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Morpho-Physiological Traits, Phytochemical Composition, and Antioxidant Activity of Canephora Coffee Leaves at Various Stages

Yudithia Maxiselly [1,2], Pisamai Anusornwanit [1], Adirek Rugkong [1], Rawee Chiarawipa [1,*] and Pin Chanjula [3]

[1] Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand; yudithia.maxiselly@unpad.ac.id (Y.M.); 6010620028@psu.ac.th (P.A.); adirek.r@psu.ac.th (A.R.)
[2] Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Bandung 45363, Indonesia
[3] Animal Production Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand; pin.c@psu.ac.th
* Correspondence: rawee.c@psu.ac.th

Abstract: Coffee leaves contain a wide range of leaf compounds, which vary by growth stage. Recently, the importance of coffee leaf metabolites with beneficial phytochemicals has been widely identified. This research investigated Canephora coffee's morphological and physiological development and analyzed the phytochemical composition of the main leaf stage. Canephora coffee leaves were harvested and classified into the following five growth stages: S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14 days), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days). The antioxidant activity, total phenol content, flavonoids, and tannin content of coffee leaves at different stages were observed. The results indicated that the highest values for the leaf area, dry weight, greenness, chlorophyll content, and carotenoid content were found at the last stage (S5). The specific leaf area (SLA) differences had higher values in the S3 and S1 growth stages. The youngest leaf phase (S1) was less green, more yellow, and brighter in color than the mature phase. By comparing the assays, it was found that a significant increase in the antioxidant activity and the contents of phenolic compounds, flavonoids, and tannins were observed in the S1 and S2 growth stages.

Keywords: leaf growth phase; coffee by-products; coffee leaf tea; functional beverage; *Coffea canephora*

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1. Introduction

Coffee is a crucial plant-based beverage commodity and is ranked as the second most traded product in the world after fuel. One of the most famous coffee species is *Coffea canephora*, which is typically called Robusta or Canephora. Canephora contains a high amount of caffeine along with other phytochemical components, such as tannins, saponins, and chlorogenic acid, which have antioxidant effects [1,2]. Canephora can also adapt well to marginal environments with low altitudes and high temperatures compared with the other famous coffee species, Arabica [3]. Thus, Canephora coffee has the potential to be cultivated in a wide range of areas.

One factor that is important for growing plants is leaf development. According to Campa et al. [4], coffee leaves' light-absorbing capacity significantly influences its photosynthetic activity, consequently improving the quantity and quality of the coffee product. The stages of leaf development also result in various compositions of chlorophyll and phenolic contents because a leaf's surface area influences the nitrogen concentration (a component of chlorophyll) and photosynthetic capacity [5]. Young coffee leaves possess less chlorophyll compared with mature coffee leaves because chlorophyll is found mostly in the parenchyma cells of mature leaves [4]. The amount of chlorophyll and other phytochemicals

differs depending on the stage of the coffee leaf's development [1]. Coffee leaves are also known to store many substances, including phytochemical compounds, such as phenolic acids (e.g., chlorogenic acid, carotenoids, catechins, and anthocyanins), which have benefits for human health [6,7]. Some coffee leaves exhibit a much higher composition of substances than other plant parts. For instance, Patay et al. [8] reported that coffee leaves contain higher levels of chlorogenic acid, which functions as UV protection for the skin, compared with coffee beans. Local Ethiopian coffee leaves also contained a much greater mineral content (Ca, Mg, and Fe) than coffee beans [9]. According to Ratanamarno and Surbkar [10] and Chen [9], coffee leaves are the only part of coffee that contains mangiferin, an antioxidant that functions as an anti-inflammatory agent.

Because of these health benefits, some countries have been consuming coffee leaf tea as an ethnomedicine for generations. Novita et al. [11] reported that Ethiopia, Sudan, Indonesia (West Sumatra and Java), and Jamaica use coffee leaves as herbal tea. In addition, coffee leaves are used for curing anemia and edema in Haiti, as well as intestinal pain and diarrhea in Africa. In some Central and South American countries (Cuba, Nicaragua, Peru, and Mexico), coffee leaves are applied for the amelioration of some diseases (flu, fever, and cough) [12]. In other areas of the world, pruned leaves of the coffee tree are often only processed as waste after pruning or used as organic fertilizer, such as mulch or compost [2].

Developing coffee leaf products can be a good opportunity for growers because the coffee leaves are available for harvest throughout the tree's lifespan. Thus, the valorization of coffee leaves could be expanded to other products because of their beneficial effects on human health [13,14]. This research aimed to investigate Canephora coffee's morphological and physiological development and evaluate the phytochemical composition and antioxidant activity of each leaf stage.

2. Materials and Methods

2.1. Location and Material

The experiment was performed in the experimental site from September 2017 to December 2018 in Songkhla province, Thailand (latitude 7°00′17.3 N, longitude 100°30′15.1 E, and altitude 32 m above sea level).

One variety of Thai Canephora coffee (*Coffea canephora* var. robusta), which was approved and named Chumphon 2 (FRT 65, no. 005/2009) by the Department of Agriculture, Ministry of Agriculture and Cooperatives (Thailand), was investigated.

For this investigation, 20 trees of a clonal genotype of 3-year-old Canephora coffee, which varied in leaf developmental stages, were used. Canephora coffee leaves were harvested and classified into the following five growth stages: S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days).

2.2. Chemical Reagents

The reagents 95% ethanol (Merck, Darmstadt, Germany), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), *N*,*N*-dimethylformamide (DMF) (Sigma Aldrich, Darmstadt, Germany), sodium acetate, Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeSO_4$), Folin–Ciocalteu reagent, 7.5% sodium carbonate ($NaCO_3$), sodium nitrite solvent ($NaNO_2$), 10% aluminum chloride solution ($AlCl_3$), and sodium hydroxide were purchased from Ajax Finechem Pty Limited (Ajax Finechem, Australia) and used in different stages for leaf preservation and preparation according to specific protocols.

2.3. Leaf Morpho-Physiological Traits at Different Stages in Canephora Coffee Leaves

The leaf area (LA) was measured by a leaf area meter (LI-3000C, LI-COR, Lincoln, NE, USA). Ten replicate samples per leaf stage were used to calculate the mean leaf area by which the sample leaves were harvested directly and were dried in the oven at 65 °C for 72 h. Then, the dried leaf mass was measured to determine the leaf dry weight (DW) and specific leaf area (SLA) (cm^2/g).

The analysis of the pigment extraction for chlorophyll content—chlorophyll a, chlorophyll b, and total chlorophyll (Chl_a, Chl_b, Chl_{total})—and carotenoid (Car) were performed using *N*,*N*-dimethylformamide (DMF) according to the modification method of Netto et al. [15]. In brief, the absorbances of chlorophyll a and b and carotenoids were determined at 480 nm, 647 nm, and 664 nm, respectively, using a UV spectrophotometer (Pharmacia Biotech Ultrospec 3000 UV–Visible, Thermo Scientific, Waltham, MA, USA).

For non-destructive assessment of plant status, e.g., chlorophyll and carotenoid contents, two hundred leaves at all stages were selected and measured by using the Chlorophyll Meter (SPAD-502 Plus, Minolta, Tokyo, Japan) and Chroma Meter (CR-400, Konica Minolta, Tokyo, Japan). Each leaf sample was randomized to be measured around the leaf blade at 5 positions, and the average value for the SPAD unit was determined for each leaf. Then, the linear correlations of chlorophyll and carotenoid contents with their corresponding Chlorophyll Meter readings (SPAD unit) were measured as calibration equations and then calculated with the following formulas:

$Chl_a = 0.0056x^2 + 0.3014x + 0.6767$ ($r^2 = 0.95$),
$Chl_b = 0.0014x^2 + 0.2687x - 1.1945$ ($r^2 = 0.94$),
$Chl_{total} = 0.0080x^2 + 0.5104x + 0.281$ ($r^2 = 0.95$),
and $Car = 0.0007x^2 - 0.0094x + 0.5439$ ($r^2 = 0.74$), respectively.

2.4. Analysis of Phytochemical Compositions and Antioxidant Activity in Canephora Coffee Leaves

2.4.1. Preparing the Samples

The coffee leaf samples at each stage were pooled into S1–S2, S3, and S4–S5 stages, and they were dried and then milled and extracted with 95% ethanol at a ratio of 1:10 (samples and ethanol). For instance, 10 g of samples/replications was mixed with 100 mL of 95% ethanol. The extracts were stored in a dark place before the analyzing step (less than seven days). The samples were filtered and centrifuged (Cryste, Bucheon-si, Korea) for around 15 min at 4000 rpm to separate the sediment from the liquid. Then, a rotary evaporator (Buchi, Flawil, Switzerland) and water bath (Alpha A12, Lauda, Germany) continued to evaporate and reduce the ethanol from the samples. Finally, the samples were kept in a balm bottle, around 0.025 g mixed with ethanol was taken to be one stock (3 replications were prepared for the analysis).

2.4.2. Antioxidant Activity (AA)

This parameter was analyzed by the Ferric Ion Reducing Antioxidant Power (FRAP) method based on Panda's method [16]. FRAP reagent contained ten parts 300 mM sodium acetate by adding 1 part 10 mM TPTZ solution and 1 part 20 mM $FeCl_3 \cdot 6H_2O$ solution. The reaction solution was incubated for 30 min at 37 °C. The FRAP reagent was mixed with the sample and ferrous sulfate ($FeSO_4$) solvent at many concentrations, and the solutions were used as stocks. Each 0.1 mL sample was combined with FRAP 4.5 mL, followed by shaking for 10 min at room temperature in dark conditions. After that, the mixed sample was measured by spectrophotometer (UV–VIS spectrophotometer, Ultraspec 3000 UV/Visible, Pharmaica Biotech Inc., Piscataway, NJ, USA) at a 593 nm wavelength.

2.4.3. Total Phenolic Content (TPC) and Total Tannin Content (TTC)

TPC and TTC were identified by the Folin–Ciocalteu method according to McDonald et al. [17] with minor modifications. TPC was calculated by assorted extracts (0.1 mL of 1:20, v/v) or phenolic standard combined with Folin–Ciocalteu reagent (2 mL, 1:5 diluted with distilled water) and 1.5 mL of 7.5% $NaCO_3$ shaken and kept in the dark for 2 h. TTC was estimated by the same method with a different volume of solvent. A solution extract (0.1 mL of 1:20, *v*/*v*) or tannin standard was combined with Folin–Ciocalteu reagent (1.6 mL, 1:5 diluted with distilled water) and 2 mL of 7.0% $NaCO_3$ shaken and kept in the dark for 1.5 h. Both parameters used the standard curve arranged using 25 mg gallic acid for 50 mL or 500 mg/L solvents. The absorbance of the solution was calculated by a spectrophotometer at 751 nm for TPC and 760 nm for TTC.

2.4.4. Total Flavonoid Content (TFC)

This parameter was identified using the aluminum chloride colorimetric assay based on the method of Sultana et al. [18]. A sample of around 0.4 mL was fused with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solvent ($NaNO_2$), and 0.3 mL of 10% aluminum chloride solution ($AlCl_3$) was further added after five minutes of incubation. The mixture was permitted to stand for 6 min. Then, 2 mL of 1 mol/L sodium hydroxide solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and the absorbance was estimated at 510 nm by a spectrophotometer. The TFC was predicted from a standard curve, and the results are reported as mg routine equivalent per g dry weight.

2.5. Statistical Analysis

All data are described as mean values (averages) in addition to their standard deviations (SD). ANOVA analysis was used to identify the variance in leaf morphological and phytochemical parameters in different coffee leaf stages. The *p*-values of ≤ 0.05 were regarded as significant data and followed by Duncan's New Multiple Range Test (DMRT). The results of the data analyses are presented in the tables.

3. Results

3.1. Leaf Morpho-Physiological Traits at Different Stages of Canephora Coffee Leaves

Canephora coffee leaves at various stages showed different morpho-physiological traits, such as LA, DW, and SLA (Table 1). The youngest leaves had the lowest values for all traits of the other leaf phases—the traits were significantly increased for each treatment except SLA. For SLA, the fluctuation contents for the leaves in the S3 growth stage and the medium phase were the greatest; SLA should be obtained by the ratio between LA and DW of that treatment. The highest score of all the morphology characters was observed in the mature leaves (S5). The results illustrated that S5 had the largest size of leaf formation. Morphological and physiological characteristics were the appearances that indicated plant development.

Table 1. Leaf area, leaf dry weight, and specific leaf area at different stages of Canephora coffee leaves.

Developmental Stages	LA (cm^2)	DW (g)	SLA (cm^2/g)
S1	3.57 ± 0.80 e	0.03 ± 0.01 d	115.98 ± 24.08 b
S2	12.56 ± 1.67 d	0.10 ± 0.01 c	131.83 ± 18.23 b
S3	19.32 ± 1.69 c	0.12 ± 0.01 c	161.91 ± 16.55 a
S4	36.16 ± 2.33 b	0.26 ± 0.03 b	140.52 ± 27.07 ab
S5	58.36 ± 2.26 a	0.46 ± 0.03 a	127.90 ± 11.36 b

LA (leaf area), DW (leaf dry weight), SLA (specific leaf area), S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14 days), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

Table 2 describes leaf color changes in coffee plants by SPAD values that diagnosed the greenness or relative chlorophyll content of the leaves. This table also shows the a*, b*, and L values, which indicate the intensity, tone, and value of the leaf color. The range of red to green color is illustrated by the a value; the b value indicates the color in the yellow to blue range; the brightness and darkness (white–black) are represented by the L value [19]. The youngest coffee leaves (S1) were less green, more yellow, and brighter in color than the mature leaves (S5) according to the a*, b*, and L values. Then, SPAD values were converted by the regression formula to identify the chlorophyll contents (Table 3). Table 3 shows all the chlorophyll values for each stage, namely Chl_a, Chl_b, Chl_{total}, and Car. The chlorophyll content for each phase showed a gradual linear increase. Chlorophyll a was the predominant pigment in coffee leaves and ranged from 6.31 to 22.51 mg/cm^2, while 3.05 to 12.20 mg/cm^2 was the chlorophyll b range for the lowest to the highest stages. The lowest pigment value was observed for Car, ranging from 0.5 to 1.34 mg/cm^2. The content of Chl_a

was around two times higher than that of Chl_b, indicating that the dominant color of the coffee leaves was red to dark green. The most mature leaf (S5) had the highest score, while the juvenile (S1) had the smallest one for all chlorophyll types. For instance, the values of Chl_a, Chl_b, Chl_{total}, and Car for S5 were 356%, 400%, 365%, and 239% higher than those of S1, respectively.

Table 2. Changes in leaf color ranges (leaf greenness, a*, b*, and L*) in Canephora coffee leaves at different stages.

Developmental Stages	Leaf Color			
	(SPAD Unit)	(a*)	(b*)	(L*)
S1	10.00–18.00	(−5.99)–\0.00	34.40–45.00	49.50–60.00
S2	18.01–23.99	(−10.00)–\(−6.00)	30.30–34.39	46.00–49.49
S3	24.00–27.99	(−12.59)–\(−10.01)	27.40–30.29	42.00–45.99
S4	28.00–37.99	(−18.00)–\(−12.60)	20.51–27.39	37.50–41.99
S5	38.00–45.00	(−25.00)–\(−18.01)	15.00–20.50	32.00–37.49

S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14 days), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days).

Table 3. Changes in chlorophyll and carotenoid contents in Canephora coffee leaves at different stages.

Developmental Stages	Chl_a (mg/cm^2)	Chl_b (mg/cm^2)	Chl_{total} (mg/cm^2)	Car (mg/cm^2)
S1	6.31 ± 0.73 e	3.05 ± 0.48 e	9.50 ± 1.16 e	0.56 ± 0.02 e
S2	9.90 ± 0.90 d	5.32 ± 0.55 d	15.19 ± 1.42 d	0.67 ± 0.03 d
S3	12.17 ± 0.66 c	6.66 ± 0.38 c	18.75 ± 1.03 c	0.77 ± 0.03 c
S4	16.41 ± 1.71 b	9.02 ± 0.92 b	25.36 ± 2.65 b	0.98 ± 0.09 b
S5	22.51 ± 1.87 a	12.20 ± 0.94 a	34.75 ± 2.87 a	1.34 ± 0.12 a

Means ± SD, S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14 days), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days). Means (±SD) in the same column followed by a common letter are not significantly different at the 99% level by DMRT ($p \leq 0.01$).

3.2. Phytochemical Contents and Antioxidant Activity at Different Stages of Canephora Coffee Leaves

The research identified TTC, TFC, TPC, and AA at different coffee leaf stages as phytochemical parameters. All coffee leaf stages were found to contain these phytochemicals. Additionally, all the contents showed varied values in different coffee leaf stages (Table 4). In contrast to the morphological composition of the coffee leaves, the phytochemical composition and antioxidant activity showed that the youngest leaves had the most significant value for the measurement. The parameters decreased gradually for each variable. The lowest score for all phytochemical contents and antioxidant activity were mature leaves at the S4–S5 stages. The S1–S2 stages had values almost two times higher than those in the S4–S5 stages for AA and TTC. Meanwhile, the composition of other phytochemicals, such as flavonoids, showed a slight steady increase in the values for each stage (around 3 mg increases in the later stages), and TPC was more than that, although the value was less than double.

Table 4. Phytochemical compositions and antioxidant activity at different stages of Canephora coffee leaves.

Developmental Stages	Antioxidant Activity Content (mg Fe (II) Equivalent/g DW Extract)	Phenolic Compound Content (mg Gallic Acid Equivalent/g DW Extract)	Flavonoid Content (mg Catechin Equivalent/g DW Extract)	Tannin Content (mg Tannic Acid Equivalent/g DW Extract)
S1–S2	38.73 ± 0.36 a	29.01 ± 0.28 a	20.09 ± 0.88 a	27.43 ± 1.19 a
S3	35.04 ± 0.84 b	25.68 ± 0.45 b	17.26 ± 0.49 b	25.01 ± 1.03 a
S4–S5	22.14 ± 1.48 c	18.76 ± 1.15 c	14.46 ± 1.17 c	15.36 ± 0.88 b

DW (dry Weight), S1 (leaf age of 1–4 days), S2 (leaf age of 5–8 days), S3 (leaf age of 9–14 days), S4 (leaf age of 15–20 days), and S5 (leaf age of 21–27 days). Means (±SD) in the same column followed by a common letter are not significantly different at the 95% level by DMRT ($p \leq 0.05$).

4. Discussion

4.1. Leaf Morpho-Physiological Traits at Different Stages of Canephora Coffee Leaves

LA, DW, and SLA are essential to illustrate leaf growth. LA is involved in the photosynthetic and transpiration processes, whose causes are related to the number of stomata [20]. This research reported that the development of coffee leaves gradually inclined step by step in line with growth stages. The results showed that the youngest leaves had the smallest size for all the measurements. These data are supported by Bhakta and Ganjewala [21], who observed that leaf development grew rapidly from the young leaves to the mature stage as the maximum growth. Campa et al. [4] explained that leaf morphology controlled light absorption on coffee leaves, such as leaf area.

Chlorophyll is an integral part of the leaf's ability to synthesize CO_2 and H_2O as products and oxygen with the help of sunlight in photosynthetic metabolism. Pigment groups, which absorb and reflect light in the photosynthetic process, contain various pigment types depending on the light color, such as a, b, and carotenoids [20]. This result showed that the chlorophyll contents (Chl_a, Chl_b, and Chl_{total}) and carotenoid content (Car) had variations in the treatments. These results were supported by Pan et al. [22], who explained a negative a* as green and a positive a* as red, a negative b* (from −120) as blue, and a higher value (up to +120) as yellow. Meanwhile, the darkest to the brightest colors were represented by an L value of 0–100.

Chl_a and Chl_b are the standard types of angiosperm plants, and the variation composition depends on the environment and growth conditions. The chlorophyll concentration is also related to the plant's photosynthetic capacity [23]. According to Acidri et al. [24], the total chlorophyll concentrations in coffee leaves are an accumulation of Chl_a and Chl_b and vary depending on the leaf stage. Continued from that research, the chlorophyll concentration gradually increased following the stages of leaf maturation and showed that the Chl_a value was higher than the Chl_b value for each stage. Chl_a and Chl_b are unsteady at high temperatures and light and are degraded easily [15]. On the other hand, Car is one type of chlorophyll involved as photo-protection in the photosynthetic process. This pigment also functions as an antioxidant, a plant hormone precursor, and a controller of plant growth related to abscisic acid and is involved in elongation and cell division [25]. It is also an additional plant pigment for light harvesting and is essential in environments with high light stress, salinity, and nutrient stress [26]. It describes the mature phase of Canephora coffee leaves as having better photoprotection than juvenile leaves. Young leaves are more susceptible to damage in high-light conditions due to the limitation of chlorophyll contents, and they should contain another component that also has a protection function for survival.

4.2. Phytochemical Contents and Antioxidant Activity at Different Stages of Canephora Coffee Leaves

Many parts of the coffee plant contain phytochemical contents, including the leaves. The composition of phytochemicals is influenced by the species and plant anatomy. Patay et al. [27] observed differences between coffee seeds and leaves from two coffee species,

showing that the seeds and leaves of Arabica coffee contained higher levels of polyphenols, such as caffeine, chlorogenic, ferulic, p-coumaric, and sinapic acid, than Bengal coffee using HPLC and UV detection. They also reported coffee leaves had a higher variation of polyphenols than coffee seeds. This was also reported by Acidri et al. [24], who analyzed the phytochemical profiles and antioxidant capacity of coffee plant organs and showed that the coffee leaves had higher total phenolic acid levels compared with green and roasted coffee beans. Their research used HPLC to identify phytochemical variation, analyzed the TPC and antioxidant capacity (DPPH, ABTS, and FRAP) values using chemical reagents, and performed calculations using a UV spectrophotometer. Coffee leaves observed by the HPLC method also had a greater composition of *cis* isomers relative to *trans* isomers of chlorogenic acid compared with coffee beans as UV damage protection [28]. According to Chen et al. [12], different stages of coffee leaves showed variations of compositions for phytochemicals. It was reported that the young coffee leaves had higher values of some phytochemicals, such as alkaloids, flavonoids, and phenolics, than leaves in mature phases. Additionally, Ratanamarno and Subhkar [10] revealed in the research on Arabica coffee leaves that younger leaves contained more caffeine and antioxidants than mature leaves. This was supported by Campa et al. [4], who observed juvenile, growing, and mature coffee leaves and showed that the juvenile leaves contained phenolics, namely mangiferin, caffeine, and flavonoids, at higher levels than leaves in other stages. Mangiferin is only available in the leaf organs in the coffee plant, and the youngest leaves had the highest mangiferin values [24]. Another study on leaf development also described that the phenolic compounds in the top leaves (juvenile stage) were better than the basal ones (mature) in the artichoke plant [29]. Sielicka-Różyńska et al. [30] stated that white leaf tea (made from newly budded leaves) contained higher TPC and AA than green leaf tea (made from young and mature leaves). These results support the present results, which showed various values for all the phytochemical contents, with the youngest leaves exhibiting the greatest values. Chen et al. [6] explained that the polyphenol activity in coffee leaves decreased during the leaf growth process. The youngest leaves contained a few chlorophylls that functioned as photo-protection and filtered the sunlight.

On the other hand, the high composition of phytochemicals would replace the function of protection. Flavonoids are one of the groups of phytochemicals that protect coffee leaves from UV light damage [4]. According to Close and McArthur [31], phenolics and antioxidants are needed for plants in marginal environments, namely high light intensity or full-sunlight conditions, because of their function as photo-damage protection. Some plants, such as Mahonia repens and Eucalyptus, contain phenolics and tannins at levels more than 2–3 times higher when cultivated with high light intensity.

5. Conclusions

The development stages of the Canephora coffee leaves affected the morphological and physiological characteristics. The S5 growth stage of the Canephora coffee leaves had the highest LA, DW, chlorophyll, and carotenoid contents. The youngest stage (S1) was less green, more yellow, and brighter in color than the mature leaf stage. However, the S3 stage of the Canephora coffee leaves had the highest SLA. Meanwhile, the S1 to S2 leaf development stages (juvenile) had a significant factor affecting the phytochemical contents (phenolics, tannins, and flavonoids) and antioxidant activity of Canephora coffee leaves by UV spectrophotometer calculation. On the basis of their compositions, Canephora coffee leaves could be considered to have health benefits when drinking herbal infusions that contain a high concentration of phytochemicals, especially in juvenile stages.

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