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Photoprotective Role of Quercetin to *Tetracera sarmentosa*

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Tetracera sarmentosa was studied to find out how varying light intensity affects the plant's quercetin content. It was hypothesised that a decrease in the activity of antioxidant enzymes may increase the plant's quercetin content. The study was carried out at Gunung Ledang, Johor, Malaysia because the forest provides an excellent natural experimental setup for investigating the possible effect of light intensity to the selected plant in its natural environment. Shaded, semi-shaded and sun-exposed *T. sarmentosa* were selected for the study. The photoprotective role of quercetin was supported by quantifying the malondialdehyde (MDA) content and activity of antioxidant enzymes. The results indicated that high light intensity increased the MDA content and decreased the activity of antioxidant enzymes. This increases the quercetin content which in return overcomes the extra need for reactive oxygen species (ROS) scavenging.

Keywords: antioxidant enzymes; light intensity; malondialdehyde; ROS scavenging; quercetin

I. INTRODUCTION

The environment is not having a constant stable condition, it always changes, and these changes can lead to various effects in the biochemical characteristics of a plant. One of the most important environmental factor affecting plants is light. Scientific studies have indicated the significant effect of light intensity and quality on the production of secondary metabolites like flavonoids (Bernal *et. al.*, 2014; Csepregi *et. al.,* 2017; Del Valle *et. al.,* 2018; Grifoni *et. al.,* 2016; Ilić & Fallik, 2017; Jahangir *et. al.,* 2009; La *et. al.,* 2003; Landi *et. al.,* 2015; Le´on-Chan *et. al.,* 2017; Li *et. al.,* 2018; Taulavuori *et. al.,* 2018, 2015; Zhang *et al.,* 2017). This secondary metabolites function in protecting the plants against harmful ultraviolet (UV) radiation. When the light intensity increases, the harmful UV radiation increases, and therefore the plant produce more flavonoids so as to protect itself from the radiation (Wang *et al.,* 2017). UV is an electromagnetic radiation ranging from 10nm to 400nm. About 10% of solar radiation is UV. UV-A, UV-B and UV-C are examples of UV radiation which have wavelengths of 315-400nm, 280-315nm, and 100-280nm respectively. In plants, UV light can affect nucleic acids, amino acids, proteins, plant growth regulators, lipids, pigments, membranes and photosynthesis (Hollósy, 2002). UV-B alone can impair plant photosystems and also decrease the plant chlorophyll content, RuBisCO activity and carbon dioxide (CO_2) fixation (Lingakumar and Kulandaivelu, 1993). Plants get protection from UV radiation using protective structures like hairs and waxes, enzymes or photoprotective secondary metabolites like flavonoids.

Quercetin is a flavonoid (flavonol) produced by plants. It is abundant and widely distributed in nature. The molecular structure is represented as 3, 3', 4', 5, 7 pentahydroxyflavone. The human body cannot synthesise quercetin, rather we depend on plants. Quercetin flavonol is yellow in colour, insoluble in cold water, slightly soluble in warm water and completely soluble in alcohol and lipids. Quercetin absorbs at 310-420nm and 240-280nm (Park *et al.,* 2013). Among other

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flavonoids, it is one of the most used flavonoid for the treatment of metabolic and inflammatory problems, due to its bioactivity and its availability in many plants (Panche *et al.,* 2016). Quercetin glycosides include Quercetin-3-O-rutinoside, Quercetin-3-O-a-Lrhamnoside, Quercetin-3-O-glucoside, Quercetin-3-O-gluconoride, Quercetin-3-O-arabinoside, Quercetin-3-O-galactoside, and Quercetin-4l-O-glucoside. Quercetin plays an important role in protecting plants against stress. It acts as UV-filters and antioxidant molecules (Agati *et. al.,* 2013; Brunetti *et al.,* 2013). It can protect plants against microorganisms; for example, *Vitis vinifera* accumulates quercetin for protection against *Plasmopara viticola* (Ali *et al.,* 2012). Moreover, quercetin plays a vital role in the interaction of a plant with its environment (Mierziak *et al.,* 2014). In humans, it possesses anti-inflammatory, anti-allergic, anti-ulcer, anti-viral, anti-cancer, anti-diabetic, anti-hypertensive, and anti-infective properties (Kesarkar *et al.,* 2009). Medicinal plants have high quercetin content (Kumar & Pandey, 2013).

Like other flavonoids, quercetin accumulation can be affected by various factors. For instance, light availability and quality, latitude (Jaakola & Hohtola, 2010), altitude, temperature, plant growth stage (Becker, 2014) and nutrient availability. CO_2 concentration has an effect on quercetin accumulation, with high biosynthesis when CO_2 concentration is high (Moghaddam *et al.,* 2017). The level of stress affecting a plant has an influence on quercetin production (Amalesh *et. al.,* 2011; Ramakrishna & Ravishankar, 2011; Treutter, 2005; Winkel-Shirley, 2002). Besides, a plants level of maturity also influences quercetin accumulation. For example, the accumulation of quercetin in *Rubus ideaus* (Wang *et al.,* 2009).

Studies have indicated the photoprotective role of quercetin in some plants. For example, *Lycopersicon esculentum* accumulates high amount of quercetin which enables it to acclimatize to acute UV-B stress (Shourie *et al.,* 2014). Nevertheless, quercetin can protect plants against excess light by acting as an antioxidant agent (Agati *et. al.,* 2011a; Agati *et. al.,* 2012; Agati *et. al.,* 2013; Brunetti *et. al.,* 2013; Brodowska, 2017; Ferdinando, Brunetti & Fini, 2012; Zoratti *et al.,* 2014). Quercetin-3-O-rutinoside accumulates in very high concentration in *Ligustrum vulgare* and *Phillyrea latifolia* exposed to full sunlight (Tattini *et al.,* 2005). Accordingly, when comparing shaded and sun-exposed *Camellia sinensis*, the latter contains a higher amount of quercetin (Zhang *et al,* 2017). Moreover, quercetin content of *Labisia pumila* was higher in sun-exposed compared to shaded species (Karimi *et al.,* 2013). Under full sunlight, *Cyclocarya paliurus* accumulates the highest amount of quercetin (Deng *et. al.,* 2012; Liu *et al.,* 2018). The amount of quercetin accumulated in apple skin varies depending on the position of the fruit on the tree. Fruits located at upper part of the tree receives higher light intensity, thereby accumulating more quercetin compared to fruits located at other parts of the tree (Awad *et al.,* 2001). The level of Quercetin-3-galactoside and that of Quercetin-3-glucoside decrease significantly as *Vaccinium corybosum* plant was exposed to UV-C (Wang, Chen & Wang, 2009a). Quercetin content of grapes increases as UV-B dose increases (Martínez-Lüscher *et al.,* 2014). Also, UV radiation can enhance quercetin biosynthesis but decrease xanthophyll biosynthesis (Guidi *et al.,* 2016). Quercetin and other flavonoids cause light-induced changes in the spectral features of *Phllirea latifolia*, *Ligustrum vulgare* and *myritus communis* (Agati *et al.,* 2011b). Therefore biosynthesis of quercetin can be decreased by shading (Cortell & Kennedy, 2006; Downey *et. al.,* 2004; Koyama *et al.,* 2012).

Various studies on the effect of light intensity and quality on the accumulation of flavonoids include that of *Hyptis marrubioides* (Pedroso *et al.,* 2017), *Anacardium othonianum* (Gazolla *et al.,* 2017), *Chlorella miniata* (Sozmen *et al.,* 2018), *Berberis microphylla* (Arena *et al.,* 2017), *Lactuca sativa* (Bian *et. al.,* 2018; Liu *et. al.,* 2018; Pérez-López *et al.,* 2018), *Cannabis sativa* (Gianmaria *et al.,* 2018), *Anoectochilus roxburghii* (Chen *et al.,* 2017), *Cyclocarya paliurus* (Liu *et al.,* 2018), *Abelmoschus esculentus* (Irshad *et al.,* 2018), *Elephantopus scaber* (Dawiyah et al., 2018) and *Fagopyrum esculentum* (Nam *et al.,* 2018). Some plants accumulate a higher amount of flavonoids under high light intensity, while others require moderate shading for their maximum accumulation of flavonoid. The variation in flavonoids accumulation among plant species may be due to the complex metabolism of flavonoids. In heliophytes, the activity of antioxidant enzymes decreases under lower light intensity. This increases reactive oxygen species (ROS), and consequently increase

flavonoids biosynthesis (Li *et al.,* 2016). The activity of antioxidant enzymes of a plant also varies depending on a plant species, and the amount of light it receives (Chen *et al.,* 2016). In previous studies, it was reported that superoxide dismutase (SOD) and catalase (CAT) enzymes were higher in concentration for sun-exposed *Changium smyrnioides* (Wang *et al.,* 2017), *Oryza sativa* (Gu *et al.,* 2017), while in *Dianthus caryophyllus* (Manivannan *et al.,* 2017), and *Camptotheca acuminuta* (Yu *et al.,* 2016), the antioxidant enzymes were higher under blue light emiting diode (LED) light. Variation in the antioxidant enzymes activities may be due to the level of stress encountered, either acute or slow. Due to these differences, it is hypothesised in this study that if the activity of antioxidant enzymes of a plant is low, then flavonoid biosynthesis may increase. *Tetracera sarmentosa* was selected for the study because the research is aimed at identifying the photoprotective role of quercetin to plants growing in their natural environment. Besides, the studied plant was found growing in sun-exposed, semi-shaded, and shaded regions of the mountain. Other reasons that justify selection of the plant include its medicinal application and its ability to grow in about 60 % of the forest.

II. MATERIALS AND METHOD

A. Study Area

The study was carried out at Gunung (Gn) Ledang, a mountain located at Ledang area of Johor in Malasia (02°22'27"North 102°36'28"East). The mountain is having an elevation of about 1,276 m (4186 ft) which makes it the 64th highest mountain in the country. The study was performed at Gn Ledang because the forest provides an excellent natural experimental setup for investigating the possible effect of light intensity on the selected plants (i.e., there are sun-exposed, semi-shaded, and shaded regions). Moreover, the research is aimed at identifying the photoprotective role of quercetin to *T. sarmentosa* growing in its natural environment, because most studies used a controlled environment without any fluctuation in light intensity compared to the natural environment. Light intensity received at the sun-exposed regions is above 1600 µmol. m^{-2}. s^{-1}, at the semi-shaded is below 100 µmol.m^{-2}. s^{-1}, and at the shaded region is below 20 µmol. m^{-2}. s^{-1}. The light intensities were measured using LICOR quantum sensor.

B. Selection of Plants

Tetracera sarmentosa from deeply shaded part of the forest (shaded plants), from forest edges (semi-shaded plants), and those directly under the sun (sun exposed) were selected. Factors influencing quercetin production include temperature, salinity, water, light intensity, chemical stress, mechanical stress and infection. Prior to sample extraction, the selected plants were screened for any other factor that can possibly alter the quercetin concentration. Healthy, plant free from any mechanical damage, chemical stress, and infection were used. The plants were not under flooding or drought, neither were they affected by salinity nor unfavourable temperature. The only factor affecting the plants at that point was the light intensity.

C. Determination of Quercetin Content using High-performance Liquid Chromatography (HPLC)

Shimadzu-LC system (UFLC, Shimadzu, Kyoto, Japan) equipped with LC – 20AT prominence liquid chromatograph, SIL – 20AC prominence auto sampler, SPD – M20A prominence diode array detector (DAD), CBM – 20A communication module, CTO – 10AS VP Shimadzu column oven, and DGU – 20 A5R degassing unit. Thermos hypersil Gold column C18, 5 µm (250 mm by 4.6 mm) (Fisher Scientific, UK) was inserted to Shimadzu-LC system. HPLC grade acetic acid (Fisher Scientific, UK) and methanol (Fisher Scientific, UK) were used. The plant extracts were dissolved in 80 % methanol at a concentration of 250 mg/ml and were centrifuged at 13,200 rpm for 5 min. The supernatant was collected and filtered through a 0.45 µm nylon syringe filter. Standard stock solutions of quercetin were prepared at a concentration of 2 mg/ml in 80 % methanol. The standard solutions of quercetin were serially diluted with 80 % methanol to obtain calibration standard solutions at concentrations of 20, 40, 60, 80 and 100 µg/ml.

All solvents were degassed using FB 15055 Fisher brand ultrasonic cleaner (Fisher Scientific, UK). Analysis of sample and standard solutions were done in a reverse phase HPLC method, at ambient temperature. 1 % acetic acid and methanol were prepared in a ratio (10:90), (v/v). Injection

volume was 10 µl (Dar *et al.*, 2017). Solvents eluted in an isocratic manner with 1.00 ml flow for every one minute.

D. Quantitative Determination of Lipid Peroxidation

Lipid peroxidation was estimated by quantifying the malondialdehyde (MDA) content of the studied plants. Quantification of MDA content gives an estimate of the oxidative stress effects on the lipids of the plants. The test is based on the reaction of MDA with thiobarbituric acid (TBA) to form a complex which have its maximum absorption at 532 nm. Water bath (Memmert, Germany), UV visible spectrophotometer (Thermo Scientific Spectrophotometer Biomate 3S UV PREuroplug & UK), centrifuge (MPW-351R refrigerated Laboratory Centrifuge, MPW Med Instruments, Boremlowsk Warszawa, Poland), Thiobarbituric acid (TBA) (Sigma-Aldrich, Germany), trichloroacetic acid (TCA) (Sigma-Aldrich, Germany), and MDA (Sigma-Aldrich, Germany) were used for this test.

The sample solution was prepared by grounding and homogenizing 0.1 g of leaf sample in 1 ml of 0.10 % (w/v) TCA and then centrifuged at 10,000 g for 10 minutes. Then, 20 % TCA was mixed with 5 % TBA, thereby generating the reaction mixture. MDA standard was serially diluted to generate 10, 20, 40, 60, 80 and 100 µg/ml. Both sample and standard solutions were mixed with 4 ml of the reaction mixture, boiled at 95 ^{o}C for 15 minutes, and then placed on ice. The sample and standard solutions were centrifuged at 10,000 g for 5 minutes, and the absorbances of the supernatant were recorded at 532 nm (Zhang & Rongfeng, 2013). The MDA content was calculated from the standard curve, and the results were expressed as µg/ml.

E. Determination of Antioxidant Enzymes Activity

Superoxide dismutase (SOD) was quantified using 100 mM phosphate buffer (Na2HPO4, Sigma-Aldrich, Germany; mixed with NaH2PO4 Sigma-Aldrich, Germany), 1 mM EDTA-Na2 (Sigma-Aldrich, Germany), 130 mM methionine (Sigma-Aldrich, Germany), 750 µM nitro blue tetrazolium (NBT) (Sigma-Aldrich, Germany), 20 µM riboflavin (Sigma-Aldrich, Germany), UV visible spectrophotometer, and centrifuge (MPW-351R refrigerated Laboratory Centrifuge, MPW Med Instruments, Boremlowsk Warszawa, Poland). Prior to this test, crude protein extract of each plant was prepared by grinding 0.2 g of fresh leaf sample in 3 ml of 100 mM phosphate buffer (pH 7.8). The extract was centrifuged at 10,000 g for 20 min at low temperature and the absorbance of the supernatant was recorded at 280 nm and 260 nm. The protein concentration of the crude extract was calculated using Equation 1 (Tianzi & Baolong, 2016).

The reaction solution was prepared by mixing 30 ml of 100 mM phosphate buffer with 0.6 ml of 1 mM EDTA-Na_2, 2 ml of 20 µM riboflavin, 2 ml of 750 µM NBT and 2 ml of 130 mM methionine. The sample solution was prepared by mixing 50 ml of the previously prepared crude protein with 1ml of the reaction mixture. Two types of control were made, one of which was incubated in light together with the sample mixture while the other control was kept at the dark. All incubations were ended after 15 min, and absorbance was immediately recorded at 560 nm. The second control was used for comparison only. SOD enzyme activity was calculated using equation 2 (Tianzi & Baolong, 2016).

Peroxidase (POD) were quantified using 100 mM phosphate buffer pH 7.0, 0.2 % guaiacol (Sigma-Aldrich, Germany), 30 % hydrogen peroxide (H_2O_2), and UV visible spectrophotometer. The reaction mixture used for determining the POD activity was prepared by mixing 28 µl of 0.2 % guaiacol with 50ml phosphate buffer. The reaction was heated, then cooled down before adding 19 µl of 30 % H_2O_2. 1ml of the reaction solution was further diluted with 50µl of phosphate buffer. The sample solution was prepared by mixing 50 µl of crude protein with 1ml of the previously prepared reaction solution in a cuvette and immediately recording the absorbance at 470nm. Absorbance was recorded at an interval of 15 seconds for a duration of 1 minute. Lastly, POD activity was calculated using equation 3 (Tianzi & Baolong, 2016).

Catalase (CAT) was quantified using 100 mM phosphate buffer pH 7.0, 30 % hydrogen peroxide (H_2O_2), and UV visible spectrophotometer were used for this study. The reaction solution used for determining the CAT activity was prepared by mixing 77.5 µl of 30 % H_2O_2 with 50ml 100 mM phosphate buffer. The sample solution was prepared by mixing 50 µl of crude protein with 1ml of the previously prepared reaction solution in a cuvette and immediately recording the absorbance at 240 nm. Absorbance was

recorded at an interval of 15 seconds for a duration of 1 minute. For blank solution preparation, 50 µl of crude protein was replaced with 50 µl of 100 mM phosphate buffer. Lastly, CAT activity was calculated using equation 4 (Tianzi and Baolong, 2016).

$$\text{Crude protein (Cp) (mg/ml)} = 1.55 \times A_{280} - 0.76 \times A_{260} \quad (1)$$

$$\text{SOD (U/mg)} = [(A_c - A_s \times V) / (0.5 \times A_c \times V_t)] / Cp \quad (2)$$

$$\text{POD (U/mg)} = [(\Delta A_{470} \times (V/Vt)) / (0.01 \times t)] / Cp \quad (3)$$

$$\text{CAT (U/mg)} = [(\Delta A_{240} \times (V/Vt)) / (0.01 \times t)] / Cp \quad (4)$$

Where A_c is absorbance of light incubated control, A_s is absorbance of sample, V is volume of crude protein extract solution, V_t is volume of crude protein extract used in the test, and Cp is the crude protein concentration (mg/ml), $\triangle A_{470}$ is the change of absorbance at λ 470 nm during every 15 sec, $\triangle A_{240}$ is the change of absorbance at λ 240 nm during every 15 sec.

F. Statistical Analysis

Data were reported as the mean ± standard deviation (SD), and all tests were performed using the SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). Data were tested for normality (Shapiro–Wilk normality test) prior to Pearson correlation coefficient. Quercetin content, MDA contents, and antioxidant enzymes were analysed statistically using student T-test while relationships among quercetin, MDA and antioxidant enzymes were analysed using Pearson correlation coefficient. All statistical analyses were performed at a 95 % confidence level.

III. RESULT AND DISCUSSION

A. Quercetin Content

The total quercetin content accumulated by sun-exposed, semi-shaded and shaded *Tetracera sarmentosa* are represented in Figure 1. The results show an increasing order of quercetin as light intensity increases. This indicates that light is affecting the accumulation of quercetin in the plants. From the results obtained, sun-exposed species accumulated a higher amount of quercetin, followed by semi-shaded while the least was accumulated in shaded species ($P < 0.05$).

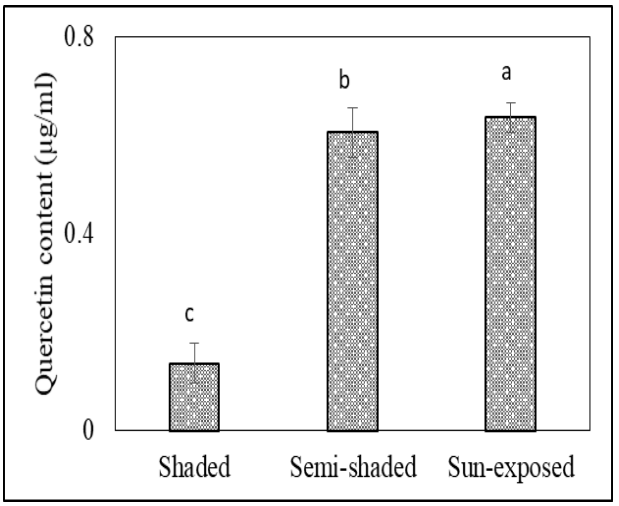

Figure 1. Quercetin content

Different small letters indicate significant differences among sun-exposed, semi-shade and shaded species ($P < 0.05$). Quercetin is a flavonoid possessing a dihydroxy-B-ring which give it a photoprotective characteristic (Agati *et. al.,* 2011; Agati *et. al.,* 2013; Brunetti *et. al.,* 2013; Edreva, 2005; Pérez-López *et. al.,* 2018; Shourie *et. al.,* 2014; Zhang *et al.,* 2017). Some studies also indicate that higher amount of quercetin was recovered under full sunlight (Debski *et. al.,* 2017; Karimi *et. al.,* 2013; Shourie *et. al.,* 2014; Tattini *et al.,* 2005). Biosynthesis of quercetin can be decreased by shading (Cortell & Kennedy, 2006; Downey *et. al.,* 2004; Koyama *et al.,* 2012). In *Zingiber officinale* (Ghasemzadeh *et al.,* 2010), and *Berberis microphylla* (Arena *et al.,* 2017), a lower amount of quercetin was recorded under high light intensity. Vacuolar quercetin in mesophyll cells of *Catharanteus rosues* increases under high sunlight (Ferreres *et al.,* 2011). Quercetin biosynthesis may have a contribution to natural selection, dynamic selection, evolutionary response and physiological response in plants (Lesne, 2008). Presence of quercetin, therefore, helps in UV-B tolerance (Jacobs & Rubery, 1988; Jansen *et al.,* 2001).

The higher amount of quercetin obtained in sun-exposed plants may be due to the photoprotective role of flavonoids (Amalesh *et. al.,* 2011; Carvalho *et. al.,* 2010; Cortell & Kennedy, 2006; Gavin & Durako, 2012; Liu *et. al.,* 2016; Re *et. al.,* 2018; Tattini *et. al.,* 2005; Treutter, 2005; Treutter, 2006; Winkel-Shirley, 2002; Yuan *et. al.,* 2015; Zlatev *et. al.,*

2012; Zoratti *et al.,* 2014). Flavonoids are produced in response to stress because they function as UV-filters and antioxidant molecules (Agati *et. al.*, 2013; Brodowska, 2017; Brunetti *et. al.*, 2013; Mierziak *et al.*, 2014). Sun-exposed leaves contain a higher amount of dihydroxy flavonoids (quercetin), indicating that the genes involved in the biosynthesis of dihydroxy flavonoids were upregulated by high light intensity (Agati *et al.*, 2013). Photoprotective role of dihydroxy flavonoids has been reported by (Agati *et. al.*, 2011; Agati *et. al.*, 2013; Brunetti *et. al.*, 2013; Edreva, 2005; Pérez-López *et. al.*, 2018; Shourie *et. al.*, 2014; Zhang *et al.*, 2017).

In sun exposed environment, when net photosynthesis of a plant reduces due to unfavourable conditions, light becomes excess due to a reduction in photosynthesis. This leads to an increase in the expression of flavonoid biosynthesis genes (Agati *et al.*, 2012), decrease in expression of photosynthetic genes (Pego *et al.*, 2000) and a decrease in activity of antioxidant enzymes (Fini *et al.*, 2011). The lower quercetin accumulation in shaded leaves may be due to continuous ROS generation induced by light. This indicates that low light and high light can cause accumulation of quercetin in increasing order of light intensity (Agati *et al.*, 2013). Flavonoids that can respond to light have catechol group in their B ring (Agati *et al.*, 2013). They can inhibit ROS generation and can reduce generated ROS. They are found in chloroplast and Vacuole. In chloroplast, flavonoids can reduce singlet oxygen (Agati *et al.*, 2007). Quercetin can scavenge ROS (Choudhary and Agrawal, 2014) and singlet oxygen (Majer *et al.,* 2014) more than kaempferol. Sun-exposed leaves of *Tilia platyphyllos* had a higher capacity to neutralise singlet oxygen compared to shaded leaves (Majer *et al.,* 2014).

B. Lipid Peroxidation

MDA content was determined because it is a measure of lipid peroxidation. The accumulation of MDA is affected by light intensity. From this study, as the light intensity increases, the accumulation of MDA also increases. Sun-exposed species accumulated higher MDA compared to semi-shaded and shaded species ($P < 0.05$) (Figure 2). The results obtained provide a negative correlation (Table 2) with antioxidant enzymes activity ($P < 0.01$).

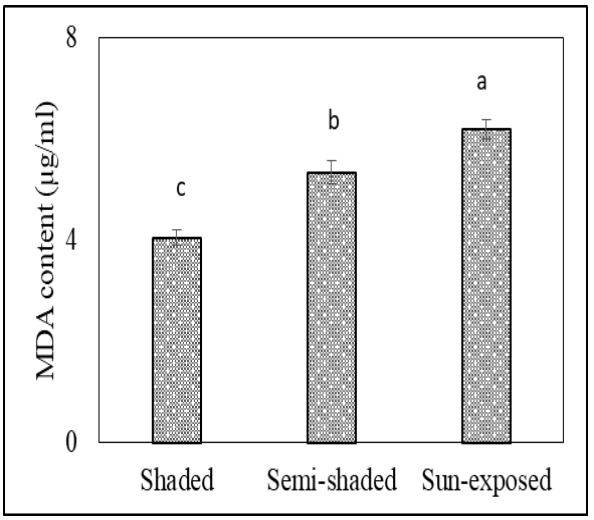

Figure 2. MDA content

Different small letters indicate significant differences among sun-exposed, semi-shade and shaded species ($P < 0.05$).

The higher amount of oxidative stress was recorded for sun-exposed species because, under high light intensity, MDA content may increase, thereby upregulating the biosynthesis of photoprotective secondary metabolites (Ibrahim and Jaafar, 2012). From this present study, the highest lipid peroxidation occurred in sun-exposed *T. sarmentosa*, while the lowest occurred in shaded species. This indicates that sun-exposed plant receives higher light intensity which in turn increases the plant stress, leading to an increase in ROS, where ROS may oxidise lipids, thereby leading to a high amount of MDA content. As the level of light intensity increases, more ROS is generated. The generated ROS can oxidise lipids (Yu *et al.,* 2016). Therefore, as the stress exposure time increases, MDA content increases. Other studies also indicates an increasing level of MDA with increasing light intensity (Agati *et. al.,* 2011; Distelbarth *et. al.,* 2013; Gu *et. al.,* 2017; Liu *et. al.,* 2013; Tang *et. al.,* 2015; Tattini *et. al.,* 2005; Wang *et al.,* 2017). Even though some studies indicate a decreasing order of MDA as light intensity increases (Ibrahim & Jaafar, 2012; Ibrahim *et. al.,* 2014; Li *et. al.,* 2016; Ma *et. al.,* 2015; Zhu *et al.,* 2017), other studies had indicated increase in MDA content under blue light (Yu *et al.,* 2016), red and blue light (Bian *et al.,* 2018) and UV light (Alexieva *et. al.,* 2001; Basahi *et. al.,* 2014; Guidi *et al.,* 2011).

C. Activity of Antioxidant Enzymes

The activity of antioxidant enzymes was affected by light

intensity. As represented in Table 1, the SOD, POD and CAT were higher under shaded conditions, followed by semi-shaded conditions while the least were obtained under sun exposed conditions. Differences in means of antioxidant enzymes are significant between shaded, semi-shaded and shaded species ($P < 0.05$).

Table 1. Antioxidant enzymes activity (U/mg protein)

Plant	Sun-exposed	Semi-shaded	Shaded
CAT	3.75±0.17 [a]	6.46±0.37 [b]	9.04±0.27 [c]
POD	59.59±2.67 [a]	93.27±5.33 [b]	118.35±3.59 [c]
SOD	2.64±0.12 [a]	3.32±0.19 [b]	3.79±0.11 [c]

Different small letters indicate significant differences among sun-exposed, semi-shade and shaded species ($P < 0.05$).

Antioxidant enzymes aid the plant in overcoming oxidative stress. Studies indicate an increasing order of their activity as the stress increases. Unfortunately, the activity of antioxidant enzymes may be inhibited if the level of stress passes a particular level, thereby increasing the ROS (Zhu *et al.,* 2017). This can increase the plants demand for more ROS scavenging metabolites like quercetin (Szymańska *et al*., 2017). As antioxidant enzymes decreases, hydrogen peroxide also reduces in the chloroplast due to the presence of chloroplast flavonoids (Agati *et al.,* 2013). Short term light stress can increase antioxidant enzymes like SOD, CAT and POD while long term stress can decrease their activity (Chen *et al.,* 2016). High light intensity can leads to an increase in the expression of flavonoid biosynthesis genes (Agati *et al.,* 2012), decrease in expression of photosynthetic genes (Pego *et al.,* 2000) and a decrease in activity of antioxidant enzymes (Fini *et al.,* 2011) when the plant is under unfavourable nutrient condition, temperature or lower CO_2 concentration. The activity of SOD may decrease due to light-induced hydrogen peroxide generation (Peltzer & Polle, 2001).

In the present study, there is a decreasing order of antioxidant enzymes activity as light intensity increases. Other studies also indicate a decreasing order of antioxidant enzymes with increasing light intensity (Chen *et. al.,* 2016; Lu *et. al.,* 2017; Ma *et al.,* 2015). Some studies had reported an increase in antioxidant enzymes with increasing light intensity (Agati *et. al.,* 2011; Gu *et. al.,* 2017; Li *et. al.,* 2016; Liu *et. al.,* 2013; Tang *et. al.,* 2015; Tattini *et. al.,* 2005; Wang *et al.,* 2017). A decreasing order of antioxidant enzymes with increasing light intensity may be due to other antioxidant mechanisms which usually dominates the early phase of light stress before flavonoid biosynthesis occurs (Carletti *et al.,* 2003). Daily variation in sunlight radiation can affect the antioxidant system because high light stress can decrease the activity of primary antioxidants and increase secondary antioxidants (Brunetti *et al.,* 2015). UV-B can decrease SOD activity under low temperature (Xu *et al.,* 2008). UV light can increase antioxidant enzymes activity (Alexieva *et. al.,* 2001; Basahi *et al.,* 2014). Likewise, blue light can increase oxidative stress in plants, thereby increasing antioxidant enzymes activity (Yu *et al.,* 2016).

D. Photoprotective Role of Quercetin

The photoprotective role of quercetin to the selected plant (Figure 3) can be supported by deducing the correlation coefficients among MDA, SOD, POD, CAT and quercetin content. From the results obtained, it can be seen that there is a strong negative correlation among quercetin contents and antioxidant enzymes ($P < 0.01$) (Table 2). Moreover, as MDA increases, SOD, POD and CAT decreases ($P < 0.01$), indicating a strong negative correlation. A positive correlation exists between quercetin content and MDA content ($P< 0.01$).

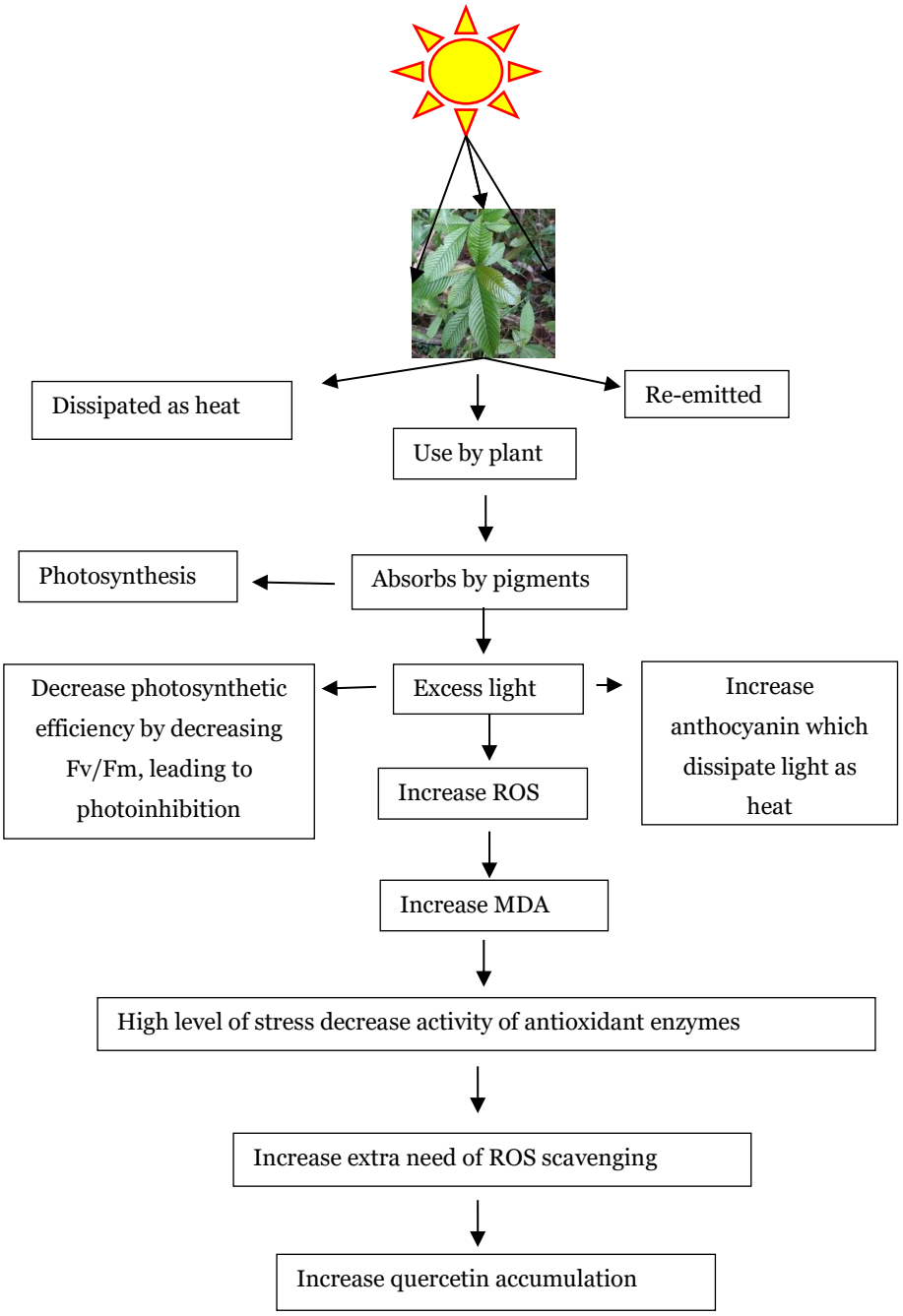

Figure 3. The photoprotective role of quercetin

From Table 2, as the light intensity increases, antioxidant enzymes activity increased, but due to high light stress, the activity of antioxidant enzymes become inhibited or reduced. This leads to an increase in ROS. The generated ROS then oxidises lipids contents of the plants otherwise referred to as lipid peroxidation. This leads to a rise in MDA content. At this point, the plant became in need of extra ROS scavenging because the activity of antioxidant enzymes is low. Therefore, the plants increase the accumulation of quercetin so as to conquer the light stress. This leads to the acceptance of the hypothesis generated at the beginning of the study which states that "if the activity of antioxidant enzymes of a plant is low, then quercetin flavonoid biosynthesis may increase". The quercetin content of *T. sarmentosa* increased as a result of an increase in MDA content which was due to a decrease in the activity of antioxidant enzymes.

Table 2. Correlation coefficients among MDA, SOD, POD, CAT and quercetin content

Coefficients	MDA	Quercetin	SOD	POD	CAT
MDA	1	.923**	-.899**	-.937**	-.956**
Quercetin	.923**	1	-.814**	-.843**	-.880**
SOD	-.899**	-.814**	1	.994**	.986**
POD	-.937**	-.843**	.994**	1	.997**
CAT	-.956**	-.880**	.986**	.997**	1

** Correlation is significant at the 0.01 level (2-tailed)

IV. CONCLUSION

The quercetin content of sun-exposed *T. sarmentosa* was higher than that of semi-shaded and shaded species, thereby indicating a photoprotective role. To support the evidence of photoprotection, lipid peroxidation and activity of antioxidant enzymes were quantified. Lipid peroxidation was higher at sun-exposed species while the activity of antioxidant enzymes was higher at shaded species. This shows that at higher light intensity, the plants antioxidant enzymes activity reached a certain level, and then declined due to a high level of stress. This increases the ROS which may oxidise lipids, thereby increasing the MDA content of the plants. In return, quercetin production increased so as to overcome the extra need for ROS scavenging. The hypothesis generated for the study was accepted because quercetin content was higher when the activity of antioxidant enzymes was low.

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VI. REFERENCES

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