การประเมินความหลากหลาย ปริมาณสารอาหาร และฤทธิ์ต้านอนุมูลอิสระของผักพื้นบ้านในเมนูขนมจีนเมืองนครศรีธรรมราช ประเทศไทย

Assessment of Species Diversity, Nutritional Content, and Antioxidant Activity of Indigenous Vegetables in the Rice Noodles with Fish Curry Sauce Dish from Nakhon Si Thammarat, Thailand

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินความหลากหลายของชนิดพืช ปริมาณสารอาหาร และฤทธิ์ต้านอนุมูลอิสระของผักพื้นบ้าน 25 ชนิด ที่ใช้ในเมนูขนมจีนแกงป่าในจังหวัดนครศรีธรรมราช ประเทศไทย ผลการศึกษาพบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในด้านพลังงาน โปรตีน ไขมัน คาร์โบไฮเดรต และเถ้า (*ash content*) โดยใบของหมุรุย (*Clausena cambodiana*) มีปริมาณพลังงานสูงที่สุด (448.53±34.44 กิโลแคลอรีต่อ 100 กรัม) ในขณะที่ใบของกระสัง (*Peperomia pellucida*) มีค่าต่ำที่สุด (288.77±0.02 กิโลแคลอรีต่อ 100 กรัม) เมล็ดสะตอ (*Parkia speciosa*) มีปริมาณโปรตีนสูงที่สุด (28.61±0.05 กรัมต่อ 100 กรัม) ในขณะที่ผลของข่าป่า (*Alpinia malaccensis*) มีค่าต่ำที่สุด (7.43±0.04 กรัมต่อ 100 กรัม) ปริมาณไขมันสูงที่สุดในเมล็ด

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สะตอ (17.18±0.07 กรัมต่อ 100 กรัม) ขณะที่เปลือกไม่มีไขมัน (0.00±0.00 กรัมต่อ 100 กรัม) ปริมาณคาร์โบไฮเดรตสูงที่สุดในผลของมะไฟ (*Baccaurea ramiflora*) (82.05±0.12 กรัมต่อ 100 กรัม) และต่ำที่สุดในใบของกระสัง (48.85±0.08 กรัมต่อ 100 กรัม) ใบของกระสังยังมีปริมาณเถ้าสูงที่สุด (23.84±0.01 กรัมต่อ 100 กรัม) อีกด้วย ความสามารถในการต้านอนุมูลอิสระซึ่งวิเคราะห์โดยวิธี ORAC FRAP และ DPPH มีความแตกต่างกันอย่างชัดเจนขึ้นอยู่กับส่วนของพืช เช่น ใบของทัมมัง (*Litsea petiolate*) และยอดอ่อนของมะม่วงหิมพานต์ (*Anacardium occidentale*) แสดงฤทธิ์ต้านอนุมูลอิสระสูงสุด ซึ่งสัมพันธ์กับปริมาณฟลาโวนอยด์และสารฟีนอลิกที่สูง ใบของมะกอก (*Spondias pinnata*) และหมุรุยก็มีค่าการทดสอบ ORAC และ FRAP สูงเช่นกัน ผลไม้ เช่น ลูกชิ้ง (*Ficus botryocarpa*) และมะไฟ แสดงฤทธิ์ต้านอนุมูลอิสระในระดับปานกลาง ขณะที่เมล็ดสะตอมีค่าต่ำกว่า อย่างไรก็ตามเปลือกของสะตอมีฤทธิ์สูงกว่าเมล็ด โดยเฉพาะในการทดสอบด้วยวิธี FRAP และ DPPH ดอกไม้ ซึ่งในที่นี้ คือ ตัวอย่างของดาหลา (*Etlingera elatior*) พบว่ามีระดับสารอาหารและฤทธิ์ต้านอนุมูลอิสระค่อนข้างต่ำ ผลการศึกษานี้แสดงให้เห็นว่าใบและยอดอ่อนมีคุณค่าทางโภชนาการและฤทธิ์ต้านอนุมูลอิสระสูงกว่าส่วนอื่นของพืช จึงมีความสำคัญในการนำมาเป็นวัตถุดิบที่มีศักยภาพสำหรับการพัฒนาอาหารฟังก์ชันและการประยุกต์ใช้ด้านโภชนาการ

คำสำคัญ: ขนมจีน ภูมิปัญญา ผักพื้นบ้าน ปริมาณสารอาหาร สารต้านอนุมูลอิสระ

Abstract

This study assessed the species diversity, nutritional content, and antioxidant activity of 25 indigenous vegetables used with a traditional dish of rice noodles with fish curry sauce from Nakhon Si Thammarat, Thailand. Significant differences ($p < 0.05$) were found in energy, protein, fat, carbohydrate, and ash contents. *Clausena cambodiana* leaves had the highest energy content (448.53±34.44 kcal/100 g), while *Peperomia pellucida* leaves had the lowest (288.77±0.02 kcal/100 g). *Parkia speciosa* seeds exhibited the highest protein content (28.61±0.05 g/100 g), while *Alpinia malaccensis* fruits had the lowest (7.43±0.04 g/100 g). Fat content was highest in *P. speciosa seeds* (17.18±0.07 g/100 g) and undetectable in its peel (0.00±0.00 g/100 g). Carbohydrates content was highest in *Baccaurea ramiflora* fruits (82.05±0.12 g/100 g) and lowest in *P. pellucida* leaves (48.85±0.08 g/100 g). *P. pellucida* leaves also showed the highest ash content (23.84±0.01 g/100 g). Antioxidant capacity, analyzed by ORAC, FRAP, and DPPH assays, showed distinct variations depending on the plant part. Leaves and young shoots, such as those from *Litsea petiolata* and *Anacardium occidentale*, demonstrated the highest antioxidant capacity, which correlated with their high flavonoid and phenolic compound content. *Spondias pinnata* and *Clausena cambodiana* leaves also showed high ORAC and FRAP values. Fruits, including *Ficus botryocarpa* and *Baccaurea ramiflora*, had moderate antioxidant activity, while *P. speciosa* seeds displayed lower values. The

peel of *P. speciosa* performed better than its seed, particularly in the FRAP and DPPH assays. Flowers, represented by *Etlingera elatior*, exhibited relatively low antioxidant and nutrient levels. These findings highlight the superior nutritional and antioxidant properties of leaves and young shoots, emphasizing their importance for functional food and dietary applications.

Keywords: Fish curry sauce dish, Traditional knowledge, Vegetable, Nutrition content, Antioxidant

Introduction

The incorporation of fresh vegetables with spicy foods in Southeast Asian cuisine can be attributed to multifaceted factors, Including culinary, cultural, and climatic considerations (Putra *et al*., 2023). The culinary rationale behind this practice is to achieving a sensorially balanced meal, in which the milder and often cooling attributes of fresh vegetables counteract the robust flavors inherent in spicy dishes (Fieldhouse, 2002). This combination provides a textural contrast, enhancing the overall gustatory experience, but also imparts a refreshing effect, particularly salient in the context of the warm and humid climate prevalent in the region. Furthermore, the nutritional enrichment afforded by the vitamins, minerals, and fiber present in fresh vegetables contributes to a comprehensive and balanced dietary profile (Pem and Jeewon, 2015). The prevalence of locally available, diverse, and tropical vegetables further underscores the practicality and sustainability of this culinary tradition. Rooted in historical culinary practices, the integration of fresh herbs and vegetables into Southeast Asian cuisines serves as a cultural cornerstone. This tradition is perpetuated through successive generations, shaping the gastronomic identity of the region (Zocchi *et al*., 2024). Moreover, the incorporation of flavorful dipping sauces and condiments, often featuring fresh herbs, citrus, and vegetables, is emblematic of the meticulous attention to taste and presentation in Southeast Asian culinary traditions. In a broader context, the utilization of fresh vegetables with spicy foods not only conforms to traditional practices but also aligns with a holistic approach to culinary aesthetics, nutritional value, and local agricultural abundance (Varzakas and Antoniadou, 2024). This is particularly evident in Nakhon Si Thammarat in southern of Thailand, where culturally ingrained culinary practices consistently incorporate vegetables into daily dietary habits. This cultural phenomenon reflects an intricate interplay among nutritional habits, agricultural practices, and regional cultural norms. The practice of including vegetables in every meal is a cultural hallmark, symbolizing a profound connection with the land and its agricultural heritage (Thomas *et al*., 2022). The ubiquity of vegetables aligns with the region's robust agricultural

practices, contributing to a distinctive cultural identity tied to agrarian traditions. The integration of vegetables into daily culinary practices acts as a conduit for the transmission of cultural values across generations, evident in familial and communal settings where meals become cultural rituals (Kennedy *et al*., 2021).

The preparation of rice noodles in fish curry sauce, a traditional Thai dish in Nakhon Si Thammarat, consistently incorporates locally sourced vegetables, emphasizing the importance of indigenous produce in maintaining the authenticity of the culinary tradition (Chamnian *et al*., 2024). This culinary practice aligns with the region's agricultural richness, where locally grown vegetables contribute not only to the dish's flavor profile but also to its cultural and historical roots (Cole *et al*., 2023). The use of these local vegetables underscores a sustainable and regionally distinct approach to culinary creation, ensuring that rice noodles in fish curry sauce remains a true reflection of Nakhon Si Thammarat's unique gastronomic identity (Chamnian *et al*., 2024). The rice noodles in fish curry sauce dish embodies profound cultural significance, serving as a culinary representation of the region's rich heritage and traditions. Beyond its role in daily cuisine, the dish holds cultural importance in social gatherings and ceremonial occasions, fostering community and contributing to the festive cultural fabric. Its use of locally sourced ingredients, diverse toppings, and harmonious flavor profiles reflect both the agricultural abundance of the region and the culinary creativity ingrained in local practices (Aster *et al*., 2023).

This study aimed to systematically assess the species diversity, nutritional composition, and antioxidant properties of indigenous vegetables commonly consumed with rice noodles in fish curry sauce in Nakhon Si Thammarat, Thailand. The research focuses on identifying and categorizing the plant species used in this traditional dish, analyzing their nutritional value, and evaluating their antioxidant potential. By highlighting the significance of these vegetables in local dietary habits, the study seeks to provide scientific insights into their health benefits and culinary importance. The findings will contribute to promoting the use of indigenous vegetables, supporting sustainable dietary practices, and preserving cultural food traditions in the region.

Research Methods

1. Sample collection sites

Plant samples were collected from residue-free gardens associated with a group of 11 entrepreneurs in Nakhon Si Thammarat Province, who are involved in rice noodle and fish curry sauce businesses. The entrepreneurs, with operational experience ranging from 3 to 25 years, operate under three business models: sole proprietorship, family business, and community business.

2. The plant collection and taxonomic identification

The procedure involves a sequential series of steps commencing with detailed observations of plant features, including leaves, flowers, stems, and reproductive structures. The following stages include collecting plant specimens, thoroughly documenting their characteristics, using botanical keys for identification, and conducting a comparative analysis with herbarium specimens or databases (Bailey, 1963). Expert confirmation from the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation in Bangkok, Thailand, is sought for accurate identification, particularly for complex plant groups or when uncertainties arise.

3. Plant sample preparation

For macronutrient analysis, plant samples were dried at 50 ℃ in an incubator for 48 hours and subsequently ground into a powder before analysis according to the next step. For antioxidant analysis, the dried samples (prepared following the same procedure) were extracted using methanol in a ratio of 1:10 (weight: volume) and refluxed using the T100 (BUCHI) apparatus at 70 ℃ for 2 hours. The resulting solution was transferred into a new extraction tube and evaporated to dryness at 70 ℃ for 10 minutes. The extract was then redissolved in 2 mL of phosphate buffer and centrifuged at 10,000 rpm for 5 minutes. The supernatant was subsequently transferred into a new 2-mL microtube. For phytochemical screening using thin-layer chromatography (TLC), The extract was reconstituted with 2 mL of methanol in a 2-mL microtube and centrifuged at 10,000 rpm for 5 minutes to remove debris.

4. Nutritional analysis

The Kjeldahl method for protein determination involves three key steps (Jung *et al*., 2003). In step 1: Mineralization, the sample was weighed and combined with a catalyst tablet, antifoam tablet, and sulfuric acid before being heated in stages (150 ℃, 250 ℃, and 350 ℃, respectively) over 5 hours to digest the organic matter. After cooling, water was added to the mineralized sample. Step 2: Distillation involves checking water and sodium hydroxide levels before distilling the mineralized solution for 8 minutes into an Erlenmeyer flask containing HCl, methyl red, and water. Step 3: Determination consists of titrating the unreacted HCl with sodium hydroxide to calculate the nitrogen content, from which the protein percentage was deduced. A control sample was also prepared under the same conditions to ensure accuracy.

For total fat, the procedure begins by accurately weighing 5 g of the sample into a thimble or flask and was dried in an oven at 102 ℃ for 5 hours. After drying, the thimble was placed in a Soxhlet liquid/solid extractor and approximately 90 mL of petroleum ether was added. The extraction unit is assembled over an electric heating mantle or water bath, and the solvent was heated until it boils, adjusting the heat source so that the solvent drips into the sample chamber at a rate of about 6 drops

per second. The extraction process was continued for 6 hours. After extraction, the unit was removed from the heat source, and the extractor and condenser are detached. The flask was returned to the heat source to evaporate the solvent in an oven at 60 to 80 ℃ and dried until a constant weight, which typically takes 1 to 2 hours. The flask was cooled in a desiccator and weighed with the contents. To calculate the fat percentage, the following formula was used Equation (1).

$$\text{Crude fat (\%)} = (W2 - W1) \times 100 / S \quad (1)$$

where W1 is the weight of the empty flask, W2 is the weight of the flask with the extracted fat, and S is the weight of the sample.

The protocol for carbohydrate content analysis in McLoughlin *et al*. (2023) involved defatting samples with over 10% fat, followed by incubation with pancreatic α-amylase and amyloglucosidase at pH 6.0 and 37 ℃ for 4 hours to simulate small intestine conditions, hydrolyzing digestible starch to glucose and maltose. After centrifugation, the supernatant is incubated with enzymes like sucrase, maltase, β-galactosidase, and oligo-1,6-α-glucosidase to hydrolyze sucrose, maltose, lactose, and isomaltose into glucose and fructose, 2-molecules of glucose and galactose, respectively. These monosaccharides were quantified using spectrophotometry based on NADPH formation at 340 nm, and their concentrations were summed to determine the total available carbohydrates in the sample (McLoughlin *et al*., 2023).

5. Phytochemical profile screening

Two microliters of the extract were spotted onto the 10 cm width x 20 cm length TLC plate using the LINOMAT5 (CAMAG) by VisionCATs program (ver.13.0) to control the position and spotting. The plate was set up for 15 samples with the starting point from the rim is 0.8 mm and solvent front set to 80 mm. The spotted plate was then developed in CAMAG ADC2 Automatic Developing Chamber containing the mobile phase as toluene, acetonitrile, and acetate in a ratio of 35:5:15 (Sintupachee *et al*., 2022). The developed plate was then documentation under 254 nm, 366 nm, and white light (the wavelength of was 400 - 700 nm). The plate then screened for the group of flavonoid and phenol by derivatization with NP-PEG reagent and Aluminium chloride reagent, respectively, and documentation under 366 nm.

6. Antioxidant activity

Oxygen Radical Absorbance Capacity (ORAC) measurement was conducted according to the procedure described by Ou *et al*. (2001). Briefly, the assay was performed in a Costar®96-well black opaque plate (Corning Costar). Each well contained 150 µL of 10 mM Fluorescein Sodium solution (Sigma-Aldrich). For the standard curve, 25 µL of various dilutions (200 µM - 12.5 µM) of 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (Sigma-Aldrich) were added. For samples, 25 µL of sample

dilutions prepared in phosphate buffer (10 mM, pH 7.4) were added. For the blank, 25 µL of phosphate buffer (10 mM, pH 7.4) was used. Each reaction mixture was homogenized, and fluorescence readings were immediately taken using a fluorescent microplate reader at 37°C, with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. The wells were read at intervals of 1 to 5 minutes for a total duration of 60 minutes. The antioxidant capacity relating to Trolox is calculated according to the following Equation (2)

$$\text{Antioxidant capacity} = \frac{AUC_{sample} - AUC_{blank}}{AUC_{std.} - AUC_{blank}} \quad (2)$$

Where AUC is Area Under the Curve.

L (+)- Ascorbic acid, epicatechin gallate, [2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH)] were obtained from Sigma-Aldrich. Different dilutions of Trolox® (200 µM - 12.5 µM) and sample compounds (ascorbic acid and epicatechin gallate, two known antioxidants) in 96-well microplate. In every working well the following was pipetted in triplicate. Ferric Reducing Antioxidant Power (FRAP) was done based on Benzie and Strain (1996) method. Briefly, the mixture of the reaction was added 190 µL of reaction mix into well containing the standard, positive control, and sample. Measure the absorbance immediately at 594 nm (A_{594}) at the kinetic mode for 60 min at 37°C.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted according to the method described by Gulcin and Alwasel, (2023). Briefly, each well of a 96-well microplate contained 200 µL of reaction mixture composed of 20 µL of sample (in 10 mM phosphate buffer, pH 7.4) or blank (using methanol), 0.1 M Tris-HCl buffer (pH 7.4), and 100 µL of DPPH solution (0.2 mM DPPH in 99.5% methanol). The inhibition ratio was calculated using Equation (3)

$$\text{Inhibitor ratio (\%)} = \frac{A594_{control} - A594_{sample}}{A594_{control}} \times 100 \quad (3)$$

Where A594 means for absorbance at 594 nm

A regression curve of the form y = ax + b was used to estimate the IC_{50} value, where IC_{50} = (50 - b) / a. Subsequently, the Trolox Equivalent Antioxidant Capacity (TEAC) was determined in $mg{\cdot}mL^{-1}$ = IC_{50} of Trolox/ IC_{50} of sample.

7. Statistical analysis

All analyses were conducted in triplicate. One-way ANOVA was performed to compare nutrient and antioxidant levels among different vegetable components, followed by Tukey's post-hoc test for significant differences ($p < 0.05$) using IBM® SPSS® software. Multiple regression analysis was employed to identify the key vegetable contributors to nutrient and antioxidant profiles.

8. Ethics

The research initiatives, endorsed by the Human Research ethics Committee Certificate COA No. REC073/65 form Nakhon Si Thammarat Rajabhat University, demonstrated adherence to universally accepted human research ethical norms.

Results

1. Plant diversity

A diverse collection of plants highlights the broad distribution of edible species across various families, genera, and plant parts used in the rice noodle with fish curry sauce dish. This diversity reflects both the ecological range and the functional roles these plants serve in local diets and traditions. Twenty-nine species of plants from 14 families were identified (Table 1). The plants used in the dish primarily come from leaves and young shoots (15 species), young leaves (8 species), seeds (3 species), fruits (2 species), and flowers (1 species). Plant taxonomy identification is a scientific process that categorizes and names plants based on morphological and genetic attributes, essential for understanding the diversity and usage of these species. Some of the plants presented in the rice noodle with fish curry sauce dish are rare and highly valued, making them standout items in local markets, but availability may be limited for future analysis.

The Table lists plants from various families, along with their scientific names, common names in Thai or English, and the plant parts used. Species from families such as Anacardiaceae, Asteraceae, Euphorbiaceae, and Zingiberaceae are commonly used, including plants like *Anacardium occidentale* (cashew nut tree) and *Spondias pinnata* (Jew's plum) for their leaves and shoots. The Zingiberaceae family is represented by plants like *Alpinia malaccensis* and *Zingiber zerumbet*, known for their young fruits and aerial parts, contributing aromatic and medicinal qualities. Additionally, Apiaceae plants, such as *Oenanthe javanica* (water celery) and *Centella asiatica* (Asiatic pennywort), provide their aerial parts for the dish.

Due to the limited availability of certain plant species, which are rare and typically collected by villagers from the mountains for sale (and cannot be cultivated locally), only 25 out of the 29 plant species were selected for comprehensive nutritional analysis. The remaining four species did not have enough material for a thorough nutritional evaluation. However, all 29 species were included in the phytochemical analysis using thin-layer chromatography (TLC). Despite the smaller sample sizes for some species, TLC proved effective due to its low material requirements, allowing for the detection and identification of phytochemicals in all samples. This method enabled a broad chemical analysis while accommodating the constraints on sample quantity.

2. Nutrition

The macronutrient content, including protein, carbohydrate, fat, and ash, in 25 vegetable samples was statistically analyzed using one way ANOVA. Significant differences were found at p-value < 0.05. For protein content, the analysis showed D_F = 24, F(24,25) = 1.740, p < 0.001; for carbohydrate content, D_F = 24, F(24,25) = 3.093, p < 0.001; for total fat content, D_F = 24, F(24,25) = 12.100, p < 0.001; and for ash content, D_F = 24, F(24,25) = 20.710, p < 0.001. The energy content also showed a significant difference, with D_F = 24, F(24,25) = 2.542, p < 0.0121.

The nutrient analysis of 25 plant species demonstrated distinct trends in energy, protein, fat, carbohydrate, and ash contents. Energy content was highest in CCa (448.53±34.44 kcal/100 g) and lowest in PP (288.77±0.02 kcal/100 g), with most species exhibiting energy values around 350 to 370 kcal/100 g. Protein content peaked in PSseed (28.61±0.05 g/100 g) and was lowest in AM (7.43±0.04 g/100 g), with a general trend of higher protein levels in seeds and lower in aerial parts. Fat content varied widely, with PSseed having the highest fat content (17.18±0.07 g/100 g), while PSpeel had undetectable fat levels (0.00±0.00 g/100 g); most species exhibited low fat levels below 2.5 g/100 g. Carbohydrate levels were highest in BR (82.05±0.12 g/100 g) and lowest in PP (48.85±0.08 g/100 g), with a trend toward higher values in fruit and peel-based samples. Ash content was most abundant in PP (23.84±0.01 g/100 g) and least in PSpeel (3.93±0.01 g/100 g), indicating a broad variation linked to plant part type and nutrient density (Table 2).

The nutrient composition from Table 2 shows clear correlations with the parts of the plants analyzed. Leaves and young shoots, which were the most frequently assessed plant parts (including species such as GL, LP, and SP), typically exhibited moderate to high protein contents, with energy values ranging from 322.32 to 368.28 kcal/100 g dry weight. These parts generally had low fat levels, often below 2 g/100 g, which aligns with their natural role as foliage with limited lipid reserves. In contrast, seeds such as those from PS demonstrated higher fat content, notably 17.18±0.07 g/100 g, as seeds tend to store oils for germination. Additionally, carbohydrate content was notably higher in fruit-derived samples like BR (82.05±0.12 g/100 g), reflecting the energy-rich nature of fruit tissues. Flowers, as represented by EE, had balanced but lower nutrient profiles due to their reproductive function. The ash content was highest in young leaves, particularly PP (23.84±0.01 g/100 g), suggesting a higher mineral concentration in these plant tissues. These correlations indicate that the plant parts analyzed exhibit characteristic nutrient profiles, aligning with their biological functions and typical metabolic compositions.

Table 1 Plant species and their part uses

No.	Plant Family	Scientific Name	Common Name Thai/ English	Part of Plant
1	Euphorbiaceae	*Glochidion littorale* Blume Baill: GL	มันปู/ Mon-Pu	Leaves young shoots
2	Lauraceae	*Litsea petiolata* Hook. f.: LP	ทัมมัง/ Litsea	Leaves young shoots
3	Anacardiaerae	*A. occidentale* L.: AO	มะม่วงหิมพานต์/ Cashew nut tree	Leaves young shoots
4	Moraceae	*Ficus botryocarpa* Miq.: FB	ลูกชิ้ง/ Duea ching	Fruits
5	Asteraceae	*Crassocephalum crepidioides* (Benth.) S.Moore: CC	ผักแกด/ Thickhead weed, Redflower rag leaf, Fireweed	Leaves
6	Anacardiaceae	*S. pinnata* (L.f.) Kurz.: SP	มะกอก/ Jew's plum	Leaves young shoots
7	Rutaceae	*C. cambodiana* Guill.: CCa	หมุรุย	Leaves
8	Euphorbiaceae	*B. ramiflora* Lour.: BR	มะไฟ/ Burmese grape	Young fruits
9	Apiaceae	*O. javanica* (Blume) DC.: OJ	ชีล้อม/ Water celery	Young leaves
10	Amaranthace	*Alternanthera sissoo* hort.: AS	ผักเป็ดญี่ปุ่น/ Brazilian spinach	Young leaves
11	Asteraceae	*Emilia sonchifolia* (L.) DC. ex Wight: ES	ผักกาดนกเขา/ Phak nok khao	Young leaves
12	Stilaginaceae	*Antidesma velutinosum* Blume.: AV	มะเม่า	Leaves
13	Asteraceae	*Eupatorium capillifolium* (Lam.) Small ex Porter & Britton: EC	โกศจุฬา/ Dogfennel	Young leaves
14	Apiaceae	*C. asiatica* Urban.: CA	บัวบก/ Asiatic pennywort	Young leaves
15	Gnetaceae	*Gnetum gnemon* Linn. var. tenerum Markgr.: GG	เหลียง เขลียง	Leaves young shoots
16	Alismataceae	*Limnocharis flava* (L.) Buchenau: LF	ผักพายใหญ่/ Yellow velvetleaf	Young leaves
17	Zingiberaceae	*A. malaccensis* (Burm.) Roscoe: AM	ข่าป่า/ Arrow root	Young fruits
18	Rutaceae	*Feroniella lucida* (Scheff.) Swingle: FL	มะสัง/ Wood apple	Leaves seeds
19	Zingiberaceae	*Z. zerumbet* (Linn.) Smith. ZZ	ทือ/ Wild ginger	Young leaves
20	Leguminosae	*Leucaena leucocephala* (Lamk.) de Wit.: LL	กระถิน/ White popinac	Leaves seeds

Table 1 (continued)

No.	Plant Family	Scientific Name	Common Name Thai/ English	Part of Plant
21	Zingiberaceae	*Etlingera elatio*r [Jack] R. M. Smith.: EE	ดาหลา/ Torch ginger	Flower
22	Scrophulariaceae	*Limnophila rugosa* (Roth) Merr.: LR	ราน้ำ/ Phak kachom	Young leaves
23	Leguminosae	*Archidendron jiringa* Nielsen: AJ	เนียง/ Jering	Fruits
24	Rutaceae	*Toddalia asiatica* (L.) Lam.: TA	เล็บรอก/ Forest pepper	Leave
25	Mimosoideae	*P. speciosa* Hassk.: PS	สะตอ/ Nitta tree	Seed
26	Fabaceae	*Parkia timoriana* (DC.) Merr.: PT	เหรียง/ Tree bean	Seed
27	Zingiberaceae	*Alpinia mutica* Roxb.: AMm	ปุด/ Orchid ginger	Young leaves
28	Piperaceae	*P. pellucida* (L.) Humb; Bonpl & Kunth.: PP	กระสัง/ Phak kra sang	Young leaves
29	Anacardiaceae	*Schinus terebinthifolius* Raddi: ST	มะตูมแขก/ Brazilian peppertree	Leaves

Table 2 Nutrient composition of 25 plant species used in rice noodles with fish curry sauce (per 100 g dry weight)

Vegetable species	Energy (kcal/100 g dry weight)	Protein (N x 6.25) (g/100 g dry weight)	Fat (g/100 g dry weight)	Carbohydrate (g/100 g dry weight)	Ash (g/100 g dry weight)
GL	356.37±0.14	15.85±0.41	0.59±0.01	71.90±0.53	5.51±0.01
LP	357.75±0.09	15.05±0.40	2.27±0.08*	69.27±0.59	4.34±0.11
AO	368.28±0.55	25.06±0.21**	1.56±0.05	63.48±0.18	4.48±0.01
FB	352.96±0.14	10.15±0.03	1.74±0.01	74.16±0.05*	8.57±0.07
CC	328.19±0.73	22.73±0.35	1.83±0.00	55.19±0.53	14.76±0.16*
SP	364.92±0.41*	12.48±0.37	1.24±0.02	75.95±0.32*	4.99±0.06
CCa	448.53±34.44**	16.10±0.01	0.64±0.01	70.98±0.20	6.99±0.03
BR	367.18±0.38	7.88±0.01	0.82±0.01	82.05±0.12**	5.54±0.01
OJ	346.69±0.14	15.31±0.11	2.11±0.06	66.62±0.20	12.44±0.04
AS	323.85±0.24	22.86±0.19*	1.47±0.04	54.79±0.23	17.37±0.05
ES	331.33±0.73	16.34±0.05	1.65±0.06	62.77±0.11	13.34±0.03
AV	350.06±0.07	16.19±0.08	0.92±0.01	69.24±0.12	6.44±0.02
AM	352.14±0.99	7.43±0.04	1.34±0.04	77.58±0.13	6.78±0.00
CA	340.68±0.16	15.18±0.06	1.02±0.03	67.69±0.17	11.61±0.03
GG	363.01±0.75	23.58±0.04*	2.06±0.05	62.52±0.04	5.75±0.02
LF	332.46±0.26	21.74±0.23	2.31±0.08**	56.19±0.01	14.35±0.03*
EC	342.64±0.46	21.74±0.16	2.46±0.04**	58.38±0.18	10.51±0.02
EE	322.32±0.04	24.54±0.32**	0.46±0.01	55.00±0.30	13.64±0.03
PP	288.77±0.02	21.06±0.01	1.01±0.03	48.85±0.08	23.84±0.01**
FL	350.20±0.55	17.74±0.22	1.10±0.02	67.32±0.13	7.83±0.02
ST	356.37±0.14	14.58±0.03	1.57±0.06	72.22±0.02	5.95±0.04
PSseed	357.75±0.09	28.61±0.05**	17.18±0.07	44.27±0.17	4.56±0.01
PSpeel	368.28±0.55*	8.17±0.25	0.00±0.00	81.17±0.37**	3.93±0.01
ZZ	352.96±0.14	12.12±0.01	0.55±0.25	76.86±0.05*	4.47±0.01
AMm	328.19±0.73	11.32±0.04	6.73±0.08	64.77±0.04	11.05±0.01

Note: - Values are expressed as mean ± standard deviation.

- Statistically significant differences were determined by Tukey's multiple comparison test (* $p < 0.05$; ** $p < 0.01$).

3. Phytochemical screening using the thin layer chromatography

A TLC plate where different phytochemicals are separated across the lanes. Each lane (numbered 1 to 28 according to the Figure 1) corresponds to different plant

extracts or samples. The bands represent various compounds separated based on their interaction with the TLC solvent system. The darker or more pronounced bands indicate a higher concentration of specific compounds. The intensity and distribution of bands vary across the samples, suggesting significant differences in the composition of phytochemicals. The movement of the bands up the plate reflects the relative polarity of the compounds, with less polar compounds migrating higher up the plate. The banding patterns show a range of compounds, indicating a diversity of chemical constituents in the different plant extracts. Figure 1 (a), the TLC plate has been visualized under specific conditions (likely after spraying with a reagent or under UV light) to highlight phenolic and flavonoid compounds. These compounds often fluoresce or change color when visualized under UV or treated with specific chemicals. These colors represent the reaction of phenolic or flavonoid groups, which are characteristic of many plant metabolites involved in antioxidant activity. Some lanes show prominent yellow or orange bands, particularly in lanes SP, OJ, AS, EC, and PSseed. This indicates that these plant extracts have high concentrations of flavonoids or phenolic compounds. Other lanes, such as LP, CC, and CA, show fainter yellow or orange bands, suggesting a lower concentration of these compounds. Flavonoid-rich extracts: Based on the lower panel, samples in lanes CCa, AS, AM, EE, and PSpeel show distinct yellow-orange fluorescence, indicating they may be particularly rich in flavonoids. The distribution of phenolic compounds varies widely, with some extracts showing strong signals and others having minimal or no detectable phenolics. This variation highlights the chemical diversity across the different samples. The clear differentiation of bands across lanes suggests the presence of unique phytochemical profiles among the samples, particularly in lanes with stronger fluorescence or more distinct patterns. This TLC phytochemical analysis shows considerable variation in the phenolic and flavonoid content across the different samples. The presence of yellow or orange bands in the lower panel indicates that several of the extracts contain significant levels of flavonoids and phenolic compounds, which may correlate with their antioxidant or medicinal properties. Samples in lanes with strong bands are particularly rich in these compounds, while others show lower or negligible amounts.

4. Antioxidant activity

The antioxidant analysis using ORAC and FRAP revealed that all 25 types of vegetables showed significantly different values according to the one way ANOVA test (p-value < 0.05). For ORAC, $F(23, 24) = 7{,}560$, $p < 0.001$, and for FRAP, $F(23, 24) = 762.3$, $p < 0.001$. Similarly, the DPPH test showed $F(23, 24) = 1{,}809$, $p < 0.001$.

The Table 3 presents the antioxidant capacity of various samples measured using three different assays: ORAC, FRAP, and DPPH, expressed in micromoles of Trolox

equivalents (μmoles TE). Among the samples, LP (2,226,877.70 μmoles TE) and AO (2,029,005.34 μmoles TE) exhibit the highest ORAC values, indicating their superior oxygen radical absorbance capacity. In the FRAP assay, AO (127,025.02 μmoles TE) and PSpeel (119,129.44 μmoles TE) show the highest ferric reducing power. The DPPH assay, which measures free radical scavenging activity, highlights AO (202,077.54 μmoles TE) and ST (172,049.59 μmoles TE) as the most effective samples. Conversely, GG (2,696.88 μmoles TE) and PSseed (1,738.24 μmoles TE) exhibit the lowest DPPH values, suggesting weak radical scavenging abilities. Samples like AS and PSseed show consistently low values across all assays, whereas ST and PSpeel perform strongly in multiple tests. The variability in results across different methods underscores the complexity of antioxidant activity, with some samples excelling in one assay but not others. These findings highlight significant differences in antioxidant properties among the tested samples, which could be valuable for nutritional and pharmaceutical applications.

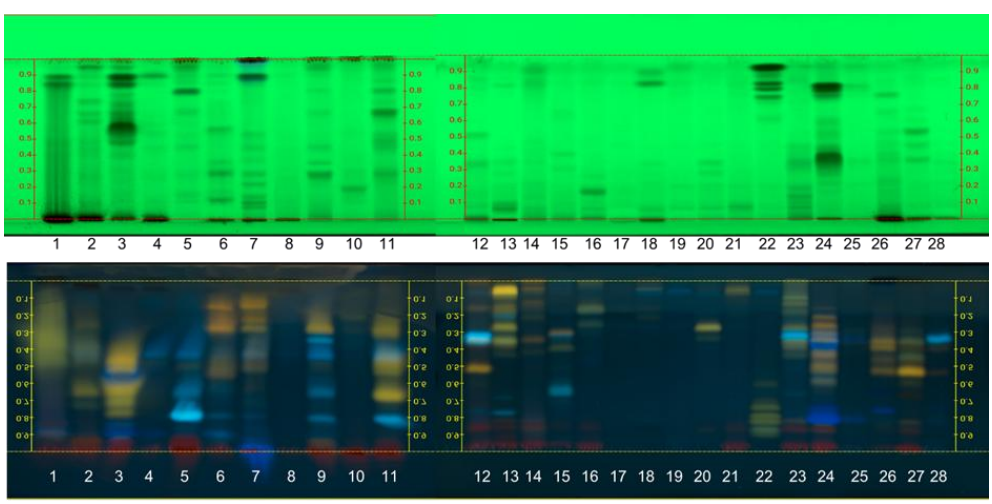

Figure 1 Phytochemical profile on TLC plate under 254 nm UV luminescent (a) and the chemical reaction show the antioxidant activity visualized under 366 nm (b). lane 1: GL, lane 2: LP, lane 3: AO, lane 4: FB, lane 5: CC, lane 6: SP, lane 7: CCa, lane 8: BR, lane 9: OJ, lane 10: AS, lane 11: ES, lane 12: AV, lane 13: AM, lane 14: CA, lane 15: GG, lane 16: LF, lane 17: EC, lane 18: EE, lane 19: PP, lane 20: FL, lane 21: ST, lane 22: PSseed, lane 23: PSpeel, lane 24: LR, lane 25: LL, lane 26: TA, lane 27: ZZ, lane 28: Amm

Table 3 Antioxidant capacity of 25 plant species measured using ORAC, FRAP, and DPPH assays (μmoles TE)

Vegetable species	ORAC (μmoles TE)	FRAP (μmoles TE)	DPPH (μmoles TE)
GL	90,152.94±273.67	87,007.63±118.08*	23,195.47±371.26
LP	2,226,877.70±24,050.39**	81,082.32±2,259.33	153,529.96±3,042.65*
AO	2,029,005.34±35,663.08**	127,025.02±129.43	202,077.54±5,136.78**
FB	100,204.73±2,307.55	76,690.27±1,024.83	91,447.21±1,266.15
CC	80,922.04±380.76	17,135.65±454.68	11,028.01±88.44
SP	95,239.86±1,531.88	37,084.13±1,210.98	25,126.12±556.80
CCa	148,870.65±6,472.70	88,007.36±2,229.80*	80,146.35±375.23
BR	80,663.77±3,357.15	14,842.84±94.57	12,165.81±291.24
OJ	125,748.09±1,434.46	20,017.48±256.06	20,951.36±280.73
AS	76,588.35±2,669.91	2,790.93±52.86	4,586.95±113.63
ES	114,769.01±3,584.66	27,546.12±1,383.86	12,668.62±53.61
AV	175,301.73±6,265.86*	76,613.04±1,426.61	45,841.06±1,084.11
AM	41,343.16±276.13	21,006.61±321.39	18,549.35±124.20
CA	38,049.40±199.74	11,898.98±43.69	8,135.53±136.05
GG	25,880.96±1,069.26	4,766.22±149.50	2,696.88±37.89
LF	27,926.87±223.27	7,275.84±320.74	3,705.74±56.08
EC	34,040.82±1,572.67	9,662.06±159.19	3,978.11±78.55
EE	23,874.13±1,096.92	6,748.32±354.21	4,322.02±79.18
PP	32,127.30±972.50	7,479.89±227.86	3,475.13±61.91
FL	173,554.74±10,051.06*	15,175.10±425.35	7,791.63±67.51
ST	128,574.43±4,012.84	114,892.53±6,347.96**	172,049.59±2,346.93*
PSseed	10,422.38±542.50	2,420.52±70.93	1,738.24±52.48
PSpeel	95,870.00±5,037.50	119,129.44±6,520.55**	135,391.63±7,357.64*
ZZ	125,766.19±1,532.68	30,614.09±1,397.57	24,463.93±390.80
AMm	131,580.93±2,702.06	25,519.87±1,429.60	6,609.44±141.46

Note: - Values are expressed as mean ± standard deviation.
- Statistically significant differences were determined by Tukey's multiple comparison test (* $p < 0.05$; ** $p < 0.01$).

The antioxidant activity of different plant parts was analyzed using ORAC, FRAP, and DPPH assays, highlighting variations among leaves, young shoots, fruits, seeds, and flowers. Among the leaves and young shoots, LP and AO exhibited the highest ORAC values, suggesting strong radical absorption capacities. Cashew Nut Tree (AO) also showed the highest DPPH value, indicating potent free radical scavenging ability. The SP and CCa leaves also demonstrated high antioxidant capacity, particularly in ORAC and FRAP assays. Fruits, such as FB and BR, exhibited moderate antioxidant activity, while

seeds of PS and PT showed lower values across all assays, indicating weaker antioxidant properties. The peel of PSpeel performed significantly better than its seed, especially in FRAP and DPPH assays. *A. malaccensis* (AM) and *A. mutica* (AMm), both from the Zingiberaceae family, displayed moderate antioxidant potential, with AMm having higher ORAC and FRAP values compared to AM. The flower of EE showed relatively low antioxidant capacity compared to other plant parts, suggesting that leaves and young shoots generally contain higher antioxidant compounds. Overall, the results indicate that plant parts such as leaves and young shoots possess the highest antioxidant activity, whereas seeds and flowers exhibit relatively lower potential. This suggests that the choice of plant part plays a crucial role in determining antioxidant benefits for nutritional or pharmaceutical applications.

Discussion

The findings of this study highlight the significant variations in antioxidant activity among different plant species, emphasizing the influence of plant parts on antioxidant potential. The results align with previous studies demonstrating that leaves and young shoots generally exhibit higher antioxidant capacities compared to seeds, fruits, and flowers (Fieldhourse, 2002; Kennedy *et al*., 2021; Yakoh, 2023; Zocchi *et al*., 2024). The high ORAC and DPPH values observed in *L. petiolata* (LP) and *A. occidentale* (AO) can be attributed to their high flavonoid and phenolic compound content, as confirmed by thin-layer chromatography (TLC) analysis. These results are consistent with findings from previous research indicating that polyphenols and flavonoids contribute significantly to antioxidant activity (Sintupachee *et al*., 2022).

The variation in antioxidant potential among plant parts suggests a metabolic adaptation in different plant structures. Leaves, being primary photosynthetic organs, accumulate high levels of antioxidants to counteract oxidative stress caused by environmental factors such as UV radiation and pathogens (Thomas *et al*., 2022). In contrast, seeds and fruits tend to have lower antioxidant activity, as they are primarily storage organs with different metabolic functions. This observation is supported by the relatively low antioxidant values seen in *P. speciosa* (PSseed) and *F. lucida* (FL), which were among the lowest in all three assays (ORAC, FRAP, and DPPH).

The strong antioxidant performance of *P. speciosa* peel (PSpeel) over its seed further corroborates the role of plant parts in determining antioxidant potential. The outer layers of fruits and seeds are often rich in phenolic compounds, which act as protective barriers against environmental stressors (Cole *et al*., 2023; Szerlauth *et al*., 2019). This is reflected in the significantly higher FRAP and DPPH values of PSpeel compared to PSseed. These findings suggest that plant by-products such as peels could be valuable sources of natural antioxidants for functional food applications.

In comparison to other studies focusing on indigenous vegetables, our results align with research on Southeast Asian plants, which frequently demonstrate high antioxidant potential due to their diverse phytochemical composition (Varzakas and Antoniadou, 2024; Putra *et al*., 2023). The traditional consumption of these plants in Thai cuisine, particularly in dishes like rice noodles with fish curry sauce, not only enhances flavor profiles but also contributes to health benefits by providing dietary antioxidants (Fieldhouse, 2002).

The results of this study also have implications for dietary recommendations and functional food development. The high antioxidant values observed in LP, AO, and AV suggest that these plants could be incorporated into functional foods or nutraceutical products. Further studies should focus on isolating the specific bioactive compounds responsible for the high antioxidant activity in these plants and evaluating their bioavailability and health benefits in human studies.

Overall, this study underscores the importance of plant selection and part utilization in maximizing antioxidant intake. The results support the notion that incorporating a diversity of plant parts, particularly leaves and young shoots, into the diet can significantly enhance antioxidant consumption. Future research should explore the commercial potential of these plants in the food and pharmaceutical industries and their role in mitigating oxidative stress-related diseases.

Conclusion

This study assessed the diversity, nutritional content, and antioxidant activity of indigenous vegetables commonly used in Thai cuisine, particularly in rice noodles with fish curry sauce. The findings revealed significant variability in antioxidant capacity among plant species, with leaves and young shoots exhibiting the highest levels of antioxidant activity. *L. petiolata* (LP) and *A. occidentale* (AO) have significant ORAC and DPPH values, indicating their potential as natural antioxidants and strengthening their position in functional food applications. The concept for this study came from a profound love for traditional Thai culinary traditions, in which locally obtained veggies are vital components of many recipes. By scientifically confirming the antioxidant qualities of these plants, this study promotes the preservation and promotion of Thai culinary traditions with a health focus. Incorporating antioxidant-rich vegetables into traditional cuisines not only maintains cultural history, but it also provides a long-term solution for boosting dietary health. These findings provide valuable insights for the food industry, promoting the use of indigenous Thai vegetables in nutraceutical development and dietary recommendations. Future research should focus on the bioavailability of these antioxidants and their potential role in disease prevention,

further bridging the gap between traditional culinary wisdom and modern nutritional science.

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