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Chemical constituents, antioxidant, and anticancer activities of bee pollen from various floral sources in Taiwan

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Abstract

Bee pollen has been traditionally used for health promotion. However, the chemical constituents and pharmaceutical effects of bee pollen strongly depend on their sources. This study determined chemical compositions and evaluated the antioxidant and anticancer activity of six bee pollen samples from Taiwan. The Cs sample contained the highest amounts of carotenoid (417.67 mg/g DW) and anthocyanin (10.96 μmol/g DW) while the Nn sample showed the highest content of chlorophyll (Chl) *a* (23.39 mg/g DW) and Chl *b* (39.17 mg/g DW). The highest flavonoid (11.69 mg QE/g DW) and phenolic content (42.91 mg GAE/g DW) were found in Bp and Pm samples, respectively. The highest Fe^{2+}-chelating ability was observed in Bp (IC_{50} value of 6.28 mg/mL), while Bn exhibited the most effective in scavenging DPPH radical with IC_{50} value of 3.96 mg/mL. The Bp sample also showed the highest activity against three breast cancer cell lines, MCF-7 (cell viability of 43.5%), BT-20 (cell viability of 0%), and Hs 578T (cell viability of 0%). This study suggested that the level of bioactive compounds and biological activity of bee pollen significantly differ among their sources and the Bp is a potent antioxidant and anticancer agent for medicinal use.

Keywords: anticancer; antioxidant; bee pollen; chemical constituents

Introduction

Carcinogenesis and oxidative damage may cause the formation of cancer (Pham *et al.*, 2020). Currently, breast cancer is one of the most common diseases and become the second leading cause of death in women worldwide (Rashid *et al.*, 2021). To overcome these health problems, many synthetic drugs have been widely

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used for treatment. Although this chemotherapy exhibits the most efficiency, the use of such chemically synthesized agents potentially causes unfavourable side effects on human health (Nguyen *et al.*, 2018). Consequently, herbal medicine has been increasingly considered as a promising alternative to synthetic drugs to ensure safety for the patient (Nguyen *et al.*, 2021b). Many natural compounds isolated from various sources demonstrate potential pharmaceutical activities, such as anticancer, anti-inflammatory, and antioxidant activities (Keser and Kak, 2021; Nguyen *et al.*, 2021a). Hence, the search for new natural products with potent biological activities is required.

Bee pollen is a bee product produced by worker honeybees as a staple food for growing larvae (Nainu *et al.*, 2021). It is a mixture of flower pollen, enzymes, floral nectar, and honeybee salivary substances. Bee pollen is a rich source of carbohydrate (35-61%), protein and amino acid (14-30%), lipid and free fatty acid (1-13%), minerals, and vitamins (vitamin B, C, E, D, and P) (Nainu *et al.*, 2021). In addition, bee pollen contains various bioactive compounds such as phenolic and flavonoid compounds (Aylanc *et al.*, 2021; Dundar, 2021). Consequently, bee pollen has been used as a valuable dietary supplement with various medicinal benefits such as immunomodulatory, hepatoprotective, antibacterial, antioxidant, and anticancer activities (Khalifa *et al.*, 2021).

The pharmaceutical effect of bee pollen is due to the presence of various flavonoids and phenolic compounds with high antioxidant activities (Omar *et al.*, 2016). The antioxidant activity of bee pollen attributes to the ability to neutralize active oxygen species (Omar *et al.*, 2016). In addition, studies have reported that these compounds in bee pollen exhibit antiproliferative activities that can induce cell apoptosis and regulate cell proliferation (Omar *et al.*, 2016). Consequently, bee pollen can inhibit the growth of tumor cells and alleviate the pain of chemotherapy in cancer patients (Omar *et al.*, 2016). However, the pharmaceutical effect of bee pollen depends on its bioactive compositions and levels, which are strongly affected by weather conditions, bee activities, plant and bee species (Amalia *et al.*, 2020; Nainu *et al.*, 2021). Therefore, there is an urgent need to determine the chemical constituents and biological activity of bee pollen derived from different sources.

Taiwan has a tropical and subtropical climate, leading to diverse plant and animal species and abundant their based-products. Particularly, more than 700 tons of bee pollen are annually produced by honey bee *Apis mellifera* (Hsu *et al.*, 2021). The main floral sources of pollen for honey bees in Taiwan are Japanese apricot (*Prunus mume*), beggartick (*Bidens pilosa* var. *radiata*), tea tree (*Camellia sinensis*), rape (*Brassica napus*), lotus (*Nelumbo nucifera*), and Formosan gum (*Liquidambar formosana*) (Hsu *et al.*, 2021). Our previous has shown that the bee pollens derived from these sources are rich in protein, carbohydrate, and essential amino acids (Hsu *et al.*, 2021). These bee pollen samples are also considered as a potential source of bioactive compounds and consequently have a promising biological activity. However, no studies have reported the bioactive compounds, antioxidant and anticancer activity of the bee pollen derived from the aforementioned floral sources in Taiwan.

The current study examined the chlorophyll *a* (Chl *a*), Chl *b*, carotenoid (Car), total flavonoids, and phenolic contents in bee pollen collected from different floral sources in Taiwan. Subsequently, the antioxidant activity of these bee pollen samples was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay and ferrous iron-chelating ability. Finally, the anticancer activity against 3 human breast cancer cell lines (MCF-7, BT-20, and Hs 578T) was evaluated using a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Materials and Methods

Chemicals

Methanol (HPLC grade), ethanol (HPLC grade), acetone (HPLC grade), HCl, $AlCl_3$, CH_3COOK, Na_2CO_3, $FeCl_2{\cdot}4H_2O$, Folin-Ciocalteu reagent, quercetin (>95%), gallic acid (99.1%), DPPH,

ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich (PA, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), antibiotics, and other reagents were provided by Gibco (Waltham, MA, USA).

Sample collection

Be pollen samples were provided by Yong Shyang Apiculture Co. Ltd. (Taiwan). Six monofloral bee pollen samples (purity of >98%), namely Pm (from *Prunus mume*), Bp (*Bidens pilosa* var. *radiata*), Cs (*Camellia sinensis*), Bn (*Brassica napus*), Nn (*Nelumbo nucifera*), and Lf (*Liquidambar formosana*) were collected from different floral origins and identified in our previous study (Hsu *et al.*, 2021). These samples were dried, sieved using a 35-mesh stainless (wire diameter of 0.5 mm), manually pulverized, and stored at -18 °C for further experiments.

Determination of Chl, Car, anthocyanin, total flavonoid, and phenolic contents

Chl and Car contents of the bee pollen samples were determined based on the method of Nguyen *et al.* (2018) with slight modifications. Briefly, 0.2 g of bee pollen sample was mixed with 80% acetone (5 mL) at 4 °C for overnight before centrifugation at 15000 ×g for 10 min. The supernatant was then obtained and measured the absorbance at 440.5 ($A_{440.5}$), 646.6 ($A_{646.6}$), and 663.6 nm ($A_{663.6}$) using a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The contents of Chl *a*, Chl *b*, and Car were then calculated as follows:

$$Chl\ a = (12.25 \times A_{663.6} - 2.55 \times A_{646.6}) \times \frac{Volume\ of\ supernatant\ (mL)}{Sample\ weight\ (g)}$$

$$Chl\ b = (20.31 \times A_{646.6} - 4.91 \times A_{663.6}) \times \frac{Volume\ of\ supernatant\ (mL)}{Sample\ weight\ (g)}$$

$$Car = \frac{4.69 \times A_{440.5} \times volume\ of\ supernatant\ (mL)}{Sample\ weight\ (g)} - 0.267 \times (Chl\ a + Chl\ b)$$

Anthocyanin content was determined using the method described by Mancinelli *et al.* (1975) with slight modifications. The samples (0.2 g) were extracted with 5 mL of 99% methanol solution containing 1% HCl for 1 h at room temperature. The mixture was subsequently subjected to centrifugation at 15000 ×g for 10 min at 4 °C. The supernatant was then collected and used for measuring the absorbance at 530 nm and 657 nm using a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The anthocyanin content was then calculated using the following equation:

$$Anthocyanin\ content\ (\mu mol/g) = \left(A_{530} - \frac{0.33 \times A_{657}}{31.6}\right) \times \frac{Volume\ of\ supernatant\ (mL)}{Sample\ weight\ (g)}$$

Total flavonoid content of the bee pollen samples was determined using the method described by Zhishen *et al.* (1999) with slight modifications. Briefly, 0.2 g of sample was extracted with 5 mL of 99% ethanol solution containing 1% HCl for 1 h at room temperature. The mixture was subsequently subjected to centrifugation at 15000 ×g for 10 min at 4 °C. The absorbance was measured at 540 nm using a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Quercetin was used as a reference standard to establish the standard curve ($y = 790.36x + 28.51$; $R^2 = 0.9974$). The total flavonoid content was then calculated using the standard curve and presented as milligrams quercetin equivalent per gram of dried sample (mg QE/g DW).

The phenolic content of the extract was determined using the method of Singleton and Rossi (1965) with slight modifications. The sample (0.2 g) was extracted with 5 mL of 60% methanol solution containing 3% HCl for 1 h at room temperature. The mixture was subsequently subjected to centrifugation at 15000 ×g for 10 min at 4 °C. An aliquot of the extract (100 µL) was mixed with 2 mL of 2% Na_2CO_3 at room temperature for 2 min. The 50% Folin-Ciocalteu reagent (2 mL) was then added to the mixture and incubated at room temperature for 30 min. The mixture was measured the absorbance at 750 nm using the spectrometer (Hitachi U-2000, Tokyo, Japan). The standard curve for gallic acid ($y = 2.3142x + 0.461$; $R^2 = 0.9987$) was established

and used for calculating the phenolic content. Total phenolic content was presented in milligrams gallic acid equivalent per gram of dry weight of the sample (mg GAE/g DW).

Determination of antioxidant activity

Preparation of extracts

The extract was prepared by immersing 1 g of bee pollen in 1 mL of absolute methanol at room temperature for 4 h. The mixture was then shaken at 4 °C for overnight, followed by centrifugation at 15000 ×g for 10 min to collect the supernatant. The supernatant was then separated from the cell debris through filtration using filter paper (Whatman No. 1) to obtain the extract.

DPPH radical-scavenging activity assay

The DPPH radical-scavenging activity of bee pollen extract was evaluated using the method described by Shimada *et al.* (1992) with minor modifications. Aliquots of serial dilutions of methanol extract of bee pollen (0.05 mL) were added to 0.15 mL of DPPH solution (0.4 mM in methanol). The mixture was well mixed and incubated for 90 min at room temperature. The mixture was then measured the absorbance at 517 nm using a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The DPPH radical scavenging activity of the extract was quantified as follows:

$$DPPH\ scavenging\ activity\ (\%) = (1 - \frac{A_S}{A_c}) \times 100$$

where Ac and As are the absorbances of the DPPH solution without extract and the extract, respectively. BHT was used as a positive control. The concentration required for 50% inhibition of DPPH radicals (IC_{50}) was then calculated by plotting the DPPH scavenging activity against the extract concentration.

Determination of the ferrous iron-chelating ability

The Fe^{2+}-chelating ability of extract was determined using the method described by Dinis *et al.* (1994) with minor modifications. A reaction mixture was prepared by adding 1 mL of serial dilutions of the methanol extract to 3.7 mL of 80% methanol solution containing 100 µL of 2 mM $FeCl_2{\cdot}4H_2O$. The mixture was placed for 30 s before 5 mM ferrozine solution (200 µL) was added and subsequently incubated at room temperature for 10 min. The mixture was measured the absorbance at 562 nm. The Fe^{2+}-chelating ability was then calculated using the following equation:

$$Fe^{2+} - chelating\ ability\ (\%) = (1 - \frac{A_S}{A_c}) \times 100$$

where Ac and As are the absorbances of the reaction mixture without extract and the reaction mixture containing extract, respectively. EDTA was used as a positive control. The concentration required for 50% inhibition of DPPH radicals (IC_{50}) was then calculated by plotting the Fe^{2+}-chelating ability against the extract concentration.

Determination of anticancer activity

Cell culture

Human breast cancer cell lines (MCF-7, BT-20, and Hs 578T) were originally acquired from American Type Culture Collection (ATCC, Rockville, MD, USA). These cells were grown in DMEM medium supplemented with 10% FBS (v/v), 1% antibiotics (100 µg/mL of streptomycin and 100 U/mL of penicillin). The cell lines were kept at 37 °C in an incubator with a humidified atmosphere of 5% CO_2.

MTT Assay

Cell viability was achieved using a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay described by Kumar *et al.* (2018). Briefly, MCF-7, BT-20 cell lines (5 x 10^4 cells/well), and Hs 578 (2.5 x 10^4 cells/well) were seeded in a 96-well plate and grown for 24 h at 37 °C in a humidified atmosphere

of 5% CO_2. Cells were then treated with different bee pollen extracts (10 µg/mL) for 24~72 h. The cells were washed with PBS three times before adding 200 µL of DMEM medium containing MTT (0.25 mg/mL). The cells were subsequently incubated at 37 °C for 4 h before a solution of SDS/0.01 N HCl (100 µL/well) was added to solubilize formazan. The absorbance was then measured at 570 nm using a microplate reader (Biochrom EZ Read 400, Biochrom Ltd., Cambridge, United Kingdom). The cell viability was calculated as follows:

$$Cell\ viability\ (\%) = \frac{Absorbance\ value\ for\ the\ treated\ samples}{Absorbance\ value\ for\ the\ control} \times 100$$

Statistical analysis

Data are presented as mean ± standard deviation (SD). The data were analysed using the SAS v.8 (SAS Institute, USA) with Student's *t*-test or one-way analysis of variance (ANOVA). Differences were designated as statistically significant when $p < 0.05$.

Results and Discussion

The content of Chl a, Chl b, Car, anthocyanin, flavonoid, and phenolic compounds

Table 1 shows the contents of Chl *a*, Chl *b*, carotenoids, anthocyanin, phenolics, and flavonoids in different bee pollen samples. The content of these compounds significantly varied. Among the sample tested, the Cs sample showed the highest content of Car (417.67 mg/g DW) and anthocyanin (10.96 µmol/g DW), but it contained the lowest amount of phenolic compounds (22.89 mg GAE/g DW) and a low amount of Chl *a* (6.14 mg/g DW). Different from the Cs sample, the Lf sample contained the highest amount of Chl *a* (21.79 mg/g DW) and a high amount of phenolic compounds (33.99 mg GAE/g DW), but the lowest amount of anthocyanin (1.34 µmol/g DW) was found in this bee pollen sample. The highest contents of Chl *b* (39.17 mg/g DW) and Chl *a* (23.39 mg/g DW) were obtained in the Nn sample. This sample was also found to contain considerable high amounts of carotenoid (253.99 mg/g DW) and anthocyanin (6.75 µmol/g DW), but relatively low contents of flavonoids (2.02 mg QE/g DW) and phenolic compounds (24.28 mg GAE/g DW) compared to other samples. Notably, the Bp sample contained the highest flavonoid content (11.69 mg QE/g DW) and a relatively high amount of remaining chemical constituents determined whereas Bn showed the lowest contents of Chl *a*, Chl *b*, Car, and flavonoids.

Table 1. Content of pigments, phenolic, flavonoid, and anthocyanin in different bee pollen samples

Samples	Chl *a* (mg/g DW)	Chl *b* (mg/g DW)	Carotenoid (mg/g DW)	Anthocyanin (µmol/g DW)	Flavonoids (mg QE/g DW)	Phenolics (mg GAE/g DW)
Pm	8.82 ± 1.41^{b}	19.29 ± 0.65^{b}	44.79 ± 8.47^{d}	3.29 ± 0.36^{d}	5.15 ± 1.17^{b}	42.91 ± 3.06^{a}
Bp	7.59 ± 0.50^{b}	21.39 ± 5.69^{a}	341.65 ±29.82^{b}	5.52 ± 0.20^{c}	11.69 ± 1.30^{a}	27.61 ± 1.81^{d}
Cs	6.14 ± 1.47^{b}	17.47 ± 1.63^{b}	417.67 ±9.55^{a}	10.96 ± 1.06^{a}	4.54 ± 1.00^{b}	22.89 ± 2.10
Bn	2.87 ± 0.15^{c}	4.96 ± 1.17^{c}	5.88 ± 0.59^{e}	2.23 ± 0.78^{de}	1.55 ± 0.33^{d}	36.30 ± 0.62^{b}
Nn	23.39 ± 10.56^{a}	39.17 ± 15.60^{a}	253.99 ± 4.39^{c}	6.75 ± 0.55^{b}	2.02 ± 1.41^{cd}	24.28 ± 2.31^{d}
Lf	21.79 ± 2.84^{a}	25.66 ± 10.76^{a}	37.51 ± 5.12^{d}	1.34 ± 0.78^{e}	2.60 ± 0.24^{c}	33.99 ± 1.83^{c}

Data are presented as mean ± SD (n = 3). Different letters within the column indicate a significant difference at $p < 0.05$.

Previous studies have shown that the bee pollen from China, Brazil, Vietnam, Spain, New Zealand, Turkey, and Portugal has total flavonoid contents ranging between 1.0 and 17.5 mg QE/g and total phenolic contents ranging between 0.5 and 213 mg GAE/g (Mărgăoan *et al.*, 2021; Quoc, 2021). The phenolic content of the Pm sample in this study was observed to be higher than that of several bee pollen samples in Turkey

(16.4-38.06 mg GAE/g DW), Romania (17.96-38.53 mg GAE/g DW), and coffee bee pollen in Vietnam (10.62 mg GAE/g DW) (Quoc, 2021). Bp samples (bee pollen collected from *Bidens pilosa* var. *radiata*) contained higher flavonoids content than bee pollen obtained from *Lamiacea* (7.16 mg QE/g), *Castanea* sp. (4.79 mg QE/g), and *Fabaceae* (2.5 mg QE/g) (Mărgăoan *et al.*, 2021). It could be explained that the differences in the phytochemical constituents of these bee pollen samples might depend on various factors such as botanical origin, climatic conditions, beekeeping activity, plant and bee species (Mărgăoan *et al.*, 2021). Consequently, this study confirms a great variation in the phytochemical constituents of the bee pollen samples obtained from different sources.

Antioxidant activity of bee pollen samples

The antioxidant activities of bee pollen were evaluated as DPPH radical-scavenging ability and the Fe^{2+} iron-chelating activity. As shown in Figure 1, the bee pollen extracts strongly scavenged the free radical and the DPPH radical-scavenging activity significantly differed among the bee pollen samples. Among the samples tested, Bn showed the highest activity to scavenge DPPH radical with IC_{50} value of 3.96 mg/mL, followed by Lf (IC_{50} value of 7.82 mg/mL), Pm (IC_{50} value of 8.84 mg/mL), Cs (IC_{50} value of 8.92 mg/mL), and Bp (IC_{50} value of 13.46 mg/mL), whereas the lowest DPPH radical scavenging activity was found in Nn sample with IC_{50} values of 98.61 mg/mL (Table 2). There was no significant difference in DPPH radical scavenging activity between Cs and Pm samples.

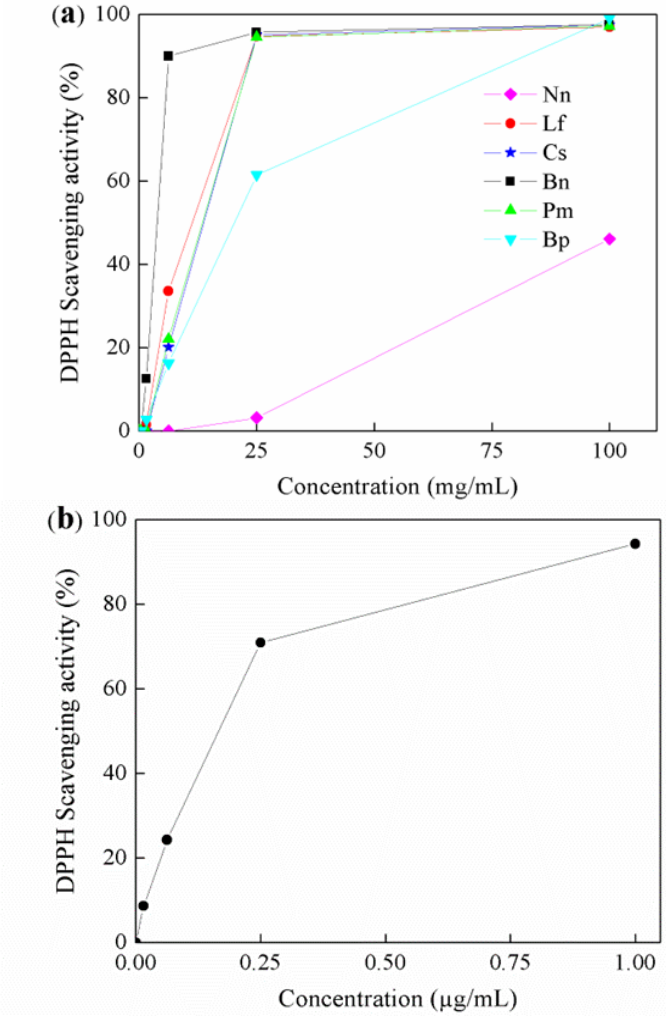

Figure 1. DPPH scavenging activity of different bee pollen (a) and BHT (b)

Figure 2 shows the Fe^{2+}-chelating activity of the bee pollen samples with a significant difference observed among the samples. The highest Fe^{2+}-chelating rate was observed in the Bp sample (IC_{50} value of 6.29 mg/mL) whereas the lowest Fe^{2+}-chelating effect was found in Bn and Nn samples with IC_{50} values of 25.42 mg/mL and 25.35 mg/mL, respectively (Table 2). There was no significant difference in Fe^{2+}-chelating effect between Lf (IC_{50} value of 13.78 mg/mL) and Cs (IC_{50} value of 13.55) samples, but their activity was significantly higher than that of the Pm sample (IC_{50} value of 20.34 mg/mL).

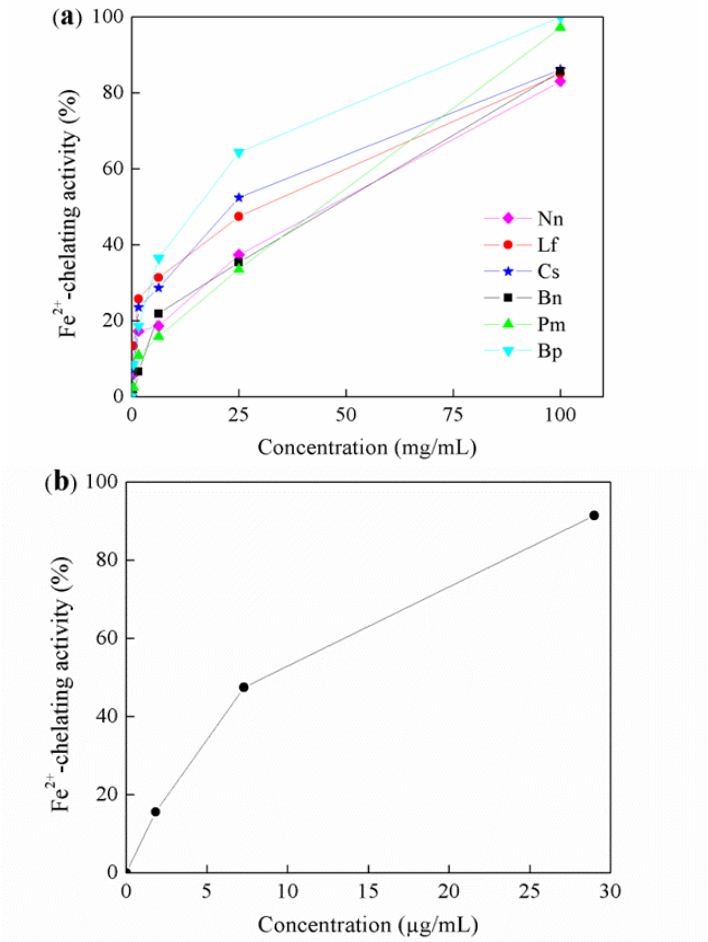

Figure 2. Fe^{2+}-chelating activity of different bee pollen samples (a) and EDTA (b)

There has been a growing interest in using antioxidants from natural sources as nutraceutical and pharmaceutical products for promoting health and preventing diseases (Nguyen *et al.*, 2020). Consequently, various plant- and animal-based products have been studied for their antioxidant activities (Do *et al.*, 2020; Qiao *et al.*, 2021). Particularly, bee pollen, one of the bee products, has increasingly gained considerable attention for its antioxidant activity. In this work, the antioxidant activities of six bee pollen samples collected from different origins were examined using DPPH-scavenging activity assay and Fe^{2+}-chelating assay. The antioxidant activity varied among the samples tested. Bn exhibited the highest scavenging activity against DPPH while Bp was the best chelator of Fe^{2+}. The differences in the antioxidant activities of the bee pollen samples might be due to the variation of phytochemical compositions contained in bee pollen, especially flavonoids and phenolic compounds. A high amount of phenolics and flavonoids in Bp and Bn samples are considered as the main contributors to the highest antioxidant activity of these samples. Similar to previous studies, the bee pollen samples obtained in this work had potential antioxidant activities compared to other bee

pollen from other countries (Dulger *et al.*, 2020; Spulber *et al.*, 2020; Soares de Arruda *et al.*, 2021). This study suggested that Bp and Bn samples were promising antioxidant agents for further applications.

Table 2. The IC_{50} values of DPPH radical-scavenging and Fe^{2+}-chelating activities

Samples	IC_{50} value (mg/mL)	
	DPPH scavenging activity	Fe^{2+} chelating ability
Pm	8.84 ± 0.17^{d}	20.34 ± 1.07^{d}
Bp	13.46 ± 1.94^{e}	6.29 ± 0.55^{b}
Cs	8.92 ± 0.23^{d}	13.55 ± 0.64^{c}
Bn	3.96 ± 0.09^{b}	25.42 ± 3.87^{e}
Nn	98.61 ± 1.7^{f}	25.35 ± 2.98^{e}
Lf	7.82 ± 0.21^{c}	13.78 ± 0.88^{c}
Standard (μg/mL)	0.17 ± 0.001^{a}	7.61 ± 0.13^{a}

Data are presented as mean ± SD (n = 3). Means within a column with different letters significantly differ at $p < 0.05$.

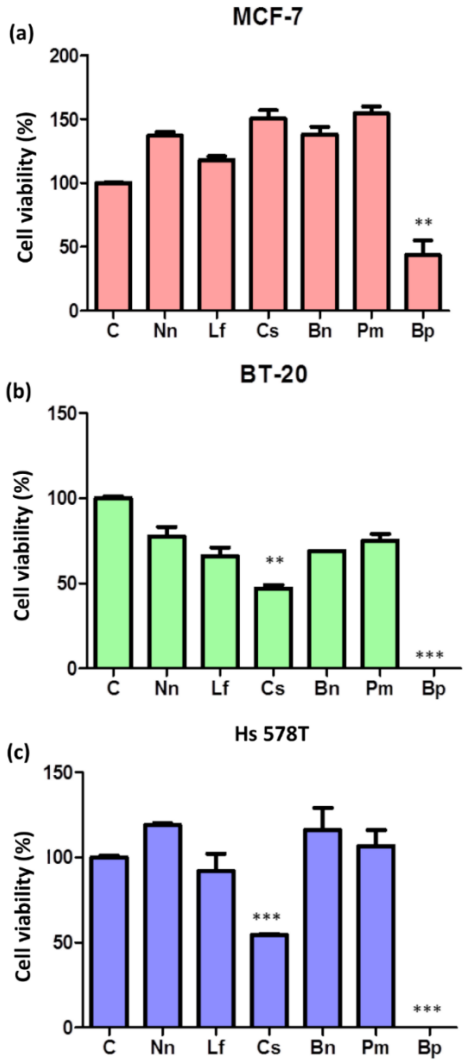

Figure 3. The cytotoxicity of different bee pollen samples on breast cancer cell lines: MCF-7 (a), BT-20 (b), and Hs 578T (c)

C, medium only; Pm, *Prunus mume*; Bp, *Bidens pilosa* var. *radiate*; Cs, *Camelia sinensis*; Bn, *Brasica napus*; Nn, *Nelumbo nucifera*; Lf, *Liquidambar formosana*

Anticancer activity

This study evaluated the anticancer activity of bee pollen against three human breast cancer cell lines. As shown in Figure 3, the Bp sample strongly inhibited the growth of all cancer cell lines tested and exhibited the highest anticancer activity against MCF-7 (cell viability of 43.5%), BT-20 (cell viability of 0%), and Hs 578T (cell viability of 0%). Cs samples also significantly induced the cytotoxicity in BT-20 and Hs 578T cell lines with the cell viability of 47% and 54.5%, respectively, whereas other samples showed no inhibitory effect on the three cancer cell lines. The high-anticancer activity against MCF-7, BT-20, and Hs 578T of Bp and Cs samples might be attributed to the presence of high levels of flavonoids, anthocyanin, and Car content in these samples. The synergetic effect of these secondary metabolite compounds may also enhance their anticancer activity (Balakrishna and Kumar, 2015). Such findings suggested that Bp is the most potent bee pollen with the highest antioxidant and anticancer activity and therefore this sample is promising for further medical applications.

Conclusions

This study reports the chemical constituents and biological activities of different bee pollen samples collected in Taiwan. The contents of Chl *a*, Chl *b*, Car, anthocyanin, flavonoids, and phenolic compounds significantly differed among the bee pollens tested. Bp and Bn samples exhibited the highest antioxidant activity in the Fe^{2+}-chelating assay (IC_{50} of 6.29 mg/mL) and DPPH radical scavenging assay (IC_{50} of 3.96 mg/mL), respectively. Notably, the Bp sample possessed strong cytotoxicity to three breast cancer cell lines, MCF-7, BT-20, and Hs 578T with cell viability of 43.5%, 0%, and 0%, respectively. This study suggested that bee pollen derived from different floral sources possessed different biological activity and the bee pollen collected from *Bidens pilosa* var. *radiata* is a promising natural antioxidant and anticancer agent for medicinal application.

Authors' Contributions

Conceptualization: HCN, CYY, and MYH; Funding acquisition: MYH and CYY; Investigation: HCN, LCL, JWL, MCW and TPL; Methodology: HCN, LCL, CYY, and MYH; Project administration: CYY and MYH; Supervision: MYH; Validation: HCN, LCL, CYY, MCW, and MYH; Writing - original draft: HCN; Writing - review and editing: HCN, CYY, and MYH. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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