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Research Article

Free Radical-Scavenging, Anti-Inflammatory, and Antibacterial Activities of Water and Ethanol Extracts Prepared from Compressional-Puffing Pretreated Mango (*Mangifera indica* L.) Peels

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During the processing of mango, a huge amount of peel is generated, which is environmentally problematic. In the present study, a compressional-puffing process was adopted to pretreat the peels of various mango cultivars, and then the bioactive compounds of mango peels were extracted by water or ethanol. The phenolic compound compositions as well as the free radical-scavenging, anti-inflammatory, and antibacterial activities of water extract (WE) and ethanol extract (EE) from nonpuffed (NP) and compressional-puffed (CP) mango peels were further evaluated. It was found that compressional-puffing could increase the yield of extracts obtained from most mango varieties and could augment the polyphenol content of extracts from Jinhwang and Tainoung number 1 (TN1) cultivars. The WE and EE from TN1 exhibited the highest polyphenol content and the greatest free radical-scavenging activities among the mango cultivars tested. Seven phenolic compounds (gallic acid, pyrogallol, chlorogenic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ECG, and CG) were detected in CPWE (compressional-puffed water extract) and CPEE (compressional-puffed ethanol extract) from TN1, and antioxidant stability of both CPWE and CPEE was higher than that of vitamin C. Further biological experiments revealed that CPEE from TN1 possessed the strongest anti-inflammatory and antibacterial activities, and thus it is recommended as a multibioactive agent, which may have applications in the food, cosmetic, and nutraceutical industries.

1. Introduction

Mango (*Mangifera indica* L.) is recognized as one of the most economically productive fruits in tropical and subtropical areas throughout the globe. Mango has excellent nutritional value and health-promoting properties. A variety of studies have been performed showing high concentrations of antioxidants including ascorbic acid, carotenoids, and phenolic compounds in mango [1]. Mango fruit is the main edible part and is usually processed into various products such as puree, nectar, jam, leather, pickles, chutney, frozen mango, dehydrated products, and canned slices. During the processing of mango, a huge amount of peel is generated, which constitutes approximately 15–20% of the mango fruit [2]. Mango peel is a waste by-product, and its disposal may have a substantial impact on the environment. Previous studies reported that mango peel contains a variety of valuable compounds such as polyphenols, carotenoids, enzymes, and dietary fiber [2]. Extracts from mango peel also exhibit antioxidant activity [3], anti-inflammatory activity [4], protection against membrane protein degradation and morphological changes in rat erythrocytes caused by hydrogen peroxide (H_2O_2) [5], antibacterial activity [6], and anticancer activity [7]. Hence, the utilization of mango peels may be an economical means of ameliorating the problem of waste disposal from mango production factories, as well as converting a by-product into material for food, cosmetic, and pharmaceutical industrial usages.

Free radicals, including superoxide anion radical ($O_2^{\bullet-}$), hydroperoxyl radical ($HO_2^\bullet$), hydroxyl radical ($HO^\bullet$),

peroxyl radical (ROO$^\bullet$), and alkoxyl radical (RO$^\bullet$), are defined as any molecules or atoms with one or more unpaired electrons and are often involved in human diseases [8]. Many studies have shown that free radicals in living organisms cause oxidative damage to different molecules such as lipids, proteins, and nucleic acids and these are involved in the interaction phases of many diseases such as cancer, atherosclerosis, respiratory ailments, and even neuronal death [9]. Antioxidants are substances that delay or prevent the oxidation of cellular oxidisable substrates. They exert their effect by scavenging reactive oxygen species (ROS) or preventing the generation of ROS [10]. Synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have potent antioxidant activity and are commonly used in processed foods. However, they have been restricted because of their carcinogenicity and other toxic properties [11, 12]. Thus, in recent years, there has been considerable interest in natural antioxidants derived from biological materials because of their presumed safety and potential nutritional and therapeutic value.

A large number of publications have suggested that fruit polyphenols are related to immunomodulatory and anti-inflammatory properties via *in vitro* and animal studies [13]. Inflammation is a complicated physiological phenomenon that occurs when the immune system in the body is activated to counter threats such as injury, infection, and stress. Macrophages often play a unique role in the immune system because they not only elicit an innate immune response but also act as effector cells in inflammation and infection. When macrophages encounter bacterial endotoxin lipopolysaccharide (LPS), they can be stimulated to generate a variety of inflammatory mediators such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, prostaglandin E2 (PGE2), and adhesion molecules to help eradicate the bacterial assault [14]. Generally, substances with inhibitory effects on the expression and activity of enzymes (e.g., inducible NO synthase (iNOS)) involved in the generation of inflammatory mediators such as NO in the mouse macrophage-like cell line RAW 264.7 are considered to possess immunomodulatory activity [15]. Since a variety of polyphenols exist in mango peels, further research on the use of mango peel extracts as immunomodulatory or anti-inflammatory agents is warranted.

Antibacterial agents are the synthetic or natural compounds that interfere with the growth and division of bacteria. A number of studies have shown that pathogenic microorganisms in humans and various animal species have developed resistance to drugs. This drug resistance is due to the random or otherwise inappropriate usage of commercial antimicrobial agents. As such, there is an urgent need for new antibacterial agents. In addition, synthetic antibiotics have been known to induce side effects such as the appearance of resistant bacteria, skin irritation, organ damage, and immunohypersensitivity [16]. Accordingly, many studies have attempted to develop new agents with high antibacterial activity but with fewer or possibly even no side effects. There is a particular demand for antibacterial compounds from natural resources [17]. Plants produce a range of antimicrobial

compounds in various parts such as bark, stalk, leaves, roots, flowers, pods, seeds, stems, hull, latex, and fruit rind [6]. Fruit peel is the outer covering of a fruit, which functions as a physical barrier. It also serves as a chemical barrier by virtue of the presence of many antimicrobial constituents, which protect the fruit from exposure to external pathogens or other factors that may tend to decrease the quality of the fruit. Therefore, fruit peels are good sources for obtaining natural antibacterial agents.

Bioactive compounds in mango peel are generally extracted via the following methods: extraction with 80% ethanol by sonication for 3 days at room temperature [18]; extraction performed three times with methanol, for 3 h per time [19]; extraction with 95% ethanol three times, 72 h per time [20]; extraction with acetone or ethyl acetate for up to 20 h [21–23]; extraction by microwave-assisted method [24, 25]; or extraction with supercritical CO_2, followed by pressurized ethanol [26]. However, these methods generally involve the use of a large volume of solvents, require a long extraction time, consume a lot of energy, are costly, and sometimes are not eco-friendly. The present study builds upon on the research reported in our previous investigation [27]. In brief, we previously developed a compressional-puffing process that has been successfully implemented to increase the extraction yield of fucoidan from brown seaweed [27, 28] and augment the extraction yields of total phenolics and total flavonoids from pine needles [29, 30]. Compressional-puffing can be utilized as a pretreatment step to disrupt the cellular structure of samples, thereby better enabling the release of bioactive compounds by solvent extraction [27]. In this study, compressional-puffing was utilized for pretreatment of mango peels, and water extract (WE) and ethanol extract (EE) extracted from nonpuffed (NP) and compressional-puffed (CP) mango peels were compared. The phenolic compound composition and the free radical-scavenging, anti-inflammatory, and antibacterial activities of WE and EE from mango peels were also evaluated. To the best of the authors' admittedly limited knowledge, this is the first study to elucidate the free radical-scavenging, anti-inflammatory and antibacterial activities of WE and EE extracted from compressional-puffed mango peels. The recovered WE and EE are expected to possess multifunctional activities providing a wide range of benefits. The utilization of mango peel will also help to play a role in minimizing the generation of waste worldwide.

2. Materials and Methods

2.1. Materials. Folin-Ciocalteu's phenol reagent, gallic acid, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, pyrogallol, caffeic acid, mangiferin, epicatechin, *p*-coumaric acid, ferulic acid, epicatechin gallate (ECG), catechin gallate (CG), ellagic acid, rutin, quercetin, kaempferol, homogentisic acid, tannic acid, vanillic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitrite, LPS, dimethyl sulfoxide (DMSO), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
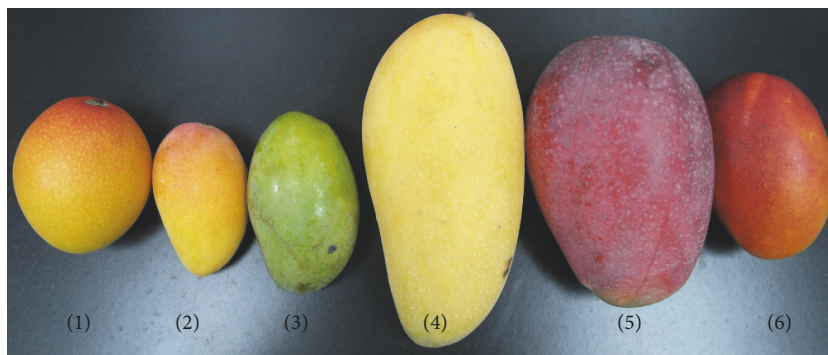
FIGURE 1: Appearance of various Taiwanese mango varieties. (1) Haden; (2) Tainoung number 1; (3) Tu; (4) Jinhwang; (5) Yuwen; (6) Irwin.

(MTT) was purchased from Calbiochem (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM), trypsin/EDTA, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco Laboratories (Grand Island, NY, USA). Methanol, acetic acid, and potassium persulfate were obtained from Nihon Shiyaku Industries, Ltd. (Tokyo, Japan). All other reagents if not declared were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were all of analytical grade.

2.2. Mango Fruits and Peels. Six mango cultivars, produced in Tainan City, Taiwan, were utilized in this study. The varieties, namely, Jinhwang, Tainoung number 1 (TN1), Irwin, Yuwen, Haden, and Tu (Figure 1), were collected from a local grocery market in Xinhua District, Tainan City, Taiwan. The fruits were used after they had completed ripening. Samples of peels were separated manually from six varieties of mango fruits and were then oven-dried and stored in aluminum bags at 4°C until use.

2.3. Compressional-Puffing Procedure. A compressional-puffing method [27, 28, 31] with minor modification was adopted to pretreat mango peels. In brief, the dried peel samples were crumbled and sieved using a 20-mesh screen. The portion retained by the screen was collected and then compressional-puffed using a continuous compressional-puffing machine with the temperature set at 220°C. The corresponding mechanical compression pressure and steam pressure levels inside the chamber are listed in Table 1. After the compressional-puffing, the peel samples were ground into fine particles and stored at 4°C for further extraction experiments.

2.4. Extraction Procedure. We followed the methods of Yang et al. (2017) [28]. Briefly, the nonpuffed and compressional-puffed peel samples were pulverized and sieved using a 20-mesh screen. The portion passed through the screen was collected and extracted by 95% ethanol (w/v = 1:10) for 4 h at 25°C with shaking. The resultant solution was then centrifuged at 9,170 ×g for 10 min and the supernatant was collected. NPEE (nonpuffed ethanol extract) and CPEE (compressional-puffed ethanol extract) were thus obtained after oven-drying the supernatant at 40°C. In addition, the precipitates after 95% ethanol extraction were further

extracted by double-distilled water (w/v = 1:10) for 1 h at 70°C with shaking. Then the mixture was centrifuged at 9,170 ×g for 10 min and the supernatant was collected. NPWE (nonpuffed water extract) and CPWE (compressional-puffed water extract) were obtained after oven-drying the supernatant at 50°C. All dried extracts were milled to fine particles and stored at 4°C for further analyses. The combined compressional-puffing pretreatment and extraction process is depicted in detail in Figure 2. The extraction yield was calculated using the following equation:

$$\text{extraction yield } (\%) = (g_A/g_B) \times 100, \tag{1}$$

where g_A represents the dry mass weight of the extract and g_B is the weight of the mango peel sample on a dry basis.

2.5. Determination of Polyphenol Content. Polyphenol content was estimated by the Folin-Ciocalteu colorimetric method based on the procedure of Singleton and Rossi (1965) [32] and using gallic acid as the standard agent.

2.6. High-Performance Liquid Chromatography (HPLC) Analysis of Total Phenolic Compound Composition. The separation of total phenolic compounds was performed by the method of Schieber et al. (2000) [33] and using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with a UV-vis detector. A reversed-phase Inspire C18 column (250 mm × 4.6 mm, id 5 μm) purchased from Dikma Technologies (USA) was used for all chromatographic separations. The column was operated at 25°C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A), 0.5% acetic acid in water, and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 20–55% B (50 min), 55–100% B (10 min), and 100–20% B (5 min). The injection volume of all samples was 20 μl. The spectra were monitored at 280 nm and performed at a flow rate of 1 ml/min. Gallic acid, pyrogallol, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, mangiferin, epicatechin, *p*-coumaric acid, ferulic acid, ECG, CG, ellagic acid, rutin, quercetin, kaempferol, homogentisic acid, tannic acid, and vanillic acid were used as standards for HPLC analyses.

2.7. DPPH Radical-Scavenging Activity. The scavenging activity of the DPPH radical in the samples was determined

TABLE 1: Process variables for compressional-puffing and extraction and extraction yields for various Taiwanese mango peel extracts.

Operational variables		NPWE	CPWE	NPEE	CPEE
Mechanical compression	Pressure (kg/cm^2)	0	5	0	5
	Number of compression times	0	3	0	3
Puffing	Temperature (°C)	0	220	0	220
	Pressure (kg/cm^2)	0	11	0	11
	Time (sec)	0	10	0	10
Pretreatment	Solvent	95% EtOH	95% EtOH	NA*	NA
	Temperature (°C)	25	25	NA	NA
	Time (h)	4	4	NA	NA
Extraction	Solvent	ddH$_2$O	ddH$_2$O	95% EtOH	95% EtOH
	Temperature (°C)	70	70	25	25
	Time (h)	1	1	4	4
Extraction yield of extract (%)**		NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar		33.5 ± 0.4^{cBC***}	36.6 ± 2.3^{bC}	23.4 ± 1.2^{bA}	30.2 ± 1.0^{cB}
Tainoung number 1 cultivar		29.5 ± 1.2^{bA}	34.8 ± 1.0^{bB}	29.2 ± 0.7^{dA}	33.7 ± 0.9^{dB}
Irwin cultivar		30.9 ± 0.9^{bcB}	40.0 ± 2.2^{bC}	22.6 ± 0.3^{bA}	29.6 ± 0.4^{cB}
Yuwen cultivar		31.2 ± 1.4^{bcB}	37.0 ± 1.8^{bC}	26.3 ± 0.9^{cA}	37.4 ± 1.0^{eC}
Haden cultivar		25.5 ± 1.5^{aB}	28.6 ± 2.7^{aB}	18.8 ± 0.8^{aA}	20.4 ± 0.5^{aA}
Tu cultivar		25.9 ± 0.3^{aB}	29.1 ± 0.1^{aD}	22.9 ± 0.5^{bA}	27.0 ± 0.1^{bC}

*NA: not applicable. **Extraction yield of extract (%) = ($g_{\text{solid extract, dry basis}}/g_{\text{mango peel sample, dry basis}}$) × 100. ***Values are mean ± SD ($n = 3$); values in the same column with different letters (in a, b, c, d, and e) and in the same row with different letters (in A, B, C, and D) are significantly different ($p < 0.05$).
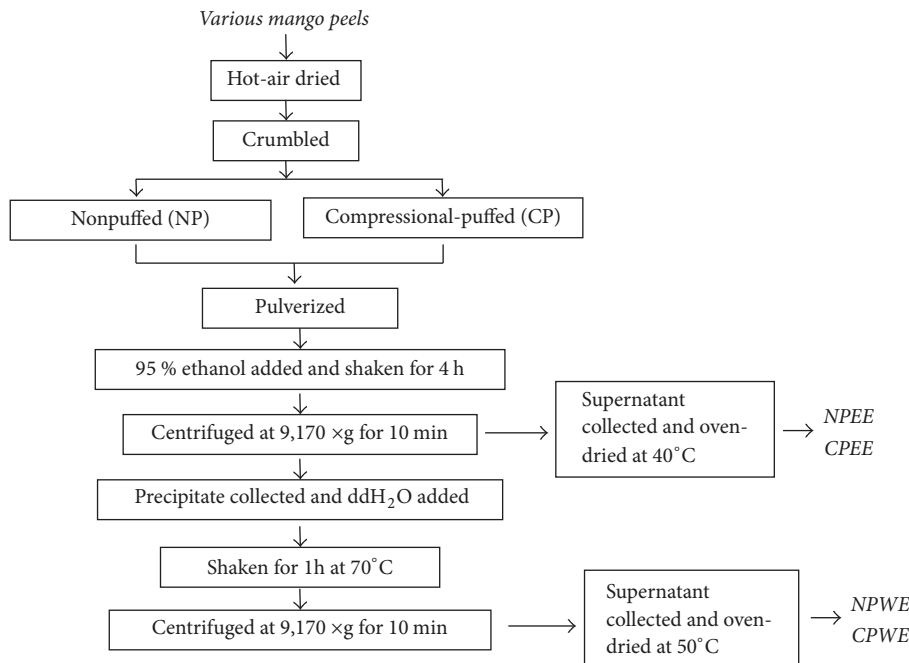

FIGURE 2: Flowchart of the compressional-puffing process and extraction methods for NPEE, CPEE, NPWE, and CPWE.

using the method described previously [28, 34]. In brief, 50 μl of mango peel extract (concentrations ranging from 0 to 300 μg/ml for Tainoung number 1 and Haden cultivars; 0–600 μg/ml for Jinhwang and Tu cultivars; and 0–900 μg/ml for Irwin and Yuwen cultivars) was added to 200 μl 0.1 mM DPPH solution (in methanol). The mixture was shaken vigorously for 1 min and left to stand for 30 min in the dark at room temperature. After the reaction, the absorbance of all sample solutions was then measured at 517 nm using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA). The radical-scavenging activity was calculated as the percentage inhibition using the following equation:

$$\text{DPPH}_{\text{radical-scavenging}} (\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100, \quad (2)$$

where A_{sample} is the absorbance of the methanol solution of DPPH with tested samples and A_{control} represents the absorbance of the methanol solution of DPPH without the sample.

2.8. ABTS Radical Cation-Scavenging Activity.

The ABTS radical cation-scavenging activity was performed according to the method described previously [28, 34]. The ABTS$^{\bullet+}$ solution was produced by mixing 5 ml of 7 mM ABTS solution with 88 μl of 140 mM potassium persulfate and allowing the mixture to stand in the dark for 16 h at room temperature before use. The ABTS$^{\bullet+}$ solution was diluted with 95% ethanol so that its absorbance at 734 nm was adjusted to 0.70 ± 0.05. To determine the scavenging activity, 100 μl diluted ABTS$^{\bullet+}$ solution was mixed with 100 μl of mango peel extract (concentrations ranging from 0 to 100 μg/ml for Tainoung number 1 and Haden cultivars; 0–300 μg/ml for Irwin, Yuwen, and Tu cultivars; and 0–500 μg/ml for Jinhwang cultivar) and the mixture was allowed to react at room temperature for 6 min. After the reaction, the absorbance of all sample solutions was then measured at 734 nm using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA). The blank was prepared in the same manner, except that distilled water was used instead of the sample. The scavenging activity of ABTS$^{\bullet+}$ was calculated using the following equation:

$$\text{ABTS}_{\text{radical cation-scavenging}} (\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100, \quad (3)$$

where A_{sample} is the absorbance of ABTS with tested samples and A_{control} represents the absorbance of ABTS without the sample.

2.9. Cell Line and Culture.

Murine macrophage cell lines RAW 264.7 were obtained from the Bioresource Collection and Research Center, the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). The cells were grown in DMEM supplemented with 10% FBS and 100 U/ml penicillin-streptomycin solution at 37°C in a humidified chamber with 5% CO_2. The medium was changed every two days.

2.10. Measurement of Cell Viability.

The MTT assay was used to evaluate cell viability. Briefly, RAW 264.7 cells (2×10^5/ml in a 96-well plate) were plated with culture medium and incubated for 24 h at 37°C, with 5% CO_2 in a humidified atmosphere. The medium was removed and fresh serum-free medium containing different concentrations of mango peel extracts (concentrations ranging from 0 to 25 μg/ml for CPEE of TN1 and CPWE of TN1) was added. After 24 h of incubation at 37°C, with 5% CO_2, the MTT reagent (0.1 mg/ml) was added. After incubating at 37°C for 4 h, the MTT reagent was removed and DMSO (100 μl) was added to each well and thoroughly mixed by pipetting to dissolve the MTT-formazan crystals. The absorbance was then determined by an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA) at a wavelength of 570 nm. The cell viability (%) was calculated using the following equation:

$$\text{Cell viability } (\%) = \left(\frac{T}{C} \right) \times 100, \quad (4)$$

where T is the absorbance in the test and C is the absorbance for the control.

2.11. Measurement of Nitrite Oxide in Culture Media.

RAW 264.7 cells (2×10^5 cells/ml) were seeded in a 96-well flat bottom plate for 24 h at 37°C with 5% CO_2. The culture medium was removed and replaced with fresh medium containing tested samples at various concentrations prior to challenging with 1 μg/ml of LPS. The nitrite concentration was measured in the culture supernatant after 24 h of coincubation. In brief, 50 μl of the cultured supernatants was added in the 96-well plate and 100 μl of Griess reagent was added to each well and allowed to stand for 10 min at room temperature. The absorbance at 540 nm was measured using an ELISA reader (PowerWave 340, BioTek Instruments, Winooski, VT, USA), and the quantification of nitrite was standardized with NaNO$_2$ at 0–100 μM concentrations [35].

2.12. Zone of Inhibition.

Five bacteria were tested for antibacterial activity of mango peel extracts. These were three Gram-negative bacteria (*Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 13311, and *Vibrio parahaemolyticus* ATCC 17802) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 12600 and *Bacillus cereus* ATCC 14579), which were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu, Taiwan. Antibacterial activity was measured using the standard method of diffusion disc plates on agar [36]. In brief, *E. coli*, *S. typhimurium*, *S. aureus*, and *B. cereus* were grown in tryptic soy broth (TSB) medium (Difco Laboratories, Detroit, MI, USA) and *V. parahaemolyticus* was grown in TSB medium + 3% NaCl for 24 h at 37°C, and 0.1 ml of each culture of bacteria at proper cell density was spread on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) plate surfaces (3% NaCl was added to TSA for *V. parahaemolyticus*). Paper disc (8 mm in diameter) was placed on the agar medium to load 50 μl containing 2 mg of mango peel extract (4%, w/v, in 0.05 M acetate buffer, pH 6.0). Control paper discs were prepared by infusing with 50 μl Antibiotic-Antimycotic Solution (containing 10,000 units/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin) (Corning, Corning, NY, USA) or 50 μl 0.05 M acetate buffer. The plates were incubated at 37°C for 24 h. After 24 h, antibacterial activity of the extracts against the test bacteria was observed by growth-free zone of inhibition near the respective disc and the inhibition diameters were measured.

2.13. Statistical Analysis.

Experiments were performed at least three times. Values represent the means ± standard deviation (SD). Statistical analyses were done using the Statistical Package for the Social Sciences (SPSS). The results obtained were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range tests. $p < 0.05$ was considered statistically significant. Correlation analyses were performed using the square of Pearson's correlation coefficient (R^2).

3. Results and Discussion

3.1. Effects of Mango Varieties, Compressional-Puffing, and Extraction Methods on Extraction Yields of Peel Extracts. Six varieties of mango fruits, namely, Jinhwang, Tainoung number 1 (TN1), Irwin, Yuwen, Haden, and Tu, were collected from a local grocery market in Xinhua District, Tainan City, Taiwan. Samples of peels were separated manually and the peels were oven-dried till the moisture content reached 4–7% (wet basis). The dried peel samples were crumbled and sieved using a 20-mesh screen, and the portion retained by the screen was collected and compressional-puffed according to the technique developed previously [27]. Compressional-puffing applies a mechanical compression force of approximately $5 \, kg/cm^2$ to the sample three times before puffing, which can account for the difference between compressional-puffing and the conventional puffing gun process. The puffing temperatures were set at 220°C, and the corresponding pressure level inside the chamber was found to be $11 \, kg/cm^2$ (Table 1). The NP and CP peel samples were ground and sieved using a 20-mesh screen. The portion passing through the screen was collected and then the bioactive compounds were extracted by either ethanol or hot water as shown in Figure 2. In the preliminary experiment, we extracted puffed peel sample directly using 70°C hot water and found that the extract, after being dried, exhibited a stone-like hard structure, which stuck tightly to the inner surfaces of the container and was difficult to dislodge. Thus, the 70°C hot water extraction condition was not adopted in the present study. After extraction, four peel extracts, namely, NPWE (nonpuffed water extract), CPWE (compressional-puffed water extract), NPEE (nonpuffed ethanol extract), and CPEE (compressional-puffed ethanol extract), were obtained according to their puffing pretreatments and extraction methods for each mango cultivar (Figure 2). The yields of these extracts are indicated in Table 1. In the comparison of extraction yields among different mango varieties for these four extracts, it was found that the yields of extracts for the tested mango cultivars were similar, except that Haden and Tu cultivars had relatively lower extraction yields. Thus, the peels of Jinhwang, TN1, Irwin, and Yuwen cultivars with higher yields of extracts would have advantages for further commercial production. It was reported that compressional-puffing could primarily rupture the structure of the puffed samples and then augment the extraction yield of crude fucoidan from brown algae [27, 28] and increase the extraction yields of total phenolics and total flavonoids from pine needles [29, 30]. In the present study, we also found that compressional-puffing could rupture the structure of mango peel (data not shown) and increase the extraction yields in both CPWE and CPEE as compared to NPWE and NPEE, respectively (Table 1). Therefore, compressional-puffing can also be effectively used in mango peels to facilitate the release of bioactive compounds by simple extraction operations. A comparison of the extraction yields between water and ethanol extractions revealed that water extraction tended to have higher yields of extracts as compared to ethanol extraction. A higher yield of extract has the potential for commercialized production. In addition, previous reports revealed that the composition of mango peel extract is complicated, and it may contain polyphenols, flavonoids, carotenoids, vitamin E, vitamin C, pectin, unsaturated fatty acids, and other biologically active components that positively influence health [25, 37–39]. Mango peel extract has also exhibited biological functions such as antioxidant properties [25, 39] and inhibition of HeLa human cervical carcinoma cell proliferation [38]. Generally, phenolic compounds are the major bioactive components of mango peels [18] and these have exhibited antioxidant activity and an antiproliferative effect on HeLa cells [25, 37–39]. Thus, the phenolic compound composition in our mango peel extracts and their effects on biological functions warrant further examination. Taken together, peel extracts from Jinhwang, TN1, Irwin, and Yuwen cultivars had higher extraction yields than those of Haden and Tu cultivars. Compressional-puffing pretreatment resulted in a worthwhile incremental increase in the extraction yields of mango peel extracts. Water extraction tended to have higher yields of extracts as compared to ethanol extraction, which would be beneficial in commercialized production. The phenolic compound composition and biological functions of mango peel extracts require further characterization.

3.2. Polyphenol Contents and Free Radical-Scavenging Activities of Peel Extracts from Various Mango Cultivars. Phenolic compounds are reported to be the major bioactive components that exist in mango peels [18]. In the present study, four peel extracts (NPWE, CPWE, NPEE, and CPEE) from six mango cultivars were utilized to determine their polyphenol contents by the Folin-Ciocalteu colorimetric method. The results presented in Table 2 suggest that peel extracts from the TN1 cultivar possessed the highest amount of total phenolic compounds as compared to other peel extracts. Thus, it is reasonable to postulate that peel extracts of the TN1 may exhibit high biological activities, and therefore further investigation is warranted. Moreover, a comparison of the polyphenol contents between NPWE and CPWE in all mango cultivars revealed that polyphenol content of CPWE was higher than that of NPWE (Table 2), indicating that compressional-puffing could increase the polyphenol content of water extracts in all mango cultivars. However, in the case of ethanol extracts, only CPEEs from Jinhwang and TN1 had higher polyphenol contents than those of NPEEs from Jinhwang and TN1 (Table 2). Moreover, for all mango cultivars, polyphenol contents of ethanol extracts were higher than those of water extracts (Table 2), indicating that ethanol extraction was effective in the extraction of polyphenols. Polyphenols are well known to exhibit antioxidant activity due to their ability to scavenge free radicals via hydrogen donation or electron donation and the reactivity of the phenol moiety [40]. Accordingly, the antioxidant capacities of NPWE, CPWE, NPEE, and CPEE of six mango peels were characterized using DPPH and ABTS radical-scavenging assays. DPPH is a stable free radical and is widely used to evaluate the antioxidant activity in a relatively short time compared to other methods [41]. The SC_{50} values (concentration of mango peel extract capable of scavenging 50% of DPPH radical) of the peel extracts (NPWE, CPWE, NPEE, and CPEE) from six mango cultivars for DPPH

TABLE 2: Polyphenol content, DPPH radical-scavenging activity, and ABTS radical cation-scavenging activity of extracts from various Taiwanese mango peels.

Polyphenols (%)[*]	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	$1.40 \pm 0.11^{aA****}$	2.11 ± 0.24^{aB}	5.31 ± 0.25^{aC}	9.13 ± 0.16^{cD}
Tainoung number 1 cultivar	15.9 ± 0.9^{eA}	16.6 ± 1.1^{eA}	23.5 ± 0.4^{eB}	28.5 ± 0.7^{eC}
Irwin cultivar	3.09 ± 0.18^{bA}	2.92 ± 0.19^{aA}	7.06 ± 0.29^{bC}	5.07 ± 0.11^{aB}
Yuwen cultivar	2.36 ± 0.25^{bA}	4.63 ± 0.90^{bB}	7.21 ± 0.05^{bD}	6.26 ± 0.05^{bC}
Haden cultivar	6.41 ± 0.20^{dA}	7.31 ± 0.19^{cB}	18.9 ± 0.3^{dD}	13.4 ± 0.3^{dC}
Tu cultivar	5.25 ± 0.27^{cA}	10.1 ± 1.6^{dB}	14.7 ± 0.2^{cD}	13.0 ± 0.5^{dC}

DPPH, SC$_{50}$ values (μg/ml)[**]	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	499 ± 7^{fD}	368 ± 13^{fC}	197 ± 12^{dA}	251 ± 0^{fB}
Tainoung number 1 cultivar	57.0 ± 2.2^{aC}	67.0 ± 2.2^{aD}	46.0 ± 1.4^{aB}	41.7 ± 1.3^{aA}
Irwin cultivar	368 ± 11^{eC}	255 ± 2^{dB}	195 ± 9^{dA}	222 ± 8^{eA}
Yuwen cultivar	324 ± 3^{dD}	303 ± 5^{eC}	165 ± 5^{cA}	206 ± 4^{dB}
Haden cultivar	124 ± 3^{bD}	101 ± 5^{bC}	69 ± 5^{bA}	86 ± 4^{bB}
Tu cultivar	183 ± 2^{cD}	158 ± 5^{cC}	78.3 ± 4.9^{bA}	96.7 ± 4.7^{cB}
Vitamin C	11.3 ± 0.1			

ABTS, SC$_{50}$ values (μg/ml)[***]	NPWE	CPWE	NPEE	CPEE
Jinhwang cultivar	186 ± 0^{eD}	139 ± 0^{eC}	70.0 ± 0.0^{fB}	54.0 ± 3.3^{cA}
Tainoung number 1 cultivar	28.2 ± 3.8^{aC}	23.3 ± 0.5^{aB}	15.7 ± 0.9^{aA}	13.0 ± 0.8^{aA}
Irwin cultivar	113 ± 7^{cC}	101 ± 6^{dC}	59.0 ± 0.8^{eA}	76.3 ± 2.8^{eB}
Yuwen cultivar	137 ± 2^{dC}	102 ± 2^{dB}	52.0 ± 0.9^{dA}	62.0 ± 1.7^{dA}
Haden cultivar	55.3 ± 1.7^{bC}	37.3 ± 2.4^{bB}	27.3 ± 0.9^{cA}	30.7 ± 1.7^{bA}
Tu cultivar	115 ± 5^{cD}	77.3 ± 3.4^{cC}	24.7 ± 1.3^{bA}	34.0 ± 1.6^{bB}
Vitamin C	3.58 ± 0.07			

[*]Polyphenols (%) = $(g/g_{\text{solid extract, dry basis}}) \times 100$. [**]SC$_{50}$ values (concentration of mango peel extract capable of scavenging 50% of DPPH radical) for DPPH radical-scavenging of different mango peel extracts. [***]SC$_{50}$ values (concentration of mango peel extract capable of scavenging 50% of ABTS cation radical) for ABTS radical cation-scavenging of different mango peel extracts. [****]Values are mean $\pm$ SD ($n = 3$); values in the same column with different letters (in a, b, c, d, e, and f) and in the same row with different letters (in A, B, C, and D) are significantly different ($p < 0.05$).

radical-scavenging activity are presented in Table 2. As shown in Table 2, all peel extracts from TN1 exhibited the most DPPH radical-scavenging activity as compared to other mango cultivars, and the most potent was CPEE of TN1 with an SC$_{50}$ value of 41.7 $\pm$ 1.3 μg/ml. Kim et al. (2010) reported that the SC$_{50}$ value of the DPPH radical-scavenging activity of Irwin mango peel ethanol extract was about 40 μg/ml [18], which was similar to the SC$_{50}$ value of CPEE of TN1 reported here. A comparison of the DPPH radical-scavenging activities of the CPWE group with those of the NPWE group revealed that compressional-puffing could increase the DPPH radical-scavenging activities of peel extracts (Table 2). Moreover, DPPH radical-scavenging activity of all EE groups (including NPEE and CPEE) was greater than that of the WE groups (NPWE and CPWE), which appeared to be positively correlated with the higher polyphenol amount in the EE groups as shown in Table 2. Regarding the scavenging activity of ABTS$^{\bullet+}$, the relatively long-lived ABTS$^{\bullet+}$ was decolorized during the reaction with hydrogen-donating antioxidant [42]. The SC$_{50}$ values (concentration of mango peel extract capable of scavenging 50% of ABTS radical cation) of the peel extracts (NPEE, CPEE, NPWE, and CPWE) from six mango cultivars for ABTS radical cation-scavenging activity are also presented in Table 2. The results show that, among the extracts from six mango cultivars, peel extracts from the TN1 exhibited the most ABTS radical cation-scavenging

activity, and the SC$_{50}$ value for the most potent CPEE of TN1 was 13.0 $\pm$ 0.8 μg/ml. Kim et al. (2010) reported that the SC$_{50}$ value of the ABTS radical cation-scavenging activity for Irwin mango peel ethanol extract was about 200 μg/ml [18], which was less effective in ABTS radical cation-scavenging capacity as compared to our CPEE of TN1. Regarding NPWE and CPWE, compressional-puffing could increase the ABTS radical cation-scavenging activity in CPWE of mango cultivars, which was similar to the finding for DPPH radical-scavenging activity. All EEs (including NPEE and CPEE) had greater ABTS radical cation-scavenging activity compared to WEs (including NPWE and CPWE) (Table 2). To better understand the relationship between polyphenol contents and free radical-scavenging activities of peel extracts, a correlation plot was performed and the results are shown in Figure 3. A high correlation between the polyphenol contents of peel extracts and their corresponding free radical-scavenging activities (DPPH and ABTS radical-scavenging activities) was found in NPWE, CPWE, NPEE, and CPEE, which was also consistent with previously reported observations [43]. In summary, peel extracts from the TN1 had the highest amount of total phenolic compounds and possessed the most DPPH and ABTS free radical-scavenging activities. For all water extracts, compressional-puffing had a tendency to increase the contents of total phenolic compounds in CPWEs and resulted in an incremental increase in free
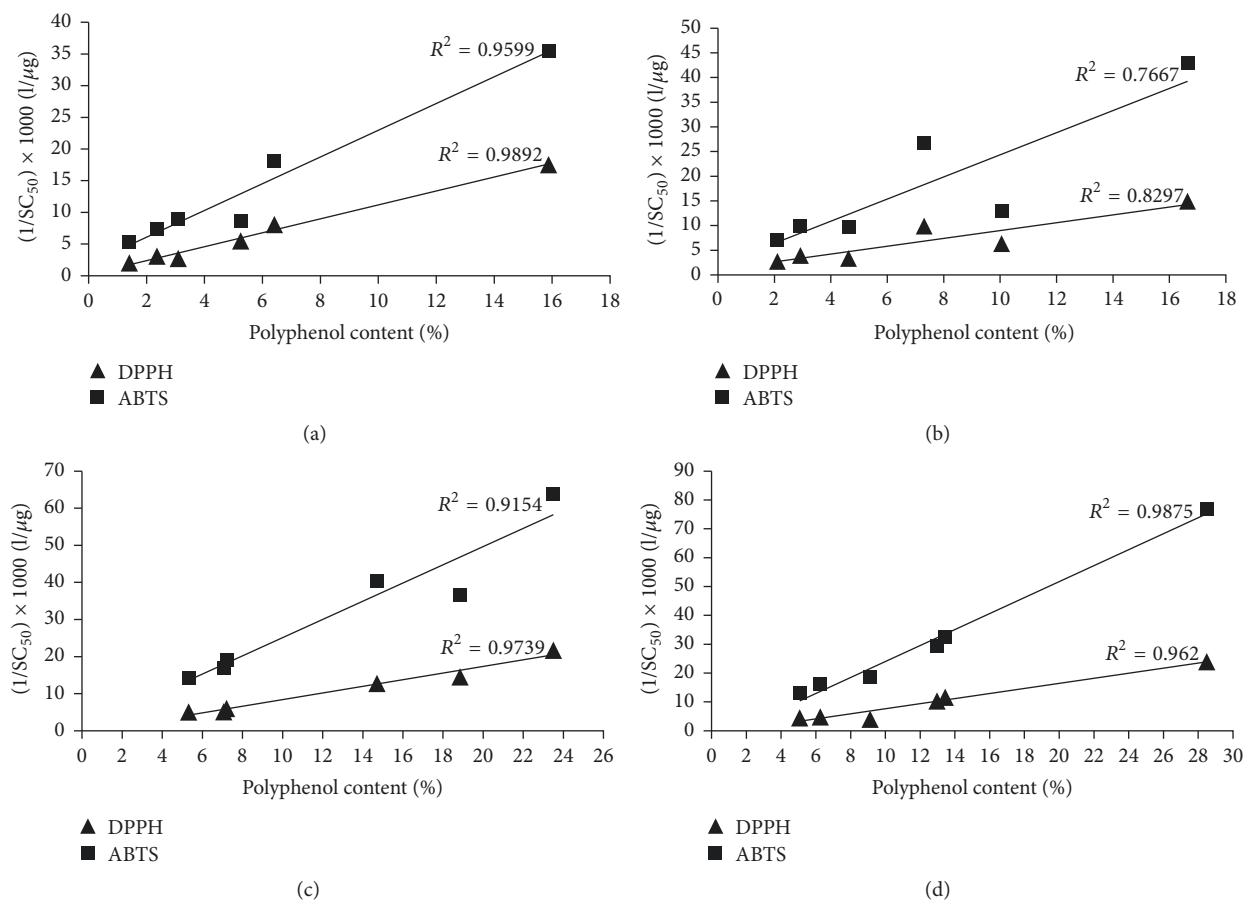
Figure 3: Association between polyphenol content and DPPH/ABTS radical-scavenging activities of mango peel extracts. (a) NPWE; (b) CPWE; (c) NPEE; (d) CPEE. SC_{50}: concentration for scavenging 50% of DPPH or ABTS free radicals.

radical-scavenging activities as compared to NPWEs. For all ethanol extracts, only CPEE of TN1 had a higher content of total phenolic compounds and possessed higher free radical-scavenging activities as compared to NPEE of TN1. Moreover, for all extracts, ethanol extracts generally had a higher amount of total phenolic compounds and caused greater free radical-scavenging activities as compared to water extracts. Therefore, in summary, both CPWE and CPEE of the TN1 cultivar warrant further analyses of the phenolic compound composition and storage stability of their antioxidant capacity, as well as their anti-inflammatory and antibacterial activities.

3.3. Analysis of Phenolic Compound Composition, Storage Stability of Antioxidant Capacity, Anti-Inflammatory Activity, and Antibacterial Activity in CPWE and CPEE of TN1 Cultivar.
Peel extracts of TN1 cultivar have the highest amount of total phenolic compounds and the most free radical-scavenging activities. Moreover, CPWE and CPEE from TN1 had higher extraction yields and greater polyphenol contents as compared to NPWE and NPEE from TN1. Therefore, the phenolic compound composition of CPWE and CPEE from TN1 was analyzed by RP-HPLC coupled with UV-vis detector. The results are shown in Figure 4 and Table 3. In Figure 4, it can be seen that seven phenolic compounds, namely, gallic

Table 3: Phenolic compound composition in the CPWE and CPEE of Tainoung number 1 cultivar.

Compound	Tainoung number 1 cultivar	
	CPWE (mg/100 g)*	CPEE (mg/100 g)
p-Hydroxybenzoic acid	1863 ± 318	3313 ± 2
Gallic acid	579 ± 72	1052 ± 1
Pyrogallol	566 ± 55	930 ± 90
Chlorogenic acid	125 ± 8	245 ± 7
Catechin gallate (CG)	125 ± 43	189 ± 52
p-Coumaric acid	68.9 ± 9.4	131 ± 0
Epicatechin gallate (ECG)	32.0 ± 3.9	50.8 ± 7.0

*The concentration of phenolic compound is expressed as mg/100 g peel weight, dry basis.

acid, pyrogallol, chlorogenic acid, p-hydroxybenzoic acid, p-coumaric acid, ECG, and CG, were tentatively identified in CPWE and CPEE of TN1 by HPLC analysis. Table 3 shows the quantitative data of phenolic compound composition in the CPWE and CPEE of TN1. It was found that both CPWE and CPEE of TN1 contained large amounts of p-hydroxybenzoic acid, gallic acid, and pyrogallol and smaller amounts of chlorogenic acid, CG, p-coumaric acid, and ECG. A comparison of the phenolic compound composition in
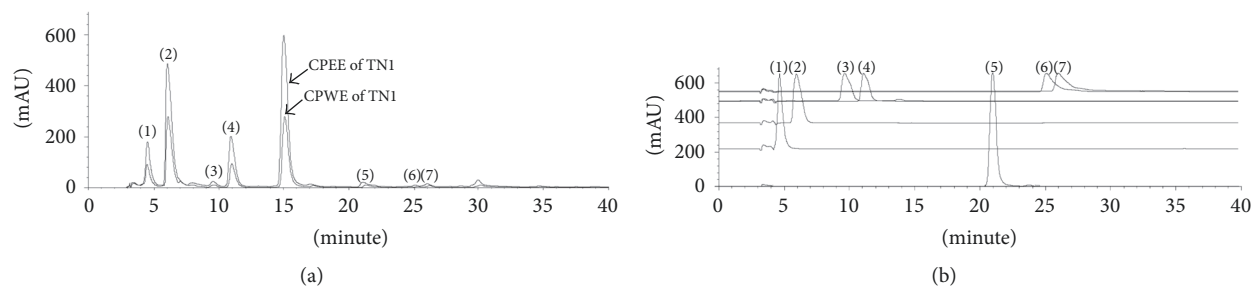
Figure 4: (a) High-performance liquid chromatography of peel extracts (CPWE and CPEE) of Tainoung number 1 cultivar; (b) high-performance liquid chromatography of polyphenol standards: gallic acid (1), pyrogallol (2), chlorogenic acid (3), *p*-hydroxybenzoic acid (4), *p*-coumaric acid (5), ECG (6), and CG (7).

CPWE and CPEE revealed that CPEE of TN1 had greater amounts of *p*-hydroxybenzoic acid, gallic acid, pyrogallol, chlorogenic acid, CG, *p*-coumaric acid, and ECG than those of CPWE (Table 3). These results are consistent with the data shown in Table 2, which illustrates that CPEE of TN1 has higher total phenolic compounds compared to CPWE of TN1. We found that *p*-hydroxybenzoic acid was the predominant phenolic compound detected (up to 3313 ± 2 mg/100 g peel weight, dry basis) in CPEE of TN1, and the results were also supported by other studies reporting that *p*-hydroxybenzoic acid could be detected in the extract of mango cultivar [44]. The concentrations of gallic acid for CPWE and CPEE of TN1 were recorded as 579 ± 72 and 1052 ± 1 mg/100 g peel weight, dry basis, respectively. These data are comparably higher than those reported previously for the ethanol extract of mango peel, with an average gallic acid concentration of 152.20 ± 0.14 mg/100 g mango peel, dry weight [45]. Previous studies suggested that pyrogallol can be detected in the ethanolic extract of mango kernel (the mango tested was purchased from an Egyptian local market) with a concentration of 1337.9 ± 0.31 mg/100 g mango kernel, dry weight, but it was absent in the ethanolic extract of mango peel [45]. However, we found that pyrogallol could be detected in CPWE and CPEE of TN1 with a concentration of 566 ± 55 and 930 ± 90 mg/100 g peel weight, dry basis, respectively. We speculate the reason may be due to differences between the tested mango varieties. Structurally, *p*-hydroxybenzoic acid, gallic acid, and pyrogallol are monophenolic compounds, which exhibit antioxidant activity owing to their hydrogen-donating or electron-donating properties [46]. Therefore, the high free radical-scavenging activities of CPWE and CPEE of TN1 may be attributed to the high contents of *p*-hydroxybenzoic acid, gallic acid, and pyrogallol. Besides phenolic compounds, previous studies reported that a synergistic effect of combinations of phytochemicals may also result in beneficial biological functions such as inhibition of proliferation of human cancer cells [38, 47]. Thus, the synergistic effects of constituents in CPWE and CPEE of TN1 with respect to their effects on biological functions warrant further investigation. The storage stability of antioxidant agent is important with respect to its potential industrial application. Here, we evaluated the storage stability of vitamin C, CPWE of TN1, and CPEE of TN1 by DPPH radical-scavenging assay. The test sample powders were redissolved in double-distilled water at various concentrations and the sample solutions were stored at room temperature for 1, 2, 4, and 8 hours, and then the corresponding DPPH radical-scavenging activities were determined. The data presented in Figure 5(a) suggest that the well-known natural antioxidant vitamin C would dramatically reduce its DPPH radical-scavenging activity after 1–8 hours' storage. However, the DPPH radical-scavenging activities in either CPWE of TN1 or CPEE of TN1 were not obviously changed after 1–8 hours' storage (Figures 5(b) and 5(c)). These findings clearly indicate that the peel extracts of mango exhibited a high storage stability in terms of antioxidant activity. Fruit polyphenols have been reported to be related to immunomodulatory and anti-inflammatory properties via *in vitro* and animal studies [13]. NO is an inflammatory mediator induced by inflammatory cytokines or bacterial LPS in various cell types including macrophages [48]. Samples with NO inhibitory activity thus have the potential to possess anti-inflammatory activity. CPEE and CPWE from TN1 were tested for their anti-inflammatory activities by investigating their effects on NO production in LPS-induced RAW264.7 macrophages. Neither CPEE nor CPWE obviously affected the viability of RAW264.7 cells at the 6.25–25 μg/ml concentrations that were tested, in the presence of 1 μg/ml LPS (Figure 6(a)). As shown in Figure 6(b), when RAW264.7 cells were treated with 1 μg/ml LPS, the NO production was increased from 3.11 ± 0.25 μM to 12.8 ± 0.1 μM. Moreover, when RAW264.7 cells were treated with 1 μg/ml LPS in the presence of various concentrations of CPEE, it was found that NO production was significantly decreased from 12.8 ± 0.1 μM to 9.54 ± 0.08 μM, whereas in the presence of various concentrations of CPWE, NO production was only slightly reduced. These results indicate that CPEE of TN1 had apparent anti-inflammatory activity, and thus it may have potential as a natural and safe agent in the protection of human health by modulating the immune system. Previous studies demonstrated that extracts with high polyphenol content exhibited high antibacterial activity [49]. As such, we evaluated the antibacterial activity of CPEE and CPWE of TN1 by the diffusion disc method. Five bacteria, three Gram-negative bacteria (*E. coli*, *S. typhimurium*, and *V. parahaemolyticus*) and two Gram-positive bacteria (*S. aureus* and *B. cereus*), were adopted to assess the antibacterial properties. As can be seen in Figures 7(a)–7(f), both CPEE and CPWE of TN1 exhibited antibacterial activities against the
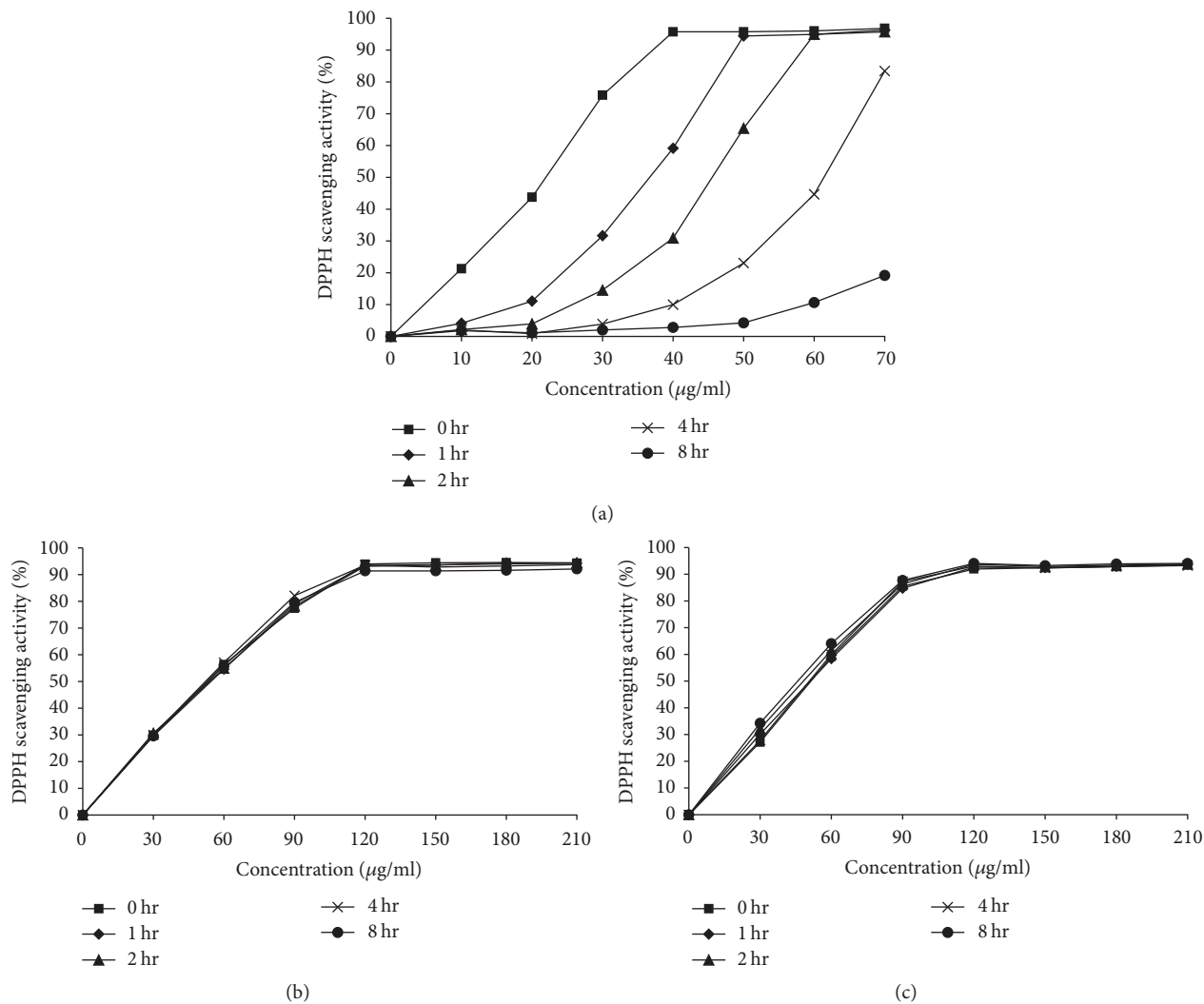
(a)

(b)

(c)

Figure 5: DPPH scavenging activities of vitamin C, CPWE of TN1, and CPEE of TN1 under different storage times. (a) Vitamin C; (b) CPWE of TN1; (c) CPEE of TN1.
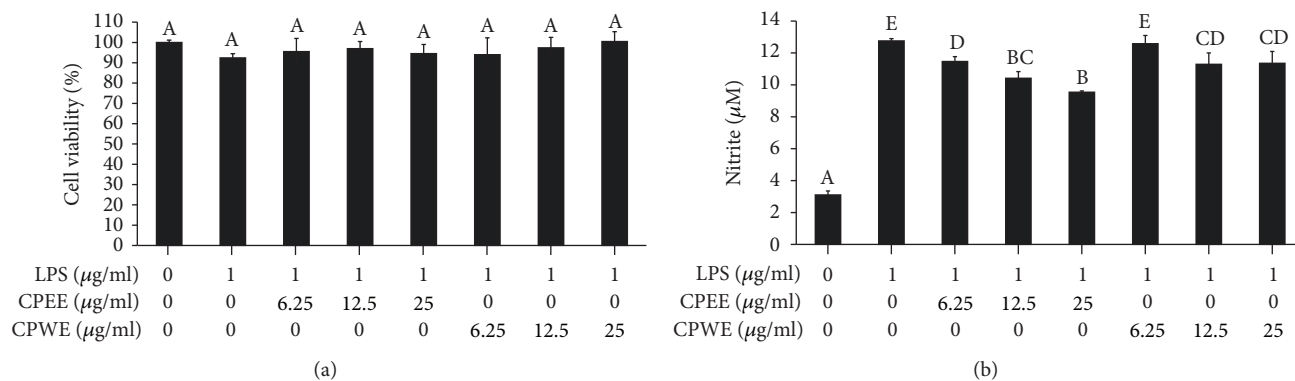

(a)

(b)

Figure 6: (a) Effects of CPEE of TN1, CPWE of TN1, and LPS on cell viability of RAW 264.7 cells. (b) Effects of CPEE of TN1, CPWE of TN1, and LPS on NO secretion in RAW 264.7 cells. The data are the means ± SD of triplicate samples. Bars with different letters are significantly different ($p < 0.05$).
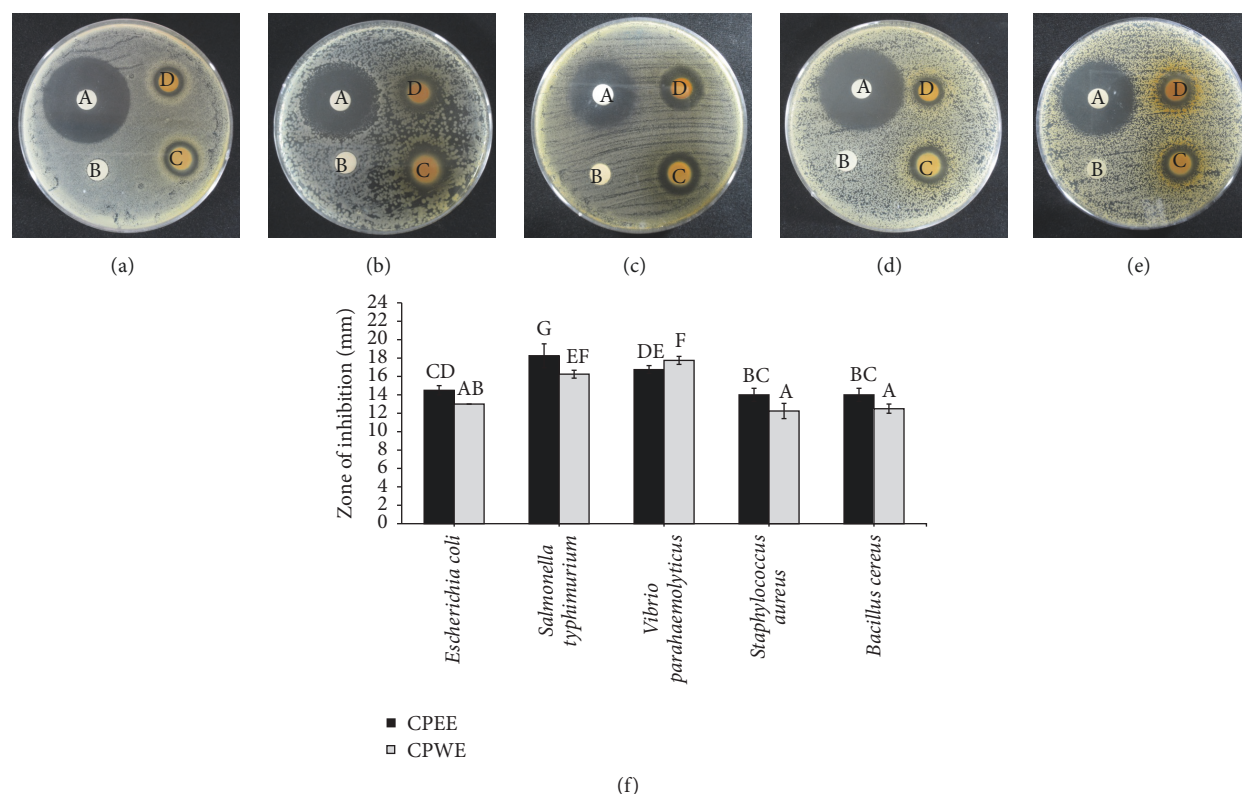
(a)

(b)

(c)

(d)

(e)

(f)

FIGURE 7: Zone of inhibition of CPEE of TN1 and CPWE of TN1 at concentration of 4%, w/v, in 0.05 M acetate buffer, pH 6.0, against (a) *Escherichia coli*, (b) *Salmonella typhimurium*, (c) *Vibrio parahaemolyticus*, (d) *Staphylococcus aureus*; and (e) *Bacillus cereus*. In each dish, A, B, C, and D represent antibiotic, acetate buffer, CPEE of TN1, and CPWE of TN1, respectively. (f) The bar graph summarizes the four separate antibacterial experiments and shows the zone of inhibition according to treatments. Values are expressed as the mean ± SD ($n = 4$). The means that have at least one common letter do not differ significantly ($p > 0.05$).

five bacteria tested. The Gram-negative bacteria were more sensitive than Gram-positive ones to CPEE and CPWE of TN1 (Figure 7(f)). In addition, for these five bacteria, except *V. parahaemolyticus*, CPEE exhibited higher antibacterial activity compared to CPWE. These results may be attributed to the higher polyphenol content detected in CPEE (Table 2), which is also consistent with previous findings [50]. Interestingly, for *V. parahaemolyticus*, CPEE had less antibacterial activity compared to CPWE. We speculate the reason may be due to the presence of 3% NaCl in the medium of *V. parahaemolyticus*. However, further experimental studies are needed to elucidate the mechanism of action. In summary, the present study demonstrated that CPEE and CPWE from TN1 had high amounts of phenolic compounds, possessed good and stable free radical-scavenging activities, and exhibited anti-inflammatory and antibacterial activities. CPEE of TN1 exhibited the most antioxidant, anti-inflammatory, and antibacterial properties and thus has potential for use in the food, cosmetics, and nutraceutical industries.

4. Conclusion

In this study, we employed a compressional-puffing pretreatment process and two extraction methods to extract bioactive compounds from six Taiwanese mango peels. The compressional-puffing process increases the extraction yields and polyphenol contents of peel extracts. Ethanol extracts of peels had higher amounts of total phenolic compounds and greater free radical-scavenging activities as compared to water extracts of peels. The polyphenol contents of extracts positively correlated to the free radical-scavenging activities of extracts. Among these extracts, CPEE of TN1 exhibited the most antioxidant, anti-inflammatory, and antibacterial properties. Thus it is suggested as a natural, safe, and stable antioxidant agent with anti-inflammatory and antibacterial properties, which may have a wide range of applications in food, cosmetics, and nutraceuticals. Future studies on the polyphenol composition and biological activities of mango peel extracts after an *in vitro* digestion as well as investigations of the *in vivo* biological activities of mango peel extracts are warranted.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Chun-Yung Huang and Chia-Hung Kuo contributed equally to this work.

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