3.91 cm^2). Thus, leaf area tends to grow larger in the B+R lighting system compared to other lighting systems.

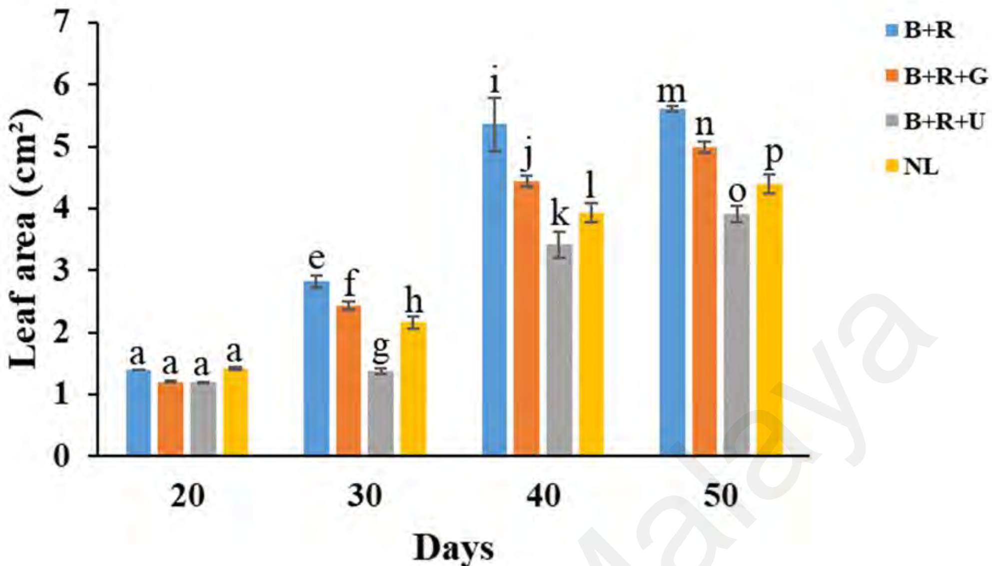

Figure 4.4: Leaf area of *H. bonariensis* under different lighting conditions.
NL: Natural light, B+R: Blue + Red B+R+U: Blue + Red + Ultraviolet, B+R+G: Blue + Red + Green. Each bar represent the mean from measurement of 5 plants and error bars represent the standard errors of the experiment. Different letters on the top of each bar indicate significant differences at $p \leq 0.05$.

4.4 Effect of different lighting systems on biomass of *H. bonariensis*

The biomass (fresh and dry) content of *H. bonariensis* was found to be highly influenced by the different lighting systems (B+R), (B+R+U), (B+R+G) and NL for 20, 30, 40 and 50 days (Table 4.1). At 20 days of the treatment, plants grown under B+R showed highest fresh and dry biomass yields (fresh biomass: 170.50 mg; dry biomass: 15.40 mg), followed by B+R+G (fresh biomass: 130.36 mg; dry biomass: 12.32 mg), B+R+U (fresh biomass: 92.02 mg; dry biomass: 9.84 mg) and NL (fresh biomass: 80.78 mg; dry biomass: 5.06 mg). After 30, 40 and 50 days of the treatment, the fresh and dry biomass yields had increased but there was no significant difference between plants grown under B+R+U and NL lighting. For all treatment durations, plants grown under

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B+R showed the highest biomass (Table 4.1) indicating that a B+R lighting system is a suitable light combination for enhancement of growth in *H. bonariensis*.

4.5 Effect of different lighting systems on the photosynthetic pigments of *H. bonariensis*

An estimation of the photosynthetic capacity of *H. bonariensis* grown under different lighting conditions (B+R, B+R+U, B+R+G and NL) was determined by quantifying the photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Table 4.2). At 20 days of the treatment, plants grown under B+R showed highest photosynthetic pigment content (Chl a: 13.32 µg/µl; Chl b: 6.27 µg/µl; carotenoids: 2.74 µg/µl), followed by B+R+G (Chl a: 10.68 µg/µl; Chl b: 5.12 µg/µl; carotenoids: 1.79 µg/µl), NL (Chl a: 7.09 µg/µl; Chl b: 2.64 µg/µl; carotenoids:1.29 µg/µl) and B+R+U (Chl a: 7.06 µg/µl; Chl b: 2.16 µg/µl; carotenoids:1.04 µg/µl). No significant difference was found between B+R+U and NL at 20 days of the treatment.

At 30 days of the treatment, plants grown under B+R showed the highest content for all photosynthetic pigments (Chl a: 14.44 µg/µl; Chl b: 6.55 µg/µl; carotenoids: 2.87 µg/µl) compared to other light treatments. However, no significant difference in pigment content was observed between plants grown under B+R+U and NL for Chl a content and between plants grown under B+R+G and NL for carotenoids at 30 days of the treatment. At 40 days of the treatment, the photosynthetic pigment contents of plants grown under B+R was significantly higher (Chl a: 15.38 µg/µl; Chl b: 6.98 µg/µl; carotenoids: 3.32 µg/µl) compared to other lighting systems. Among all lighting

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Table 4.1: Fresh and dry weight in leaf biomass of *Hydrocotyle bonariensis* grown under different LED system and natural light for a period of 20, 30, 40 and 50 days.

Light treatment	Days							
	20		30		40		50	
	FW (mg)	DW (mg)	FW (mg)	DW (mg)	FW (mg)	DW (mg)	FW (mg)	DW (mg)
B+R	170.50± 0.03^{a}	15.40± 0.02^{e}	260.96± 0.07^{i}	21.98± 0.02^{m}	529.04± 0.02^{q}	68.08± 0.02^{u}	1180.04± 0.05^{y}	190.86± 0.02^{c*}
B+R+G	130.36± 0.04^{ab}	12.32± 0.03^{ef}	190.08± 0.05^{j}	19.00± 0.06^{n}	343.10± 0.01^{r}	61.00± 0.03^{u}	665.84± 0.03^{z}	156.56± 0.04^{d*}
B+R+U	92.02± 0.01^{b}	9.84± 0.01^{f}	120.00± 0.03^{k}	18.50± 0.04^{o}	240.00± 0.05^{s}	32.50± 0.05^{v}	403.00± 0.02^{a*}	120.50± 0.01^{e*}
NL	80.78± 0.01^{c}	5.06± 0.05^{g}	120.50± 0.05^{k}	18.00± 0.04^{o}	250.00± 0.08^{s}	33.61± 0.05^{v}	436.00± 0.03^{a*}	110.84± 0.07^{e*}

Notes: FW= Fresh weight, DW=Dry weight. Each value represents the mean ± SD from measurement of 20 plants. Values followed by the same letters are not significantly different ($p < 0.05$) by post-hoc parametric test of Tukey HSD, $p < 0.05$.

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tested, *H. bonariensis* grown in B+R+U (Chl a: 8.04 µg/µl; Chl b: 3.80 µg/µl carotenoids: 1.90 µg/µl) showed lowest photosynthetic pigment contents at 40 days of the treatment.

At 50 days of the light treatment, the photosynthetic pigment contents of plants grown under B+R was much higher (Chl a: 17.91 µg/µl; Chl b: 8.33 µg/µl; carotenoids:3.72 µg/µl), compared to that of plants grown under B+R+G (Chl a: 12.40 µg/µl; Chl b: 7.01 µg/µl; carotenoids: 2.91 µg/µl), NL (Chl a: 14.66 µg/µl; Chl b: 6.06 µg/µl; 3.37 µg/µl) and B+R+U (Chl a: 8.74 µg/µl; Chl b: 4.58 µg/µl; carotenoids:2.00 µg/µl). However, plants grown under NL had significantly higher pigment content compared to that of plants grown under B+R+U. Comparison of photosynthetic pigments suggests that the plants had a higher photosynthetic capacity when grown under B+R than when grown under the other light treatments tested in this study.

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Table 4.2: Photosynthetic pigments (chlorophyll a, b and carotenoids) content in *Hydrocotyle bonariensis* grown under different LED system and natural light for a period of 20, 30, 40 and 50 days.

Light treatment	Days											
	20			30			40			50		
	Ch-a	Ch-b	Carotenoids	Ch-a	Ch-b	Carotenoids	Ch-a	Ch-b	Carotenoids	Ch-a	Ch-b	Carotenoids
B+R	13.32± 0.32^{a}	6.27± 0.14^{e}	2.75± 0.25^{i}	14.44± 0.27^{m}	6.55± 0.33^{q}	2.87± 0.19^{u}	15.38± 0.35^{y}	6.98± 0.45^{c*}	3.33± 0.17^{g*}	17.91± 0.143^{k*}	8.33± 0.19^{o*}	3.72± 0.38^{s*}
B+R+G	10.38± 0.15^{b}	5.13± 0.11^{f}	1.79± 0.29^{j}	12.08± 0.02^{n}	5.43± 0.29^{r}	2.12± 0.08^{v}	12.15± 0.67^{z}	5.94± 0.72^{d*}	2.70± 0.23h*	12.40± 0.36^{l*}	7.01± 0.25^{p*}	2.91± 0.40^{t*}
B+R+U	7.06± 0.07^{c}	2.16± 0.24^{g}	1.04± 0.11^{k}	7.98± 0.24^{o}	2.43± 0.31^{s}	1.32± 0.19^{w}	8.03± 0.30^{a*}	3.80± 0.45^{e*}	1.90± 0.14^{i*}	8.75± 0.34^{m*}	4.58± 0.13^{q*}	2.00± 0.13^{u*}
NL	7.09± 0.16^{c}	2.64± 0.23^{g}	1.29± 0.13^{k}	8.41± 0.41^{o}	4.24± 0.41^{t}	2.11± 0.15^{v}	13.22± 0.49^{b*}	5.94± 0.23^{f*}	2.82± 0.08^{h*}	14.66± 0.59^{n*}	6.06± 0.40^{r*}	3.37± 0.13^{v*}

Notes: Ch-a = Chlorophyll a, Ch-b = Chlorophyll b. Each value represents the mean ± SD from measurement of 20 plants. Values followed by the same letters are not significantly different ($p < 0.05$) by post-hoc parametric test of Tukey HSD, $p < 0.05$.

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CHAPTER 5: DISCUSSION

More than 100 pennywort species have been identified and few of the species are well known for their medicinal properties (http://www.efloras.org). *H. bonariensis* is one of such pennywort species, which is abundantly grown in Malaysia (Maulidiani et al., 2012). The non-medicinal and medicinal use of this species have been well studied (literature review: Table 1-3). The awareness for *H. bonariensis* is increasing in the local market (https://www.thestar.com.my) for its high antioxidant and pharmacological values (literature review: Table 1-3) and hence, demand. However, *H. bonariensis* is mainly sourced from natural habitats. Sourcing plant materials from natural habitats is not an economically viable proposition since it could not sustain the demand in pennyworth production (Hashim, 2011). Hence, sustainable large-scale cultivation is necessary to meet the steadily increasing demand of the industry, while protecting natural habitats from over-exploitation.

Based on the problem encountered by the sourcing of *H. bonariensis*, this research was designed to establish an efficient, scalable and sustainable indoor cultivation with an optimised LED lightening system. Light plays an important role in determining photosynthesis and plant growth (Evans, 2013). Hence, optimised lighting conditions are crucial for indoor cultivation to promote desired morphology and biomass production of the plant (Morrow, 2008). LED is one of the most popular artificial lighting systems due to its high flexibility for customising spectrum, lower thermal radiation emission and lower energy requirement as well as a high safety performance (Singh et al., 2015).

The primary focus of the study was to compare the effect of different combinations of LED light wavelengths and natural daylight on the photosynthetic activity and the

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biomass yield of *H. bonariensis*. Three different combination of LED light wavelength were tested on *H. bonariensis* which included 1) Blue and Red (B+R); 2) Blue, Red and Ultraviolet (B+R+U); and 3) Blue, Red and Green (B+R+G).

The plants grown under B+R showed a significantly higher plant height and number of leaves at 20, 30, 40 and 50 days of treatments compared to the other lighting systems testing in the study (Figure 4.2-4.3). Leaf area was also higher for plants grown under B+R at 30, 40 and 50 days of the treatment compared to the other lighting systems (Figure 4.4). A similar effect of B+R was also reported by Choi et al. (2015), where strawberry cultivation under the illumination of B+R light showed a significantly higher number of petioles compared to blue or red light alone. Strawberry plants grown for 40 days under B+R showed longer petioles and higher leaf area compared to red or blue light alone (Folta & Childers, 2008). The similar effect of B+R was also reported in chrysanthemum species grown *in vitro* condition where higher leaf area was reported in B+R compared to B+ far-R, R + far-R, red, blue and fluorescent light (Kim et al., 2004).

For most of the treatment durations, *H. bonariensis* grown under B+R lighting system showed the highest fresh and dry biomass yields compared to other lighting systems (Figure 4.5), which was consistent with the greater height, leaf number and leaf area for those plants. *H. bonariensis* at 50 days of the B+R lighting treatment, showed significantly higher fresh and dry biomass yields compared to other lighting systems tested (Figure 4.5). A similar effect of B+R was also reported in *Lactuca sativa* (lettuce), where biomass yields of plants grown was higher under B+R light compared to plants grown under high pressure sodium (HPS) lamps alone (Wojciechowska et al., 2015). In

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another similar study, young lettuce grown under B+R showed higher fresh biomass compared with the plants grown in red light alone (Jokhan et al., 2010).

H. bonariensis grown under B+R lighting system at 20, 30, 40 and 50 days showed significantly higher photosynthetic pigment content (Chl a, Chl b, and carotenoids) compared to plants grown under the other lighting systems (Figure 4.6). Similar effect of B+R on photosynthetic pigment was also reported in strawberry plants where cultivation under the B+R light treatment significantly elevated the chlorophyll a and total chlorophyll contents compared to blue and red light alone (Choi et al., 2015). Higher chlorophyll and carotenoid content in plants grown under B+R light was also reported for *Brassica campestris* (Chinese cabbage) compared to plants grown under red light alone or fluorescent light (Li et al., 2012).

The red and blue regions regarded as main sources of energy for photosynthesis and CO_2 absorption in the plants (Pennisi et al., 2019). Photosynthetic pigments such as Chl a, b and carotenoid mainly absorbed the blue and red wavelengths in the light spectrum (Bayat et al., 2018). Plants responses to light spectra are mediated by series of complex processes and mainly associated with the specificity of light-sensing photoreceptor in plants (Javanmardi & Emami, 2013) and downstream signalling genes (Bayat et al., 2018). The phytochrome is the photoreceptor for red light responses (reviewed in Metallo et al., 2018). Phytochromes are known to respond to cell elongation and to induce greater leaf surface area (reviewed in Pierik & de Wit, 2013). The photoreceptors used by blue light known as cryptochromes and phototropins, which mediate phototropism, stomatal opening and the intracellular positioning of chloroplasts to increase light absorption (reviewed in Christie et al., 2014) and thus control water loss and the uptake of carbon

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dioxide (Hiyama et al., 2017). Hence, the application of B+R light treatment might activate the response of both of these photoreceptors in *H. bonariensis*, which resulted in improved growth, greater leaf area, increased yield and higher photosynthetic pigment content.

The B+R+G lighting system was found to be the second most efficient lighting treatment for *H. bonariensis* in terms of the morphological parameters compared to B+R+U and NL (Figure 4.2-4.4). Kim et al. (2006) reported that treatment of lettuce with a combination of B+R+G improved leaf number over that of plants grown under green or white light alone. Studies on *Cucumis sativus* (cucumber) hybrid (Novičkovas et al., 2012), tomato and sweet pepper (Samuoliene et al., 2012c) showed supplementation of green light (505 nm and 530 nm) with HPS lamps increased the leaf area compared to HPS lamps alone or a combination of HPS lamps with blue light (455 nm and 470 nm).

Under the B+R+G lighting system, comparatively higher fresh and dry biomass yields of *H. bonariensis* were observed compared to those of plants grown under B+R+U and NL (Figure 4.5). This result is consistent with that reported in previous research which states that green light wavelengths of 505 nm and 530 nm with HPS lamps led to accumulation of more fresh and dry biomass compared to HPS lamps alone in tomato, sweet pepper and cucumber (Novičkovas et al., 2012; Samuoliene et al., 2012c).

In most of the treatment periods, the plants grown under the B+R+G lighting system accumulated a higher photosynthetic pigment content compared to the plants grown under B+R+U or NL (Figure 4.6). This result is consistent with that reported for cucumber transplants grown under green light supplemented with HPS lamps that showed significantly higher content of chlorophyll a and chlorophyll b compared to plants grown

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under HPS lamps alone (Samuoliene et al., 2012c). In another study on lettuce plant, green light supplementation with B+R light produced a higher single leaf photosynthetic rate compared to green fluorescent lamps and cool white fluorescent lamps (Kim et al., 2006).

Studies conducted with green light showed it worked best when applied in supplementation as when applied alone, green light is not able to support the growth of plants because the plant absorbs little of this spectrum and most is reflected (Singh et al., 2015). That finding is consistent with earlier studies that reported the application of monochromatic green light showed a reduction in chlorophyll content and inhibition of stomatal opening in red leaf and green leaf lettuce (Son and Oh, 2013) and broad bean, Asiatic dayflower, wild tobacco and Arabidopsis (Talbott et al., 2002). The information for the photoreceptor of green light still not clearly reported in plants (Zhang & Folta, 2012). However, it was reported that green light tends to oppose blue or red light-induced responses (Bouly et al., 2007). That could be the reason why *H. bonariensis* grown under the B+R lighting system showed better growth morphology, higher biomass yields and higher photosynthetic pigments content compared to plants grown under the B+R+G lighting system.

Plants grown under the B+R+U lighting system showed significantly inferior values for morphological parameters compared to plants grown under the other lighting systems tested (Figure 4.2-4.4). The plants showed stunted plant height, lower number of leaves and smaller average leaf area irrespective of the presence of the favourable B+R light spectra. Also, the fresh and dry biomass yields and photosynthetic pigment content showed lower values compared to the other lighting systems at most of the treatment

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durations (Figure 4.5-4.6). There are three different types of UV represented by their range of wavelengths, which are UV-A (315-399nm), UV-B (280-315 nm) and UV-C (< 280 nm) (Caldwell, 1971). Most of the previous studies on the effect of UV radiation on the growth and development of plants used UV-B as a source. The irradiated UV-B showed a negative impact on plant growth with an increase in the intensity of the UV light (Afreen et al., 2005; Sakalauskaitė et al., 2012; Zuk-Golaszewska et al., 2012). Moreover, the supplementation of UV-B showed reduced leaf area in sweet basil and perilla (Johnson et al., 1999; Nishimura et al., 2008), reduced fresh and dry weights in sweet basil (Sakalauskaite et al., 2012) and reduced chlorophyll contents in some annual desert plants (Salama et al., 2011).

In the present study, the UV light was from a UV-A source with range of 380-399 nm and was used in combination with the blue and red lights. The effect of UV-A in plants is comparatively less studied than the effect of UV-B. Regarding UV-A, there are contradictory reports on the effects on the plant growth and development with only two studies reporting a positive effect: The inclusion of UV-A (with the help of UV filter) along with natural daylight showed an increased level of chlorophyll a and chlorophyll b content in lettuce compared to natural light without any UV (Krizek et al., 1998). In another similar study, carotenoids and chlorophyll development were greatly enhanced by UV-A radiation (Godnev et al., 1959). Such differential response of UV-A exposure in plant species was reported to be dependent on plant genotype, developmental stage of the plant and the intensity and duration of exposure (reviewed in Neugart et al., 2018). Hence, it was our interest to see the effect of UV-A on *H. bonariensis*. Our result demonstrated that including UV light resulted in stunted growth and is not suitable for indoor cultivation of *H. bonariensis*.

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In the present study, natural daylight was used as a comparator for the other lighting systems tested. As Malaysia is located near the equator, its climate is hot and humid throughout the year (https://www.worldatlas.com). Thus Malaysia has 12 hours of daylight year-round which represent a wide range of wavelengths at almost unchanging day length. In the current study, the lighting systems which provided specific wavelengths were set up to 16 hours per day as this is commonly practiced for indoor plant cultivation (reviewed in Adams & Langton, 2005). The difference in the photoperiod between the other lighting systems and NL could be one of the factors resulting in better growth performance in the LED lighting systems, especially the B+R and B+R+G compared to the performance in the plants in the NL group.

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CHAPTER 6: CONCLUSIONS

Hydrocotyle bonariensis was successfully grown under three different LED lighting systems by indoor cultivation with controlled watering conditions. The results demonstrated the B+R lighting to be the most efficient to enhance photosynthetic activity and biomass yield production of *H. bonariensis* and to give better yield compared to the other lighting systems tested and to the natural daylight control condition. This finding is important for indoor cultivation of *H. bonariensis* and can be of benefit to farmers and other herb growers. This knowledge can also be exploited as supplemental LED light to obtain superior quality of plant material. In the future, in depth study on the phytochemical and pharmacology aspects of *H. bonariensis* grown under different lighting combinations should be carried out to find out the optimal spectral quality that can enhance the phytochemical composition.

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