Journal of Plant Growth Regulation (2024) 43:3171–3191
https://doi.org/10.1007/s00344-023-11076-y

Acclimation of *Polygonum minus* Huds. to Low Light Irradiance and Its Effects on Growth, Leaf Gas Exchange and Antioxidant Defense

Fairuz Fatini Mohd Yusof[1] · Nurul Amalina Mohd Zain[1] · Normaniza Osman[1] · Phaik Eem Lim[3] · Acga Cheng[1] · Ayushi Gautam[4] · Praveen Guleria[4] · Vineet Kumar[5] · Jamilah Syafawati Yaacob[1,2]

Received: 21 December 2022 / Accepted: 6 July 2023 / Published online: 31 July 2023

Abstract

Polygonum minus Huds. has massive potential to be commercialized following many current discoveries of its medicinal properties. We investigated the growth and physiology changes of *P. minus* under different shading and treatment durations. The effects of four shading levels: 0% (T1), 30% (T2), 50% (T3) and 70% (T4) and two treatment durations of 10 weeks and 20 weeks were studied. Significant effects of shading levels and treatment durations were observed on the transpiration rate (E), leaf resistance, pigment content in leaves (Chl a, Chl b and Car), carbohydrate, ascorbic acid, total anthocyanin, phenolic and flavonoid contents. Enzyme activity of superoxide dismutase and ascorbate peroxidase were also significantly affected by shading levels and treatment durations. The highest antioxidant activities were recorded under T4 of both treatment durations along with the high content of Chl a, Chl b and Car. The leaf, stem and root extracts of *P. minus* were also subjected to HPLC analysis for quantification of selected flavonoid compounds. It was observed that longer treatment duration of 20 weeks reduced the amount of flavonoid.

Keywords *Polygonum minus* Huds · Shading levels · Treatment duration · Growth · Leaf gas exchange · Antioxidant activities

Introduction

Polygonum minus Huds is a common medicinal herb grown as a native plant in the Southeast Asian countries. In Malaysia, it is locally known as *kesum* or *daun laksa.* It has been widely used as a natural flavoring agent due to the significant amount of aldehydes that contributes to its flavor (Baharum et al. 2010). Various antioxidant, anti-inflammatory, antimicrobial, antiulcer and analgesic properties of *P. minus* had been reported, which were contributed by its high level of flavonoid and phenolic compounds (Suhailah et al. 2012; Christapher et al. 2015). A study has reported that *P. minus* showed anti-proliferative activity in several cancer cell lines (Mohd Ghazali et al. 2014). Polygonumins A, a novel compound that was isolated from the stem of *P. minus* was also reported to be able to inhibit cancer cells without harming the normal cells (Ahmad et al. 2018). In addition, *P. minus* also exhibit notable ROS scavenging activities (Maizura et al. (2011), which make it as one of natural medicinal resources that has been traditionally used by the locals to treat digestive problems, dandruffs, and body ache (Christapher et al. 2015). *P. minus* was also reported

Handling Editor: Vijay kumar.

✉ Nurul Amalina Mohd Zain
amalina86@um.edu.my

✉ Jamilah Syafawati Yaacob
jamilahsyafawati@um.edu.my

[1] Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[2] Centre for Research in Biotechnology for Agriculture (CEBAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[3] Institute of Ocean and Earth Sciences, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[4] Plant Biotechnology & Genetic Engineering Lab, Department of Biotechnology, DAV University, Jalandhar, Punjab 144012, India

[5] Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab 144111, India

to be able to improve cognitive function and exhibit neuroprotective effect (You et al. 2018). Most recently, *P. minus* was reported to be able to reduce anorexia and improves the anhedonia among stressed mice (Bashir et al. 2022a). It was also reported that aqueous extract of *P. minus* exhibited antidepressant effects, decreased immobility time, increased spatial memory, reduced corticosterone, increased the brain-derived neurotrophic factor level, and reduced monomamine oxidase- A level, coupled with increasing monoamines (serotonin and norepinephrine) in the hippocampus of a chronic ultra-mild stress-induced mice model (Bashir et al. (2022b).

Various studies have reported on the use of different light intensities to influence both the morphological and physiological properties of plants. Shading was reported to be able to increase the leaf mass per unit area and leaf thickness due to the elongation of palisade cells in *Acer pseudoplatanus* L. and the mature leaves may re-acclimate anatomically to increase irradiance (Wyka et al. 2022). A study reported that shading results in higher synthesis of essential oils in lemon balm, mint and sweet basil (Ilić et al. 2022). The same study also reported that shading significantly increased the antioxidant activities of basil leaves (Ilić et al. 2022). In a different study, Ghasemzadeh et al. (2010) reported that light can stimulate the production of secondary metabolites such as gingerol and zingiberine in *Zingiber officinale.* This result is in contrast with the study by Ibrahim et al. (2014), where low light intensity along with high CO_2 level were found to increase the secondary metabolites content in *Labisia pumila.* This indicates that the effects of irradiance levels vary with different plant species. In the study of *P. minus*, intermediate shading onto the plant increases its pigment contents, improve photosynthesis rate, anthocyanin and the phenolic compounds (Mohd Yusof et al. 2021).

Although various reports can be found on the chemical profiles and antioxidative effects of *P. minus* (Baharum et al. 2010; Suhailah et al. 2012), very few studies had been conducted to assess the benefits of micro-environmental manipulation (such as the effects of shading) on the production of secondary metabolites and antioxidant activity in *P. minus* and subjected to different treatment durations. By incorporating improvements and another variable from the previous study by Mohd Yusof et al. (2021), this study will be able to yield valuable knowledge on the role of shading levels and durations on the production of secondary metabolites and antioxidant activities of *Polygonum minus* Huds.

Materials and Methods

Experimental Design

This study was designed based on the outcomes obtained from our previous study (Mohd Yusof et al. 2021), where we had reported that shading levels (T1 = 0%, T2 = 50% and T3 = 70%) positively affected the growth, physiological properties, and the production of secondary metabolites of *P. minus*. The current study was carried out to further improve the research parameter, where the plants were grown under different shade levels (T1 = 0%, T2 = 30%, T3 = 50% and T4 = 70%) and subjected to two treatment durations (10 weeks and 20 weeks). The effects of these treatments on plant growth, physiological properties and production of bioactive secondary metabolites were thoroughly and carefully monitored throughout the duration of the treatments. Under each treatment, the light intensities were also continuously monitored by using a LI-COR LI-250A light meter, 3 times a day throughout the 12 h daytime (7 a.m. to 7 p.m.) at the location where the study was carried out. The average light intensity (in μmol m^{-2} s^{-1}) recorded under T1 (0% shading; control) was 160.254 ± 8.770^{a}, T2 (30% shading) recorded $50.922b \pm 3.603^{b}$, T3 (50% shading) recorded 39.423 ± 2.781^{bc} and T4 (70% shading) recorded 23.328 ± 2.302^{c} (the different letters on top indicate significant difference at $p \leq 0.05$ according to Tukey's test). Instead of looking at the single factor of different shade levels and treatment durations, the interaction or relationship effects between the different shade levels and treatment durations exerted on the plants were also studied. Figure 1 shows the set up of the study.

Media Preparation and Treatment Details

This study was carried out in a greenhouse at Rimba Ilmu Botanical Garden, Universiti Malaya. *P. minus* plants were propagated for two weeks in small pots before being transplanted into small sized polyethylene bags filled with a soilless mixture of burnt rice husk and coco peat (ratio 3:1),

Fig. 1 The glasshouse and the wall-to-wall installation of the black nylon netting, forming horizontal shading above the plants in each treatment row

following the same procedures reported in Mohd Yusof et al. (2021). The shade levels were prepared in rows, where the shade levels were created by black nylon netting canvas with different mesh percentages. The canvas was installed from wall to wall of the greenhouse, forming horizontal shading above the plants in each row. Inner walls were also installed around the shaded compartments, to make sure plants were shaded from all directions. The light intensities under each shading levels were continuously monitored using a LI-COR LI-250A light. The plants were arranged in RCBD with 4 shade levels × 2 treatment durations × 4 blocks × 4 replicates.

Fertilization and Plant Maintenance

All the plants were watered every morning and fertilized by using NPK green once per week and organic fertilizer twice per week. In order to prevent the attack from fungus and mold, chalk powder was scattered around the plots area (Mohd Yusof et al. 2021).

Data Collection

Plant Growth

Plant height (PH) and number of leaves (LN) were measured every two days until the 25th day after transplant. Plant height measurements were taken on the main stem starting from the reference point on the soil surface towards the tip of the stem by using a measuring tape with an accuracy of ± 1 mm. The number of total leaves were recorded per plant.

Biomass and Root–Shoot Ratio

The plants were harvested and used to obtain the biomass by dividing the plant parts into three different categories which were the leaves, stems and roots. All three parts were subjected to the process of freeze-drying using Labconco freeze dryer (Labconco Corporation, MO 64132 USA) at − 50 °C. The dry weight of the plants was recorded as the biomass dry weight; leaf dry weight (LDW), stem dry weight (SDW) and root dry weight (RDW). Root-shoot ratio was calculated by dividing root dry weight with the above ground dry weight.

Leaf Gas Exchange

The measurements of photosynthesis rate (A), intercellular CO_2 (C_i), leaf temperature (Tleaves), transpiration rate (E) and stomatal conductance (Gs) were obtained by a closed infrared gas analyser LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA). The measurements used optimal condition set of 400 µmol mol^{-1} CO_2 flux, 30 °C standard cuvette temperature and PAR of 1,200 µmol m^{-2} s^{-1}. Gas exchange measurements were carried out between 10:00 a.m. to 11:00 a.m., on fully expanded young leaves numbered 3 from the plant apex. Data were recorded in triplicates from each treatment within each block.

In-situ Chlorophyll Content

In-situ chlorophyll content (SPAD) was assessed using the Chlorophyll Meter SPAD-502 (Minolta, Japan) once every three days on a mature expanded leaf of each plant. The equipment was calibrated prior to the measurements. The data were recorded in triplicates from each treatment within each block.

Chlorophyll Fluorescence

Measurements of chlorophyll fluorescence were taken from fully expanded second leaves by using Handy-PEA (Hansatech, Kings Lynn, UK). Leaves were darkened for 15 min by attaching light-exclusion clips to the central region of the leaf surface. Measurement of F_0 (initial fluorescence), F_m (maximum fluorescence) and F_v (variable fluorescence) were obtained from this procedure. The use of minimal (F_0) and maximal (F_m) fluorescence leads to the well accepted expression for the maximum quantum yield of primary photochemistry (TR_0/ABS) equals to $1-(F_0/F_m)=F_v/F_m$.

Leaf Chlorophyll and Carotenoid Analysis

The chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) were analysed from mature expanded leaves. Leaf samples (0.1 g) from each treatment from each block were crushed in liquid nitrogen by using a mortar and pestle. The sample was ground in 10 mL absolute methanol using a chilled mortar and pestle before incubated under -20 °C in the dark for 24 h. The chlorophyll and carotenoid levels were measured in triplicates with a spectrophotometer model UV160U at 665.2, 652.4, and 470 nm wavelengths after tenfold dilution. The pigment concentrations of chlorophyll a, chlorophyll b and carotenoid were calculated following the methods by Lichtenthaler and Buschmann (2001) and expressed as mg chlorophyll g^{-1} tissue fresh weight (mg g^{-1} FW).

$$\text{Chl a } (\mu g/\text{ml}) : 16.72(A665.2) - 9.16(A652.4)$$

$$\text{Chl b } (\mu g/\text{ml}) : 34.09(A652.4) - 15.28(A665.2)$$

$$\text{Car } (\mu g/\text{ml}) : (1000(A470) - (1.63(\text{Chl a}) - 104.96(\text{Chl b}))/221$$

Anthocyanin Content

The total anthocyanin content (TAC) was determined using the pH differential method. The methanolic extracts were

diluted separately with two types of buffers: potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5 using the ratio 1:4 (1-part test portion and 4 parts buffer). The absorbance of the samples was measured at 510 and 700 nm. The TAC was calculated using the following formula:

$$\text{Total anthocyanin content (mg cyd 3 - glu/L)} = (\text{Ab} \times \text{MW} \times df \times 1000)/(\varepsilon \times L \times m)$$

where: Ab = (A510–A700) pH1.0 -(A510–A700) pH4.5, MW = Molecular weight of cyd 3 − glu (449.2 g/mol), df = the dilution factor, ε = Extraction coefficient (29,600 mol/g).

Total Phenolics and Total Flavonoids Quantification

Folin-Ciocalteu reagent (10%) was used to determine the total phenolics content of the leaf samples. Approximately 0.1 mL of the sample extract was mixed with Folin–Ciocalteau reagent (0.75 mL). Then, 2% aqueous sodium carbonate (0.75 mL) was added into a test tube incubated in the dark for 45 min. Blanks were prepared by the addition of 70% methanol instead of the sample. The absorbance was measured at 765 nm. The TPC was then determined from the standard calibration curve prepared with a series of gallic acid standards (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg/mL). The results were represented as mg gallic acid equivalent per 100 g dry weight (mg GAE/100 g dry sample).

For flavonoid quantification, the sample (0.5 mL) was mixed with 1.5 mL of 70% methanol in a test tube covered with aluminum foil, and then was left for 5 min. Next, 10% 0.10 mL of $AlCl_3$ ($AlCl_3.6H_2O$) was added followed by addition of 1 M NaOH (0.1 mL) and 2.8 mL of distilled water. Later, the absorbance was measured at 415 nm using a spectrophotometer after 40 min of incubation with quercetin as the standard and the results were expressed as mg g^{-1} dry sample.

DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity of each sample was determined according to the standard protocol. 50 µL of extract at six different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/mL) was added to 150 µL of DPPH solution (60 mM) in each well of a 96-well plate. The change in absorbance at 515 nm was measured after 30 min incubation at room temperature. The obtained data were then used to determine the concentration of the sample required to scavenge 50% of the DPPH free radicals (IC_{50}). The percentage of inhibition was plotted against the concentration and the IC_{50} was obtained from the fitted linear curve. A lower IC_{50} denotes a more potent antioxidant.

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was performed based on the method described by Benzie and Strain (1999) with slight modifications. About 300 µL of extract or methanol (control) was mixed with 10 µL FRAP reagent and was incubated in microplate wells at room temperature in the dark for 30 min. The absorbance was recorded at 593 nm. A series of stock solution at 100, 10, 1, 0.1 and 0.01 mM of aqueous ascorbic acid were prepared to generate a standard curve ($r^2 = 0.9944$). The results obtained were expressed as mM of ascorbic acid equivalent per gram of dried samples.

Carbohydrates Estimation

The carbohydrates were estimated following an earlier described method (Sharma et al. 2022). Briefly, 100 mg of variously treated fresh samples were extracted overnight with methanol. The supernatant isolated from the extract was used for carbohydrate estimation. The reaction mixture was prepared by adding 5% aqueous phenol and concentrated H_2SO_4 to the supernatant, followed by incubation, and boiling. The carbohydrates were quantified by UV–Visible absorbance measured at 490 nm.

Activity of Antioxidant Enzymes

The sample (100 mg) was extracted with a chilled extraction buffer constituted of 100 mM potassium phosphate pH 7.0 and 0.1 mM EDTA to estimate the enzyme activity of superoxide dismutase (SOD) (Sharma et al. 2022). The reaction was initiated by adding 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 0.1 µM EDTA, 75 µM nitroblue tetrazolium, and 2 µM riboflavin to 50 µl of the supernatant acting as crude enzyme extract. The reaction mix was illuminated with 20-W fluorescent tubes and the absorbance was recorded at 560 nm.

100 mg sample was also extracted in 50 mM phosphate buffer (pH 7.8) to evaluate the enzyme activity of ascorbate peroxidase (APX) (Sharma et al. 2022). The reaction was initiated by adding the crude extract to a reaction mix comprising 50 mM phosphate buffer, 0.2 mM EDTA, 0.5 mM ascorbic acid, and 0.25 mM hydrogen peroxide. The absorbance was recorded at the start and end of the reaction at 290 nm. The molar extinction coefficient used for the calculation was 2.8 mM^{-1} cm^{-1}.

Determination of Lipid Peroxidation

Lipid peroxidation was estimated in terms of malondialdehyde (MDA) equivalent accumulation in the samples. The sample was homogenized in 0.1% trichloroacetic acid (TCA) followed by centrifugation. To the supernatant, 20% TCA containing 0.5% thiobarbituric acid was added, heated for

30 min at 95 °C, and cooled quickly. After centrifugation, the absorbance of the supernatant was recorded at 532 nm and 600 nm. Malondialdehyde (MDA) was quantified using extinction coefficient of 155 mM^{-1} cm^{-1} (Sharma et al. 2022).

Ascorbic Acid Estimation

The dried sample (100 mg) was crushed into 6% trichloro acetic acid and extracted. In the reaction mixture, 2% 2,4-dinitrophenylhydrazine and 10% thiourea was added. The reaction mixture was incubated at 37 °C for 15 min. After addition of 80% sulphuric acid to the reaction mixture, its absorbance was read at 530 nm (Panwar et al. 2016).

Hydrogen Peroxide Determination

100 mg of sample was crushed in 5 mL of 0.1% trichloro acetic acid and centrifuged to yield the extract. To the 0.5 mL of supernatant, 0.5 mL of 10 mM postassium phosphate buffer and 1 mL KI was added and incubated in the dark for 1 h. The absorbance of the reaction mix was measured at 390 nm using UV–Visible spectrophotometer (Wang and Jiao 2000).

HPLC Quantification of Flavonoids

A HPLC analysis was carried out to determine the amounts of flavonoid under different irradiance, following the methods by Qader et al. (2012). The phytochemical analysis was conducted using the high-performance liquid chromatography (HPLC) method, using an LC-20AD apparatus (Shimadzu Corp, Kyoto, Japan).

The experiments were carried out using a reversed phase C18 Hypersil GOLD column (250 mm × 4.6 mm; Thermo Fisher Scientific). The samples were eluted with a stepwise elution of the mobile phase. The mobile phase consisted of solvent A [H_2O + 0.1% trifluoroacetic acid (TFA)] and solvent B (methanol + 0.1% TFA), at a unique flow rate of 1 mL/min. The gradient elution program was as follows: 0-min 90% A, 10% B; 5 min 55% A, 45% B; 20 min 50% A, 50% B; 21 min 40% A, 60% B and 22 min 90% A, 10% B. The sample injection volume was 20 µL followed by equilibration run of 15 min and was monitored with an SPD-20 A (UV) at 280 nm.

Statistical Analysis

Data obtained from the study was analyzed using two-way analysis of variance (two-way ANOVA) and means separation tests between treatments were performed using Duncan multiple range test (DMRT) for the significant interaction effect, while Tukey's post hoc test was used to determine the significance of single effect. The standard error of differences between means was calculated with the assumption that data were normally distributed and equally replicated. The relationships between parameters were analyzed by using Pearson's correlation. A multivariate analysis was also done to determine two principle relationships: the relationship between the growth performance and physiological components of the plant and the relationship between the antioxidant activities with the secondary metabolites content. These relationships were determined by using the statistical method of Partial Least Squares Regression (PLSR) using XLSTAT version 2021.1 (Addinsoft Inc., Paris, France). The importance of the physiological components and the secondary metabolites content were expressed by Variable Importance in Projection (VIP) scores, where a VIP score of more than 0.8 (90% confidence interval; CI) was rated as important, whereas a VIP score of more than 1 (95% CI) was deemed as the most important. The said relationships were shown by using a PLSR correlation plot and the summary of the two-way ANOVA table with shading level and treatment durations as the main factors is also provided.

Results

Plant Growth

Plant height (PH) and number of leaves (LN) of *P. minus* were both significantly influenced by the single effect of shade levels. There was no significant interaction observed in PH and LN between the shade levels and treatment durations (Supplementary Table S1). After the 29th day, the highest PH was obtained under T1 (29.384 cm ± 2.381) followed by T3 (22.797 cm ± 2.411) > T2 (21.394 cm ± 2.103) and the lowest plant height was found under T4 (15.969 cm ± 3.221) (Fig. 2a). Similarly, T1 recorded the highest LN compared to other treatments (Fig. 2b). This confirms that high light intensity resulted in high growth of leaves. LN under T3 and T4 were observed to constantly decrease from day 11 until the 29th day. LN was also strongly correlated with PH ($r = 0.674$, $p \leq 0.01$). This shows that the increase in PH will also increase the number of leaves.

Biomass and Root–Shoot Ratio

Data analysis showed that there was no significant interaction between the treatment durations and different shade levels on plant biomass (Supplementary Table S1). However, shade levels were found to significantly influence the LDW, SDW and RDW (Fig. 3a). T1 was observed to yield the highest leaf dry weight (LDW), stem dry weight (SDW), root dry

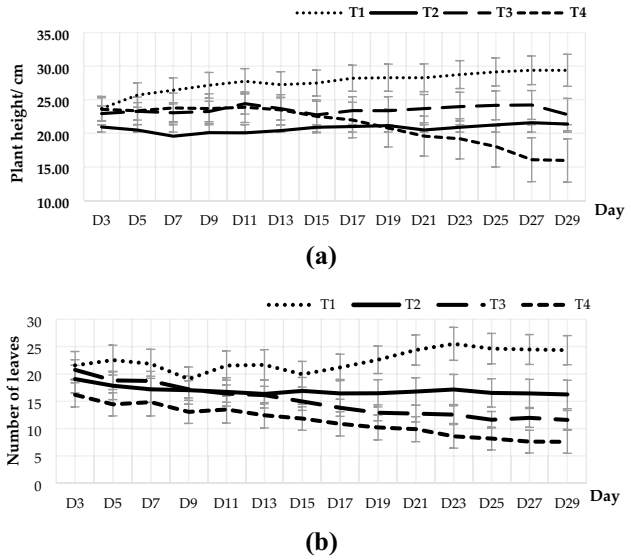


Fig. 2 **a** Effect of shade levels on plant height; **b** effect of shade levels on number of leaves. [T1 = 0% Shaded; T2 = 30% Shaded; T3 = 50% Shaded and T4 = 70% Shaded. Data are means of treatments; Small bars represent standard error]

weight (RDW) and root-shoot ratio (RSR) compared to other treatments (Fig. 3a). In comparison, lower irradiance level (under 50% and 70% shade levels) showed low biomass dry weight than T1. Meanwhile, 30% shade level (T2) produced LDW of approximately 0.511 g, which was not significantly different compared to the control (0.595 g).

Meanwhile, the SDW was observed to be significantly influenced by the single factor of treatment durations, but not LDW, RDW and RSR (Fig. 3b). Treatment duration of 20 weeks was observed to produce higher biomass (dry weight), with lower RSR compared to 10 weeks duration. Longer treatment duration was also found to increase the plant biomass under all treatments (Fig. 3b).

Leaf Gas Exchange, Leaf Pigments and Chlorophyll Fluorescence

The highest photosynthesis rate (A) was observed under T2 (30% shade level) (21.916 ± 0.419 µ mol CO_2 m^{-2} s^{-1}) followed by T3 > T4 = T1 (Table 1). Meanwhile, the lowest intercellular CO_2 (C_i) was observed under 50% shade level (T3), followed by T2 > T4 > T1 (Table 1). Stomatal conductance (Gs) were also found to be significantly influenced by different shade levels ($p < 0.01$). The highest Gs (2.012 ± 0.033 mol H_2O m^{-2} s^{-1}) were recorded under T1 > T2 > T4 > T3.

Besides, the interaction between shade levels and treatment durations was found to significantly influence the transpiration rate (E), leaf resistance (RL) and leaf pigments content ($p < 0.05$) (Supplementary Table S1). The transpiration rate (E) was found to be the highest in T3 at 20 weeks, followed by T4 at 10 weeks > T3 at 10 weeks = T1 at 10 weeks > T2 at 10 weeks, while the lowest E value was obtained by T4 at 20 weeks (Fig. 4a). RL was found highest under T4 with treatment duration of 20 weeks, followed by T1 at 20 weeks > T2 at 20 weeks and the lowest was recorded by T4 with treatment duration of 10 weeks (Fig. 4a).

The highest chlorophyll a (Chl a) (2.431 ± 0.224 mg g^{-1} FW), chlorophyll b (Chl b) (1.919 ± 0.156 mg g^{-1} FW) and total chlorophyll (total Chl) (4.349 ± 0.376 mg g^{-1} FW) were obtained under T4 at 20 weeks of treatment duration, followed by T3 at 10 weeks = T2 at 20 weeks, while the lowest was recorded by T1 with treatment duration of 20 weeks (Fig. 4b). Meanwhile, chlorophyll a/b (Chl a/b) ratio was found to be highest under T1 with treatment duration of 20 weeks, followed by T3 at 20 weeks = T4 at 20 weeks, while the lowest a/b ratio was recorded by T1 with treatment duration 10 weeks (Fig. 4b).

The highest carotenoid (Car) value was obtained under T3 after 20 weeks (1.225 ± 0.103 mg g^{-1} FW), parallel to the highest amount of chlorophyll obtained. The highest in-situ chlorophyll (SPAD) value was obtained under T3 with treatment duration of 20 weeks (35.717 ± 0.841), but the value was not significantly different compared to T4 with treatment duration of 20 weeks (35.042 ± 1.167) (Fig. 4b). These were parallel with the chlorophyll content that was found to be the highest under T4 under the same treatment duration. On the other hand, chlorophyll fluorescence shows non-significant difference on the interaction effect, neither the single effect (Supplementary Table S1). The results of chlorophyll fluorescence are presented in Table 2.

Relationships Between Growth Performance and Physiological Components of Plant

The significant Pearson's correlations between the plant growth and physiological parameters described in earlier paragraphs are summarized in Table 3. Moreover, a multivariate analysis (PLSR) was also conducted to evaluate the contributions or relationships between the physiological parameters of the plant (X variables) on plant growth performance (Y). As observed in the PLSR correlation plot in Fig. 5a, the chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll a + b (total a + b), in-situ chlorophyll content (SPAD), carotenoid (Car), photosynthesis rate (A), transpiration rate (E), maximal fluorescence (F_m) and maximum quantum yield of PSII (F_v/F_m) are all negatively loaded along Dimension 1. Meanwhile, the intercellular CO_2 (Ci), chlorophyll a/b ratio (Chl a/b), relative humidity (RH), stomatal conductance (Gs), minimal fluorescence (F_o), and

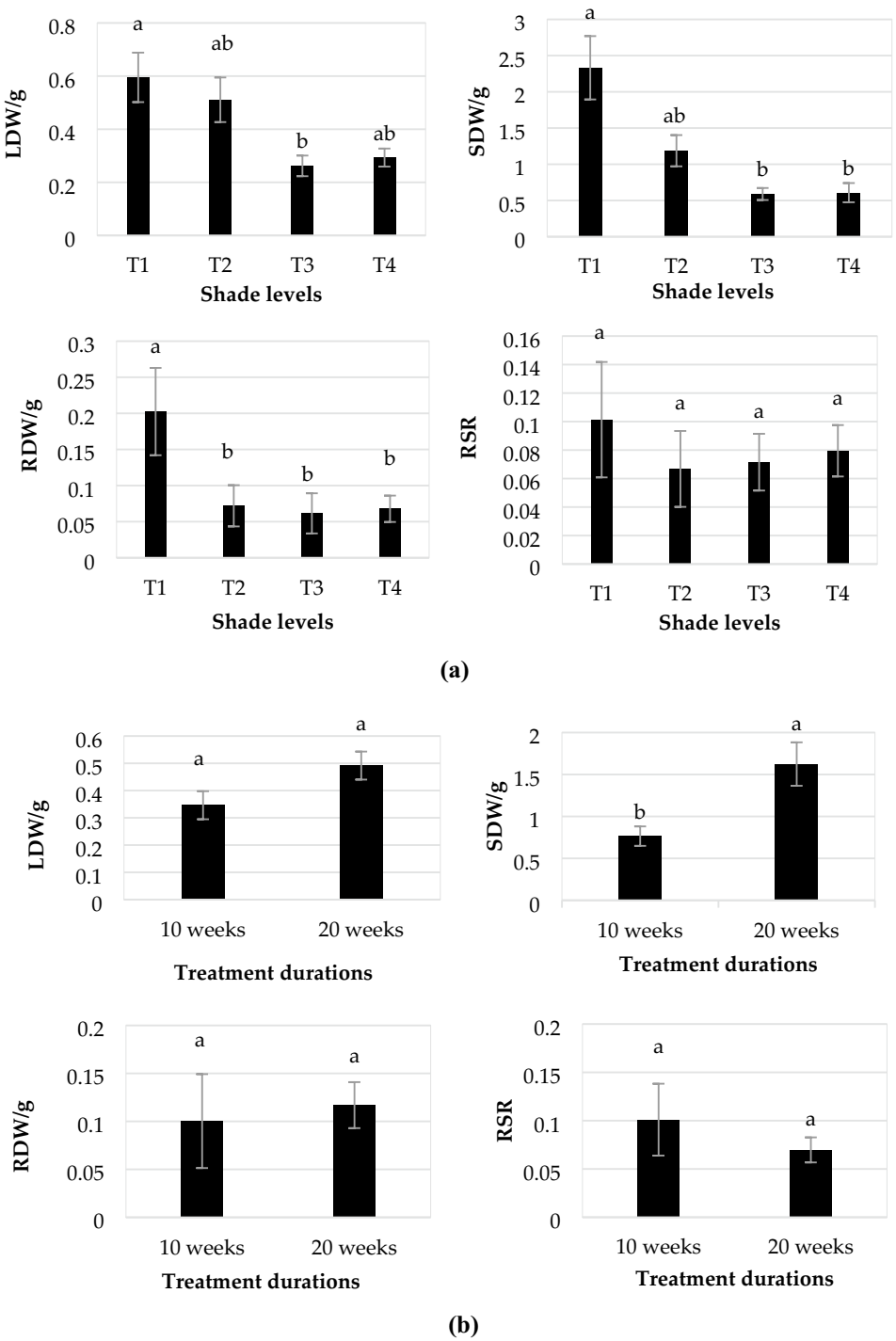


Fig. 3 **a** Effects of different shade levels on leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW) and root-shoot ratio (RSR); **b** effects of treatment durations on leaf dry weight (LDW), shoot dry weight (SDW), root dry weight (RDW) and root-shoot ratio (RSR). [T1 = 0% Shaded; T2 = 30% Shaded; T3 = 50% Shaded and T4 = 70% Shaded. Data are means of treatments; Small bars represent standard error]

ratio of variable fluorescence (F_o/F_m) are positively loaded along Dimension 1 (Fig. 5a). Additionally, based on the computed VIP values, the pigment content (Chl a, Car, total a + b and SPAD), Gs and Ci recorded VIP scores of > 1.0, which indicate that these are the parameters that gave major contribution to the growth performance of *P. minus*. The VIPs values are illustrated in Fig. 5b.

Total Anthocyanin, Phenolic and Flavonoid Contents

The total anthocyanin content (TAC), total phenolic content (TPC) and total flavonoid content (TFC) of *P. minus* leaves were all significantly influenced by the interaction effect of shade levels and treatment durations (Supplementary Table S2). For plants that were harvested after 10 weeks, T3 recorded the highest TAC (306.592 ± 7.13 μg g^{-1} DW)

Table 1 The effects of the different shade levels on the leaf gas exchange of *P. minus*

Shade levels		T1	T2	T3	T4
Leaf gas exchange	A (μ mol CO_2 m^{-2} s^{-1})	14.849 ± 0.209^{c}	21.916 ± 0.419^{a}	16.597 ± 0.338^{b}	15.126 ± 0.443^{c}
	C_i (μ mol CO_2 mol^{-1})	370.712 ± 0.504^{a}	349.841 ± 0.902^{c}	329.972 ± 13.565^{d}	362.012 ± 0.44^{b}
	Tleaves (°C)	31.5 ± 0.348^{a}	31.575 ± 0.321^{a}	31.608 ± 0.309^{a}	31.725 ± 0.287^{a}
	RH	78.886 ± 0.112^{a}	78.568 ± 0.546^{b}	74.268 ± 1.408^{d}	77.215 ± 0.054^{c}
	Gs (mol H_2O m^{-2} s^{-1})	2.012 ± 0.033^{a}	1.354 ± 0.065^{b}	1.086 ± 0.161^{d}	1.186 ± 0.026^{c}

Means with different letters on top of each bar are significantly different at $p < 0.05$ according to Tukey's test

A photosynthesis rate, *Ci* intercellular CO_2, *Tleaves* leaf temperature, *RH* relative humidity, *Gs* stomatal conductance

followed by T1 (287.221 ± 6.92 μg g^{-1} DW) and the lowest was obtained by T4 (203.822 ± 6.59 μg g^{-1} DW) (Fig. 6a). However, when the plants were exposed with longer treatment duration of 20 weeks, T2 was observed to yield the highest TAC (360.695 ± 18.07 μg g^{-1} DW) followed by T4 > T3.

In terms of the total phenolic content, T1 was observed to yield the highest TPC (395.491 ± 10.37 mg g^{-1} DW) after 10 weeks of treatment, followed by T3 > T4 > T2. Meanwhile, for plants harvested after 20 weeks, the highest TPC was recorded under T2, with values of 643.013 ± 25.64 mg g^{-1} DW (Fig. 6b), similar to that recorded for TAC (Fig. 6a). On the other hand, T4 recorded the lowest TPC after 20 weeks of treatment duration (248.163 ± 19.83 mg g^{-1} DW) (Fig. 6b). Overall, the TPC in all treatments was observed to increase with treatment duration (Fig. 6b). The same trend was observed for TFC, where the TFC of all treatments was observed to also increase with treatment duration (Fig. 6c). After 10 weeks of treatment, the highest TFC was observed in T1 (201.904 ± 1.42 mg g^{-1} DW), followed by T3 > T4 > T2. These observations were similar to that obtained in TPC after 10 weeks of treatment (Fig. 6b). Meanwhile, the TFC of T2 and T4 at 10 weeks were observed to be not significantly different. In addition, after 20 weeks of treatment, T1 was also observed to produce the highest TFC (387.606 ± 17.78 mg g^{-1} DW), followed by T2 > T4 > T3. Nevertheless, the TFC of T1, T2 and T4 was not significantly different.

Antioxidant Assays

The highest FRAP reducing power was recorded under T4 of 10 weeks (7.145 ± 0.01 mg g^{-1} DE) and under treatment duration of 20 weeks (7.628 ± 0.44 mg g^{-1} DE). T4 also showed excellent DPPH radical scavenging activities at both treatment durations, where T4 leaf extract subjected to 10 weeks of treatment needed only 1.873 mg mL^{-1} of the extract to inhibit 50% of the DPPH radicals, and 2.507 mg mL^{-1} for T4 leaf extracts subjected to 20 weeks treatment. These results are shown in Table 4.

A correlation analysis was also conducted to determine the relationships between the secondary metabolites and antioxidant activities observed (Table 5). Significantly strong correlations were observed between Chl a with Chl b ($r = 0.862$, $p < 0.01$), Car ($r = 0.982$, $p < 0.01$) and between Chl b with Car ($r = 0.923$, $p < 0.01$). Meanwhile, significantly moderate correlations were observed between these three pigments with TAC (Table 5), indicating that the increase in Chl a, Chl b and Car pigments will lead to an increase in TAC. Similarly, a moderate but significant correlation was also observed between TFC and TPC ($r = 0.465$, $p < 0.01$). Nevertheless, only weak but significant correlations were observed between FRAP with Chl a ($r = 0.196$; $p < 0.05$) as well as between DPPH with TPC ($r = -0.240$, $p < 0.01$) and TFC ($r = -0.230$, $p < 0.01$).

Carbohydrates Estimation

Total carbohydrate content (TCC) was recorded to be significantly affected by the interaction of different shade levels and treatment durations (Supplementary Table S2). The highest TCC was recorded under shade level of T2 of 20 weeks (49.712 ± 0.611 mg g^{-1} DW) followed by T4 of 20 weeks (41.882 ± 0.015 mg g^{-1} DW) which are not significantly different with the total carbohydrate content recorded under T2 of 10 weeks (41.346 ± 0.415 mg g^{-1} DW) and the lowest was recorded under T3 of 20 weeks (28.327 ± 0.158 mg g^{-1} DW). Under treatment duration of 10 weeks, the TCC recorded under 0% shade levels or T1 (31.634 ± 0.5 mg g^{-1} DW) was not significantly different compared to the data obtained under 70% shade levels (T4) (34.458 ± 0.151 mg g^{-1} DW). These data are represented in Fig. 7.

Activity of Antioxidant Enzymes

The SOD and APX enzyme activities were all recorded to be significantly affected by the interaction effect of shade levels and treatment durations (Supplementary Table S2). Under treatment durations of 10 weeks, the SOD obtained between all treatments were not significantly different

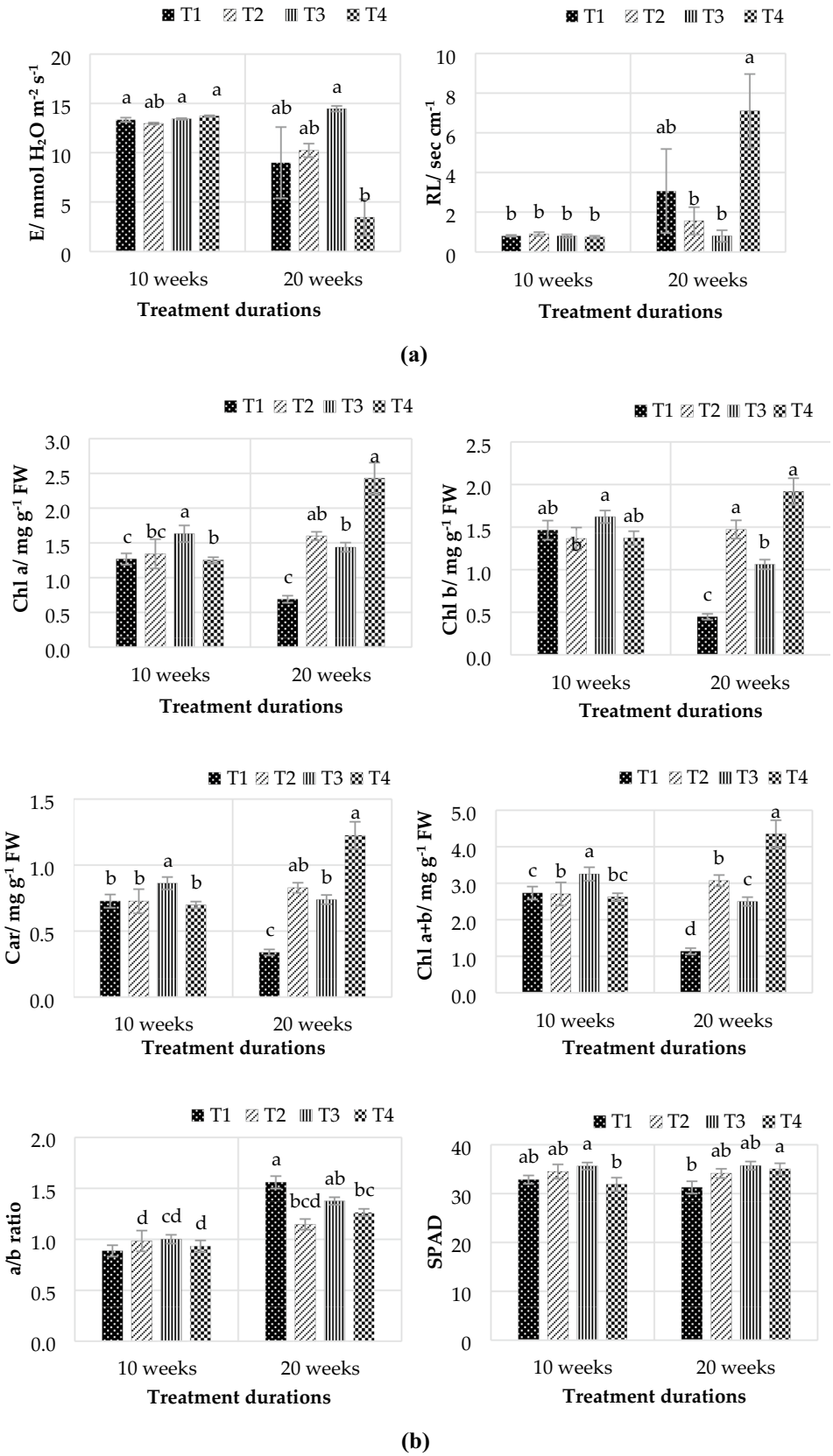


Fig. 4 **a** Effects of different shade levels on transpiration rate (E) and leaf resistance (RL); **b** effects of different shade levels on the leaf pigments (Chl a, Chl b, Car, Chl a + b, Chl a/b ratio and SPAD value). [T1 = 0% Shaded; T2 = 30% Shaded; T3 = 50% Shaded and T4 = 70% Shaded. Data are means of treatments; Small bars represent standard error]

Table 2 The effects of the different shade levels and treatment durations on leaf fluorescence of *P. minus*

Shade Levels		T1		T2		T3		T4	
Treatment Durations		10 Weeks	20 Weeks	10 Weeks	20 Weeks	10 Weeks	20 Weeks	10 Weeks	20 Weeks
Chlorophyll Fluorescence	F_0	5996.88 ± 485.777[a]	5226.75 ± 165.728[a]	5562.083 ± 314.531[a]	5664.25 ± 136.636[a]	5705.0 ± 128.347[a]	5923.0 ± 246.287[a]	5599.38 ± 532.954[a]	5931.0 ± 304.521[a]
	F_m	29,580.5 ± 2118.779[a]	26,936.5 ± 1852.598[a]	28,993.0 ± 1139.336	31,406.5 ± 614.723[a]	30,867.0 ± 735.18[a]	30,353.0 ± 1758.76[a]	26,824.75 ± 3152.609[a]	31,563.0 ± 507.502[a]
	F_0/F_m	0.2017 ± 0.006[a]	0.1961 ± 0.011[a]	0.1897 ± 0.007[a]	0.1804 ± 0.004[a]	0.1853 ± 0.005[a]	0.1962 ± 0.008[a]	0.188 ± 0.007[a]	0.1998 ± 0.005[a]
	F_v/F_m	0.798 ± 0.006[a]	0.804 ± 0.011[a]	0.81 ± 0.007[a]	0.820 ± 0.004[a]	0.815 ± 0.005[a]	0.804 ± 0.007[a]	0.774 ± 0.021[a]	0.812 ± 0.007[a]

Means with different letters on top of each bar are significantly different at $p < 0.05$ according to Tukey's test. [Note: F_0 = Minimal fluorescence, F_m = Maximal fluorescence, F_0/F_m = Ratio of variable fluorescence, F_v/F_m = Maximum quantum yield of PSII]

with each other, where the highest reading obtained under T1 (35.586 ± 0.433 units mg^{-1} DW) and the lowest was obtained under T4 (33.634 ± 0.173 units mg^{-1} DW). However, under the treatment durations of 20 weeks, the highest SOD enzyme activity was recorded under T1 (38.288 ± 0.433 units mg^{-1} DW) but the value was not significantly different with the one recorded under T4 (36.036 ± 1.040 units mg^{-1} DW). The lowest SOD enzyme activity was recorded under T2 (33.633 ± 0.347 units mg^{-1} DW). These results are shown in Fig. 8a.

Moreover, significant interaction effects between the shade levels and treatment duration were also observed in the ascorbate peroxidase (APX) enzyme activity (Supplementary Table S2), where under the treatment duration of 10 weeks, the same trend as observed in the SOD enzyme activity was obtained. The highest APX enzyme activity was recorded under T1 (1.277 ± 0.145 nmole min^{-1} mg^{-1} DW) followed by T2 > T3 and the lowest was recorded under T4 (0.461 ± 0.247 nmole min^{-1} mg^{-1} DW). However, when the treatment duration was prolonged to 20 weeks, the highest enzyme activity was recorded under T4 (1.631 ± 0.034 nmole min^{-1} mg^{-1} DW) followed by T1 > T3 and the lowest was observed under T2 (0.725 ± 0.183 nmole min^{-1} mg^{-1} DW). The results of APX enzyme activities are shown in Fig. 8b.

Ascorbic Acid Estimation

The ascorbic acid (AsA) content was significantly affected by the interaction of shade levels and treatment durations (Supplementary Table S2). The highest AsA content was observed under T4 of treatment duration of 10 weeks (19.984 ± 0.212 µg mg^{-1} DE), followed by T2 (17.224 ± 0.069 µg mg^{-1} DE) and the lowest was recorded under T1 (7.904 ± 0.055 µg mg^{-1} DE). Meanwhile, under treatment duration of 20 weeks, the highest AsA was recorded under T3 (17.808 ± 0.055 µg mg^{-1} DE) and the lowest was recorded under T4 (12.592 ± 0.055 µg mg^{-1} DE), completely in contrast to when the plant was exposed to treatment duration of 10 weeks, however the values were not significantly different with the AsA recorded under T1 (12.736 ± 0.018 µg mg^{-1} DE). The effects of the treatments and treatment durations on the AsA contents were shown in Fig. 9.

Hydrogen Peroxide and Lipid Peroxidation Determination

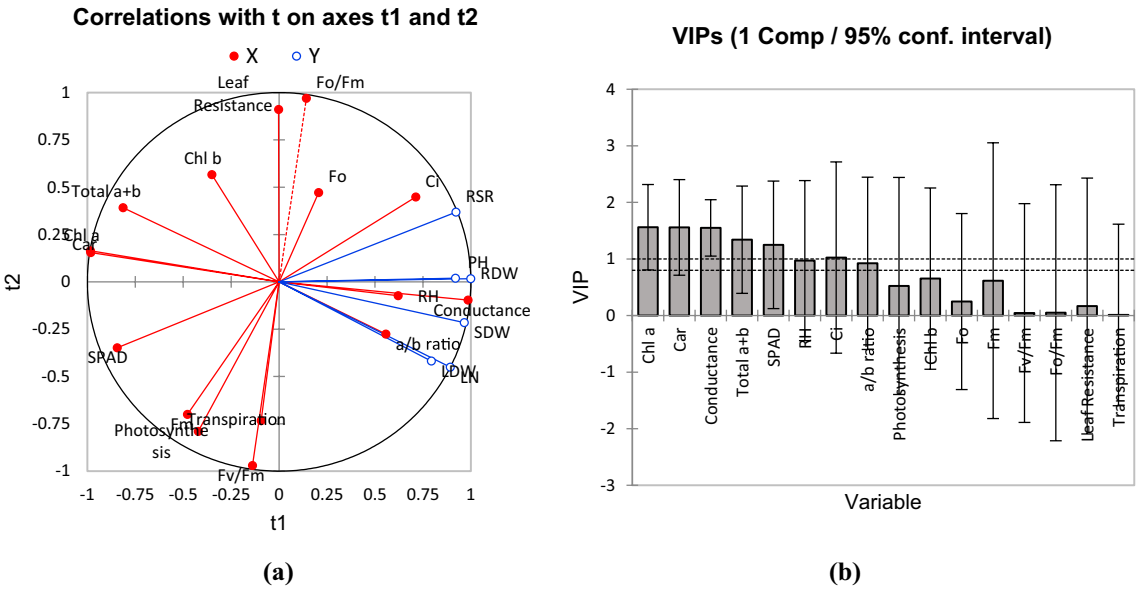
The concentration of hydrogen peroxide (H_2O_2) in *P. minus* was found to be significantly affected by the interaction effect of shade levels and treatment durations (Supplementary Table S2). Under the treatment

Table 3 Significant Pearson's correlation between parameters measured in this phase

	1 PH	2 LN	3 LDW	4 SDW	5 RDW	6 RSR	7 A	8 Ci	9 E	10 Gs	11 RH	12 Tleaves	13 R_L	14 Chl a	15 Chl b	16 Car	17 Total Chl	18 a/b ratio	19 SPAD	20 F_o	21 F_m	22 F_o/F_m	23 F_v/F_m
1	1																						
2	0.674**	1																					
3	0.033	0.114	1																				
4	0.225*	0.225*	0.546**	1																			
5	0.123	0.100	0.287**	0.318**	1																		
6	-0.017	−0.024	0.091	0.026	0.854**	1																	
7	−0.021	−0.123	0.371**	0.192*	0.037	0.128	1																
8	0.039	−0.083	0.371**	0.219*	0.088	0.175	0.946**	1															
9	0.088	−0.017	0.405**	0.282**	0.148	0.192*	0.847**	0.950**	1														
10	0.089	−0.009	0.416**	0.299**	0.150	0.180	0.826**	0.927**	0.995**	1													
11	0.029	−0.091	0.372**	0.214*	0.079	0.168	0.963**	0.997**	0.934**	0.910**	1												
12	−0.117	−0.152	0.231	0.163	0.118	0.101	−0.068	0.115	0.119	0.082	0.108	1											
13	0.048	−0.043	0.052	−0.005	−0.012	0.023	0.292**	0.336**	0.305**	0.269**	0.331**	0.173	1										
14	0.001	−0.059	−0.097	−0.096	−0.122	−0.060	0.202*	0.196*	0.131	0.108	0.200*	−0.230	0.019	1									
15	−0.001	−0.078	−0.089	−0.174	−0.118	0.003	0.350**	0.333**	0.260**	0.230*	0.341**	−0.186	0.141	0.862**	1								
16	−0.017	−0.085	−0.123	−0.134	−0.137	−0.043	0.245*	0.241*	0.175	0.150	0.245*	−0.211	0.052	0.982**	0.923**	1							
17	0.000	−0.070	−0.097	−0.142	−0.125	−0.030	0.286**	0.274**	0.202*	0.175	0.280**	−0.235	0.084	0.969**	0.961**	0.989**	1						
18	0.040	0.096	0.155	0.282**	0.092	−0.006	−0.104	−0.070	−0.043	−0.029	−0.078	−0.095	−0.145	0.241**	−0.112	0.152	0.077	1					
19	−0.189*	−0.253**	0.074	0.040	0.036	0.015	0.151	0.120	0.106	0.102	0.127	−0.196	0.037	0.112	0.134	0.129	0.127	−0.038	1				
20	0.005	−0.040	0.217*	0.074	0.034	0.177	0.798**	0.804**	0.693**	0.653**	0.814**	−0.021	0.308**	0.348**	0.600**	0.421**	0.493**	−0.246*	0.112	1			
21	0.012	−0.035	0.223*	0.072	0.004	0.142	0.810**	0.800**	0.684**	0.647**	0.812**	−0.136	0.268*	0.378**	0.613**	0.444**	0.515**	−0.229*	0.115	0.992**	1		
22	−0.029	−0.082	0.228*	0.107	0.029	0.155	0.797**	0.810**	0.704**	0.661**	0.817**	0.238	0.469**	0.318**	0.578**	0.393**	0.466**	−0.254*	0.113	0.934**	0.912**	1	
23	−0.010	−0.063	0.240*	0.100	−0.009	0.122	0.834**	0.825**	0.713**	0.677**	0.837**	−0.238	0.340**	0.369**	0.616**	0.440**	0.512**	−0.239*	0.124	0.975**	0.979**	0.961**	1

The (*) indicates significant difference at $p < 0.05$ while (**) indicates significant different at $p < 0.01$

PH plant height, *LN* number of leaves, *LDW* leaf dry weight, *SDW* stem dry weight, *RDW* root dry weight, *RSR* root–shoot ratio, *A* photosynthesis rate, *Ci* intercellular CO_2, *E* transpiration rate, *Gs* stomatal conductance, *RH* relative humidity, *Tleaves* leaf temperature, *RL* leaf resistance, *Chl a* chlorophyll a, *Chl b* chorophyll b, *Total chl* total chlorophyll content, *Car* carotenoid, *a/b ratio* chlorophyll a and b ratio, *SPAD* in-situ chlorophyll, *F0* minimal fluorescence, *Fm* maximal fluorescence, *F0/Fm* ratio of variable fluorescence, *Fv/Fm* maximum quantum yield of PSII total anthocyanin, phenolic and flavonoid content



(a) (b)

Fig. 5 Partial Least Squares Regression (PLSR) analysis to determine the relationship between the growth performance and the physiological parameters of *P. minus*; **a** PLSR correlation plot; **b** VIPs score. [Small bars represent standard error, dotted line indicates threshold values of 0.8 and 1.0]

duration of 10 weeks, the highest H_2O_2 concentration was recorded under T1 (30.959 ± 0.118 µmol mg^{-1} DE) followed by T3 (30.745 ± 0.133 µmol mg^{-1} DE), T2 (23.882 ± 0.059 µmol mg^{-1} DE) and the lowest concentration was found under T4 (19.933 ± 0.015 µmol mg^{-1} DE). These results contrasted with the values of APX enzyme activity recorded under the treatment duration of 10 weeks, where the highest was recorded under T1. Under the treatment duration of 20 weeks, the highest concentration of H_2O_2 was recorded under T4 (38.215 ± 0.163 µmol mg^{-1} DE) but the value was not significantly different than the one recorded under T1 (37.908 ± 0.104 µmol mg^{-1} DE). The lowest concentration of H_2O_2 recorded under the treatment duration of 20 weeks was under T2 (24.574 ± 0.163 µmol mg^{-1} DE). The effects of the treatments and treatment durations on H_2O_2 concentration in *P. minus* are recorded in Fig. 10. *P. minus* was also subjected to the analysis of malondialdehyde concentration (MDA) to study the effects of shade levels and treatment duration on the lipid peroxidation of *P. minus*. However, the values recorded for MDA levels were too low to be detected.

Relationships Between Antioxidant Activities and Secondary Metabolites Content

The relationships or contributions of the secondary metabolites content and antioxidant enzymes (TAC, TPC, TFC, TCC, AsA, APX and SOD as the X variables) on the antioxidant activities (Y) of the extracts were elucidated using PLSR. Figure 11a shows that the TAC, TPC, TFC, SOD and APX are all negatively loaded along Dimension 1, while the intercellular TCC and AsA are positively loaded along Dimension 1. Meanwhile, based on the computed VIP values, the antioxidant enzymes (APX and SOD) and AsA recorded VIP scores of > 1.0, which indicated that these three variables were the most important factors that influenced the antioxidant activities of the plants. The VIPs values are illustrated in Fig. 11b.

HPLC Quantification of Individual Flavonoids

The extracts from different plant parts (leaf, stem and roots) were also subjected to HPLC analysis to screen and quantify the amount of selected flavonoids. The leaf extracts were observed to contain kaempherol, kaempherol 3-glucoside, myricetin, quercetin and rutin, with myricetin and rutin found in a significant amount (Table 6). However, the amounts of flavonoids in the leaf extracts were observed to reduce after a longer treatment duration (20 weeks). The content of kaempherol 3-glucoside in the leaf samples were also observed to diminish after 20 weeks of treatment duration (Table 6). This contrasts with the observation found on the stem extracts, where synthesis of kaempherol 3-glucoside was only observed at 20 weeks of treatment duration and was not detected at 10 weeks of treatment. Not only that, the stem extracts were also observed to contain very little amounts of flavonoids, in contrast to the amounts recorded in the leaf extracts (Table 7). Similar observations were also recorded in the root extracts, where root extracts obtained from plants harvested after 10 weeks of treatment mostly

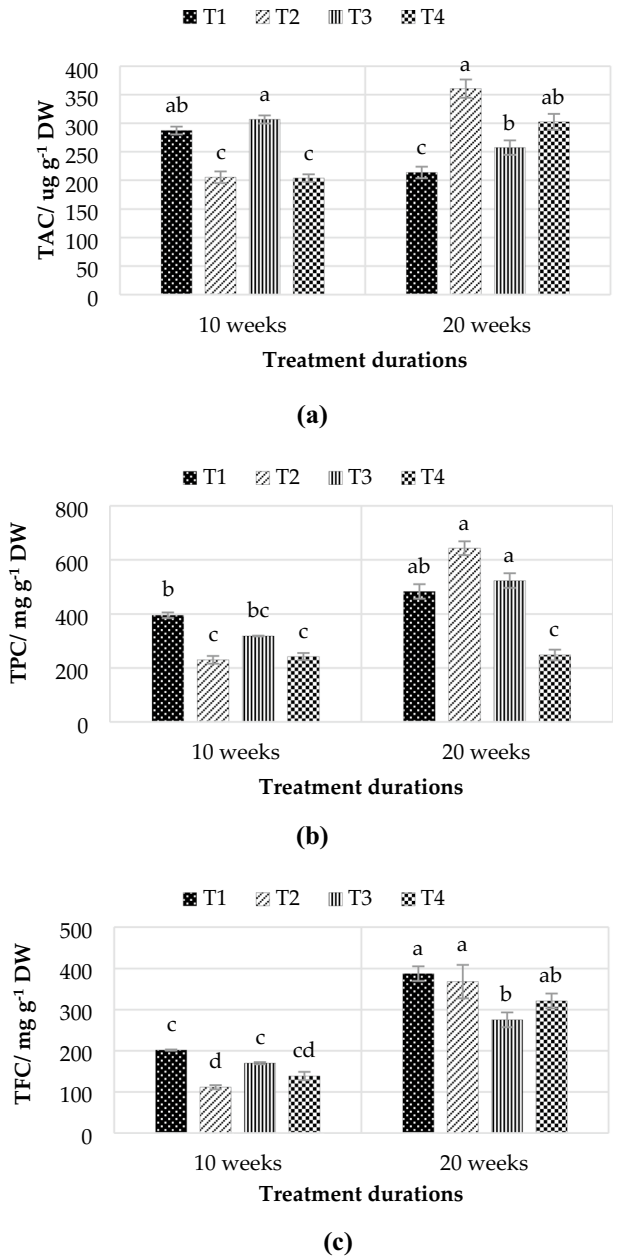


Fig. 6 Effects of different shade levels on the **a** total anthocyanin; **b** total phenolic and; **c** total flavonoid contents of *P. minus*. [T1 = 0% Shaded; T2 = 30% Shaded; T3 = 50% Shaded and T4 = 70% Shaded. Data are means of treatments; Small bars represent standard error]

consists of kaempherol 3-glucoside (Table 8). However, its synthesis was found to be stopped at longer treatment duration (20 weeks). Besides that, root extracts were also observed to be lacking in quercetin and rutin compounds, at both treatment durations (10 and 20 weeks). Two-way ANOVA analysis showed that quercetin production in leaves and stem of *P. minus* is significantly influenced by the interaction effect of shading levels and treatment durations (Supplementary Table S2). Meanwhile, myricetin is recorded to be significantly influenced by the interaction effect of the two factors in root of *P. minus* (Supplementary Table S2).

Discussion

The data obtained in this study was analyzed for the interaction effects between the shading levels and the treatment durations exposed onto the plants. It was reported that both PH and LN were significantly influenced by single factor shade levels. Both PH and LV have the highest value under T1 and the lowest under T4, after 20 days of exposure. This result is in contrast with the one observed in the study of Bhatt et al. (2002) on 17 tropical grasses and legumes where the plant height was high under low light condition, at maximum of 50% shading. The growth of *P. minus* was also observed to reduce at low irradiance level (under 50% and 70% shade levels) compared to under T1, which indicates that low light intensity indeed limits the growth of *P. minus*. Under 30% shade level (T2), the LDW recorded was not significantly different compared to the LDW recorded under control or 0% shade level (T1). In line with this observation, the study on *Castana dentata* seedlings also showed that 32% light intensity yielded high weight of leaves and were not significantly different with 100% light intensity (Wang et al. 2006). This was different to the one reported in the study on *Mitragyna speciosa* Korth. where it was shown that low light conditions would most likely maximize plant biomass and total leaf alkaloid production (Zhang et al. 2022). Analysis showed that LDW was positively correlated with SDW ($r = 0.546$, $p \leq 0.01$). Besides that, LDW also showed significant correlation with A ($r = 0.371$, $p \leq 0.01$), Ci ($r = 0.371$, $p \leq 0.01$), E ($r = 0.405$, $p \leq 0.01$) and Gs ($r = 0.416$, $p \leq 0.01$). The association between plant yield and photosynthetic performance have long been studied especially under controlled growth conditions, as light conditions under natural environments are often unpredictable and changed rapidly. However, plant's ability to respond and adapt to the differences in light intensities are also highly demonstrated in many studies and some proved that the ability to increase photosynthetic performance has significant potential in increasing plant yield (Lawson et al. 2012).

Significant interaction effects between shading levels and treatment durations were observed in E, RL, leaf pigment contant, i.e. Chl a, Chl b and Car, TAC, TPC and TFC. The intercellular CO_2 concentration of the leaves is a critical indicator in photosynthesis as the mesophyll cells of leaves consume CO_2 during photosynthesis (Tominaga et al. 2018). This is parallel to the significant correlation shown between photosynthesis rate and internal CO_2 concentration ($r = 0.946$, $p < 0.01$) in Table 3. Higher CO_2 assimilation rates and stomatal conductance under lower irradiance level had been reported in a study by Medina et al.

Table 4 The effects of the different shade levels on antioxidant activities of *P. minus* extract

Treatments	FRAP (mg g^{-1} DE)		DPPH IC_{50} (mg mL^{-1})	
	10 weeks	20 weeks	10 weeks	20 weeks
T1	3.588 ± 0.12^{c}	6.434 ± 0.22^{b}	1.152 ± 0.09^{c}	2.531 ± 0.07^{a}
T2	3.244 ± 0.30^{c}	4.063 ± 0.30^{c}	4.704 ± 0.12^{a}	2.367 ± 0.09^{a}
T3	5.108 ± 0.03^{b}	3.131 ± 0.27^{d}	4.631 ± 0.45^{a}	2.592 ± 0.09^{a}
T4	7.145 ± 0.01^{a}	7.628 ± 0.44^{a}	1.873 ± 0.11^{b}	2.507 ± 0.04^{a}

Data are means of treatments; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and, T4 = 70% Shading; DE: Dry Extract; means with different letters within a column are significantly different at $p \leq 0.05$ between shade levels

Table 5 Significant Pearson's correlation between secondary metabolites and antioxidant activities

	1 Chl a	2 Chl b	3 Car	4 TAC	5 TPC	6 TFC	7 FRAP	8 DPPH
1	1							
2	0.862**	1						
3	0.982**	0.923**	1					
4	0.448**	0.421**	0.442**	1				
5	−0.240**	−0.377**	−0.285**	0.397**	1			
6	0.030	−0.272**	−0.055	0.162	0.465**	1		
7	0.196*	0.081	0.161	−0.018	−0.174	0.063	1	
8	0.093	0.090	0.075	−0.097	−0.240**	−0.230**	-0.136	1

The (*) indicates significant difference at $p \leq 0.05$ while (**) indicates significant different at $p \leq 0.01$

Chl a chlorophyll a, *Chl b* chorophyll b, *Car* carotenoid, *TAC* total anthocyanin content, *TPC* total phenolic content, *TFC* total flavonoid content

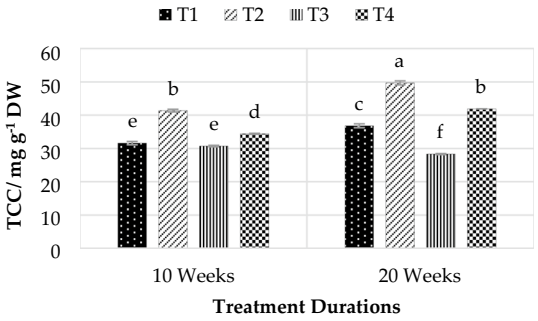
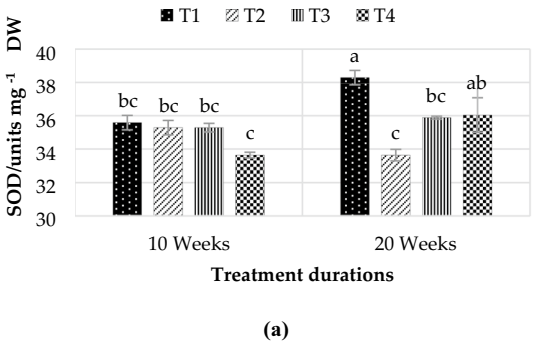


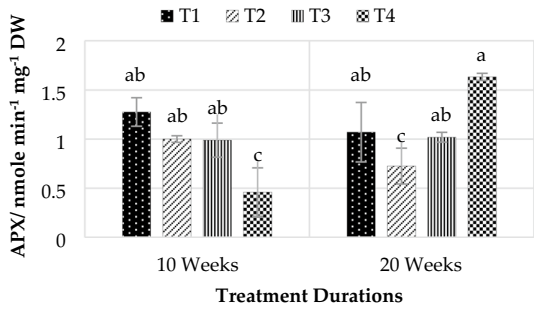
Fig. 7 Effects of different shade levels on the total carbohydrate content of *P. minus*. [Data are means of treatments; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and T4 = 70% Shading. Means with different letters on top of each bar are significantly different at $p \leq 0.05$]

(2002). Correlation between Gs and A was significantly high ($r = 0.826$, $p < 0.01$) (Table 3), whereby the high Gs also indicated the increase of photosynthesis rate. Meanwhile, the leaf temperatures (Tleaves) were not significantly influenced by shade levels in this experiment. The transpiration rate (E) that was found highest under 70% shade levels and being exposed for 20 weeks indicate the importance of shade levels and its significant influence on transpiration rate, where it will aid in reducing the transpiration rate as the plants became adapted to the growth conditions and also due to maturing leaves. Transpiration rate showed a strong correlation with Gs ($r = 0.927$, $p < 0.01$) (Table 3) due to the opening and closing of stomata which would directly influence the transpiration rate and stomatal conductance, where both were greatly depending on leaf characteristics and features (Miyashita et al. 2005), along with differences in mechanical and performance characteristics that may change throughout the treatment duration (Franks and Farquhar 2007). Meanwhile, low but significant correlation was recorded between RL and E ($r = 0.305$, $p \leq 0.01$) and between RL and Gs ($r = 0.269$, $p \leq 0.01$) (Table 3). This shows that the increase in transpiration rate should progressively lower the RL recorded. RL was also influenced by the leaf characteristics and features, and with transpiration rate and stomatal conductance.

Low chl a/b ratio was attributed to the increase in chlorophyll b proportion compared to chlorophyll a and usually obtained under shaded environment, to enhance the efficiency of blue light absorption (Beneragama and Goto 2010). The lowest a/b ratio obtained under T1 of treatment duration 10 weeks may be due to the reduction in chlorophyll b due to interconversion of chlorophyll a and chlorophyll b which is believed to facilitate the regulation



(a)



(b)

Fig. 8 Effects of different shade levels on **a** superoxide dismutase (SOD); **b** ascorbate peroxidase (APX) enzyme activities of *P. minus*. [Data are means of treatments; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and T4 = 70% Shading. Means with different letters on top of each bar are significantly different at $p \leq 0.05$]

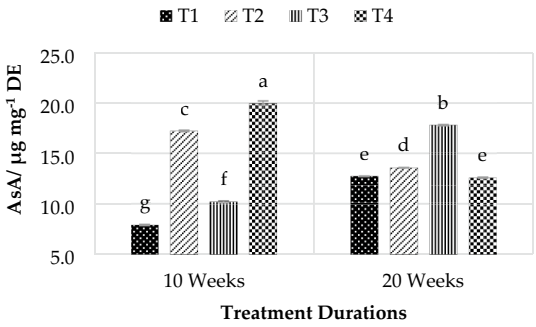


Fig. 9 Effects of different shade levels on the ascorbate acid content activity of *P. minus*

of the chlorophyll composition in a fluctuating light environment and throughout developmental stages (Sato et al. 2015). A strong positive correlation was observed between Chl a and Chl b ($r = 0.862$, $p \leq 0.01$) which showed that the increase of Chl a and Chl b occurred at a similar particular time and the decrease of Chl a would also reduce Chl b pigment. Car showed a strong correlation with Chl a ($r = 0.982$, $p \leq 0.01$) and Chl b ($r = 0.923$, $p \leq 0.01$), which proved that

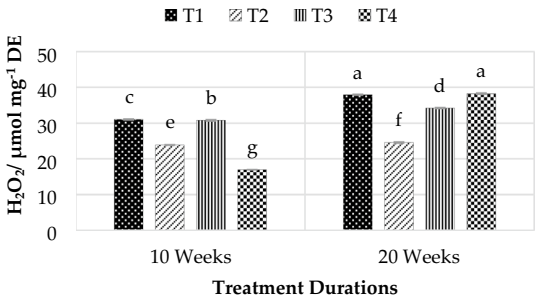


Fig. 10 Effects of different shade levels on the H_2O_2 concentration in *P. minus*

the carotenoid composition of *P. minus* was influenced by the production of chlorophylls in this study.

According to Walters (2005), during the determination of changes in the constituents of the photosynthetic apparatus at a cellular level, it may be more useful to express photosynthesis and chloroplast components relative to chlorophyll, therefore it could reflect the balance in the investment between the capture and utilization of light in photosynthesis process. Degradation of chlorophyll b under high light condition was also reported in a study by Sato et al. (2015). Plants may develop acclimation over time to cope with different irradiance levels in its anatomical, morphological, physiological and biochemical aspects (Zervoudakis et al. 2012). According to Walters (2005), acclimation acts as a homeostatic mechanism, where it is able to reverse the changes from environmental challenges and therefore, maintaining efficient photosynthesis. The present study shows that *P. minus* may have adapted to the low irradiance condition with the increase of treatment duration by developing more photosynthetic pigments in order to utilize more sunlight for photosynthesis process. Again, in understanding acclimation of plants, Walters (2005) also stated that plants with strong acclimation are more likely to succeed in variable conditions, under both exposed and shaded environments. The present study suggests that for 10 weeks of treatment exposure (or treatment duration), 50% shade level was the most optimal condition in the leaf gas exchange traits and the production of chlorophyll in *P. minus* plants. In contrast, 70% was the most optimum shade level for *P. minus* plants exposed to 20 weeks of treatment.

Chlorophyll fluorescence showed non-significant difference on the interaction effect neither the single effect (Supplementary Table S1). However, the chlorophyll fluorescence showed significantly high correlations with A, Ci, E and Gs with r values of more than 0.600, $p < 0.01$ (Table 3). These correlations are common, as chlorophyll fluorescence would indeed provide the information on the efficiency of the phytochemistry process and the process of

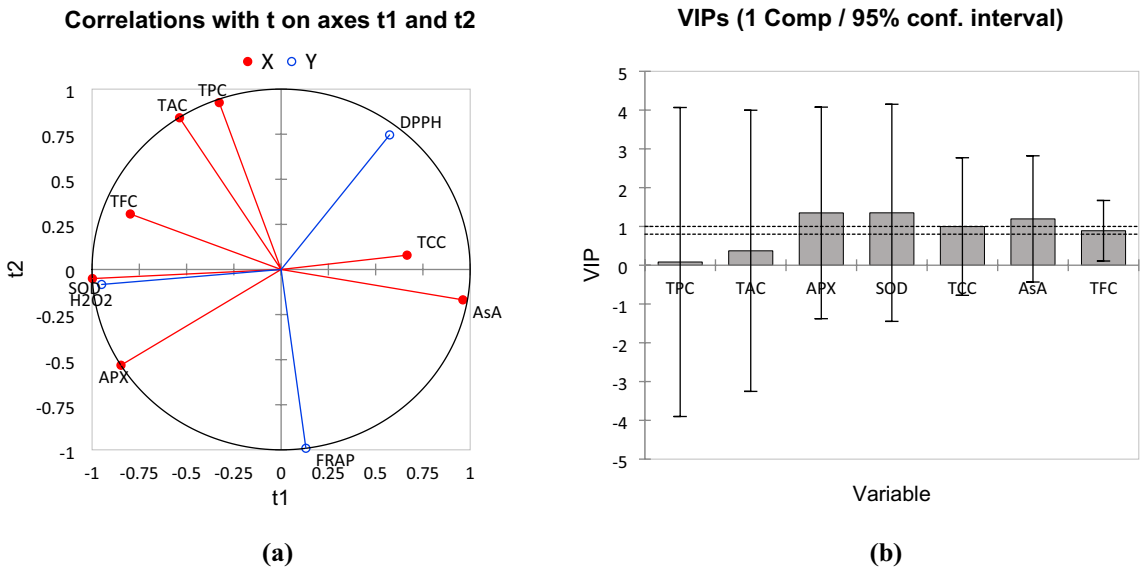


Fig. 11 Partial least squares regression (PLSR) analysis to determine the relationship between the secondary metabolites content and antioxidant activity of *P. minus*; **a** PLSR correlation plot, **b** VIPs score. [Small bars represent standard error, dotted line indicates threshold values of 0.8 and 1.0]

heat dissipation happened in the plants (Murchie and Lawson 2013).

PLSR analysis showed that pigments content in the leaves is one of the major parameters that impacted the growth development of *P. minus* positively (Fig. 5b). This means that the higher the content of the chlorophyll and carotenoid pigments in the leaves, the higher the growth performance of the plants. Stomatal conductance was also seen to highly impact the growth of *P. minus* with a VIP score value of > 1.0. Many studies have proven the indirect affect of stomatal conductance activities that will affect the growth or yield of plant. A study showed that the blocking of stomatal conductance had caused the reduction of yield in cotton plant by 28% (Zia-Khan et al. 2014). This was probably due to the direct effect of stomatal conductance and the photosynthesis rate of plant (Inoue et al. 2021), which was supported by the Pearson's correlation results with r of 0.826, $p < 0.01$ (Table 3).

Overall, the TPC in all treatments was observed to increase with treatment duration (Fig. 6b). The same trend was observed for TFC, where the TFC of all treatments was observed to also increase with treatment duration (Fig. 6c). The results of TPC, TFC and TAC suggested that the shading level of 30% (T2) for 20 weeks was the best treatment to produce high amounts of TPC, TFC and TAC in *P. minus*.

However, the results of FRAP reducing potential of the T1 extracts after 10 weeks of treatment were in direct contrast to that observed in its TPC and TFC contents, where it exhibited the highest TPC and TFC contents after 10 weeks of treatment but showed lower FRAP reducing potential than other treatments. Nevertheless, the high TPC and TFC contents in T1 (after 10 weeks of treatment) was observed to result in the highest scavenging activity against DPPH radicals (Table 4). On the other hand, T4 resulted in high FRAP reducing potential and DPPH scavenging activities after 10 and 20 weeks of treatment duration, parallel to its high contents of Chl a, Chl b and Car, which suggested that the high antioxidant properties of the extracts were also influenced by the plant photosynthetic pigments, not just due to its phenolic contents, as has been reported by Mohd Yusof et al. (2021). Significantly strong correlations were observed between Chl a with Chl b ($r = 0.862$, $p < 0.01$), Car ($r = 0.982$, $p < 0.01$) and between Chl b with Car ($r = 0.923$, $p < 0.01$). These observations were due to the fact that these three pigments work hand in hand in capturing sunlight for photosynthesis. Meanwhile, significantly moderate correlations were observed between Chl a, Chl b and Car with TAC (Table 5), indicating that the increase in Chl a, Chl b and Car pigments will lead to an increase in TAC. Similarly, a moderate but significant correlation was also observed between TFC and TPC ($r = 0.465$, $p < 0.01$). Nevertheless, only weak but significant correlations were observed between FRAP with Chl a ($r = 0.196$; $p < 0.05$) as well as between DPPH with TPC ($r = -0.240$, $p < 0.01$) and TFC ($r = -0.230$, $p < 0.01$). These suggests that there were other compounds that were present in the extracts that could also contributed to its high antioxidant properties. A study by Nam et al. (2012) has demonstrated that there is an abundance of antioxidant compounds that can present in a plant, while Wei and Shibamoto (2007) demonstrated significant

Table 6 The effects of the different shade levels on individual flavonoid composition of *P. minus* leaf extracts

Treatment	Flavonoids (mg g^{-1} DW)									
	Kaempherol		Kaempherol 3-glucoside		Myricetin		Quercetin		Rutin	
	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks
T1	0.0668 ± 0.03^{a}	0.0612 ± 0.03	ND	ND	7.8626 ± 0.13^{a}	13.5096 ± 0.01^{a}	0.7383 ± 0.06^{d}	0.5801 ± 0.01^{a}	0.6375 ± 0.32^{c}	0.1608 ± 0.16^{b}
T2	0.1122 ± 0.06^{a}	0.1462 ± 0.01^{a}	11.6800 ± 0.24^{b}	ND	57.3010 ± 0.01^{a}	12.2901 ± 0.04^{a}	2.0140 ± 0.01^{c}	0.6612 ± 0.02^{a}	156.4646 ± 9.23^{b}	0.6897 ± 0.36^{b}
T3	0.4392 ± 0.22^{a}	0.0986 ± 0.03^{ab}	ND	ND	51.9690 ± 0.01^{a}	6.6943 ± 0.06^{a}	2.3973 ± 0.01^{b}	0.6305 ± 0.01^{a}	ND	2.6078 ± 0.38^{a}
T4	0.3161 ± 0.20^{a}	ND	16.7776 ± 0.29^{a}	ND	58.3018 ± 29.15^{a}	34.5307 ± 17.26^{a}	3.1464 ± 0.02^{a}	1.2250 ± 0.61^{a}	204.3429 ± 10.29^{a}	ND

Data are means of treatments, N = 36; Rep = 3; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and T4 = 70% Shading; DE: Dry Extract; ND: Not detected; means with different letters within a column are significantly different at $p \leq 0.05$ between shade levels

Table 7 The effects of the different shade levels on individual flavonoid composition of *P. minus* stem extracts

Treatment	Flavonoids (mg g^{-1} DW)									
	Kaempherol		Kaempherol 3-glucoside		Myricetin		Quercetin		Rutin	
	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks
T1	ND	ND	ND	ND	4.6817 ± 2.34^{a}	ND	0.2562 ± 0.00^{b}	ND	ND	ND
T2	0.0369 ± 0.00^{a}	0.0116 ± 0.01^{a}	ND	0.1414 ± 0.10^{a}	ND	3.1223 ± 0.00	0.3534 ± 0.00^{a}	0.1173 ± 0.00	ND	0.0157 ± 0.01^{a}
T3	0.0086 ± 0.01^{b}	ND	ND	ND	2.5224 ± 1.26^{a}	ND	0.1725 ± 0.00^{c}	ND	ND	ND
T4	0.0180 ± 0.01^{b}	0.0032 ± 0.00^{a}	ND	0.1630 ± 0.08^{a}	2.8032 ± 1.40^{a}	ND	0.1709 ± 0.01^{c}	ND	0.2802 ± 0.18	0.0092 ± 0.00^{a}

Data are means of treatments, N = 36; Rep = 3; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and, T4 = 70% Shading; DE: Dry Extract; ND: Not detected; means with different letters within a column are significantly different at $p \leq 0.05$ between shade levels

Table 8 The effects of the different shade levels on individual flavonoid composition of *P. minus* root extracts

Treatment	Flavonoids (mg g^{-1} DW)									
	Kaempherol		Kaempherol 3-glucoside		Myricetin		Quercetin		Rutin	
	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks	10 weeks	20 weeks
T1	0.1894 ± 0.02^{ab}	0.1289 ± 0.03^{a}	8.8908 ± 0.00^{ab}	11.5457 ± 0.02	0.3164 ± 0.00^{b}	0.4048 ± 0.00^{ab}	ND	ND	ND	ND
T2	0.1028 ± 0.05^{ab}	ND	15.6170 ± 0.00^{a}	ND	ND	1.2728 ± 0.64^{a}	ND	ND	ND	ND
T3	0.2495 ± 0.13^{a}	ND	6.2421 ± 0.02^{ab}	ND	0.6564 ± 0.00^{a}	ND	ND	ND	ND	ND
T4	ND	0.1987 ± 0.09^{a}	ND	ND	ND	0.7123 ± 0.00^{ab}	ND	ND	ND	ND

Data are means of treatments, N = 36; Rep = 3; Control, T1 = 0% Shading; T2 = 30% Shading; T3 = 50% Shading; and, T4 = 70% Shading; DE: Dry Extract; ND: Not detected; means with different letters within a column are significantly different at $p \leq 0.05$ between shade levels

antioxidant activities from essential oil in plants such as aldehydes and carboxylic acid.

Plants provide one of the biggest sources of carbohydrates, and it is one of the elicitors of plant defenses involved in plant immunity (Trouvelot et al. 2014). The sugar content in plants is heavily affected by the photosynthesis fixation of CO_2 by the leaves, which in turn is affected by other leaf gas exchange processes such as the opening and closing of stomata (Krasavina et al. 2014). In the study, it was observed that the highest carbohydrate content (TCC) recorded was under T2, for both duration of 10 and 20 weeks (Fig. 7). This result is parallel to the photosynthesis rate which was observed to be the highest under T2 of 30% shade levels. The relationship between photosynthetic activity and carbohydrate content in plants can also be seen in a study on *Chrysanthemum morifolium* Ramat., which shows that lighting conditions affect the epinastic movement of chrysanthemum leaves, thus increasing the light absorption area which in turn increased the content of carbohydrates due to the higher photosynthetic activity in the plant (Yang et al. 2022). A study on *Rhynchelytrum repens* also showed that natural irradiance increased the content of soluble carbohydrates compared to the plants exposed to low irradiance (Souza et al. 2004). High TCC observed under T2 of 20 weeks was also similar with the trend exhibited by TPC, which was found to be the highest under T2 of 20 weeks (Fig. 6b). It is said that carbohydrates are the basic compounds for the production of phenolic compounds via the shikimic acid pathway, where extra carbohydrates derived from glycolysis and the penthose-phosphate pathway are converted into aromatic amino acids (Mattson et al. 2005).

The increase of H_2O_2 concentration was reported to happen when plants are exposed to biotic and/or abiotic stresses, such as pathogen attack, wounding, UV irradiation, exposure to intense light, drought, salinity, and/or chilling (Sofo et al. 2015). The concentration of H_2O_2 along with other ROS are a good marker of the oxidative stress occurrence (Sofo et al. 2015). The highest concentration of H_2O_2 was recorded under both low (T4) and high irradiance level (T1) when the planting period of *P. minus* was prolonged until 20 weeks. Meanwhile, under the treatment duration of 10 weeks, the lowest concentration of H_2O_2 was recorded under low irradiance level of 70% shade level (T4). This result is parallel to the antioxidant enzyme activities recorded under 10 weeks of treatment duration, where the lowest APX and SOD enzyme activities were the observed under T4. The APX response had been reported to involve directly in protecting the plants from environmental damages and it plays a key role in catalyzing the conversion of H_2O_2 into H_2O (Caverzan et al. 2012). High APX enzyme activities were also recorded under T4 of 20 weeks (Fig. 8b), parallel to the high concentration of H_2O_2 recorded. Sofo et al. (2015) also stated in his study that the balance of APX enzyme activity

is crucial for the suppression of toxic H_2O_2 levels in plants. This result is in contrast with the one recorded on the purple pak choi seedlings where the antioxidant activities were the highest under low light stress at 5 days, but when the study was prolonged to 15 days, the antioxidant activities of the seedlings decreased significantly (Zhu et al. 2017). Meanwhile, the superoxide dismutase (SOD) enzyme activities were recorded to be the highest under no shading environment (T1) when exposed for a longer treatment duration of 20 weeks (Fig. 8a). SOD activity is said to be the first line of defense in plants as it functions to catalyze the dismutation of superoxide radicals into O_2 and H_2O_2 to stop cell injury and the dysfunctional of plant tissues and therefore reducing the oxidative stress in plants, as high light condition may induce stress, resulting in high production of ROS. In addition, PLSR analysis showed that there were positive relationships between APX and SOD with H_2O_2 (Fig. 11a) which indicated that these enzymes were rapidly employed to scavenge against ROS (H_2O_2) when *P. minus* plants were under stress.

Ascorbic acid (AsA) synthesis in plants is also one of the response indicators of abiotic stresses and pathogen infections, where it plays multiple roles in metabolism of plants (Ishikawa et al. 2006). This plant-derived antioxidant is also one of the major sources of Vitamin C in human's diet. It is also an important chemical that is involved in a wide range of crucial function in plants from antioxidant defense system and photosynthesis to growth regulation (Smirnoff 1996). AsA synthesis has also been studied in tomato in response to different lighting conditions (Zushi et al. 2020). This study showed that low light intensity under planting duration of 10 weeks can enhance the production of AsA in *P. minus*, in contrast to the result obtained by Lado et al. (2015) which showed that light avoidance would reduce and inhibit the accumulation of AsA in the peels of *Citrus* fruits. A study on basil plant also showed that high light intensity applied before harvest managed to increase the total ascorbic acid content in the plant (Larsen et al. 2022). Nevertheless, different results were obtained when the treatment duration was prolonged to 20 weeks, where under intermediate light intensity (50% shading), the AsA produced was found to be the highest. A study on the *Fragaria x ananassa* or strawberry shows that the harvest time of the plant will influence its antioxidant capacity, which appeared to be more important than genotype factor (Pincemail et al. 2012). However, a study by Kevers et al. (2011) on apples and pears showed that harvest time (at normal ripeness) had only limited impact, but the year to year variations were significant. These studies showed that the effect of light conditions and harvest time exposed onto the plants will influence the production of AsA.

The extracts from different plant organs (leaf, stem and roots) were also subjected to HPLC analysis to screen and quantify the amount of selected flavonoids. The leaf extracts were observed to contain kaempherol, kaempherol 3-glucoside, myricetin, quercetin and rutin, with myricetin and rutin found in a significant amounts (Table 6). Similar results were also observed in a study by Tzanova et al. (2018) where the largest amount of rutin was found in *Betonica bulgarica*. The study also suggested that specific growth conditions would significantly affect the flavonoid content in different parts of plants. Meanwhile, high level of myricetin was found together with low level of kaempherol. A study has reported that high level of kaempherol which produced upstream in the metabolic pathway may prevent the accumulation of myricetin (Carvalho et al. 2010). However, the amounts of flavonoids in the leaf extracts were observed to reduce after a longer treatment duration (20 weeks). The content of kaempherol 3-glucoside in the leaf samples were also observed to diminish after 20 weeks of treatment duration (Table 6). These contrast with the observation found on the stem extracts (Table 7), where synthesis of kaempherol 3-glucoside was only observed at 20 weeks of treatment duration and was not detected at 10 weeks of treatment. Not only that, the stem extract was also observed to contain very little amounts of flavonoids, in contrast to the amounts recorded in the leaves. Similar observations were also recorded in the root extracts, where root extracts obtained from plants harvested after 10 weeks of treatment mostly consists of kaempherol 3-glucoside (Table 8). However, its synthesis was found to be stopped at longer treatment duration (20 weeks). Besides that, root extracts were also observed to be lacking in quercetin and rutin compounds, at both treatment durations (10 and 20 weeks). Overall, the results recorded in this study were similar to the one reported by Karimi et al. (2013) on *Labisia pumila* and Sembiring et al. (2018) on *Caesalpinia bonduc* L., where the highest amount of flavonoid was significantly found in leaves followed by the stems and then roots. Besides specific growth condition that affect the production of phytochemicals in plants, a study by Madike et al. (2017) on *Tulbaghia violacea* highlights on other factors that could affect the composition and amounts of phytocompounds such as extraction methods and types of solvent used.

Conclusions

From the data recorded in the study, it was observed that the interaction effect of shade levels and treatment duration would significantly affect the growth, production of secondary metabolites, antioxidant activities and the antioxidant defense system of *P. minus*. After 10 weeks, the growth of *P. minus* was observed to strive the best under high light condition, but its antioxidant activities and antioxidant defense

system performed better under low light condition (70% shading). However, when the duration was prolonged until 20 weeks, data analysis showed that *P. minus* adapt better under high light condition (no shading and 30% shading) in terms of its production of secondary metabolites and its antioxidant activities. This can be seen from the high production of phenolic, flavonoid and anthocyanin compounds in *P. minus*, as well as its high FRAP reducing power and high superoxide dismutase enzyme activity. Low H_2O_2 concentration was also recorded under 30% shading level when the treatment duration was prolonged until 20 weeks.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-023-11076-y.

Acknowledgements We sincerely thank Universiti Malaya for the facilities provided.

Author Contributions FFMY, NAMZ and JSY conceived and designed the experiments. FFMY conducted the experiments, collected the data and performed the statistical analysis. AG, PG and VK assisted in the analysis of antioxidant enzyme activities and ROS content. NAMZ and JSY advised on the preparation of materials. NO, LPE and AC provided the facilities for measurement. FFMY wrote the manuscript. NAMZ and JSY read an edited the manuscript. All authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by Long Term Research Grant Scheme (LRGS) (LR003-2020), and Research University Grant (GPF009B-2018).

Data Availability Not applicable.

Declarations

Conflict of interest There is no conflict of interest for this journal article.

References

Ahmad R, Sahidin I, Taher M, Low C, Noor NM, Sillapachaiyaporn C, Chuchawankul S, Sarachana T, Tencomnao T, Iskandar F (2018) Polygonumins A, a newly isolated compound from the stem of Polygonum minus Huds with potential medicinal activities. Sci Rep 8(1):1–15

Baharum SN, Bunawan H, MaA G, Mustapha WAW, Noor NM (2010) Analysis of the chemical composition of the essential oil of *Polygonum minus* Huds using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC-TOF MS). Molecules 15(10):7006–7015

Bashir MI, Abdul Aziz NHK, Noor DAM (2022a) Antidepressant-like effects of polygonum minus aqueous extract in chronic ultra-mild stress-induced depressive mice model. Behav Sci 12(6):196

Bashir MI, Aziz NHKA, Noor DAM (2022b) *Polygonum minus* aqueous extract supplement reduces stress-induced anorexia and anhedonia in mice. Pak J Med Health Sci 16(1):696–701

Beneragama C, Goto K (2010) Chlorophyll a: b ratio increases under low-light inshade-tolerant'Euglena gracilis

Benzie IF, Strain J (1999) [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology, vol 299. Elsevier, Amsterdam, pp 15–27

Bhatt R, Misra L, Tiwari H (2002) Growth and biomass production in tropical range grasses and legumes under light stress environment. Indian J Plant Physiol 7(4):349–353

Carvalho IS, Cavaco T, Carvalho LM, Duque P (2010) Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. Food Chem 118(2):384–390

Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M (2012) Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. Genet Mol Biol 35:1011–1019

Christapher PV, Parasuraman S, Christina JMA, Asmawi MZ, Vikneswaran M (2015) Review on *Polygonum minus* Huds, a commonly used food additive in Southeast Asia. Pharmacogn Res 7(1):1

Franks PJ, Farquhar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol 143(1):78–87

Ghasemzadeh A, Jaafar HZ, Rahmat A, Wahab PEM, Halim MRA (2010) Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). Int J Mol Sci 11(10):3885–3897

Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2014) Allocation of secondary metabolites, photosynthetic capacity, and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) in response to and light intensity. Sci World J 2014:360290

Ilić ZS, Milenković L, Tmušić N, Stanojević L, Stanojević J, Cvetković D (2022) Essential oils content, composition and antioxidant activity of lemon balm, mint and sweet basil from Serbia. LWT 153:112210

Ishikawa T, Dowdle J, Smirnoff N (2006) Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. Physiol Plant 126(3):343–355

Karimi E, Jaafar HZ, Ghasemzadeh A, Ibrahim MH (2013) Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. Aust J Crop Sci 7(7):1016

Kevers C, Jl P, Tabart J, Defraigne J-O, Dommes J (2011) Influence of cultivar, harvest time, storage conditions, and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears. J Agric Food Chem 59(11):6165–6171

Krasavina MS, Burmistrova NA, Raldugina GN (2014) The role of carbohydrates in plant resistance to abiotic stresses. Emerging technologies and management of crop stress tolerance. Elsevier, Amsterdam, pp 229–270

Lado J, Alós E, Rodrigo MJ, Zacarías L (2015) Light avoidance reduces ascorbic acid accumulation in the peel of Citrus fruit. Plant Sci 231:138–147

Larsen DH, Li H, van de Peppel AC, Nicole CC, Marcelis LF, Woltering EJ (2022) High light intensity at end-of-production improves the nutritional value of basil but does not affect postharvest chilling tolerance. Food Chem 369:130913

Lawson T, Kramer DM, Raines CA (2012) Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. Curr Opin Biotechnol 23(2):215–220

Lichtenthaler HK, Buschmann C (2001) Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Current Protoc Food Anal Chem 1(1):31–38

Madike LN, Takaidza S, Pillay M (2017) Preliminary phytochemical screening of crude extracts from the leaves, stems, and

roots of *Tulbaghia violacea*. Int J Pharmacogn Phytochem Res 9(10):1300–1308
Maizura M, Aminah A, Wan Aida W (2011) Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. Int Food Res J 18(2):45–53
Mattson J, Julkunen-Tiitto R, Herms DA (2005) CO_2 enrichment and carbon partitioning to phenolics: do plant responses accord better with the protein competition or the growth differentiation balance models? Oikos 111(2):337–347
Medina CL, Souza RP, Machado EC, Ribeiro RV, Silva JA (2002) Photosynthetic response of citrus grown under reflective aluminized polypropylene shading nets. Sci Hortic 96(1–4):115–125
Miyashita K, Tanakamaru S, Maitani T, Kimura K (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ Exp Bot 53(2):205–214
Mohd Ghazali MA, Al-Naqeb G, Krishnan Selvarajan K, Hazizul Hasan M, Adam A (2014) Apoptosis induction by Polygonum minus is related to antioxidant capacity, alterations in expression of apoptotic-related genes, and S-phase cell cycle arrest in HepG2 cell line. BioMed Res Int 2014:539607
Mohd Yusof FF, Yaacob JS, Osman N, Ibrahim MH, Wan-Mohtar WAAQI, Berahim Z, Mohd Zain NA (2021) Shading effects on leaf gas exchange, leaf pigments and secondary metabolites of *Polygonum minus* Huds, an aromatic medicinal herb. Plants 10(3):608
Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. J Exp Bot 64(13):3983–3998
Nam S, Jang HW, Shibamoto T (2012) Antioxidant activities of extracts from teas prepared from medicinal plants, *Morus alba* L., *Camellia sinensis* L., and *Cudrania tricuspidata*, and their volatile components. J Agric Food Chem 60(36):9097–9105
Panwar M, Tewari R, Nayyar H (2016) Native halo-tolerant plant growth promoting rhizobacteria Enterococcus and *Pantoea* sp. improve seed yield of Mungbean (*Vigna radiata* L.) under soil salinity by reducing sodium uptake and stress injury. Physiol Mol Biol Plants 22(4):445–459
Pincemail J, Kevers C, Tabart J, Defraigne JO, Dommes J (2012) Cultivars, culture conditions, and harvest time influence phenolic and ascorbic acid contents and antioxidant capacity of strawberry (Fragaria x ananassa). J Food Sci 77(2):C205–C210
Qader SW, Abdulla MA, Chua LS, Sirat HM, Hamdan S (2012) Pharmacological mechanisms underlying gastroprotective activities of the fractions obtained from *Polygonum minus* in Sprague Dawley rats. Int J Mol Sci 13(2):1481–1496
Sato R, Ito H, Tanaka A (2015) Chlorophyll b degradation by chlorophyll b reductase under high-light conditions. Photosynth Res 126(2):249–259
Sembiring EN, Elya B, Sauriasari R (2018) Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacogn J 10(1):123–127
Sharma P, Gautam A, Kumar V, Guleria P (2022) In vitro exposed magnesium oxide nanoparticles enhanced the growth of legume *Macrotyloma uniflorum*. Environ Sci Pollut Res 29(9):13635–13645
Smirnoff N (1996) Botanical briefing: the function and metabolism of ascorbic acid in plants. Ann Bot 78(6):661–669
Sofo A, Scopa A, Nuzzaci M, Vitti A (2015) Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. Int J Mol Sci 16(6):13561–13578
Souza A, De Paula A, Figueiredo-Ribeiro R (2004) Effects of irradiance on non-structural carbohydrates, growth, and hypoglycemic activity of *Rhynchelytrum repens* (Willd.) CE Hubb. (Poaceae). Braz J Biol 64:697–706
Suhailah WQ, Mahmood AA, Lee SC, Salehhuddin H (2012) Potential bioactive property of *Polygonum minus* Huds (kesum) review. Sci Res Essays 7(2):90–93
Tominaga J, Shimada H, Kawamitsu Y (2018) Direct measurement of intercellular CO_2 concentration in a gas-exchange system resolves overestimation using the standard method. J Exp Bot 69(8):1981–1991
Trouvelot S, Héloir M-C, Poinssot B, Gauthier A, Paris F, Guillier C, Combier M, Trdá L, Daire X, Adrian M (2014) Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Front Plant Sci. https://doi.org/10.3389/fpls.2014.00592
Tzanova M, Grozeva N, Gerdzhikova M, Argirova M, Pavlov D, Terzieva S (2018) Flavonoid content and antioxidant activity of *Betonica bulgarica* Degen et Neič. Bull Chem Commun 50:90–97
Walters RG (2005) Towards an understanding of photosynthetic acclimation. J Exp Bot 56(411):435–447
Wang SY, Jiao H (2000) Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J Agric Food Chem 48(11):5677–5684
Wang GG, Bauerle WL, Mudder BT (2006) Effects of light acclimation on the photosynthesis, growth, and biomass allocation in American chestnut (*Castanea dentata*) seedlings. For Ecol Manage 226(1–3):173–180
Wei A, Shibamoto T (2007) Antioxidant activities and volatile constituents of various essential oils. J Agric Food Chem 55(5):1737–1742
Wyka TP, Robakowski P, Żytkowiak R, Oleksyn J (2022) Anatomical acclimation of mature leaves to increased irradiance in sycamore maple (*Acer pseudoplatanus* L.). Photosynth Res 154(1):41–55
Yang J, Song J, Jeong BR (2022) Lighting from top and side enhances photosynthesis and plant performance by improving light usage efficiency. Int J Mol Sci 23(5):2448
You Y, Shahar S, Haron H, Yahya H (2018) More ulam for your brain: a review on the potential role of ulam in protecting against cognitive decline. Sains Malaysiana 47(11):2713–2729
Zervoudakis G, Salahas G, Kaspiris G, Konstantopoulou E (2012) Influence of light intensity on growth and physiological characteristics of common sage (*Salvia officinalis* L.). Braz Arch Biol Technol 55:89–95
Zhang M, Sharma A, León F, Avery B, Kjelgren R, McCurdy CR, Pearson BJ (2022) Plant growth and phytoactive alkaloid synthesis in kratom [*Mitragyna speciosa* (Korth)] in response to varying radiance. PLoS ONE 17(4):e0259326
Zhu H, Li X, Zhai W, Liu Y, Gao Q, Liu J, Ren L, Chen H, Zhu Y (2017) Effects of low light on photosynthetic properties, antioxidant enzyme activity, and anthocyanin accumulation in purple pak-choi (*Brassica campestris* ssp. Chinensis Makino). PLoS ONE 12(6):e0179305
Zia-Khan S, Spreer W, Pengnian Y, Zhao X, Othmanli H, He X, & Müller J (2014) Effect of dust deposition on stomatal conductance and leaf temperature of cotton in northwest China. Water 7(1):116–131
Zushi K, Suehara C, Shirai M (2020) Effect of light intensity and wavelengths on ascorbic acid content and the antioxidant system in tomato fruit grown in vitro. Sci Hortic 274:109673