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Total phenolic, flavonoid contents, and antioxidant activity of strawberries and local medicinal plants

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Abstract

In this study, we assess and compare the total phenolic content (TPC), total flavonoid content (TFC), tannin content (TC), free radical scavenging activity, and ferric reducing antioxidant power in seven local plants. Two strawberry cultivars (*Fragaria x ananassa* Duch.) with five native medicinal plants, namely, *Pandanus amaryllifolius*, *Cymbopogon citratus*, *Centella asiatica*, *Tiliacora triandra*, and *Melissa officinalis*, are investigated. All the plant extracts were found to contain phytochemical content with antioxidant activity. The leaves of the strawberry "American jumbo" exhibit the highest levels of TPC and TC, while *C. asiatica* shows the highest levels of TFC ($p < 0.05$). No significant difference was observed between the free radical scavenging activities of both strawberry cultivars. However, these cultivars exhibited a significant difference ($p < 0.05$) from others based on the 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay. Therefore, this study clearly reveals that water-soluble extracts of strawberry "Pharachatan 80" leaves is the most promising source of potential natural antioxidants among these plant extracts.

Keywords: DPPH, FRAP, Medicinal plants, Phenolic compound, Strawberry "Pharachatan 80"

1. Introduction

Medicinal plants contain high levels of antioxidants and are utilized in pharmaceuticals, aroma compounds, and food additives [1]. The majority of phytochemical compounds are widely used as medications or recreational substances. Phenolic and flavonoid chemicals are natural antioxidants found in plants and are a frequent secondary metabolite category with significant pharmacological activity. Numerous studies revealed that flavonoids, including rutin and catechin, can inhibit the proliferation of cancer cells in the human body [2, 3]. The most prominent dietary phenolic compounds produced by plants are classified as phenolic acids, tannins, and flavonoids. Some phenolic compounds dissolve in organic solvents such as ethanol and acetone, while others are soluble in water; however, the main class of phenolic molecules is insoluble in water [4]. Furthermore, phenolic compounds are actively involved in crop plant defense mechanisms against biotic and abiotic stress, and they are strong against the effects of reactive oxygen species (ROS) [5]. A free radical can be converted into a stable molecule by a hydrogen atom in its hydroxyl (OH) group, which is contained in the phenolic compound's structure [6].

Strawberry (*Fragaria x ananassa* Duch.) is one of the world's most economically and commercially important crops. Moreover, its products, especially berries, have high concentrations of bioactive molecules and contain health-promoting components such as phenolic and antioxidant compounds. Both fresh and processed strawberries are suitable not only for making yogurt, jam, and jellies but also for medicinal purposes [7]. However, strawberry leaves were found to have a higher antioxidant capacity than berries, and the extract of strawberry leaves has also demonstrated direct, endothelium-dependent vasodilator activity [8]. This is possibly due to the antioxidant properties of strawberries as well as those of other medicinal plants used in herbal medicine products [9]. Accordingly, strawberry consumption is continuing to increase in prominence among consumer demand. However, evaluating the abundance of bioactive compounds and their activities in several plants is difficult because they

are related to the cultivated variety of plants and are affected by the natural environmental conditions of the growing region [9].

In Thailand, the cool northern regions are the most suitable for commercially growing strawberries, as they produce a continuous crop throughout the growing season. Strawberries are the most valuable small fruit with significant nutraceutical content, which consumers appreciate. Therefore, in the recent past, strawberries have been extensively studied, but only in the fruit part. In contrast, there has been limited research on the phytochemical substances and antioxidant activity of other components of each strawberry cultivar that could potentially be advantageous for human health. Most studies focused on the concentrations of catechin, agrimoniin, quercetin glycoside, and kaempferol diglucoside in leaves. Unfortunately, the phenolic compounds have not been examined practically. Moreover, strawberry extraction methods, including the content of phenolic compounds and antioxidants, are untested and poorly understood. As mentioned above, there is also still insufficient information concerning the quantity of phenolic compounds and antioxidants in the different vegetative parts of strawberries, including native medicinal plants, for effective pharmaceutical preparations in the future.

Our study emphasizes the antioxidant evaluation of strawberry leaf extracts, including local medicinal plant species, namely, *Pandanus amaryllifolius* Roxb., *Cymbopogon citratus* DC., *Centella asiatica* L., *Tiliacora triandra* Colebr., and *Melissa officinalis* L., because of their common properties and growth in local and regional conditions and their potential benefits in the production of pharmaceuticals and plant-based supplement options in the future. Additionally, a comparison of reference antioxidants between strawberries and local medicinal plants has not been studied yet. For the present study, two cultivars of strawberries, "Pharachatan 80" and "American jumbo," were selected based on their ability to grow well in both the northern area and the highlands of the central region of the country, including the southern regions, where there is mild weather and abundant rainfall with low humidity. Moreover, the two cultivars also had a remarkably high total soluble solid content [10]. The present study aims to determine the antioxidant activity and total phenolic content (TPC) of strawberry leaves in "Pharachatan 80" and "American jumbo" and five local medicinal plants in water-soluble extract conditions. Furthermore, the correlations between antioxidant activities and phytochemical constituents were measured to serve as a basis for developing naturally antioxidant-enriched fruit for consumption and use in pharmaceutical preparations for future research.

2. Materials and methods

2.1 Plant sample preparation

The plant parts and the profiles of the parts used in this study are listed in Table 1. Two strawberry cultivars were grown under greenhouse conditions at Srinakharinwirot University in Bangkok, Central Thailand, for 30 days at a controlled temperature and humidity of approximately 20–25°C and 65%–75%, respectively. The cultivars were selected for uniformity based on diameter and stem height. Five medicinal plants were obtained from local markets in Bangkok. A completely randomized design with five replications was used. Plant samples were dried for one week in a hot air oven at 30°C before being blended with a blender. One-gram powdered samples of each plant were packed and sealed in plastic bags for future infusion.

Table 1 Profiles of plant parts extracted and evaluated for antioxidant activity and total phenolic content (TPC).

Common name	Scientific name	Taxonomic family	Plant parts
Strawberry "Pharachatan 80" (St.P80)	*Fragaria x* ananassa Duch.	Rosaceae	leaf
Strawberry "America jumbo" (St.AJ)	*Fragaria x* ananassa Duch.	Rosaceae	leaf
Pandan (PD)	*Pandanus amaryllifolius* Roxb.	Pandanaceae	leaf
Lemongrass (LG)	*Cymbopogon citratus* DC.	Poaceae	leaf and stem
Centella (Cen)	*Centella asiatica* L.	Apiaceae	leaf
Bamboo grass (BG)	*Tiliacora triandra* Colebr.	Menispermaceae	leaf
Mint (M)	*Melissa officinalis* L.	Lamiaceae	leaf

2.2 Plant preparation of water-soluble extracts

All plant extracts were obtained using the water-soluble extraction method, according to the modified method of Chan et al. [15]. An aqueous extract was performed by mixing 1 g of each dried powdered sample in 100 mL of distilled water at 80°C for 1 h. The extracts were filtered and stored at -20°C for future analysis. Five replications of the study were performed, and all parameters were measured.

2.3 Total phenolic content (TPC) determination

The TPC of all plant extracts was determined through some modifications to the Folin–Ciocalteu assay as described by Chan et al. [11]. Briefly, 0.3 mL of sample extract and 10% (v/v) Folin–Ciocalteu phenol reagent (1.5 mL) were placed in a test tube and mixed in a vortex. After 3 min of incubation, 1.2 mL of 7.5% (w/v) Na_2CO_3

was added to neutralize the reaction, and then 8 mL of distilled water was added. The test tubes were incubated at room temperature for 30 min in the dark. Then, a diode array spectrophotometer (DU 800 Series, Beckman Coulter, Inc., Fullerton, California, USA) was used to measure the absorbance of the blue color of the supernatant at 765 nm. The TPC in all plant extracts was calculated and expressed as milligrams of gallic acid equivalent (GAE) per 100 mL of plant extract sample.

2.4 Total flavonoid content (TFC) determination

The TFC of plant extracts was determined using the modified method of Zhishen et al. [12] after mixing 0.5 mL of the extract and 0.3 mL of 5% (w/v) $NaNO_2$ for 6 min. Then, each tube was filled with 0.15 mL of 10% (w/v) $AlCl_3$. After 5 min, 0.5 mL of NaOH (1 M) and 2.5 mL of distilled water were added and incubated at room temperature for 30 min in the dark. The absorbance was measured at 510 nm against a blank devoid of plant extracts prepared in distilled water. Subsequently, the TFC of a plant extract sample was determined using a catechin standard curve and expressed as milligrams of catechin equivalents (CEs) per 100 mL.

2.5 Tannin content (TC) determination

The Folin–Ciocalteu assay, modified by the methods of Siddhuraju and Manian [13], was used to determine the TC. The plant sample was mixed with 0.3 mL of 100 mg/mL polyvinyl polypyrrolidone and centrifuged at 4,000 rpm for 10 min. Then, 0.3 mL of supernatant was mixed with 10% (v/v) Folin–Ciocalteu reagent and 7.5% (w/v) $NaCO_3$ to determine free phenolic content in a water-soluble extract of the plant sample and the TC in the sample was calculated as follows:

$$TC = TPC - \text{free phenolic content.}$$

The result was expressed as milligrams of GAE per 100 mL of water-soluble plant extracts.

2.6 Free radical scavenging activity determination

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to determine the free radical scavenging activity. The DPPH assays were conducted using the method modifications described by Pham et al. [14]. From each extract, 2.0 mL was vigorously vortexed with DPPH solution (2.8 mL/0.1 mM). After 30 min at room temperature for the mixture's incubation, the absorbance was measured at 515 nm. Percentage (%) inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(Ac - As)/Ac] \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the sample in the presence of extracts. Each extract's antioxidant activity was expressed in milligrams of ascorbic acid equivalents (mgAAE) per 100 mL of the sample solution.

2.7 Determination of ferric reducing antioxidant power (FRAP)

With a few modifications, the FRAP of plant extracts was assessed using the Benzie and Strain method [15]. A FRAP reagent was prepared by mixing acetate buffer (300 mM; pH 3.6), a solution of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine in 40 mM HCl and 20 mM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. The FRAP reagent (2.8 mL) and sample solutions (0.2 mL each) were added to each well and mixed thoroughly. After 30 min, absorbance was measured at 593 nm and calculated using an ascorbic acid standard curve. The results were expressed as mgAAE per 100 mL of plant-soluble extract.

2.8 Statistical analysis

One-way analysis of variance, calculated using SPSS 23 (SPSS Inc., Chicago, IL, USA), was used to examine the differences between processes. Data were presented as mean ± standard error for all five replicated analyses, and the mean difference was calculated through Duncan's multiple comparison test. *P* values less than 0.05 were considered statistically significant. Experimental results were further analyzed for the Pearson correlation coefficient (r) between TPC and antioxidant activity. Furthermore, different antioxidant assays and their significance were tested using a Student's t-test ($p < 0.05$).

3. Results and discussion

3.1 Levels of TPC, TFC, and TC in plants

Table 2 demonstrates that TPC was the predominant phenolic compound in all plant extracts. The highest TPC level was found in strawberry "American jumbo" (37.25 mg of GAE/100 mL), whereas the lowest level of TPC was in pandan (7.18 mg of GAE/100 mL). In the case of TFC, the highest level was observed in centella (9.77 mg of CE/100 mL). Other plants had relatively low levels of TFC within the range of 1.02–5.00 mg of CE/100 mL. The TC levels were found in the following order: strawberry "American jumbo" (17.57 mg of GAE/100 mL) > mint (17.13 mg of GAE/100 mL) > strawberry "Pharachatan 80" (13.35 mg of GAE/100 mL) > centella (9.69 mg of GAE/100 mL) > bamboo grass (3.52 mg of GAE/100 mL) > lemon grass (3.19 mg of GAE/100 mL) > pandan (3.16 mg of GAE/100 mL). Recent investigations revealed that all the evaluated plants were relatively high but not extremely high in TFC and TC. In our comparison of two cultivars of strawberries, however, "American jumbo" exhibited higher levels of TPC, TFC, and TC than did "Pharachatan 80" ($p < 0.05$).

Table 2 Total phenolic content (TPC), total flavonoid content (TFC), tannin content (TC), radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP) and IC_{50} of water-soluble extracts from strawberry leaves, and local medicinal plants.

Cultivar	TPC (mgGAE/100 mL)	TFC (mgCE/100 mL)	TC (mgGAE/100 mL)	DPPH (mgAAE/100 mL)	IC_{50} (mg/mL)	FRAP (mgAAE/100 mL)
St.P80	24.31 ±1.80	3.38 ±0.19^{A}	13.35 ±1.61	12.80 ±0.05^{B}	1.95 ±0.17^{A}	34.11 ±1.91
St.AJ	37.25 ±1.35^{B}	5.00 ±0.16^{B}	17.57 ±1.07^{B}	12.48 ±0.02^{B}	1.01 ±0.05^{A}	49.87 ±1.10
PD	7.18 ±0.27^{A}	1.02 ±0.04	3.16 ±0.26^{A}	7.68 ±0.40	9.74 ±0.33^{B}	3.68 ±0.05
LG	8.22 ±0.24^{A}	3.20 ±0.13^{A}	3.19 ±0.24^{A}	11.01 ±0.20^{A}	7.20 ±0.13	8.49 ±0.58^{A}
Cen	19.40 ±0.64	9.77 ±0.16	9.69 ±0.56	11.99 ±0.14	2.99 ±0.04	18.27 ±0.59
BG	8.47 ±0.29^{A}	2.90 ±0.13^{A}	3.52 ±0.34^{A}	10.69 ±0.38^{A}	9.55 ±0.49^{B}	9.19 ±0.37^{A}
Mint	35.85 ±0.60^{B}	4.92 ±0.12^{B}	17.13 ±1.16^{B}	10.79 ±0.09^{A}	1.62 ±0.03^{A}	39.16 ±0.86

The values are mean ± SE (n = 5). The same letter within a column is not significantly different ($p \geq 0.05$).

Strawberry and mint are sun plants that absorb significant amounts of radiation; thus, in this study, the leaves of both strawberry cultivars and mint had significantly high TPC. Because they are exposed to bright sunlight, which requires the ability to protect against ROS in the cell, phenolic compounds with a higher content of hydroxy groups have effective ROS scavenging ability [16]. Similarly, Oviedo-Solís et al. [17] reported that UV-B radiation absorption increased the content of phenolic compounds in strawberries. Meanwhile, flavonoids are widely known to be an essential component of phenolic compounds, which are common antioxidants with electron-donating abilities and can be found in all plant parts [18]. Several studies revealed that the leaves of various plant species have a high content of phenolic compounds, which may be related to their phenolic content and antioxidant activity. Additionally, phenolic compounds and flavonoids were found in higher concentrations in organs with higher UV-B absorption and younger leaves [19]. Phenolic compounds are widely used to detoxify free radicals, which are found more abundantly in the leaves of the plant. For instance, *Beta vulgaris* (blood turnip), *Petroselinum crispum* (parsley), and *Coriandrum sativum* (Chinese parsley) contain more phenolic compounds in their leaves than in other parts [20, 21].

Our finding in the case of local medicinal plants is in agreement with that of other authors. The leaf extract of *P. amaryllifolius*, commonly known as pandan, which grows well under naturally partial shade sunlight, exhibited the lowest total antioxidant activity when compared to other plants. It is possible that phenolic compounds did not accumulate in large amounts within the plant cell if they were grown in shaded areas [22].

In addition, the various contents of phenolic and flavonoid compounds extracted from medicinal plant species (*P. amaryllifolius*, *C. citratus*, *C. asiatica*, *T. triandra*, and *M. officinalis*) contribute to differing antioxidant potentials. Because of seasonal variations in plant growth, the amounts of different phenolic compound quantities are also different [23]. The results of this investigation agree with Muniyandi et al. [24], who discovered a strong relationship between tannin and DPPH. Based on the similarities in the phenolic, tannin, and flavonoid content of seven different cultivars, our findings may suggest that the cultivars are potent antioxidant sources.

3.2 Free radical scavenging and ferric ion reducing potential in plants

The experimental results of antioxidant activity are summarized in Table 2. High DPPH free radical scavenging activities ranging from 12.48 to 12.80 mgAAE/100 mL were observed in both strawberry cultivars; however, these activities were not statistically significant ($p > 0.05$). Based on IC_{50} values, the highest scavenging activity (lowest IC_{50} value) was recorded for water-soluble extracts of "American jumbo" (1.01 mg/mL), mint (1.62 mg/mL), and "Pharachatan 80" (1.95 mg/mL), which had a statistically significant difference ($p < 0.05$). No significant difference in IC_{50} values between strawberries was observed. "Pharachatan 80" exhibited the three highest FRAP activity values of 49.87, 39.16, and 34.11 mgAAE/100 mL, whereas pandan exhibited the lowest activity value

of 3.68 mgAAE/100 mL. In the current study, the comparative results of the seven species indicated the total antioxidant strawberry activities decreased in the following order: "American jumbo" ≥ "Pharachatan 80" > mint > centella > lemon grass > bamboo grass > pandan.

In this study, DPPH and FRAP were used to determine antioxidant activity, which provided great reliability for the antioxidant's reducing capacity of plant extracts, implying the quality of consumed food products. Our finding was that the correlation of flavonoid content at different times was not significantly related to the FRAP assay ($p \leq 0.05$); however, other studies demonstrated that many fruits and vegetables have a strong positive correlation ($p \leq 0.05$) between phenolic compound content and antioxidant activity potential [25]. Previous phytochemical studies have identified a significant concentration of phenolic components and substantial antioxidant activity in Thai herbal teas. In the case of the FRAP, flavonoid content does not seem to be associated with FRAP assay results. This is similar to the evaluation of phenolic compounds and other metal-ion chelating assays in plant cell extracts when compared to potassium ferricyanide reducing power and cupric reducing antioxidant power assays [26].

The DPPH assay showed that the DPPH molecule is a stable, synthetic-free radical. This confirms that phenolic compounds and the primary antioxidant stabilize the DPPH radical into a stable molecule. There are essential antioxidants in plants that are almost no different from other phytochemical constituents in terms of free radical scavenging [5]. Accordingly, the ability of electron donation or the antioxidants' ROS scavenging activity is due to the number of hydroxyl groups, which may be enhanced by the amount of phenolic compounds [8].

3.3 Correlation analysis

To correlate phytochemical contents with antioxidant activities, correlation coefficients (r) were calculated for the seven plants analyzed (Table 3). The antioxidant activities obtained from both the FRAP and DPPH assays were found to have a strong and significant correlation with TPC, TFC, and TC. A significant positive correlation with the TC and FRAP ($p < 0.01$) was observed. Our findings revealed that TFC has a significant positive correlation ($p < 0.05$) with TC and DPPH ($p < 0.01$). For TC, there is a significant positive correlation ($p < 0.01$) between FRAP and DPPH. It is possible that the phenolic compounds of plants are the primary components responsible for the free radical scavenging activity and do not affect the reducing ability of leaves. In addition, the FRAP ability of condensed tannins may also make tannins a vital source of antioxidant capacity [27].

This study identified a significant positive correlation between the DPPH and FRAP assays and the TC of strawberry leaves, indicating remarkably high antioxidant activity in the leaves. The flavonoids and phenolic acids are highly positively correlated with antioxidant capacity [27]. The close relationships between antioxidant activity and phenolic compounds were confirmed, particularly in the case of berry fruits such as strawberries, berries, and cherries [28–30].

Table 3 Correlation between the matrix of antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and tannin content (TC) in water-soluble extracts from strawberry leaves and local medicinal plants.

Parameters	TPC	TFC	TC	DPPH	FRAP
TPC	1	0.404*	0.980**	0.535**	0.967**
TFC		1	0.402*	0.529**	0.314
TC			1	0.555**	0.939**
DPPH				1	0.630**
FRAP					1

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

4. Conclusion

The higher phenolic contents and antioxidant activity were clearly exhibited in "Pharachatan 80" and "American Jumbo" than in local medicinal plant extracts. More importantly, leaf extracts of "Pharachatan 80" have a high content of antioxidant activities, which is correlated with the leaves' high levels of phytochemical constituents. Based on the above results, we suggest the future use of various bioactive compounds from strawberries and local medicinal plants for pharmaceuticals and natural food production. However, further investigations are warranted to establish supportive information demonstrating phenolic compounds and flavonoid compositions, antioxidant activities, physiochemical characteristics, and their relationships to environmental factors in various growing areas under natural climate conditions.

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