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Research Article
Investigation of Nutritional Content Variations between Young and Mature Leaves of *Melientha suavis* Pierre

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Abstract

Background and Objective: *Melientha suavis* Pierre is a small woody species native to limestone mountain areas of northern Vietnam and valued as a wild edible vegetable with high nutritional content. Despite its dietary importance, limited information is available on nutritional variations between leaves at different developmental stages. This study aimed to compare the nutritional composition of young and mature leaves of *M. suavis* Pierre to determine stage-specific differences and provide a basis for optimizing harvest timing. **Materials and Methods:** Leaf samples were collected at two developmental stages (young and mature) and analyzed for proximate composition, vitamin C organic acids, chlorophyll, reducing sugars, cellulose, lipids and minerals using standard analytical techniques. The data were statistically processed using Microsoft Excel and SPSS 16.0 software and differences between stages were evaluated by One-way ANOVA followed by Tukey's HSD test at a significance level of $\alpha = 0.05$. **Results:** Young leaves exhibited higher moisture content (75.68%), vitamin C (79.83 mg/100 g), total organic acids (3.45%) and potassium (407.76 mg/100 g) compared with mature leaves. In contrast, mature leaves had greater dry matter (29.31%), total chlorophyll (2.96 mg/g), reducing sugars (3.19 mg/g), cellulose (8.78%), lipids (0.65%), calcium (352.08 mg/100 g), iron (4.48 mg/100 g) and zinc (0.92 mg/100 g). **Conclusion:** The study highlights stage-specific nutrient accumulation patterns in *M. suavis* Pierre leaves. Young leaves are richer in vitamin C organic acids and potassium, while mature leaves accumulate more structural components and minerals. These findings provide a scientific basis for determining optimal harvest timing to maximize nutritional value for consumption and food processing.

Key words: *Melientha suavis* Pierre, leaf age, nutrition, wild vegetables

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Melientha suavis Pierre, a member of the family Opiliaceae, is predominantly distributed in Southeast Asia, particularly in Thailand, Malaysia, the Philippines, Laos, Cambodia and Vietnam[1]. In its natural habitat, this species typically grows under forest canopies, thriving in limestone mountains and secondary forests. *M. suavis* is a small deciduous tree (3-5 m in height) with alternate, simple leaves ranging from elliptic to obovate in shape. The young shoots and leaves of *M. suavis* are highly nutritious, containing elevated levels of vitamin C, minerals (potassium, calcium, phosphorus, magnesium, iron, zinc and copper) and natural carotenoids[2]. Additionally, the leaves are rich in bioactive compounds such as flavonoids, alkaloids, saponins, terpenoids and tannins[3]. Beyond their value as a traditional food source, *M. suavis* leaves have been investigated for their bioactive constituents, particularly antioxidants and have been utilized in natural sunscreen formulations[4].

In Vietnam, *M. suavis* is also known by local names such as "rau ngot rung", "rau mi chinh" and "lai cam". It is naturally distributed in secondary forests, at the foothills of limestone mountains and on hilly soils, mainly in the northern midland and mountainous regions, as well as the North Central Region[5,6]. The young leaves have a naturally sweet taste, tender texture and distinctive aroma, making them a popular wild vegetable consumed as food or used as a medicinal resource. However, due to overharvesting in the wild, *M. suavis* has been listed as a vulnerable species (VU B1+2e) in the Red Data Book[5].

Leaf age and developmental stage significantly affect leaf morphology, structure and physiological processes in plants. Numerous studies have reported differences in morphology, anatomy and nutrient accumulation among leaf age groups in various species. Ji *et al.*[6] observed that during leaf development, dry matter content and the mass-to-area ratio increase significantly[7]. In terms of photosynthetic capacity, leaf age markedly influences chlorophyll content and photosynthetic efficiency[8,9]. Mature leaves typically exhibit higher chlorophyll content compared to young leaves[10], resulting in enhanced photosynthetic efficiency. However, as leaves enter senescence, photosynthetic capacity tends to decline with age[11]. In *Quercus ilex*, factors such as cell wall thickness and the contact area between chloroplasts and stomata vary significantly with leaf age, affecting CO diffusion and photosynthetic rate[12]. Besides, nutrient uptake and accumulation also change across leaf developmental stages. For example, in sugar cane, young leaves contain higher concentrations of sugars and amino acids, whereas mature leaves accumulate greater amounts of phenolic compounds and flavonoids, exhibiting higher antioxidant activity[13]. In *Populus deltoides*, the concentrations of elements such as Ca and Mg tend to increase with leaf age, whereas Mo decreases; copper deficiency also alters the distribution of micronutrients like Fe and Zn across different leaf age groups[14].

Although the influence of leaf age on morphological traits and nutrient composition has been extensively studied in various leafy vegetables, corresponding data on *M. suavis* remain limited. Identifying the variation in nutrient content between young and mature leaves of *M. suavis* is essential to elucidate the physiological characteristics of this species and to provide a scientific basis for determining optimal harvest timing for consumption, preservation and processing. Therefore, this study was conducted to evaluate changes in selected biochemical parameters and major mineral nutrients in *M. suavis* leaves at different developmental stages.

MATERIALS AND METHODS

Materials: Leaves were collected from healthy *Melientha suavis* Pierre plants, aged 8-9 years, free from pests and diseases and naturally grown at the foot of limestone mountains in Yen Vy Hamlet, Huong Son Commune, Hanoi City, Vietnam (coordinates: 20°36'9"N, 105°45'48"E). This area has a typical monsoon tropical climate, with an average annual temperature of approximately 23-24°C, relative humidity around 80% and an annual rainfall of 1,800-2,000 mm (climate data), providing favorable conditions for the natural growth of *M. suavis*.

Methods

Sampling method: At the study site, 10 healthy *M. suavis* trees were randomly selected. From each tree, five different secondary branches were sampled. Young and mature leaves were collected from the same branch to ensure uniform growth conditions. Sampling was conducted in the morning (08:00-09:00) under rain-free weather conditions, specifically in spring (March 2024).

Young leaves were defined as the first 3-5 leaves from the apical meristem of the secondary branch; they were light green, with soft, non-brittle leaf blades. Mature leaves were defined as the 6th-10th leaves from the shoot apex, characterized by a dark green color, firmer leaf margins and clearly visible midribs.

After collection, the leaves were placed in labeled zip-lock bags and stored in an insulated cooler box at 4-6°C for transport to the laboratory for further analysis.

Methods for determining analytical parameters: The dry matter and water content of leaves were determined by initially drying the samples at 105°C for 2 hrs, followed by further drying at 70°C until a constant dry weight was achieved[15]. The contents were then calculated using the following formulas[16]:

$$\text{Dry matter content (\%)} = \frac{\text{Dry weight of leaf}}{\text{Fresh weight of leaf}} \times 100$$

$$\text{Water content (\%)} = \frac{\text{Fresh weight of leaf} - \text{Dry weight of leaf}}{\text{Fresh weight of leaf}} \times 100$$

The vitamin C content was determined by iodine titration; Reducing sugar content was measured using the dinitro-salicylic acid (DNS) method; Pectin content was quantified by the calcium pectate method; cellulose content was determined by treating the samples with a nitric acid-acetic acid mixture; lipid content was extracted and quantified using a Soxhlet apparatus; protein content was determined by the Kjeldahl method[17]. Total organic acid content was analyzed using the Emarcov method; chlorophyll a, b and total chlorophyll contents were determined spectrophotometrically after extraction with acetone, following the procedure described by Jiao *et al.*[18]. Mineral element contents were quantified by Atomic Absorption Spectrophotometry (AAS).

Statistical analysis: The data were statistically processed using Microsoft Excel and SPSS 16.0 software. One-way ANOVA followed by Tukey's HSD test was performed at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Influence of leaf age on water content, dry matter content and chlorophyll content: The analytical results presented in Table 1 indicate that the water content in *M. suavis* leaves ranged from 70.69 to 75.68%, which was noticeably lower than the values reported for many other leafy vegetables. Among them, young leaves exhibited a water content approximately 6% higher than that of mature leaves. Correspondingly, the dry matter content of young leaves was 24.42%, lower than that of mature leaves (29.31%).

Earlier research indicated that as leaves age, the proportion of dry matter relative to fresh weight tends to rise[7,19]. Moreover, the photosynthetic efficiency of young leaves is considerably lower compared with that of fully developed leaves[20], which may partly explain their reduced ability to accumulate dry matter. Moreover, young leaves exhibit vigorous growth, with high rates of cell division and elongation, leading to a greater demand for water exchange and, consequently, a higher proportion of water in their total mass. In contrast, mature leaves have completed their development, with fully differentiated tissues containing higher amounts of cellulose, lignin and other organic compounds, contributing to an increased proportion of dry matter and a reduced water content.

The chlorophyll content is a crucial physiological indicator that reflects the photosynthetic capacity and overall physiological status of leaves or entire plants[10]. Beyond its biological role, chlorophyll also possesses medicinal value, being recognized as a bioactive compound with potential benefits in lowering blood glucose levels, detoxification, alleviating allergic responses and promoting digestive as well as excretory functions[21,22].

As shown in Table 1, the total chlorophyll content in mature *M. suavis* leaves reached 2.96 mg/g, which was 1.77 times higher than that in young leaves (1.67 mg/g). This increase resulted from elevated levels of both chlorophyll a and chlorophyll b. However, the distribution and proportion between these two chlorophyll types varied significantly with leaf developmental stage. Specifically, in young leaves, chlorophyll a dominated over chlorophyll b with an a/b ratio of 1.83, whereas in mature leaves, chlorophyll b exceeded chlorophyll a, resulting in a reduced a/b ratio of 0.73.

The chlorophyll a/b ratio serves as an indicator of structural adjustments in the photosynthetic apparatus in response to light conditions and leaf physiological status. Young leaves, which are typically more exposed to intense light during rapid growth, tend to synthesize more chlorophyll a to optimize photochemical efficiency. In contrast, mature leaves, often subjected to lower light intensity, accumulate more chlorophyll b to broaden the light absorption spectrum, thereby maintaining photosynthetic efficiency under reduced light conditions. Such shifts in the chlorophyll a/b ratio between young leaves ($\geq$1) and mature leaves ($\leq$1) have also been reported in various plant species such as *Psidium guajava*, *Datura metel* and *Ficus benjamina*[10].

The higher chlorophyll content in mature leaves also indicates greater photosynthetic potential and biomass accumulation, as reflected in Table 1. A synthesis of data from 10 plant species[10] similarly concluded that mature leaves generally exhibit higher chlorophyll levels compared to young leaves. This trend is consistent with previous findings in several species, including tomato (*Solanum lycopersicum*), silver birch (*Betula pendula*), black alder (*Alnus incana*) and common aspen (*Populus tremula*)[20,23].

Table 1: Water content, dry matter and chlorophyll content of *Melientha suavis* Pierre leaves

Type of leaf/Parameters	Young leaves	Mature leaves
Water content (%)	75.68±0.78[a]	70.69±0.63[b]
Dry matter (%)	24.34±0.50[a]	29.31±0.50[b]
Chlorophyll a (mg/g)	1.08±0.03[a]	1.25±0.01[b]
Chlorophyll b (mg/g)	0.59±0.03[a]	1.71±0.12[b]
Total chlorophyll (mg/g)	1.67±0.06[a]	2.96±0.13[b]

Means followed by different letters in the same row are significantly different among varieties at $p \leq 0.05$

Table 2: Vitamin C, total organic acid and reducing sugar contents in *Melientha suavis* Pierre leaves

Type of leaf/Parameters	Young leaf	Mature leaf
Vitamin C content (mg/100 g fresh leaf)	79.83±0.23[a]	70.27±0.18[b]
Total organic acid (%)	3.45±0.19[a]	2.11±0.13[b]
Reducing sugar content (mg/g fresh leaf)	2.47±0.09[a]	3.19±0.10[ab]

Means followed by different letters in the same row are significantly different among varieties at $p \leq 0.05$

Effect of leaf age on the contents of vitamin C, reducing sugar and total organic acid: The vitamin C, total organic acid and reducing sugar are common indicators used to evaluate the quality of fresh vegetables and fruits. The results for these three parameters in *M. suavis* leaves are presented in Table 2.

The results in Table 2 show that the vitamin C content in young *M. suavis* leaves (79.83 mg/100 g fresh weight) was higher than that in mature leaves (70.27 mg/100 g). A similar trend of decreasing vitamin C content with leaf maturation has been reported in other leafy vegetables such as lettuce and spinach[24]. However, an opposite pattern was observed in passionfruit leaves (*Passiflora edulis*), where mature leaves contained higher vitamin C levels[25]. This indicates that the accumulation pattern of vitamin C during leaf development is species-specific and strongly influenced by genetic and physiological characteristics.

When compared to some common Thai Lan vegetables, *M. suavis* can be considered a rich natural source of vitamin C, with fresh leaves containing up to 146 mg/100 g and retaining a relatively high level even after cooking (52 mg/100 g)[2]. This highlights the potential of *M. suavis* as a valuable dietary source of vitamin C.

A similar trend was observed for total organic acids, with young leaves containing 1.63 times higher levels than mature leaves. In contrast, the reducing sugar content in mature leaves (3.19 mg/g) was about 1.3 times higher than in young leaves (2.47 mg/g). This finding is consistent with studies on passionfruit leaves, where mature leaves accumulated higher levels of total sugars as well as simple sugars such as glucose and fructose compared to young shoots[25]. This result indicates an increased accumulation of photosynthetic products in the later developmental stage of the leaves. Mature leaves act as source organs, where the photosynthetic apparatus is fully developed and highly efficient, leading to greater synthesis and storage of sugars. In contrast, young leaves function as sink organs, where sugars are rapidly consumed for intense metabolic activities, including respiration, biomass synthesis, cell division and elongation, rather than being stored[26].

Effect of leaf age on the contents of cellulose, pectin, lipid and protein: As shown in Table 3, *M. suavis* leaves contain a relatively high level of cellulose compared with several other commonly consumed vegetables.. Specifically, the cellulose content in young leaves reaches 3.28%, which is higher than that of katuk (*Sauropus androgynus*) (2.5%), sweet potato leaves (1.4%), malabar spinach (2.5%), broccoli (3.2%) and water spinach *Ipomoea aquatica* (1%)[27]. The results presented in Table 3 also show that, along with leaf development, cellulose content increases significantly, reaching 8.78% in mature leaves, approximately 2.6 times higher than in young leaves. The increase in cellulose provides maximum mechanical support, enhancing leaf rigidity and resistance. Similar findings have been observed in the leaves of many other plant species[28].

In contrast to the increasing trend of cellulose, the pectin content in young leaves (0.90%) is higher than in mature leaves (0.78%). The cell walls of young leaves usually contain higher amounts of pectin and hemicellulose, which provide the necessary flexibility for cell expansion during the growth stage. As the leaves transition to maturity, the accumulation of cellulose and lignin becomes more pronounced, contributing to the thickening of the cell wall, increasing mechanical strength and stabilizing cell shape. This explains the difference in cellulose and pectin contents between the two developmental stages of *M. suavis* leaves.

Regarding lipid content, Table 3 shows that the lipid content in *M. suavis* leaves increased from 0.47% in young leaves to 0.65% in mature leaves. Similar findings were reported for *Polyalthia longifolia*[29]. This result indicates that lipid accumulation increases with leaf age, consistent with the physiological trend of plants transitioning from a rapid growth phase to a stable phase with enhanced nutrient storage.

Table 3: Cellulose, pectin, lipid and protein contents of *Melientha suavis* Pierre leaves

Type of leaf/Parameters	Young leaf	Mature leaf
Cellulose (%)	3.28±0.14[a]	8.78±0.21[b]
Pectin (%)	0.90±0.03[a]	0.78±0.02[b]
Lipid (%)	0.47±0.09[a]	0.65±0.02[b]
Protein (%)	5.61±0.11[a]	3.82±0.20[b]

Means followed by different letters in the same row are significantly different among varieties at $p \leq 0.05$

Table 4: Calcium, potassium, iron and zinc contents in *Melientha suavis* Pierre leaves

Type of leaf/Parameters	Young leaf	Mature leaf
Ca	189.4±23.13[a]	352.08±47.76[b]
K	407.76±16.40[a]	335.34±17.51[b]
Fe	3.28±0.32[a]	4.48±0.27[b]
Zn	0.84±0.03[a]	0.92±0.01[b]

Means followed by different letters in the same row are significantly different among varieties at $p \leq 0.05$

A study on three leaf age stages of *Moringa oleifera* (buds, young leaves and old leaves) showed that protein content decreases with leaf aging. Similar trends have been observed in *Polyalthia longifolia*, tomato and rapeseed[30]. This study also recorded a comparable pattern in *M. suavis* leaves. Specifically, young leaves contained 5.61% protein, which was 1.47 times higher than that in mature leaves (Table 3), aligning with the study of Sridonpai *et al.*[2]. The reduction in protein with leaf age may be associated with protein degradation processes, where the resulting metabolites are translocated to storage organs such as fruits and seeds[31].

Effect of leaf age on the content of selected mineral nutrients: Many studies have shown that leaf age significantly affects the plant's ability to accumulate minerals[24,30]. This study determined the contents of four elements, calcium (Ca), potassium (K), iron (Fe) and zinc (Zn), in both young and mature leaves of *M. suavis*. The results presented in Table 4 also indicate clear differences in the concentrations of these elements between different leaf ages.

The calcium (Ca) content in *M. suavis* leaves varied markedly with leaf age. As shown in Table 4, mature leaves contained 352.08 mg/100 g, approximately 1.86 times higher than young leaves (189.4 mg/100 g). This increase is consistent with the distribution pattern of Ca in plants, where the element tends to accumulate in older tissues due to its low mobility in the transport system[32]. Similar results were reported in lettuce and moringa, where Ca levels in mature and old leaves were significantly higher than in young leaves[24,30], as well as in *Polyalthia sonni*, where the difference between mature and young leaves was about 1.56-fold[29].

Compared to common vegetables in Vietnam, the Ca content in young leaves of *M. suavis* (189.4 mg/100 g) surpasses many types, such as lettuce (38 mg), watercress (69 mg), water spinach (100 mg), jute mallow (182 mg) and katuk (169 mg). Meanwhile, mature leaves of *M. suavis* even contain Ca levels comparable to or higher than well-known calcium-rich vegetables such as red amaranth (288 mg), amaranth *Amaranthus tricolor* (341 mg) and Vietnamese balm *Elsholtzia ciliata* (246 mg)[27]. The high Ca content in *M. suavis* leaves may be related to specific soil conditions, as the plant often grows in limestone-rich areas and it also reflects the physiological role of Ca in stabilizing cell walls and maintaining plant tissue structure. This indicates that *M. suavis* leaves, especially mature leaves, are a potential dietary source of calcium.

The potassium (K) content in *M. suavis* leaves showed an opposite trend compared to Ca. Table 4 reveals that young leaves contained 407.76 mg/100 g, about 18% higher than mature leaves (335.34 mg/100 g). This is understandable as K is a highly mobile element, actively transported to developing tissues such as buds, young leaves and immature fruits to support metabolic activities and osmotic regulation. A similar distribution pattern of K has been reported in the leaves of bitter melon and cowpea[33].

In contrast to K, trace elements such as Iron (Fe) and Zinc (Zn) tended to accumulate more in mature leaves. Although Fe is an essential micronutrient for plant growth, it exhibits low mobility within the plant. As a result, once accumulated in the leaves, it is not readily redistributed to other organs. Analysis showed that Fe content in young leaves was only 3.28 mg/100 g, about 63.4% of that in mature leaves (4.48 mg/100 g). This is consistent with findings in *Adenia viridiflora* Craib, where Fe levels in mature leaves were higher than in young shoots[25]. The accumulation of Fe in mature leaves also helps maintain chlorophyll content, contributing to the dark green color typical of older leaves.

Similarly, Zn-an essential trace element for both plants and humans-also increased with leaf age (Table 4). The Zn content in young leaves was 0.84 mg/100 g, rising to

0.92 mg/100 g in mature leaves. Compared to common vegetables, *M. suavis* has higher Zn levels than malabar spinach (0.54 mg), lettuce (0.4 mg) and jute mallow (0.79 mg) and is similar to mustard greens (0.9 mg) and katuk (0.94 mg)[27]. However, studies on moringa leaves reported the opposite trend, where Zn content was higher in young leaves30, suggesting that environmental conditions, seasonal factors or cultivar differences may influence Zn accumulation.

In summary, the differences in mineral content between young and mature leaves clearly reflect the physiological characteristics and mobility of each element within the plant. Factors such as growth stage, tissue function and species-specific traits all influence mineral accumulation in leaves. These findings further highlight the outstanding nutritional value of M. suavis compared to many other leafy vegetables, particularly in terms of Ca, K, Fe and Zn-essential minerals for human health.

CONCLUSION

The study identified differences in the nutritional composition of *Melientha suavis* Pierre leaves at different developmental stages. Young leaves contained higher water content, vitamin C, total organic acid and potassium compared to mature leaves. In contrast, mature leaves had higher contents of dry matter, total chlorophyll, reducing sugar, cellulose, lipids, calcium, iron and zinc than young leaves. These findings provide a scientific basis for optimizing harvest timing to meet specific nutritional needs and for developing fresh or functional food products. More broadly, they enhance understanding of nutrient allocation in underutilized wild vegetables, supporting their sustainable use. Future studies should address nutrient bioavailability, health benefits and the potential integration of *M. suavis* Pierre into diets and food processing.

SIGNIFICANCE STATEMENT

This study provides the first stage-specific comparison of nutrients in *Melientha suavis* Pierre leaves, revealing distinct patterns of vitamin, mineral and structural component accumulation. The findings are significant because they guide optimal harvest timing to maximize nutritional benefits, support sustainable utilization of this wild edible vegetable and contribute to food security and dietary diversification in regions where the species is consumed.

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