Effect of Salicylic Acid on Carotenoids and Chlorophyll Content in Mas Cotek (*Ficus deltoidea* Jack var. *trengganuensis)* Leaves and its Retinol Activity Equivalents (RAE)

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Abstract: This study was conducted to determine the effect of different concentrations of salicylic acid (SA) (control, 0.01, 0.10, 1.00 mM) on carotenoids and chlorophyll content and its retinol activity equivalents (RAE) value in *Ficus deltoidea* Jack var. *trengganuensis* leaves. In this study, 12 seedlings of *Ficus deltoidea* Jack var. *trengganuensis* were sprayed with different concentrations of SA. Carotenoid content was determined using High-Performance Liquid Chromatography (HPLC) and chlorophyll content was determined using chlorophyll meter. Retinol activity equivalents were calculated using RAE formulation. From the results obtained, two types of carotenoids, lutein and β-carotene, were detected in HPLC. The highest lutein and β-carotene content present in *Ficus deltoidea* Jack var. *trengganuensis* leaves extract was found in 0.10 mM SA treatment (93.50^{b} ± 0.71 µg/g DW) for lutein and (282.00^{b} ± 46.67 µg/g DW) for β-carotene, while the lowest lutein compound was found in 1.00 mM SA treatment (30.25^{a} ± 1.77 µg/g DW) while the least β-carotenoid content was found in (1.00 mM SA) treatment (63.00^{a} ± 0.71 µg/g DW). The same goes for retinol activity equivalents, the highest retinol activity equivalents was recorded in 0.10 mM SA treatment while lowest in 1.00 mM SA treatment. For chlorophyll content, the highest reading was showed in 0.10 mM SA treatment (73.50^{b} ± 0.71 µg/g DW) while the lowest reading was in 1.00mM SA treatment (42.10^{a} ± 1.41 µg/g DW). From this study, it can be concluded that salicylic acid at a certain concentration could increase or improve the carotenoid or chlorophyll content. Thus, it could be an alternative source of carotenoid and chlorophyll for the food and pharmaceutical industry in the future.

Keywords: Salicylic acid, carotenoid content, retinol activity equivalents, *Ficus deltoidea* Jack var. *trengganuensis.*

INTRODUCTION

Mas Cotek *(Ficus deltoidea* Jack*)* is a miniature tree or perennial plant [1]. The beginning of its common name is due to its flowing epiphyte plant on larger trees [2]. Besides, Mas cotek is also recognized as Kangkalibang in Africa, Agoluran and Sempit-sempit in East Malaysia and Tabat Barito in Indonesia [3]. It is known as Mas Cotek in Peninsular Malaysia due to its fine spots on the external of the leaf with gold colour [4]. “Mas” means gold and “Cotek” means dot which provides to the name of this plant [3]. It has been used conventionally to cure diseases such as diabetes and cardiovascular diseases [1] cholesterol and lipids, migraine, menopause, cancer and high blood pressure [5] lower external glucose contents [6]. Besides, they are used by the female after delivery to tighten up the womb, to cure the problem of the menstrual cycle and to recover blood circulation [7]. It also has medicinal properties such as antinociceptive [7], antioxidant [8], anti-inflammatory [9], anti-ulcerogenic [10], antibacterial [11], anti-melanogenic [12], and anti-hyperglycemic activity [13].

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature and it is normally present in plants in quantities of a few µg/g fresh mass or less [14]. Its name is originated from the word *Salix*, the scientific name for the willow tree. In the last three decades, its role in plants has only been known and the full picture is still not well-defined until day even though the beneficial consequence of SA in humans has been well learned for about 200 years [15]. According to [16], at low concentrations (less than 1 mM) it has been reported that SA was found to be beneficial for the plant growth and development. For example, the exogenous use of SA has been reported to enrich the fruit yield of cucumber [17, 18]. Salicylic acid also has various adjusting roles in the metabolism with the gathering of effects on the morphology and physiology of plants [19, 20]. SA also found to show positive resistance effects on biotic and abiotic stress compared to other hormones [21-24].

Vitamin A is a fat-soluble vitamin attained from the diet either as preformed vitamin A (retinol and retinyl

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ISSN: 2223-3806 / E-ISSN: 1927-5951/20

esters) in foods of animal source or as a provitamin A carotenoid (mainly β-carotene) in plant-derived foods, mostly in oils, fruits and vegetables. Retinol is comprised of a β-ionone ring, a polyunsaturated side chain and a polar end group. This chemical structure makes it feebly soluble in water but easily convertible through membrane lipid bilayers. Preformed vitamin A consists primarily of retinol and retinyl esters, which are provided in the diet by animal-derived products and the most abundant forms of vitamin A in the body. Retinol is a conveyance form and an ancestor of the transcriptionally active metabolite all-trans retinoic acid [25]. Vitamin A presents many tasks in the body. It is an important micronutrient throughout the life cycle and is needed for vision, regulation of cell proliferation and differentiation and reproduction, especially for embryonic development, growth and tissue repairs. It is also imperative for continuing good nutritional grades for optimal intellectual function in the elderly [26, 27].

The U.S. Institute of Medicine (IOM) has announced a new term, retinol activity equivalent (RAE) to press out the activity of carotenoids in terms of Vitamin A, to allow for new research on the Vitamin A activity (bio-efficacy) of carotenoids [28]. IOM established the following conversion factor equivalents:

1 µg retinol = 1 µg RAE

1 µg β-carotene in oil = 0.5 µg RAE

1 µg β-carotene in mixed foods = 0.083 µg RAE

1 µg other pro-vitamin A carotenoids in mixed foods = 0.042 µg RAE

1 µg retinol= 1 RE

1 µg β-carotene= 0.167µg RE (1/6 µg)

1 µg other pro-vitamin A carotenoids= 0.084µg RE, (1/12µ)

Micronutrient deficiencies among children under 5 years old in low- and middle-income countries are common. Vitamin A (VA), iron, and zinc are specifically targeted for improvement by the World Health Organization (WHO) because their deficiencies are prevalent and lead to increased mortality and morbidity [29]. Community randomized controlled trials have shown that administering preformed VA solely or in combination with zinc to children in regions with a high prevalence of malaria, reduced morbidity [30, 31]. Targeting preschool children is of specific interest because of the long-term detrimental effects of undernutrition on cognitive development and adulthood work productivity [29]. Vitamin A deficiency (VAD) can lead to anemia, stunting, and weakened resistance to infection, blindness, and death [29].

More than 800 different carotenoids are known to be present in nature and are found in green leafy and yellow-colored vegetables and orange-colored fruits [32]. Based on their chemical structure, carotenoids are lipophilic compounds and have been distributed into carotenes and xanthophylls. The xanthophylls have oxygenated functional groups making them more polar than the carotenes whereas the carotenes are hydrocarbons. Carotenoid pigments are responsible for protection of the photosynthetic tool in plants by dispelling additional energy. They also have a major role in photosynthesis by gathering light and by stabilizing protein ruin in the photosynthetic tool. Carotenoids snuff out singlet oxygen which mainly ascends from sunlight absorption by chromospheres and thus shield chlorophylls, lipids, proteins and DNA from oxidative injury [33].

The term vitamin A is used in the context of dietary requirements to comprise provitamin A carotenoids that are dietary precursors of retinol. Of the many carotenoids in nature, several have provitamin A activity but food composition data are only promptly obtainable for α-carotene, β-carotene and β-cryptoxanthin. Preformed vitamin A is found only in animal-derived foods, whereas dietary carotenoids are found mostly in oils, fruits and vegetables.

The aim of this research was to observe the effect of salicylic acid on carotenoid and chlorophyll content of *Ficus deltoidea* Jack var. *trengganuensis* leaves. Thus, it was hoped that alternative sources of carotenoid and chlorophyll compound discovered through this research could be used in the pharmaceutical and food industry in the future.

MATERIALS AND METHODS

In order to study the effect of different concentration of salicylic acid (SA) on carotenoids and chlorophyll content in *Ficus deltoidea* Jack var. *trengganuensis* leaves, this experiment was conducted under the shed behind Plant Tissue Culture Lab in Tembila Campus at Department of Faculty Bioresources and Food Industry, University Sultan Zainal Abidin. 12 seedlings of Mas Cotek *Ficus deltoidea* Jack var. *trengganuensis* were obtained from Agriculture and Biotechnology plantations at Tembila Campus, Sultan Zainal Abidin University, in Besut, Terengganu.

The seedlings were transferred to the shed provided before the experiment started. They were arranged about 2 feet each other. There were four treatments to investigate the effect of salicylic acid on carotenoids and relative activity equivalents (RAE) in Mas Cotek leaves. The seedlings were sprayed with 0.01mM SA, 0.1mM SA, 1mM SA and 0 mM SA (control). The seedlings were sprayed every three days based on their treatments. Every treatment had three seedlings. Every week, the results were recorded until reached the fourth week. The readings were taken and recorded for every treatment. Each pot was treated as one replicate and all treatments were repeated three times. The data was analysed statistically with SPSS Statistics 20 statistical software. Means were statistically compared by One Way Anova at $p< 5\%$ level.

Method for Carotenoid Determination

Sample preparation

Firstly, *Ficus deltoidea* Jack var. *trengganuensis* leaves samples were plucked, washed, freeze-dried and ground to the fine powder prior to analysis.

Sample Extraction

The extraction procedure described by Othman (2009) was followed with slight modifications [34]. For each sample, 1g of powdered dried *Ficus deltoidea* Jack var. *trengganuensis* leaves was weighed and 50ml of an acetone and methanol mixture (7:3, v/v) was mixed into the sample to allow an efficient solvent penetration. The solution was allowed to stand overnight at room temperature under dark conditions. The next day, the samples were vortexed and centrifuged for 5 min at 9500 rpm in a refrigerated centrifuge and the supernatant was transferred into another centrifuged tube. This procedure was repeated three times or until the sample became colorless. The samples were done in triplicate. Subsequently, 10ml of petroleum ether was added in the supernatant with distilled water until reached 50ml. The solutions were then allowed to separate and the upper layers of petroleum ether containing carotenoids were collected. Vials or test tubes were then capped and sealed with a parafilm to exclude oxygen and immediately stored at -20°C for further analysis.

Saponification

Saponification was done as described by Othman (2009) with again slight modifications [34]. This step was done to eliminate chlorophylls in sample leaves. Carotenoid extracts that were dried using rotary evaporator after the extraction process were then subjected to certain steps. 20µl of ethyl acetate was added into the samples, followed with 380 µl acetonitrile: distilled water (9:1 v/v). Then, 400 µl methanolic potassium hydroxide solution (10% w/v) was added. Base carotenoids were then extracted by the addition of 2 ml hexane with 0.1% butylated hydroxytoluene (BHT), followed by 10% of sodium chloride (NaCl). Finally, the extracts were washed with distilled water, the upper layer was collected, dried and re-suspended in ethyl acetate for HPLC analysis.

High-Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis was conducted as described by Othman (2009) to quantify the carotenoid content in each treatment [34]. The HPLC analysis of carotenoids was performed using the Agilent model 2100 series which comprises a binary pump with autosampler injector, micro vacuum degassers, thermostat column compartment and a diode array detector. The column used was an HPLC column ZORBAX Eclipse XDB-C_{18} end-capped (5 µm), sized at 4.6 x 150 mm. The solvents used were (A) acetonitrile: water (9: 1 v/v) and (B) ethyl acetate. The solvent gradient used was developed as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min) , 60-100% solvent B (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min) at a flow rate of 1.0 ml min^{-1}. The temperature of the column was maintained at 20°C. The injection volume was 10 µl. Carotenoid standards of β-carotene and lutein were obtained commercially from Sigma- Aldrich. Detection for carotenoid peaks was in the range of 350 nm to 430 nm. Individual carotenoid concentration was calculated by comparing their relative proportions, as reflected by the integrated HPLC peak areas.

Calculation of Retinol Activity Equivalent (RAE)

RAE was determined by using the equation of 1 RAE = 1 µg retinol or 12 µg β-carotene or 24 µg of provitamin A carotenoids [28].

Determination of Chlorophyll Content

Chlorophyll content was determined according to [35] using SPAD-502 Plus Chlorophyll Meter. The reading of chlorophyll content was taken based on the reading appear on the instrument after clicking the leaves with chlorophyll meter.

RESULTS AND DISCUSSION

Analysis of Concentration of Salicylic Acid on Carotenoid Content

Figure **1** shows the HPLC chromatogram of carotenoids detected in *Ficus deltoidea* Jack var.

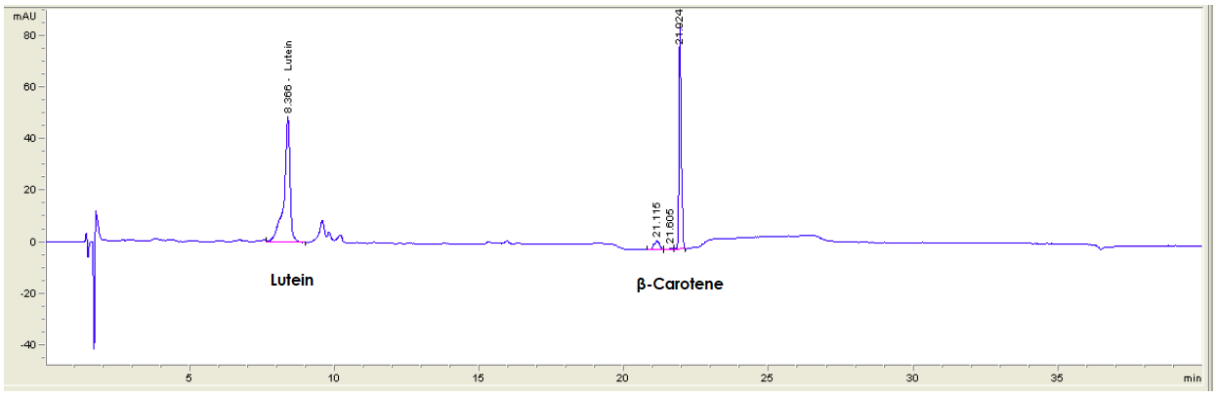

Figure 1: HPLC chromatogram of lutein and β-carotene detected in Mas Cotek leaves.

trengganuensis leaves treated with salicylic acid. Two types of carotenoids detected in HPLC were lutein and β-carotene. Table **1** shows the value of β-carotene and lutein present in *Ficus deltoidea* Jack var. *trengganuensis* leaves extracts based on HPLC analysis. The highest lutein compound was detected in 0.10 mM SA treatment (93.50 ± 0.71 µg/g DW), followed with lutein compound treated with 0.01 mM SA treatment (38.50 ± 7.78 µg/g DW) while the least lutein compound present was in 1.00 mM SA treatment (30.25 ± 1.77 µg/g DW). There was a significant difference between the treatments. Meaning here that, 0.10 mM SA was the best outcome to enhance lutein content in the samples.

Table **1** also shows the content of β-carotene content detected in *Ficus deltoidea* Jack var. *trengganuensis* leaves treated with different concentrations of Salicylic acid. The highest compound of β-carotene present in *Ficus deltoidea* Jack var. *trengganuensis* leaves extracts from 0.10 mM SA treatment was about 282.0 ± 46.67 µg/g DW, while the second-highest was control with 112.25 ± 9.55 µg/g DW and the least β-carotene compound was from 1.00 mM SA treatment (63.00[a] ± 0.71 µg/g DW).

Table 1: Effect of Different Concentrations of Salicylic Acid on Lutein and β-Carotene Content in *Ficus deltoidea* Jack var. *trengganuensis* Leaves Extract

Concentration of SA (mM)	Lutein (µg/g DW)	β-carotene (µg/g DW)
Control	32.0[a] ± 2.12	112.25[a] ± 9.55
0.01	38.50[a] ± 7.78	106.25[a] ± 7.42
0.10	93.50[b] ± 0.71	282.0[b] ± 46.67
1.00	30.25[a] ± 1.77	63.00[a] ± 0.71

Data expressed as the mean ± standard deviation (n = 4) of four samples. [a,b]Variation in the letters between samples in different columns indicate significant differences at 5% level (P<0.05) utilizing One-Way Anova.

The previous study showed that there was a positive result in sample *Wolffia arrhiza* (*Lemnaceae*) when 10^{-1} to 10^{-3} M SA strongly stimulated action on the content of chlorophyll *a* and *b* and carotenoids especially β-carotene and lutein with zeaxanthin [36]. Other than that, one study has found that when 0, 0.5, 1.0 and 1.5 mM SA used in sample tomatoes (*Solanum lycopersicum*), salicylic acid increased the carotenoid content in the tomato plants [37]. It also reported that 10^{-2} M SA in salt-stressed maize plants (*Zea mays*) increased the chlorophyll and carotenoid content in maize plants [38]. Besides, in one study using sunflower cotyledons (*Helianthus annuus L.*) as samples have found that about 10 and 0.1 µM SA concentrations accelerated carotenoid content of excised cotyledons, 1000 µM SA retarded it. Salicylic acid also affected photosynthetic pigments like chlorophyll *a*, *b*, and carotenoids significantly [39].

Salicylic acid plays a key role in a plant's growth, development, and protection responses, and is involved in some signal transduction systems to encourage certain enzymes related to the biosynthesis of secondary metabolites such as carotenoid and chlorophyll in plants [40-42]. Besides that, salicylic acid upgraded the accumulation of compounds belonging to diverse structural classes, including phenolics, terpenoids and alkaloids as an elicitor [43, 44]. In this study, 0.1 mM SA applied onto Mas cotek seedlings had increased the lutein and β-carotene content. These results were in line with those which detected that the treatment of salicylic acid increased carotenoids content as discussed.

Carotenoids and chlorophyll are well known for their antioxidant properties. In previous studies, they reported that salicylic acid had a positive effect on the antioxidant activity of various concentrations of extracts which could be an indication of the presence of chlorophyll and carotenoids in their samples treated

with SA [42, 43, 45]. From their study, salicylic acid with a quantity of 1 mM and at a concentration 300 ppm showed the highest radical scavenging activity among the different concentrations of SA, and has been demonstrated SA as an elicitor enriched secondary metabolites in plants. As a result of this compound accumulations, antioxidant activity also increased in different plants species.

The level of secondary metabolites in plants was influenced by some factors like biotic and abiotic stresses. Several factors, including the age of the plant, the season, microbial attack, cropping, radiation, competition, and nutritional status, have been proven to influence the secondary metabolite profile in higher plants [46]. Carotenoid content is also influenced by diverse environmental disorders like drought and temperature [47]. In other studies, consequences of altitude and temperature on the carotenoid content of saffron were observed [48]. [49] reported that SA is an endogenous plant growth regulator of phenolic nature, and this compound is proficient in enhancing plant growth and harvest in some plants as an elicitor. It plays an imperative role in adjusting a number of plant physiological processes, including photosynthesis and manufacture of bioactive compounds [49].

Analysis of Retinol Activity Equivalents (RAEs) in Ficus Deltoidea Jack var. Trengganuensis Leaves

Table **2** shows the relative activity equivalents (RAEs) present in *Ficus deltoidea* Jack var. *trengganuensis* leaves extracts. After treatment with salicylic acid highest reading is from 0.10 mM SA (23.5^b ± 3.89 RAE) while the second highest is from control with 9.35^a ± 0.80 RAE and the least is from 1.00 mM SA of 5.25^a ± 0.06 RAE. The results obtained were based on the calculation from the β-carotene present in *Ficus deltoidea* Jack var. *trengganuensis* leaves extract.

RAE is a measure of vitamin A activity based on the capacity of the body to convert provitamin carotenoids. Carotenoids, especially α- and β-carotene are potential provitamin A precursors that can be altered into retinol, are existent in plant products, particularly green leafy vegetables, red palm oil and yellow and orange fruits and some tubers, like sweet potato. Red palm oil is the richest unsurprisingly occurring source of β-carotene and generally contains a total of ~500-800 mg of provitamin A carotenoids/kg oil, which is ~15 times higher than the carotenoid contents of carrots on a weight-by-weight basis [52]. Carotenoids are physically less available than retinol but they are generally more reasonably priced than animal foods. Thus carotenoids stipulate most of the vitamin A activity in the diets of lower socio-economic units of the populations.

Basically, nutrient requirements for a certain group of people are different based on age, gender and health condition. Malaysian have their diet guidelines known as Recommended Nutrient Intake (RNI), and the RE (retinol equivalent) for an adult is 600 µg/day [50]. There is also an international reference such as guideline from USDA [51]. 1 RE is equivalent 1 RAE, which means in this study, the highest value of β-carotene obtained from 1g of the sample treated with 0.1 mM SA will provide 23.5 RAE (µg). Thus, 25.53 g of Mas cotek leaves extraction will provide the value of Vitamin A recommended by this RNI, which seems to be a potential source of Pro-vitamin A that could be used in the future pharmaceutical and food industry.

Table 2: Effect of Different Concentrations of Salicylic Acid on Retinol Activity Equivalents (RAEs) in *Ficus deltoidea* Jack var. *trengganuensis* Leaves Extract

Concentration of SA (mM)	Retinol Activity Equivalents (RAEs) (µg)
Control	9.35^a ± 0.80
0.01	8.85^a± 0.62
0.10	23.5^b ± 3.89
1.00	5.25^a ± 0.06

Data expressed as the mean± standard deviation (n = 4) of four samples. ^{a,b}Variation in the letters between samples indicates a significant difference at 5% level (P<0.05) utilizing One-Way Anova.

Analysis of Chlorophyll Content with Different Concentration of Salicylic Acid in Ficus Deltoidea Jack var. Trengganuensis Leaves

According to Table **3**, the range of chlorophyll content in *Ficus deltoidea* Jack var. *trengganuensis* leaves extract is from 42.10^a ± 1.41 µmol/m^2 to 73.50^b ± 0.71 µmol/m^2. The maximum chlorophyll content obtained after spraying with SA is about 73.50^b ± 0.70 µmol/m^2, in 0.10 mM SA concentration, while the lowest content of chlorophyll is shown in 1.00 mM SA concentration is around 42.10^a ± 1.41 µmol/m^2. There is a significant difference between the treatment of 0.10 mM SA and 1.00 mM SA.

There are many other factors that contribute to the performance of plant growth, yield and quality [53, 54]. Plant nutrient including ecological circumstances is among the causes that control plant growth. Nitrogen (N) and potassium (K) are the most vital nutrient elements required for plant growth and for the creation of new cells. Since N is the building block in all plant

portions and a portion of the chlorophyll molecule structure, and it is one of the key macronutrients crucial for plants, adequate N acts as a significant character in the development of chlorophyll in the photosynthetic route [55, 56].

The chlorophyll content is a revealing factor for the estimate of the N taken from the soil. K performs an imperative function in plant breathing through the stomata, enzymatic activity, diseases or drought resistance and conveying the photosynthesis products among plant tissues. Since K also builds up starch, fat and secondary metabolite compound contents of the plant, it improves the plant quality [56]. There is a relationship between the chlorophyll in the plant leaves and the complete N in all dry plant materials [57]. In this study, all seedlings were provided with sufficient nutrients needed through NPK fertilizer, thus, nutrients of N and K were assumed not to affect the production of secondary metabolites.

Previous studies reported that the exogenous application of SA was to improve plant resistance to water deficiency. SA application at different concentrations through roots, seed soaking and foliar spraying in a concentration-dependent routine reduced the negative result of water inadequacy on tissue water status, stomatal conductance, chlorophyll content, membrane properties and plants physiological actions [15, 58]. Moreover, SA is recognized as an imperative tool of photosynthesis, water relations and metabolic characteristics of plants, depending on its likenesses, concentrations, the process of application and plant type. SA is recognized to influence leaf and chloroplast structure [59], stomatal closure [60, 61], chlorophyll and carotenoid contents [62, 63] and the enzyme activities, such as Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase) and carbonic anhydrase [64-67]. However, high concentrations of SA (1 mM – 5 mM) will initiate a drop in the photosynthetic rate [68], and decline chlorophyll contents in cowpea, wheat, and Arabidopsis [62, 69, 70]. The decline in Rubisco activity credited to a 50 % reduction in protein levels, as compared to non-treated plants [71]. Exogenously applied SA will encourage alterations in leaf structure that contains a reduced width of the adaxial and abaxial epidermis, and mesophyll tissue [72]. Such changes relate ultra-physically with an increase in chloroplast volume, swelling of grana thylakoids, and gelling of the stroma [59]. Thus, the reduced photosynthetic activity at high SA concentrations was due to its effects on the thylakoid membranes and light-induced reactions related to them.

Meanwhile, in other studies, chlorophyll content was significantly improved in wheat seedlings, higher from the grains pre-treated with lower SA concentration (10 nM – 5 nM) [73]. Higher concentrations did not prove to be advantageous. In terms of treatment efficiency, foliar application of SA showed to be more productive in *Brassica napus* compared to seed-soaking treatment [74]. In line with this study, lower SA concentration (10 nM – 5 nM) sprayed on *Brassica juncea*, the chlorophyll content was significantly boosted [63]. This study was in agreement with many previous studies that exhibited low SA concentration provided the highest chlorophyll content.

Table 3: Effect of Different Concentrations of Salicylic Acid on Chlorophyll Content Activity in *Ficus deltoidea* Jack var. Trengganuensis Leaves Extract

Concentration of SA (mM)	Chlorophyll content (µmol/m^2)
Control	$72.10^{b} \pm 3.68$
0.01	$71.25^{b} \pm 4.59$
0.10	$73.50^{b} \pm 0.71$
1.00	$42.10^{a} \pm 1.41$

Data expressed as the mean± standard deviation (n = 4) of four samples.[a,b]Variation in the letters between samples indicates a significant difference at 5% level (P<0.05) utilizing One-Way Anova.

CONCLUSION

SA was found to affect the carotenoid and chlorophyll content in *Ficus deltoidea* Jack var. *trengganuensis* leaves. From the results, it can be observed that 0.10 mM SA treatment caused an increase in carotenoid and chlorophyll content of *Ficus deltoidea* Jack var. *trengganuensis* leaves extract, and thus reflected on the RAE value through the calculation of β-carotene content.

ACKNOWLEDGEMENT

This work was supported by the Research Acculturation Grant Scheme (RAGS/1/2014/SG05/ UNISZA//3) provided by the Malaysian Ministry of Education for financial support. We would like to thank the staff from the Herbarium Laboratory, KAED, IIUM for the facilities provided, as well as staff and friends from UNISZA Tembila Campus for the facilities and kind assistance provided.

REFERENCES

[1] Hakiman M, Maziah M. Non enzymatic and enzymatic anti-oxidant activities in aqueous extract of differerent Ficus-deltoidea accessions J Med Plant Res 2009; 3(3): 120-131.

[2] Fang J, Chen J, Henny R J, Chao CCT. Genetic relatedness of ornamental Ficus species and cultivars analyzed by amplified fragment length polymorphism markers. J Am Soc Hortic Sci 2007; 136: 807-815. https://doi.org/10.21273/JASHS.132.6.807

[3] Hakiman M, Syed MA, Ahmad S, Maziah M. Flavonoid content in leaf extracts of different varieties of Ficusdeltoidea Jack using High Performance Liquid Chromatograph (HPLC). UMT 11th International Annual Symposium and Sustainability Science and Management; 2012: p. 470-473.

[4] Nashriyah M, Nurrul Akmar R, Nor Zaimah AR, Norhaslinda H, Zanariah MN, Nur Fatihah HN, Abd Ghani Y, Abdul Manaf A. (Leaf morphological variations and heterophylly in *Ficus deltoidea* Jack (Moraceae). Sains Malays 2012; 41(5): 527-538.

[5] Aris SRS, Mustafa S, Ahmat N, Jaafar FM, Ahmad R. Phenolic content and antioxidant activity of *Ficus deltoidea* var. *angustifolia* sp. Malaysian J Anal Sci 2009; 13: 146-150.

[6] Aminudin N, Sin CY, Chee ES, Nee KI, Renxin L. Blood glucose lowering effect of *Ficus deltoidea* aqueous extract. Malaysian J Sci 2007; 26: 73-78.

[7] Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, Israf DA. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia 2008; 79: 557-561. https://doi.org/10.1016/j.fitote.2008.06.005

[8] Sirisha N, Sreenivalusu M, Sangeeta K, Madhusudhana CC. Antioxidant properties of Ficus species-A review. Int J PharmTech Res 2010; 2(4): 2174-2182.

[9] Zunoliza A, Khalid H, Zhari I, Rasadah MA. Anti-inflammatory activity of standardised extracts of leaves of three varieties of *Ficus deltoidea*. Int J Pharm Clin Res 2009; 1(3): 100-105.

[10] Siti Fatimah Zahra MA, Mahmood AA, Hapipah MA, Suzita MN, Salmah I. Anti-ulceragonic activity of aqueous extract of *Ficus deltoidea* against ethanol-induced gastric mucosal injury in rats. Res J Med Sci 2009; 3(2): 42-46.

[11] Suryati, Hazli N, Dachriyanus, Md Nordin HL. Structure elucidation of antibacterial compound from *Ficus deltoidea* Jack leaves. Indones J Chem 2011; 11(1): 67-70. https://doi.org/10.22146/ijc.21422

[12] Oh MJ, Abdul Hamid M, Ngadiran S, Seo YK, Sarmidi MR, Park CS. *Ficus deltoidea* (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity *in vitro* and by suppressing tyrosinase gene expression in B16F1 melanoma cells. Arch Dermatol Res 2011; 303: 161-170. https://doi.org/10.1007/s00403-010-1089-5

[13] Adam Z, Hamid M, Ismail A, Khamis S, Marsidi M. Antihyperglycemic and glucose tolerance activity of *Ficus deltoidea* ethanolic extract in diabetic rats. Malaysian J Health Sci 2010; 8(1): 25-30.

[14] Raskin I, Skubatz H, Tang W, Meeuse BJD. SA levels in thermogenic and nonthermogenic plants. Ann Bot 1990; 66: 376-383. https://doi.org/10.1093/oxfordjournals.aob.a088037

[15] Hayat Q, Hayat S, Ifran M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. Environ Exp Bot 2010; 68: 14-25. https://doi.org/10.1016/j.envexpbot.2009.08.005

[16] Rivas-San V, Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 2011; 62: 3321-3338. https://doi.org/10.1093/jxb/err031

[17] Larque-Saavedra A, Martin-Mex R. Effect of salicylic acid on the bio-productivity of plants. In: Hayat S, Ahmad A. (Eds). Salicylic Acid.A Plant Hormone. Dordrecht, The Netherlands: Springer Publishers; 2007.

[18] Martin-Mex R, Nexticapan-Garcez A, Larqué-Saavedra A. Potential benefits of SA in food production. pp 299-313, In: S. Hayat, A. Ahmad, M.N. Nasser (eds.) SA Plant Growth and Development. Dordrecht, Heidelberg: Springer 2013; p. 389. https://doi.org/10.1007/978-94-007-6428-6_13

[19] Popova L, Pancheva T. Uzunova A. Salicylicacid: Properties, biosynthesis and physiological role. Bulgarian J Plant Physiol 1997; 23: 85-93.

[20] Maghsoudia K. Arvind MJ. Salicylic acid and osmotic stress effects on seed germination and Seedling growth of wheat (Triticum aestivum L.) cultivars. Plant Ecophysiol 2010; 2: 7-11.

[21] Zahra S, Baghizadeh AB, Ali VSM, Ali Y. Mehdi Y. The salicylic acid effect on the tomato (*Lycopersicum esculentum* Mill.) sugar, protein and proline contents under salinity stress (NaCl). J Biophys Struct Biol 2010; 2: 35-41.

[22] Szepesi A, Gemes K, Orosz G, Peto A, Takacs Z, Vorak M, Tari I. Interaction between salicylic acid and polyamines and their possible roles in tomato hardening processes. Acta Biol Szeged 2011; 55: 165-166.

[23] Khan W, Prithviraj B, Smith DL. Photosynthetic responses of corn and soybean to foliar application of salicylates. J Plant Physiol 2003; 160: 485-492. https://doi.org/10.1078/0176-1617-00865

[24] Senaratna T, Touchell D, Bunn E, Dixon K. Acetylsalicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean andtomato plant. Plant Growth Regul 2000; 30: 157-161. https://doi.org/10.1023/A:1006386800974

[25] European Food Safety Authority. Scientific Opinion on Dietary Reference Values for Vitamin A. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA). *EFSA Journal* 2015; 13(3): 4028. https://doi.org/10.2903/j.efsa.2015.4028

[26] WHO/FAO. Vitamin A. In: Vitamin and Mineral Requirements in Human Nutrition (2nd Edition). Report of a Joint FAO/WHO Expert Consultation, World Health Organization, Geneva 2004; p. 17-44.

[27] IOM. Vitamin A. In: Dietary References Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Food and Nutrition Board, Institute of Medicine. Washington DC: National Academy Press 2001; p. 82-161.

[28] West CE, Eilander A, van Leishout M. Consequences of Revised Estimates of Carotenoid Bioefficacy for Dietary Control of Vitamin A Deficiency in Developing Country. J Nutr 2002; 132: 2920S- 2926S. https://doi.org/10.1093/jn/132.9.2920S

[29] Essential Nutrition Actions: Improving Maternal, Newborn, Infant and Young Child Health and Nutrition;World Health Organization: Geneva, Switzerland, 2013.

[30] Zeba AN, Sorgho H, Rouamba N, Zongo I, Rouamba J, Guiguemdé RT, Hamer DH, Mokhtar N, Ouedraogo JB. Major reduction of malaria morbidity with combined vitamin A and zinc supplementation in young children in Burkina Faso: A randomized double blind trial. Nutr J 2008; 7: 7. https://doi.org/10.1186/1475-2891-7-7

[31] Shankar AH, Genton B, Semba RD, Baisor M, Paino J, Tamja S, Adiguma T, Wu L, Rare L, Tielsch JM, Alpers MP, West KP Jr. Effect of vitamin A supplementation on morbidity due to Plasmodium falciparum inyoung children in Papua New Guinea: A randomised trial. Lancet 1999; 354: 203-209. https://doi.org/10.1016/S0140-6736(98)08293-2

[32] Stahl W, Sies H. Photoprotection by dietary carotenoids: Concept, mechanisms, evidence and future development. Mol Nutr Food Res 2012; 56(2): 287-95. https://doi.org/10.1002/mnfr.201100232

[33] Ibañez E. Cifuentes A. Benefits of using algae as natural sources of functional 431 ingredients. J Sci Food Agric 2013; 93(4): 703-709. https://doi.org/10.1002/jsfa.6023

[34] Othman R. Biochemistry and genetics of carotenoid composition in potato tubers. Lincoln University; 2009.

[35] Loh FCW, Grabosky JC, Bassuk NL. Using the SPAD 502 meter to assess chlorophyll and nitrogen content of Benjamin

Fig and Cottonwood leaves. Horttechnology 2002; 12: 682-686. https://doi.org/10.21273/HORTTECH.12.4.682

[36] Czerpak R, Dobrzyn P, Krotke A, Kicinska E. The effect of Auxins and Salicylic Acid on Chlorophyll and Carotenoid Contents in Wolffia Arrhiza (L.) Wimm. (Lemnaceae) Growing on Media of Various Trophicities. Pol J Environ Stud 2002; 11(3): 231-235.

[37] Shahba Z, Baghizadeh A. Yosefi M. The salicylic acid effect on the tomato (*lycopersicum esculentum* Mill.) germination, growth and photosynthetic pigment under salinity stress (NaCl). J Stress Physiol Biochem 2010; 6(3): 4-16.

[38] Khodary SEA. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolismvin salt-stressed maize plants. Int J Agric Biol 2004; 6: 5-8.

[39] Cag S, Cevahir-Oz G, Sarsag M, Goren-Saglam, N. Effect of salicylic acid on pigment, protein content and peroxidase activity in excised sunflower cotyledons. Pak J Bot 2009; 41(5): 2297-2303.

[40] Taguchi G, Yazawa T, Hayashida N, Okazaki M. Molecular cloning and heterologous expression of novel glucosyltransferases from tobacco cultured cells that have broad substrate specificity and are induced by salicylic acid and auxin. Eur J Biochem 2001; 268: 4086-4094. https://doi.org/10.1046/j.1432-1327.2001.02325.x

[41] Chen JY, Wen PF, Kong WF, Pan QH, Zhan JC, Li JM. Effect of salicylic acid on phenylpropanoids and phenyl-alanine ammonia-lyase in harvested grape berries. Postharvest Biol Technol 2006; 40: 64-72. https://doi.org/10.1016/j.postharvbio.2005.12.017

[42] Ghasemzadeh A, Jaafar H, Karimi E, Ibrahim M. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber* officinaleroscoe) varieties. Int J Mol Sci 2012; 13: 14828-14844. https://doi.org/10.3390/ijms131114828

[43] Idrees M, Naeem N, Aftab T, Khan MMA. Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle. Acta Physiol. Plant 2011; 33: 987-999. https://doi.org/10.1007/s11738-010-0631-6

[44] Turkyılmaz B, Aktas LY, Gu¨ven A. Salicylic acid induced some biochemical and physiological changes in *Phaseolus vulgaris* L. Sci Eng J Firat Univ 2005; 17: 319-326.

[45] Malarz J, Stojakowska A, Kisiel W. Effect of methyl jasmonate and salicylic acid on sesquiterpene lactone accumulation in hairy roots of *Cichoriumintybus*. Acta Physiol Plant 2007; 29: 127-132. https://doi.org/10.1007/s11738-006-0016-z

[46] Harborne JB. The flavonoids: Advances in research since 1980. UK: Chapman and Hall 1988. https://doi.org/10.1007/978-1-4899-2913-6

[47] Bouvier F, Backhaus R, Camara B. Induction and control of chromoplast specific carotenoids genes by oxidative stress. J Biol Chem 1998; 273: 30651-30659. https://doi.org/10.1074/jbc.273.46.30651

[48] Zarin Kamar F, Tajik S, Soleimanpour S. Effects of altitude on anatomy and concentration of crocin, picrocrocin and safranal in *Crocus sativus* L. Aust J Crop Sci 2011; 7: 831-838.

[49] Amanullah MM, Sekar S, Vincent S. Plant growth substances in crop production. Asian J Plant Sci 2010; 9: 215-222. https://doi.org/10.3923/ajps.2010.215.222

[50] Tee ES, Ismail MN, Mohd Nasir A, Khatijah I. Nutrient Composition of Malaysian Foods. 4th Edition. Malaysian Food Composition Database Programme, Kuala Lumpur: Institute for Medical Research 1997; p. 310.

[51] USDA. National Nutrient Database for Standard Reference 2016; Release 28. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. Version Current: September 2015: http://www.ars.usda.gov.

[52] Sundram K, Sambanthamurthi R, Tan YA. Palm Fruit Chemistry and Nutrition. Asia Pacific J Clin Nutr 2003; 12: 355-362.

[53] Ceylan A. Tarla Tarimi. Ege Üniversitesi Ziraat Fakültesi Yayinlari 1994; 491: 107s.

[54] Lavres Junior J, Deus Gomesdos Santos Junior J, Monteiro FA. Nitrate reductase activity and spad readings in leaf tissues of guinea grass submitted to nitrogen and potassium rates. Rev Bras Cienc Solo 2010; 34: 801-09. https://doi.org/10.1590/S0100-06832010000300022

[55] Christensen LP, Peacock WL. Mineral nutrition and fertilization. In Raisin production manual, 102-14. Oakland, CA: University of California, Agricultural and Natural Resources Publication 2000; p. 3393.

[56] Inugraha MDM, Widaryanto E. Response of Stevia (*Stevia rebaudiana* Bertoni M) to nitrogen and potassium Fertilization. IOSR J Agric Vet Sci 2014; 7: 47-55. https://doi.org/10.9790/2380-071014755

[57] Shadchina TM, Dmitrieva VV. Leaf chlorophyll content as a possible diagnostic mean for the evaluation of plant nitrogen uptake from the soil. J Plant Nutr 1995; 18: 1427-37. https://doi.org/10.1080/01904169509364992

[58] Horváth E, Szalai G, Janda T. Induction of abiotic stress tolerance by salicylic acid signaling. J Plant Growth Regul, 2007; 26: 290-300. https://doi.org/10.1007/s00344-007-9017-4

[59] Uzunova AN, Popova LP. Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. Photosynthetica 2000; 38: 243-250. https://doi.org/10.1023/A:1007226116925

[60] Mateo A, Muhlenbock P, Rusterucci C, Chang CC, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S. Lesion Simulating Disease 1 is required for acclimation to conditions that promote excess excitation energy. Plant Physiol 2004; 136: 2818-2830. https://doi.org/10.1104/pp.104.043646

[61] Melotto M, Underwood W, Koczan J, Nomura K, He SY. Plant stomata function in innate immunity against bacterial invasion. Cell 2006; 126: 969-980. https://doi.org/10.1016/j.cell.2006.06.054

[62] Chandra A, Bhatt RK. Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. Photosynthetica 1998; 35: 255-258. https://doi.org/10.1023/A:1006966908357

[63] Fariduddin Q, Hayat S, Ahmad A. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in Brassica juncea. Photosynthetica 2003; 41: 281-284. https://doi.org/10.1023/B:PHOT.0000011962.05991.6c

[64] Slaymaker DH, Navarre DA, Clark D, Del-Pozo O, Martin GB, Klessig DF. Physiological Roles in Plants. Proceedings of the National Academy of Sciences of the United States of America, 2002; 99: 11640-11645. https://doi.org/10.1073/pnas.182427699

[65] Hayat Q, Hayat S, Alyemini MN, Ahmad A. Salicylic acid mediated changes in growth, photosynthesis, nitrogen metabolism and antioxidant defense system in Cicerarietinum L. Plant Soil Environ 2012; 58: 417-423. https://doi.org/10.17221/232/2012-PSE

[66] Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q, Ahmad A. Effect of salicylic acid on salinity induced changes in Brassica juncea. J Integr Plant Biol 2008; 50: 1-4. https://doi.org/10.1111/j.1744-7909.2008.00697.x

[67] Yusuf M, Fariduddin Q, Varshney P, Ahmad A. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (Brassica juncea) through an improved antioxidant system. Environ Sci Pollut Res 2012; 19: 8-18. https://doi.org/10.1007/s11356-011-0531-3

[68] Pancheva TV, Popova LP, Uzunova AM. Effect of salicylic acid on growth and photosynthesis in barley plants. J Plant Physiol 1996; 149: 57-63. https://doi.org/10.1016/S0176-1617(96)80173-8

[69] Rao MV, Paliyath G, Ormrod DP, Murr DP, Watkins CB. Influence of salicylic acid on H2O2 production, oxidative stress, and H_2O_2-metabolizing enzymes. Plant Physiol 1997; 115: 137-149. https://doi.org/10.1104/pp.115.1.137

[70] Moharekar ST, Lokhande SD, Hara T, Tanaka R, Tanaka A, Chavan PD. Effects of salicylic acid on chlorophyll and carotenoid contents on wheat and moong seedlings. Photosynthetica 2003; 41: 315-317. https://doi.org/10.1023/B:PHOT.0000011970.62172.15

[71] Pancheva TV, Popova LP. Effect of the salicylic acid on the synthesis of ribulose- 1,5-bisphosphate carboxylase/ oxygenase in barley leaves. Plant Physiol 1998; 152: 381-386. https://doi.org/10.1016/S0176-1617(98)80251-4

[72] Rivas-San V, Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. J Exp Bot 2011; 62: 3321-3338. https://doi.org/10.1093/jxb/err031

[73] Hayat S, Fariduddin Q, Ali B, Ahmad A. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta Agron Hung 2005; 53: 433-437. https://doi.org/10.1556/AAgr.53.2005.4.9

[74] Ghai, N., Setia, R. C., & Setia, N. Effects of paclobutrazol and salicylic acid on chlorophyll content, hill activity and yield components in Brassica napus L. (cv. GSL-1). Phytomorphol 2002; 52: 83-87.

Received on 02-12-2019 Accepted on 19-01-2020 Published on 04-02-2020

DOI: https://doi.org/10.29169/1927-5951.2020.10.01.5