PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(2): 762-770
https://doi.org/10.14719/pst.2991

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Morphological and physiological properties of kratom (*Mitragyna speciosa*) leaves: Macronutrients, phytochemicals, antioxidants, and mitragynine content

Wethanee Phromchan[1], Ifwarisan Defri[1], Chutikarn Saensano[1], Anuthida Chookaew[1], Rawee Chiarawipa[1*] & Somchai Sriwiriyajan[2]

[1]Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110,Thailand
[2]Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla, 90110, Thailand

*Email: rawee.c@psu.ac.th

OPEN ACCESS

ARTICLE HISTORY

Received: 03 October 2023
Accepted: 29 March 2024

Available online
Version 1.0 : 22 May 2024
Version 2.0 : 25 May 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Phromchan W, Defri I, Saensano C, Chookaew A, Chiarawipa R, Sriwiriyajan S. Morphological and physiological properties of kratom (*Mitragyna speciosa*) leaves: Macronutrients, phytochemicals, antioxidants, and mitragynine content. Plant Science Today. 2024; 11(2): 762-770. https:/doi.org/10.14719/pst.2991

Abstract

Morpho-physiological characteristics of leaves are significantly associated with photosynthetic capacity and leaf growth. This study was designed to evaluate the relationship between leaf functional traits, nutrients, and their active compounds throughout the developmental stages of kratom leaves. Five growth stages were identified: S1 (7-15 days), S2 (15-30 days), S3 (30-45 days), S4 (45-60 days), and S5 (60-75 days). A comparison of leaf-group stages was conducted based on morpho-physiological traits, macronutrient content, phytochemical analysis, and antioxidant activity. The results revealed that leaf weight and leaf area increased from S1 to S5, with a slight decrease observed in S5. Stomatal density remained similar across all stages. In contrast, chlorophyll and carotenoid contents showed a steady increase up to the S5 stage. The maximum assimilation rate (A_{max}) and light-saturated photosynthetic rate (P_{max}) were achieved at the S2 and S3 stages. Macronutrient levels (N, P, and K) were highest in the younger leaf-group stages (S1 to S2) and significantly different from the older leaf-group stages (S4 to S5). The highest amount of phenolics, flavonoids, and antioxidant activity were found in the middle leaf-group stage (S3). However, anthocyanin content tended to decrease with leaf-group stages. Moreover, the mitragynine content continuously decreased with leaf age, with the highest content found in the young (S1 to S2) and middle (S3) group stages of leaves. Therefore, the productive phytochemical contents in the fully expanded leaves should be considered, especially mitragynine content, which is mainly used in medicinal products.

Keywords

leaf development; leaf nutrients; leaf traits; medicinal plant; photosynthetic capacity

Introduction

Kratom (*Mitragyna speciosa* (Korth.) Havil.), an indigenous tropical plant of Southeast Asia countries, is commonly used as traditional medicine (1, 2). Some people believe that kratom leaves can enhance their workability and productivity (1). It is suggested that the leaves have high potential as a valuable source of analgesic medications due to their pain-relieving properties (3). Because of these medicinal properties, the demand for kratom leaves in herbal medicine is rapidly increasing (4).

There are around 57 active compounds found in kratom leaves, of which 40 are alkaloids. The dominant alkaloids are mitragynine, which makes up 66% of the total, and 7-hydroxymitragynine, accounting for 2%

(5). Mitragynine is the primary active alkaloid, contributing to the significant medicinal benefits of the kratom leaf, such as stimulating workability and analgesic and relaxing effects (6). Various studies have reported that the alkaloid profile of kratom leaves varies significantly by different geographical locations (5, 7). Additionally, alkaloid levels in the kratom leaves are influenced by the agroclimatic environment and the leaf growth stages (8).

Through the alkaloid profiles of the kratom leaves, the explicit composition of phytochemicals is determined, including flavonoids, polyphenols, and antioxidant (9, 10). The leaves of medicinal plants are essential for their growth, function, and survival. The light-absorbing capacity of leaves influences morphological, physiological, and phytochemical traits (11, 12). Studies in Robusta coffee (13), grapevine (14), and some woody plants (15) have shown that the development of leaves depends on their age or growth stage. These factors play a crucial role in the morphological construction and physiological functions of kratom plants and serve as fundamental indicators for precise cultivation, ensuring the quality and production of kratom leaves.

This study aimed to identify the developmental stages of kratom leaves and their relationships with leaf morpho-physiological characteristics and phytochemicals. The goal is to obtain fresh raw materials of kratom leaves containing various important substances for further processing and medical use, particularly mitragynine, which is commonly used in medicinal products.

Materials and Methods

Experimental site and material

The experiment was conducted from January to December 2022 at a farm in Sichon district, Nakhon Si Thammarat province, Thailand (latitude 9°2'7.23"N, longitude 99°50'59.86" E, and altitude 10 m above sea level). Ten kratom trees, aged ten years old and exhibiting various leaf development stages, were randomly selected for leaf sample collection. The sample leaves were categorized into five growth stages based on their ages in days: S1 (7-15 days), S2 (15-30 days), S3 (30-45 days), S4 (45-60 days), and S5 (60-75 days) (Fig. 1).

Leaf area, leaf weight, and leaf stomatal density measurements

The individual leaf area (LA) was measured using a leaf area meter (LI-3000C, LI-COR, Lincoln, NE, USA). After harvesting, the sample leaves were weighed to determine their fresh weight (FW), followed by oven drying at 65 °C for 72 hours to measure the leaf dry weight (DW). The fresh and dry weights of leaves at each stage were recorded to establish their relationships. Stomatal quantification was conducted using the nail polish method (16), where nail polish was applied to the adaxial surface of each sample leaf and subsequently peeled off with clear tape. The replicas were then detached, placed on microscope slides, and examined under a compound microscope (Zeiss CP-Achromat 40x Objective) (Carl Zeiss Microscopy, USA). This method provides information about the density and distribution of stomata on the leaf surface.

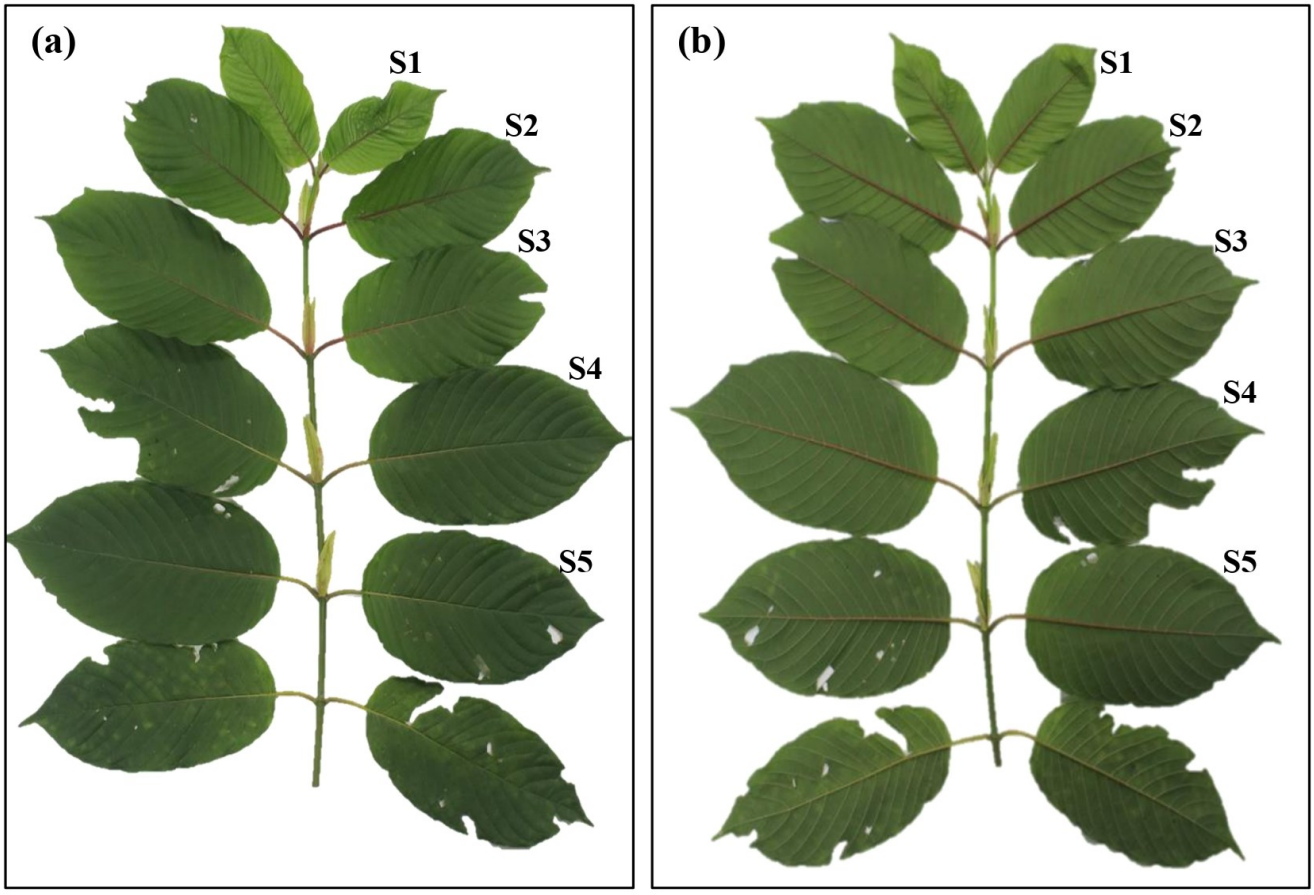

Fig. 1. The sample of growth stages of kratom leaves: S1 (7-15 days), S2 (15-30 days), S3 (30-45 days), S4 (45-60 days), and S5 (60-75 days) of adaxial (a) and abaxial (b) leaf surfaces

Leaf chlorophyll and carotenoids measurements

The photosynthetic pigments, namely, chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (Chl_{total}), as well as carotenoids (Car), were extracted using *N,N*-dimethylformamide (DMF) following a modified method of Netto et al. (17). The linear correlations between the contents of pigments (chlorophyll and carotenoid) and their respective chlorophyll meter readings (Dualex, DX19007, Force A, France) were measured and calculated using the following equations: $Chl_a = 0.5113x - 3.7332(r^2 = 0.894)$, $Chl_b = 0.194x - 1.0394(r^2 = 0.869)$, $Chl_{total} = 0.7053x - 4.7726(r^2 = 0.890)$, and $Car = 0.094x - 0.3657(r^2 = 0.941)$.

Leaf photosynthetic measurements

Leaf photosynthesis at various stages of leaf development was analyzed in field trial conditions using a portable photosynthesis system (LI-6800, Li-Cor Inc., Lincoln NE, USA). The quantitative responses associated with leaf photosynthesis were obtained for the light-saturated photosynthetic rate (P_{max}), dark respiration (R_d), light compensation point (I_c), and light saturation point (I_s). Photosynthetic curves were determined under an altered ambient LED light source at intervals of 0 to 2,000 mmol/m^2/s. The ambient CO_2 concentration and leaf chamber temperature were constantly controlled at 400 mmolCO_2/mol and 28°C, respectively. Additionally, CO_2 concentration was regulated from 0 to 2,000 mmolCO_2/mol initially. Moreover, CO_2 compensation (τ) and mesophyll conductance (g_m) were also determined using a curve fitting method (14).

Leaf macronutrients measurements

The leaf samples were collected from each treatment and dried at 65 ºC for 72 hours. The samples were then ground and sieved through 20- and 40-mesh sieves. Finely ground tissues were digested with H_2SO_4 to determine the total leaf nitrogen content (N) using the Kjeldahl method. Additionally, they were mixed with HNO_3 and $HClO_4$ (HNO_3:$HClO_4$ = 3:1 v/v) for P, K, Ca, and Mg analyses using spectrometry (18).

Analysis of phytochemical compositions and antioxidant activity in kratom leaves

Leaf samples preparation

The leaf samples from each group of stages (S1 to S2, S3, and S4 to S5) were first dried and powdered. The sample powders were then soaked in 95% ethanol solvent at a sample-to-solvent ratio of 1:10. Afterward, the solution was sieved, and the extracts and solvent were separated using a centrifuge (Cryste, Bucheon-si, Korea) at 4,000 rpm for approximately 15 minutes. The sediments were evaporated using a rotary evaporator (Buchi, Flawil, Switzerland) in a water bath (Alpha A12, Lauda, Germany) to remove the remaining ethanol. Then, the extracted samples were placed into bottles respectively. A 0.025 g sample was taken from each bottle and mixed with ethanol in preparation for analysis.

Antioxidant activity

To determine the antioxidant capacity of the sample, the Ferric Ion Reducing Antioxidant Power (FRAP) assay was used for measurement. It was prepared by mixing 300 mM sodium acetate, 10 mM TPTZ solution, and 20 mM $FeCl_3.6H_2O$ solution at a 10:1:1 ratio (19). The sample and FRAP reagent were mixed in a ratio of 0.1:4.5 ml, and the mixture was shaken for 10 min in the dark at room temperature. After 30 minutes of incubation at 37 °C, the solution's absorbance was measured at 593 nm (13).

Total phenolic content (TPC) and total tannin content (TTC)

These parameters were analyzed using the modified Folin-Ciocalteu method (13, 20). For TPC determination, the phenolic standard was mixed with 2 mL of Folin-Ciocalteu reagent and 1.5 mL of 7.5% $NaCO_3$.The solution was then shaken and kept in the dark for 2 hours. The same process was carried out for TTC, but with different solvent volumes. The tannin standard was mixed with 1.6 mL of Folin-Ciocalteu reagent and 2 mL of 7.0% $NaCO_3$, then agitated and placed in the dark for 1.5 hours. A standard curve was constructed using 25 mg of gallic acid. The absorbance of the solution was measured at 751 nm and 760 nm for TPC and TTC, respectively, using a spectrophotometer (13).

Total flavonoid content (TFC)

To analyze TFC, an aluminum chloride colorimetric assay was employed (21). The sample solution was prepared by combining 0.4 mL of the sample with 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$, and 0.3 mL of 10% $AlCl_3$. After incubating for 5 minutes, the solution was left to stand for an additional 6 minutes. Subsequently, 2 mL of 1 mol/L NaOH was added, and distilled water was added to adjust the volume to 10 mL. After allowing the solution to stand for 15 minutes, absorbance was measured using a spectrophotometer at a wavelength of 510 nm (13).

Total anthocyanin content

Anthocyanin content was determined using the spectrophotometric method (22). All samples were macerated with a mixture of 95% ethanol and 1.5 mol HCl/L in an 85:15 ratio and left to rest for 12 hours at 4 °C. The solution was then adjusted to a volume of 100 ml (23). Total anthocyanin content (mg/g) in the leaves was calculated using the following formula: Total anthocyanin = (A530 x dilution factor)/98.2, where, 98.2 is the molar absorption value of cyanidin-3-glucoside for the acid-ethanol solvent. Absorbance readings at 530 nm were taken using a UV-VIS spectrophotometer (UV-1900i, Shimadzu, Japan), then converted to mg/g using the formula (24).

Mitragynine and alkaloid contents

Each leaf-group stage (S1 to S2, S3, and S4 to S5) of leaf powder was extracted with 80% methanol (CH_3OH) at a 1:10 (w/v). The mixture was vigorously shaken and allowed to stand overnight. Subsequently, the solution was centrifuged at 3,000 rpm for 15 minutes to separate the precipitate from the extract. The methanol was evaporated using a rotary evaporator under vacuum until nearly completely evaporated, resulting in a dry crude alkaloid extract. A standard solution of mitragynine in methanol was prepared according to the kratom extraction method (25, 26). High-

Performance liquid chromatography (HPLC) at the scientific Equipment Center, Prince of Songkla University, was used to analyze mitragynine concentrations. This procedure included the use of a pump based automated injection system (Waters 2695,Waters Associates, milford, MA, USA), with photodiode array detection (Waters 996)at 225nm to monitor the column effluent.

Statistical analysis

Data were presented as mean values along with their standard deviations. ANOVA analysis was conducted to assess the variance in leaf morpho-physiological, leaf nutrients and phytochemical parameters across stages of kratom leaf development. Duncan's Multiple Range Test (DMRT) was employed for comparing the data, with significance considered at a p-value ≤ 0.05.

Results and Discussion

Leaf growth performance at different stages of kratom leaves

The leaf growth performance was assessed based on fresh weight, dry leaf weight, and leaf area. Significant relationships were observed between leaf dry weight and fresh weight of kratom leaves across various stages (Fig. 2a).The results indicated a significant influence of leaf fresh weight on its dry weight ($y = 0.2784x + 0.0437$, $r^2=0.939$). Additionally, the leaf area, measured using a leaf area meter based on width and length, exhibited a linear relationship ($y = 0.7103x + 3.3955$, $r^2=0.987$) (Fig.2b). Notably, during the initial growth stages, there was a sharply increase in leaf area (from S1 to S2), followed by steady growth in fully expanded leaves (S3) (Fig. 2c). This suggests that the fresh mass proportionately increased with the increase in leaf dry weight until the leaf area reached its maximum size before senescence due to ongoing expansion and transition acceleration (27, 28). Given the critical role of leaf area in productivity and its peak size before senescence, harvesting leaves during stages S2 to S4 is recommended.

Chlorophyll and carotenoid contents, and stomatal density in different stages of kratom leaves

Differences in chlorophyll (Chl_a, Chl_b, and Chl_{total}) and carotenoid (Car) contents across various stages (S1 to S5) of kratom leaves are depicted in Fig. 3a. Notably, all chlorophyll contents exhibited a consistent pattern, with the lowest levels observed in the youngest leaves (S1), and the highest levels in the oldest at stage S5. This trend mirror finding from a study on Robusta coffee plants (13), which reported a gradual increase in chlorophyll content in older leaves. Similarly, changes in carotenoid contents across different stages (S1 to S5) followed a pattern akin to chlorophyll content. Previous research has highlighted a correlation between carotenoid and chlorophyll levels (29). Although there were no significant difference in stomatal density among the stages, the oldest leaves (S5) exhibited higher stomatal density compared to the preceding stage (Fig. 3b). This increase in stomatal density during leaf senescence is associated with the regulatory mechanism of leaves. Stomatal density adjustment is recognized as an important trait for plant tolerance under environmental stress (16).

Physiological process of kratom leaves at different stages

Differences in leaf growth stages significantly influenced CO_2 concentration for assimilation rates. As depicted in Fig. 4a, the assimilation rate gradually increased with rising CO_2 concentration. The peak CO_2 concentration (800-1,200 μmolCO_2/mol) corresponded to a high assimilation rate (A_{max}) ranging between of 20-45 μ molCO_2/m^2/s across all leaf growth stages (S1 to S5). The highest CO2 concentration was associated with the Amax (14,30).Notably, all growth stages exhibited a similar pattern of assimilation rate, with fully expanded leaves demonstrating the highest value.

In contrast, the highest photosynthesis rate (P_{max}) varied across leaf ages, with the peak light intensity observed around 1,200 μmol/m^2/s (Fig. 4b). Interestingly, the study revealed differences in light compensation points between young and mature kratom leaves. This finding aligns with observations in grapevine leaves, where similar light responses were observed across all stages of leaf development throughout the growing season, indicating an increase in carbon assimilation with increasing Photosynthetic Photon Flux Density (PPFD) (31, 32).

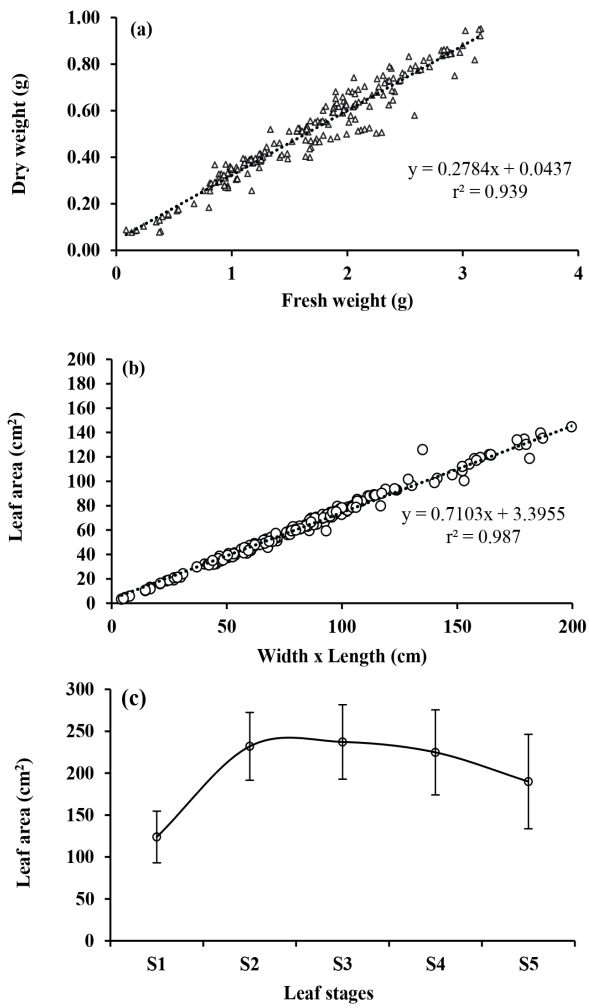

Fig. 2. The relationship between fresh weight and dry weight (a), linear regression relationships between measured leaf area (width x length, cm^2) and leaf area meter (cm^2) (b), and changes in leaf area (cm^2) in difference stages (S1-S5) of kratom leaves (c)

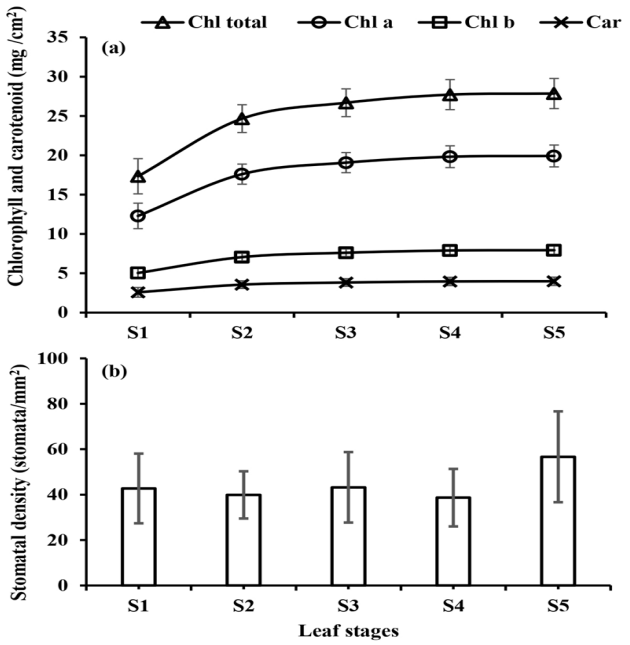

Fig. 3. Changes in the contents (a) of chlorophyll (Chl_{total}), chlorophyll a (Chl_a), chlorophyll b (Chl_b) and carotenoid (Car), and stomatal density (b) in difference stages (S1-S5) of kratom leaves

The pattern of light-saturated photosynthetic rate (P_{max}), dark respiration (R_d), light compensation point (I_c), light saturation point (I_s), CO_2 compensation point (τ), and mesophyll conductance (g_m) response across different stages of kratom leaves (Fig. 5). P_{max} exhibited a similar trend to R_d, I_c, I_s, and g_m throughout leaf development stage, with peak value observed at stages S2 to S3, followed by a gradual decline towards the end of leaf growth (S5). The distinct pattern was observed in τ during the early stage, characterized by a sharp decline from the onset of leaf development at stage S1, reaching its lowest values at stages S2 to S3. Subsequently, a gradual increase was observed until stage S5. These findings suggest that younger kratom leaves exhibit higher metabolic activity compared to older leaves, likely attributable to their greater photosynthetic capacity. This phenomenon is consistent with previous research indicating that photosynthetic capacity increases during leaf development and declines thereafter, potentially influenced by changes in mesophyll thickness and chloroplast distribution across the leaf surface (14, 33).

Macronutrient, phytochemical and mitragynine content of kratom leaves at different stages

The macronutrient content of kratom leaves at different stages is presented in Fig. 6a, encompassing N, P, K, Ca, and Mg, observed across three leaf growth stages (S1 to S2, S3, and S4 to S5) as representative groups. Significant differences were observed in all macronutrient content. Higher macronutrient values were evident in the early leaf stages (S1), while lower content was observed during leaf senescense (S5), with the exception of Ca, which exhibited a contrasting trend. The source-sink transition progresses basipetally along the leaf, coinciding with changes in physiological, biochemical, and anatomical properties (34). Variations in macronutrient content (such as N, P, K, and Mg) throughout kratom leaf development may stem

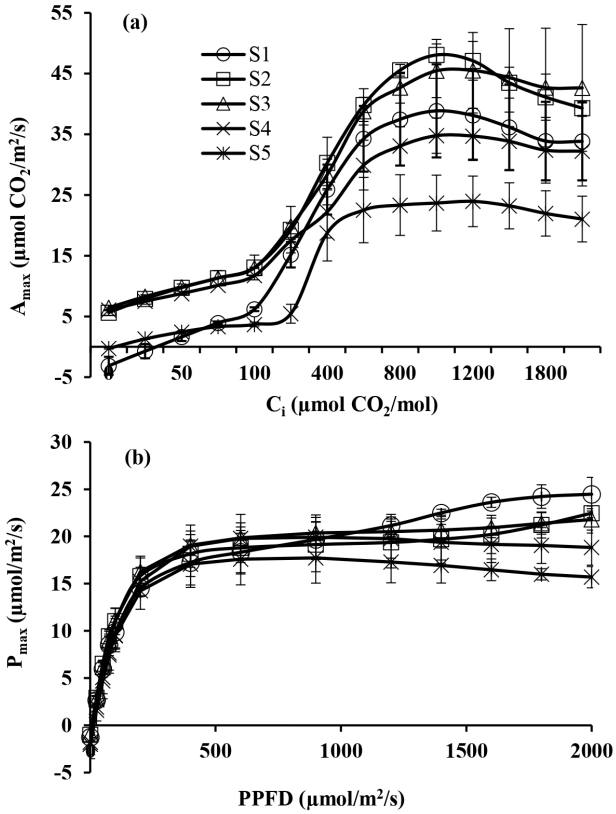

Fig. 4. Carbon response curves (A_{max}) in difference CO_2 concentration (a), and light-saturated photosynthetic response curves (P_{max}) in difference light intensity (b) in different stages (S1-S5) of kratom leaves

from the mobilization of these nutrients from older to younger leaves. Conversely, Ca, known for its relative immobility in the phloem of most plant species (35, 36, 37), showed a different distribution pattern. Furthermore, nutrient content and photosynthetic capacity in kratom leaves exhibited a parallel trend with leaf age, peaking upon full leaf expansion and declining during leaf senescence.

The content of phenolics, flavonoids, and antioxidants exhibited a consistent trend, characterized by an initial increase during the early leaf growth stages (S1 to S2, and S3), followed by a decline in the senescence stages (S4 to S5) (Fig. 6b). This pattern mirrors physiological traits such as photosynthesis, which plays a crucial role in the biosynthesis of secondary metabolites. Additionally, a significant positive correlation exists between the production of total phenolics, flavonoids, and antioxidant activity and photosynthetic functions (38, 39). The tannin content exhibited a distinct pattern compared to other phytochemicals, showing a tendency to increase up to the leaf senescence group stage (S4 to S5). This finding contrasts with observations in Robusta coffee leaves (13) and mangrove (40) leaves, where tannin content was higher in young leaves than in old leaves. In contrast, the anthocyanin content showed only a slight decline as leaves matured from stage S1 to S5. This suggests that anthocyanin production may be sustained even during senescence, unlike other compounds that are typically more susceptible to photoinhibition in mature leaves (41). Such findings underscore the dynamic nature of kratom's secondary metabolite profile throughout its leaf development.

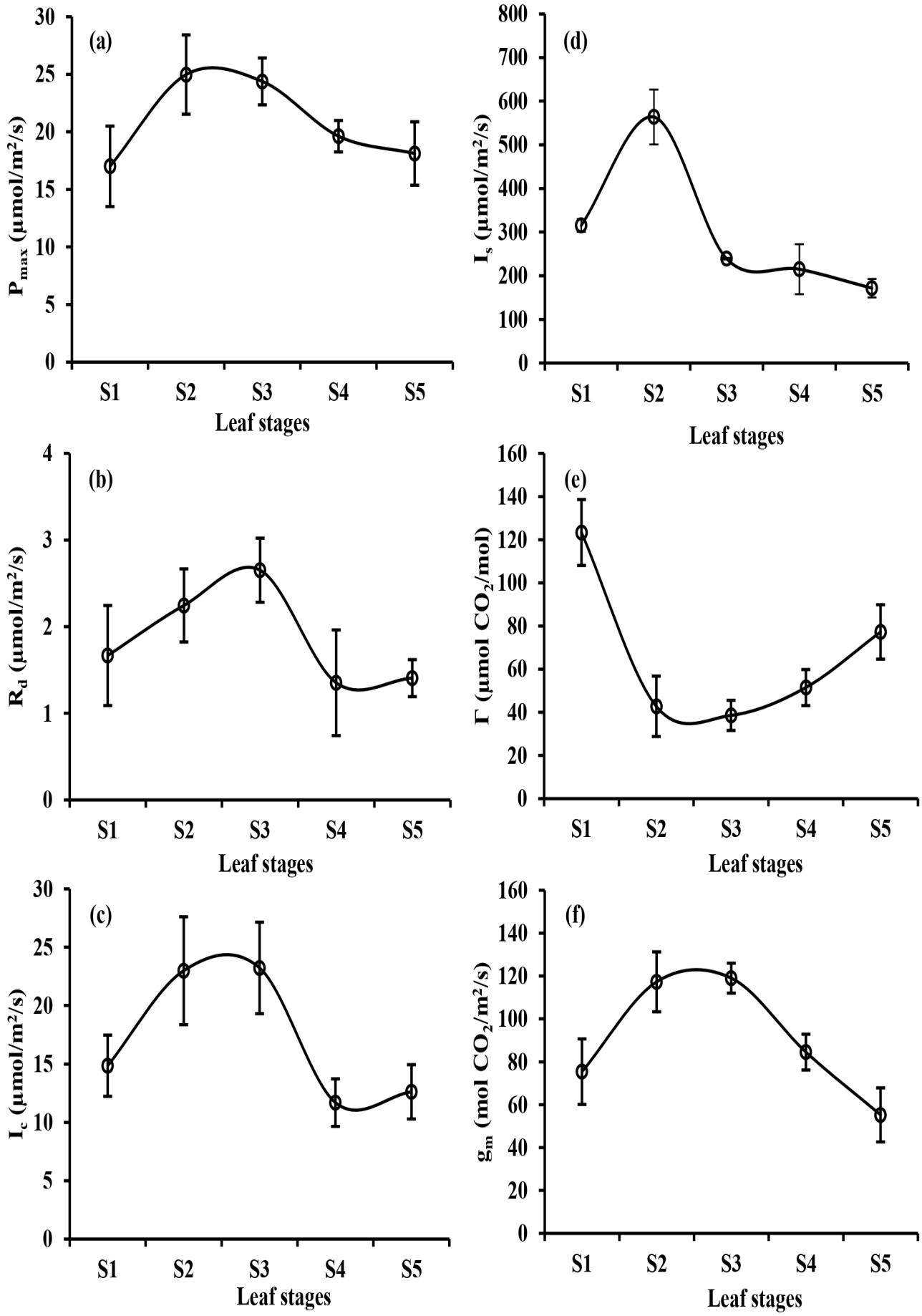

Fig. 5. Changes in light-saturated photosynthetic rate (P_{max}) (a) dark respiration (R_d) (b), light compensation point (I_c) (c), light saturation point (I_s) (d), CO_2 compensation point (τ) (e), and mesophyll conductance (g_m) (f) in different stages (S1-S5) of kratom leaves

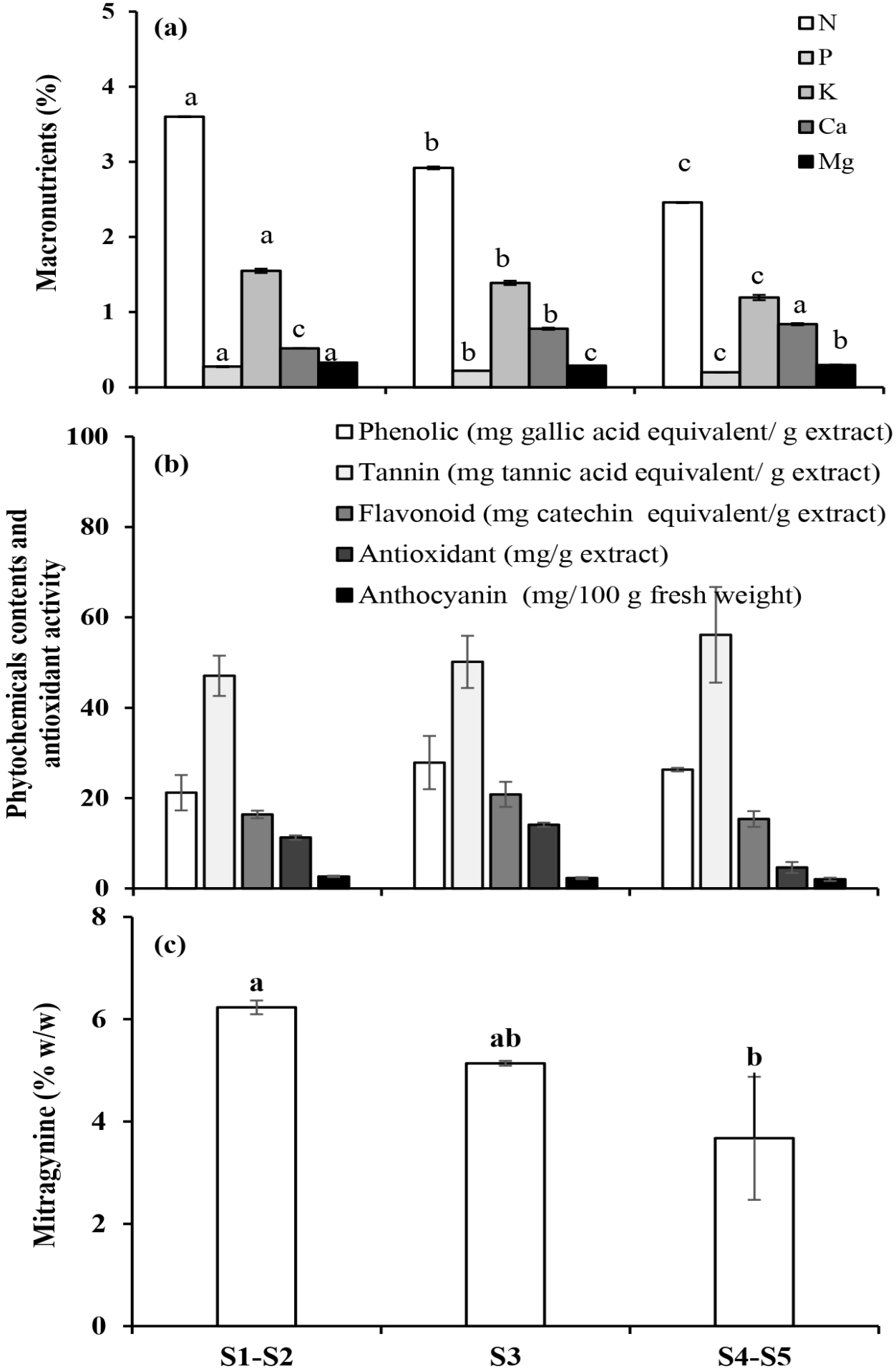

Fig. 6. The contents of macronutrients (a) phytochemicals and antioxidant activity (b), and mitragynine (c) in different stages (S1 t o S2, S3, and S4 to S5) of kratom leaves

Furthermore, the mitragynine content of kratom leaves exhibited a significant difference across various leaf stages (Fig. 6c). The content of mitragynine decreased continuously with leaf age, with the highest levels observed in young leaves and the lowest in older ones. This pattern shared similarities with the development trend of nitrogen content in kratom leaf growth stages. Previous studies have highlighted the influence of nitrogen on mitragynine levels (42). However, it's worth noting that the mitragynine content in kratom leaves is influenced by various factors, including geographical and climatic conditions, as well as leaf growth dynamics (43, 44, 45). Therefore, the variation in mitragynine content across leaf development stages underscores the importance of timing of leaf harvesting to optimize production and potency.

Hence, the distinct growth stages of kratom leaves notably influence their morpho-physiological functions, often correlating with variations in phytochemical contents. Determining the optimal harvest timing for kratom leaves should be guided by the specific active compounds desired.

Conclusion

This study examined how leaf development influences the morphological and physiological properties of kratom leaves. The findings revealed a distinct pattern: most traits increased from the youngest stage (S1) to the fully expanded leaf stage (S3), then declined during leaf senescence (S5), with peak values occurring between stages S2 and S3. Macronutrient content (N, P, and K) also varied significantly across leaf growth stages, with young leaves consistently displaying higher levels of these nutrients compared to older leaves. Notably, the content of phenolics, flavonoids, and antioxidant activity followed a different pattern, tending to increase with leaf age before decreasing during senescence. However, mitragynine content peaked in fully expanded leaves before declining in older leaves. Considering these findings, optimal harvesting of kratom leaves should be timed to maximize phytochemical and mitragynine content, with leaves harvested during the fully expanded stage (S3) likely to be most beneficial.

Acknowledgements

This research was supported by National Science, Research and Innovation Fund (NSRF), and Prince of Songkla University (Ref.No. NAT6601310S). The authors are also grateful to the Graduate School, Prince of Songkla University.

Authors' contributions

WP & ID drafted the manuscript. WP, CS, AC & RC carried out the field survey, morpho-physiological studies, and performed the statistical analysis. The conceptualization, supervision of experiments, and edited manuscript were participated by RC. Also, SS assisted in analysis of the mitragynine content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have declared no conflicts of interest.

Ethical issues: None.

References

1. Cinosi E, Martinotti G, Simonato P, Singh D, Demetrovics Z, Roman-Urrestarazu A *et al*. Following “the roots” of kratom (*Mitragyna speciosa*): The evolution of an enhancer from a traditional use to increase work and productivity in Southeast Asia to a recreational psychoactive drug in western countries. BioMed Res Int. 2015;1-11. https://doi: 10.1155/2015/968786
2. Grundmann O. Patterns of kratom use and health impact in the US–results from an online survey. Drug Alcohol Depend. 2017;176:63-70. https://doi:10.1016/j.drugalcdep. 2017.03.007
3. Halpenny GM. *Mitragyna speciosa*: Balancing potential medical benefits and abuse. ACS Med Chem Lett. 2017;8:897-99. https://doi:10.1021/acsmedchemlett.7b00298
4. Ramachandram DS, Damodaran T, Zainal H, Murugaiyah V, Ramanathan S. Pharmacokinetics and pharmacodynamics of mitragynine, the principle alkaloid of *Mitragyna speciosa*: Present knowledge and future directions in perspective of pain. J Basic Clin Physiol Pharmacol. 2019;31:20190138. https://doi:10.1515/jbcpp-2019-0138
5. Orio L, Alexandru L, Cravotto G, Mantegna S, Barge A. UAE, MAE, SFE-CO_2 and classical methods for the extraction of *Mitragyna speciosa* leaves. Ultrason Sonochem. 2012;19:591-95. https://doi: 10.1016/j.ultsonch.2011.10.001
6. Veltri C, Grundmann O. Current perspectives on the impact of kratom use. Substance Abuse and Rehabilitation. 2019;10:23-31. https://doi: 10.2147/SAR.S164261
7. Parthasarathy S, Ramanathan S, Murugaiyah V, Hamdan MR, Said MIM, Lai CS *et al*. A simple HPLC-DAD method for the detection and quantification of psychotropic mitragynine in *Mitragyna speciosa* (ketum) and its products for the application in forensic investigation. J Forensic Sci. 2013;226:183-87. https://doi.org/ 10.1016/ j.forsciint.2013.01.014
8. Zhang M, Sharma A, Leo´n F, Avery B, Kjelgren R, McCurdy CR *et al*. Plant growth and phytoactive alkaloid synthesis in kratom [*Mitragyna speciosa* (Korth)] in response to varying radiance. PLoS One. 2022;17:e0259326. https:// doi.org/ 10.1371/ journal.pone.0259326
9. Amrianto, Ishak SSO, Putra N, Salsabila S, Al Muqarrabun LMR. Mitragynine: A review of its extraction, identification and purification methods. Biosci Biotechnol Biochem. 2021;3:165-71. https://doi.org/10.5614/crbb.2021.3.1/TMPNSA4H
10. Ramanathan S, León F, Chear NJY, Yusof SR, Murugaiyah V, McMahon LR *et al*. Kratom (*Mitragyna speciosa* Korth.): A description on the ethnobotany, alkaloid chemistry and neuropharmacology. Stud Nat Prod Chem. 2021;69:195-225. https://doi: 101016/B978-0-12-819487-400003-3
11. Nicotra AB, Leigh A, Boyce CK, Jones CS, Niklas KJ, Royer DL *et al*. The evolution and functional significance of leaf shape in the angiosperms. Funct Plant Biol. 2011;38:535-52. https://doi: 101071/FP11057
12. Zhang L, Du J, Ge X, Cao D, Hu J. Leaf size development differences and comparative transcriptome analyses of two poplar genotypes. Genes. 2021;12:1775. https://doi.org/ 10.3390/ genes12111775
13. Maxiselly Y, Anusornwanit P, Rugkong A, Chiarawipa R, Chanjula P. Morpho-physiological traits phytochemical composition and antioxidant activity of canephora coffee leaves at various

stages. Int J Plant Biol. 2022;13:106-14. https://doi.org/10.3390/ijpb13020011

14. Chiarawipa R, Rueangkhanab M, Han ZH. Leaf age-related acclimation in the photosynthetic capacity and fractional investments of leaf nitrogen in grapevines of different ages. Science and Technology Asia. 2021;26:99-113. https://doi:10.14456/scitechasia.2021.50

15. Jiao Y, Niklas KJ, Wang L, Yu K, Li Y, Shi P. Influence of leaf age on the scaling relationships of lamina mass vs area. Front Plant Sci. 2022;8:860206. https://doi: 103389/fpls2022860206

16. Pathoumthong P, Zhang Z, Roy SJ, Habti AE. Rapid non-destructive method to phenotype stomatal traits. Plant Methods 2023;19:36. https://doi.org/10.1186/s13007-023-01016-y

17. Netto AT, Campostrini E, de Oliveira JG, Bressan-Smith R. Photosynthetic pigments, nitrogen chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Sci Hortic. 2005;104:199-209. https://doi.org/10.1016/j.scienta.2004.08.01

18. Sáez-Plaza P, Navas MJ, Wybraniec S, Michalowski T, Asuero AG. An overview of the kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish and quality control. Crit Rev Anal Chem. 2013;43:224-72. https://doi: 10.1080/10408347.2012.751787

19. Panda SK. Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. In: El-Missiry, MA editor. Antioxidant Enzyme. Egypt: Mansoura University. 2012; p. 381-400. https://dx.doi.org/10.5772/50782

20. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73:73-84. https://doi.org/10.1016/S0308-8146(00)00288-0

21. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14:2167-80. https://doi:10.3390/molecules14062167

22. Francis FJ. Food colorants: Anthocyanins. Crit Rev Food Sc Nutr. 1989;28:273-314. https://doi.org/10.1080/10408398909527503

23. Araújo RPD, Almeida AFD, Barroso JP, Oliveira RAD, Gomes FP, Ahnert D *et al*. Molecular and morphophysiological responses cocoa leaves with different concentrations of anthocyanin to variations in light levels. Sci Hortic. 2017;224:188-97. https://doi.org/ 10.1016/ j.scienta.2017.06.008

24. Yang Z, Zhai W. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innov Food Sci Emerg Technol. 2010;11:169-76. https://doi.org/10.1016/j.ifset.2009.08.012

25. Kikura-Hanajiri R, Kawamura M, Maruyama T, Kitajima M, Takayama H, Goda Y. Simultaneous analysis of mitragynine, 7-hydroxymitragynine and other alkaloids in the psychotropic plant "kratom" (*Mitragyna speciosa*) by LC-ESI-MS. Forensic Toxicol. 2009;27:67-74. https://doi.org/10.1007/s11419-009-0070-5

26. Janchawee B, Keawpradub N, Chittrakarn S, Prasettho S, Wararatananurak P, Sawangjareon K. A high-performance liquid chromatographic method for determination of mitragynine in serum and its application to a pharmacokinetic study in rats. Biomed Chromatogr. 2007;21:176-83. https://doi.org/10.1002/bmc.731

27. Huang W, Ratkowsky DA, Hui C, Wang P, Su J, Shi P. Leaf fresh weight versus dry weight: Which is better for describing the scaling relationship between leaf biomass and leaf area for broad-leaved plants? Forests. 2019;10:256. https://doi.org/103390/f10030256

28. Woo HR, Kim HJ, Lim PO, Nam HG. Leaf senescence: Systems and dynamics aspects. Annu Rev Plant Biol. 2019;70:347-76. https://doi.org/10.1146/annurev-arplant-050718-095859

29. Jang S, Kim GW, Han K, Kim YM, Jo J, Lee SY *et al*. Investigation of genetic factors regulating chlorophyll and carotenoid biosynthesis in red pepper fruit. Front Plant Sci. 2022;13:922963. https://doi: 103389/fpls2022922963

30. Idris A, Linatoc AC, Bakar MFBA. Effect of light intensity on the gas exchange characteristics of *Melothria pendula*. IOP Conf Ser: Earth Environ Sci. 2019;269:012021. https://doi:10.1088/1755-1315/269/1/012021

31. Chiarawipa R, Wang Y, Zhang XZ, Han ZH, Rueangkhanab M. Modeling light acclimation of photosynthetic response in different ages of vine leaves. Acta Hortic. 2012;956:255-60. https://doi:1017660/ActaHortic201295628

32. Lobo FA, de Barros MP, Dalmagro HJ, Dalmolin ÂC, Pereira WE, Souza ÉC *et al*. Fitting net photosynthetic light-response curves with Microsoft Excel-a critical look at the models. Photosynthetica. 2013;51:445-56. https://doi.org/10.1007/s11099-013-0045-y

33. Oguchi R, Hikosaka K, Hirose T. Does the photosynthetic light-acclimation need change in leaf anatomy? Plant Cell Environ. 2003;26:505-12. https://doi.org/10.1046/j.1365-3040.2003.00981.x

34. Marschner P. Mineral nutrition of higher plants. 3rd ed. Amsterdam: Elsevier Ltd; 2012.

35. Crous KY, Wujeska-Klause A, Jiang M, Medlyn BE, Ellsworth DS. Nitrogen and phosphorus retranslocation of leaves and stem wood in a mature *Eucalyptus* forest exposed to 5 years of elevated CO_2. Front Plant Sci. 2019;10:664. https://doi.org/10.3389/fpls.2019.00664

36. Himelblaua E, Amasino RM. Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. J Plant Physiol. 2001;158:1317-23. https://doi.org/10.1078/0176-1617-00608

37. Tanoi K, Kobayashi N. Leaf senescence by magnesium deficiency. Plants. 2015;4:756-72. https://doi.org/10.3390%2Fplants4040756

38. Ibrahim MH, Jaafar HZE, Karimi E, Ghasemzadeh A. Primary, secondary metabolites, photosynthetic capacity and antioxidant activity of the Malaysian herb kacip fatimah (*Labisia pumila* Benth) exposed to potassium fertilization under greenhouse conditions. Int J Mol Sci. 2012;13:15321-42. https://doi: 10.3390/ijms131115321

39. Guo F, Guo Y, Wang P, Wang Y, Ni D. Transcriptional profiling of catechins biosynthesis genes during tea plant leaf development. Planta. 2017;246:1139-52. https://doi. 10.1007/s00425-017-2760-2

40. Lin YM, Lin JW, Xiang P, Lin P, Ding ZH, Sternberg LSL. Tannins and nitrogen dinamycs in mangrove leaves at different age and decay stage (Jiulong River Estuary, China). Hydrobiologia. 2007;583:285-95. https://doi.10.1007/s10750-006-0568-3

41. Juvany M, Müller M, Munné-Bosch S. Photo-oxidative stress in emerging and senescing leaves: A mirror image? J Exp Bot. 2013;64:3087-98. https:// doi.10.1093/ jxb/ ert174

42. Zhang M, Sharma A, León F, Avery B, Kjelgren R, McCurdy CR *et al*. Effects of nutrient fertility on growth and alkaloidal content in *Mitragyna speciosa* (Kratom). Front Plant Sci. 2020;11:597696. https://doi.10.3389/fpls.2020.597696

43. Boffa L, Ghè C, Barge A, Muccioli G, Cravotto G. Alkaloid profiles and activity in different *Mitragyna Speciosa* strains. Nat Prod Comm. 2018;13:1111-16. https://doi.org/10.1177/1934578X1801300904

44. Leksungnoen N, Andriyas T, Ngernsaengsaruay C, Uthairatsamee S, Racharak P, Sonjaroon W *et al*. Variations in mitragynine content in the naturally growing Kratom (*Mitragyna speciosa*) population of Thailand. Front Plant Sci. 2022;13:1028547. https://doi: 10.3389/fpls.2022.1028547

45. Veeramohan R, Zamani AI, Azizan KA, Goh H, Aizat WH, Razak MFA *et al*. Comparative metabolomics analysis reveals alkaloid repertoires in young and mature *Mitragyna speciosa* (Korth.) Havil. leaves. PLoS ONE. 2023;18:e0283147. https://doi.org/10.1371/journal.pone.0283147