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Research Article

Functional Evaluation of Loquat (*Eriobotrya japonica* Lindl.) Flower Water Extracts and Its Potential Use in Tea

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In traditional Chinese medicine, loquat leaves have been commonly used to relieve cough and expectoration. Recently, it has been proved that loquat fruit, kernels, leaves, and flowers can be used as herbal medicines to prevent cardiovascular diseases, cancer, inflammation, and other chronic diseases. This study extracted the loquat flowers (LF) at different temperatures and times to prepare the LF water extract (LFWE). In addition, the antioxidant activity and anti-inflammatory performance of LF extract were evaluated. Moreover, the potential of developing sugar-free LF tea was evaluated. The results showed that the LFWE with a temperature above 80°C and heating for 60 min had a higher content of bioactive compounds, which was significantly different compared with other extraction conditions ($p < 0.05$); antioxidant ability trial results showed the performance of the same trend ($p < 0.05$). However, LFWE was not toxic to cell viability, and the amount of PGE_2 production was significantly lower than that of the induced group ($p < 0.05$), thus considerably inhibiting the production of PGE_2, a marker of inflammation and effectively reducing inflammatory responses. The LF tea prepared at 90°C for different times (15, 30, 45, and 60 min) showed a significant decrease in L^* value as the extraction time increased significantly to darkness. In addition, the sensory evaluation results revealed that the overall acceptability scores of LF tea extracted for 45 min were the highest, and there was a significant difference ($p < 0.05$). Hence, LF is one of the good products for functional beverages and foods.

1. Introduction

Well known as the reproductive structure of flowering plants, humans have used flowers in various food and pharmaceutical applications, therefore referred to as edible flowers while nontoxic; at the same time, it has been evaluated to have medicinal benefits, health-promoting effects, and extra nutritional advanced well with nutritional develop-ments, arousing great interest for the scientific research community [1–6]. Remarkably, it is easy for everyone who wants to buy edible flowers in supermarkets or online shopping platforms as the market expands [7]. Unique edible flowers, such as broccoli, cauliflower, chives, artichoke, and pumpkin flowers, have a long history of use as vegetables and spices, in addition to the commonly recognized ornamental flowers [5, 8].

Polyphenols are a source of antioxidants in diets, but studies showed that the stomach does not widely absorb them; in contrast, intestinal microorganisms facilitate their accumulation and intestinal health [2, 9]. Meanwhile, polyphenolic compounds have physiological effects, such as antibacterial and anti-inflammatory, preventing diabetes and inhibiting lipid formation [10–12]. Moreover, plants' flavonoids (quercetin, anthocyanidins, apigenin, and luteolin) are bioactive secondary metabolites with antioxidant and anti-inflammatory properties [10, 11, 13, 14]. Many studies have corroborated the potent medicinal qualities of the flower, attributed to its bioactive compounds that exhibit antioxidant activity [7, 15, 16]. Different plant parts may require varying levels of bioactive secondary metabolites to survive and protect themselves from reactive oxygen species (ROS) microbial, insect, and herbivore damage. These levels depend on factors such as the growing environment, fertilization, variety, genotype, and the extraction process. For instance, flowers may require higher levels of these metabolites to survive [16–18]. Concurrently, Shen et al. [19] also reported similar results, with the most significant biological activity enrichment in the four stages of the initial FL.

Loquat (*Eriobotrya japonica* (L.) Thunb.) is a medicinal and edible evergreen fruit tree that blooms in winter for 3-4 months; but to enhance the fruit quality and yield, blossom thinning is required to remove the vast amount of LF during the flowering process, thereby providing sufficient raw material for its utilization [19–23]. Since LF has been proven to contain rich biological activities (such as flavonoids, polyphenols, triterpenes, amygdalin, and oligosaccharides), in addition to being used as a traditional Chinese medicine, it also has the potential of being a homologous material for medicine and food [1, 19, 24, 25]. Loquat is becoming an increasingly popular fruit for commercial production due to its high value as an early-harvested crop, while this fruit has great potential, particularly in Mediterranean climates where traditional fruit crops may be oversaturated [21, 26, 27]. Hence, this study is aimed at determining the value of the loquat by-product, LF, to use hot water extraction for LF water extract (LFWE) as a potential natural component for the food industry, thus contributing to the improvement of human health and reducing the environmental impact.

2. Materials and Methods

2.1. Materials. LF used in this study was provided by Quhe Winery (Peinan Township, Taitung County, Taiwan) and randomly collected in November and December 2022 following blossom thinning. Mouse macrophage RAW264.7 cell line (ATCC TIB-71) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All analytical grade chemicals were purchased from Sigma-Aldrich® (Merck KGaA, Darmstadt, Germany).

2.2. Effect of Temperature and Heating Time on the Bioactivity Contents of Loquat Extracts. The extraction protocol was based on the description provided by Moreira et al. [28] with some modifications. The extracts of 1 g of dried LF were extracted with 150 mL of hot water at 60°C,

70°C, 80°C, and 90°C for 15, 30, 45, 60, and 75 min, respectively, followed by cooling down to room temperature afterward for subsequent analysis.

2.2.1. Determination of Total Phenol Content (TPC). TPC determination of the samples was performed as described by Huang et al. [29] and Li et al. [10]. The LF extracts (1 mL) were mixed with 0.5 mL of Foline-Ciocalteu reagent at room temperature for 5 min. Next, 5 mL of 5% Na_2CO_3 was added, and the reaction mixture was incubated at room temperature for 60 min. Subsequently, the absorbance was measured at 750 nm using a spectrophotometer (U-2000, Hitachi, Ltd., Tokyo, Japan). The standard curve was prepared using gallic acid (concentrations ranging from 0 to 0.025 g/L). TPC of the sample was calculated by interpolation and expressed as milligram gallic acid equivalent (mg GAE/dry weight (DW)) per milliliter of LF extract.

2.2.2. Determination of Total Flavonoid Content (TFC). The TFC of LFWE was determined as described by Huang et al. [29]. LFWE was taken 1 mL; then, 1 mL of 2% $ZrOCl_2$ and 5 mL of methanol were added and then placed in a water bath (30°C) for 1 h. Finally, the absorbance values of the samples were determined at 420 nm using a spectrophotometer (Hitachi, Ltd.). The standard curve was prepared using quercetin and calculated by interpolating the samples' quercetin content. TFC was expressed as milligram quercetin per milliliter of LFWE (mg RE/DW).

2.2.3. Quantification of Phenolic Acid Compounds. Phenolic acid compounds were determined as described by Ahumada et al. [1], with slight modifications. Briefly, samples were injected with 20 μL measurement using high-performance liquid chromatography (HPLC) (CM5000, Hitachi, Ltd., Tokyo, Japan). The mobile phase (flow rate 0.5 mL/min) composed of A 5% formic acid and B 100% acetonitrile was filtered through a 0.45 μm filter membrane and degassed by ultrasonic oscillation. The analytical temperature was set at 30°C for gradient elution, while elution conditions were as follows: 0-40 min for mobile phase B, 0%-15%; 40-45 min for mobile phase B, 15%-45%; and 45-55 min for mobile phase B, 45%-100%; the absorption wavelength was measured at 320 nm. The mobile phase (flow rate 0.5 mL/min) composed of A 5% formic acid and B 100% acetonitrile was filtered through a 0.45 μm filter membrane and degassed by ultrasonic oscillation. The analytical temperature was set at 30°C for gradient elution, while elution conditions were as follows: 0-40 min for mobile phase B, 0%-15%; 40-45 min for mobile phase B, 15%-45%; and 45-55 min for mobile phase B, 45%-100%; the absorption wavelength was measured at 320 nm. The standard curve was prepared by interpolating known phenolic acid standards (including chlorogenic acid, ferulic acid, caffeic acid, syringic acid, and p-coumaric acid). The content of each phenolic compound was calculated by interpolation, which was expressed as milligrams per dry weight.

2.3. Antioxidant Activity Analysis

2.3.1. DPPH Free Radical Scavenging Capacity Analysis. The determination of the DPPH radical scavenging capacity of

the samples was based on the description by Wang et al. [30] with appropriate modifications. The sample was taken as 0.2 mL and added to 5 mL of DPPH solution (0.2 g/L, diluted with methanol at absorbance 0.70 ± 0.01) and then reacted in a light-proof environment for 30 min. Finally, the absorbance value was measured by a spectrophotometer (U-2000, Hitachi, Ltd.) at 517 nm, and the DPPH radical scavenging rate of the sample was calculated as follows:

$$
\begin{aligned}
&\text{DPPH radical scavenging rate (\%)} \\
&= \frac{\text{A517 nm(control} - \text{sample)}}{\text{A517 nm control}} \times 100.
\end{aligned} \tag{1}
$$

2.3.2. Reducing Power. The reducing power of the samples was determined as described by Huang et al. [31], with slight modifications. The sample of 1 mL was mixed with 1 mL of 0.2 M phosphate buffer (PBS; pH 6.6) and 1% potassium ferricyanide, followed by the reaction in a water bath at 50°C for 20 min, then cooling down to room temperature. Next, 1 mL of 10% trichloroacetic acid (TCA) was added and centrifuged at $3,000 \times g$ for 10 min using a centrifuge (CN-10001, Hsiangtai, Taiwan). Afterward, $100 \mu L$ of the supernatant was mixed with $100 \mu L$ of distilled water and $100 \mu L$ of 0.1% ferric chloride ($FeCl_3$) solution and reacted for 10 min. The absorbance at 700 nm was measured using a spectrophotometer (U-2000, Hitachi, Ltd.). L-Ascorbic acid was used as the standard, while the reducing power of the sample relative to L-ascorbic acid was calculated using the following formula:

$$
\text{Reducing power (\%)} = \frac{\text{A700 nm of sample}}{\text{A700 nm of L} - \text{ascorbic acid}} \times 100. \tag{2}
$$

2.4. Analysis of Anti-Inflammatory Activity

2.4.1. Cultured RAW264.7 Cell Lines. The RAW264.7 cell line was cultured based on the protocol described by Zhou et al. [32]. Mouse macrophage RAW264.7 cell lines were cultured using Dulbecco's modified Eagle's medium (DMEM) containing 3.7 g/L sodium bicarbonate ($NaHCO_3$), 10% fetal bovine serum, and 1% penicillin/streptomycin (100 U/mL), which were incubated in a constant temperature incubator at 37°C with 5% CO_2.

2.4.2. Cell Viability Assay (MTT Assay). Cell viability was determined as described by Huang et al. [33] with some modifications. In brief, RAW264.7 cell lines were briefly incubated with 1 mL LFWE and 9 mL DMEM for 24 h. Next, the culture medium was removed and washed twice with PBS, and then, DMEM containing 0.1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added that was placed in a constant temperature incubator at 37°C with 5% CO_2 for 3 h. Subsequently, the culture medium added and isopropyl alcohol to dissolve the purple compound formazan crystals produced by the MTT reaction. Afterward, the supernatant was centrifuged for 10 min at 4°C, at $9,560 \times g$ with a microcentrifuge (CR21E, Hitachi, Japan), with the wavelength 570 nm absorbance measured by an ELISA reader (Synergy HTX, BioTek, USA), and cell viability was calculated using the following equation:

$$
\text{Cell viability (\%)} = \frac{\text{A570 nm of sample}}{\text{A570 nm of control}} \times 100. \tag{3}
$$

2.4.3. Cell Microscopic Observation. Cell microscopy was performed according to the procedure used by Ali and Ms [34]. The RAW264.7 cell line was cultivated with 2×10^5 cells per milliliter in a dish (3.5 cm diameter) and incubated in a constant temperature incubator at 37°C with 5% CO_2 for 24 h. Finally, the variation of cell morphology was observed with an inverted microscope (ECLIPSE Ts2, Nikon, Japan) and photographed.

2.5. Determination of Prostaglandin E_2 (PGE_2) Contents. The PGE_2 content was determined by the PGE_2 ELISA kit (Item No. 514010, Cayman Chemical Inc., Ann Arbor, Michigan, USA) and measured in accordance with the standard operating procedures provided by the manufacturer. The RAW264.7 cell lines were reacted with different concentrations (1, 5, and 10 mg/mL) of LFWE with inducer lipopolysaccharide (LPS) for 20 h. The culture medium was collected by centrifugation at $3,000 \times g$ for 5 min at 4°C. Then, $50 \mu L$ of the supernatant was added to the 96-well plate, followed by $50 \mu L$ of tracer and $50 \mu L$ of antibody, and the reaction solution was removed after 18 h at 4°C without light. Afterward, the reagent was washed 5 times with $200 \mu L$ of wash buffer (1 min/time), and $200 \mu L$ of Ellman's reagent was added, followed by the reaction at room temperature for 2 h. Finally, the wavelength of 412 nm was measured by an ELISA reader (Synergy HTX, BioTek) to determine the absorbance. The PGE2 standard prepared the standard curve, while the performance of PGE2 in RAW264.7 cell lines treated with LFWE at different concentrations was calculated by interpolation.

2.6. Application of LFWE. This study used 90°C hot water and different extraction times (15, 30, 45, and 60 min) as extraction conditions, depending on the extraction results, while the extracts were formulated into sugar-free LF tea. Meanwhile, the relevant physicochemical properties were determined at a room temperature of 25°C, while the soluble solids (°Brix) were determined using a hand-held sugar meter (Shimadzu, MASTER-T, Japan), and the pH value was measured using a pH meter (Suntex, SP-2500, Taiwan).

In addition, this study evaluated the appearance, color, and taste, which significantly influenced consumers' decision to purchase the LF tea and further assessed the beverage's potential for commercialization. Precisely, the appearance of the color difference was measured using a chromatic analyzer (Nippon Denshoku, SA-4000, Japan), and the samples' L^*, a^*, and b^* values were measured. The L^* value is the brightness; the closer it is to 100, the brighter the color, and the closer it is to 0, the darker the color; a^* value is the red-green color, the $+a^*$ value indicates the red color, and the $-a^*$ value indicates the green color; b^* value indicates yellow-blue, $+b^*$ value indicates yellow chroma, and $-b^*$ value indicates blue chroma.

Moreover, sensory evaluation was carried out with a 9-point scale for the consumer model ($n = 20$). Each of the five evaluation items of acceptability includes the appearance of color, smell, taste, taste persistence, and overall acceptability. The 9-point evaluation score has no decimal points, which enables repeated scoring, where 1 means light (dislike), 5 means medium (like), and 9 means strong (like very much). The overall acceptability rating depended on personal preference, with 1 indicating that it was impossible in the mouth, 5 indicating that it was willing to be put in the mouth, and 9 indicating that it was very willing to be eaten, where a higher score represented a better rating. To evaluate each sample interval, the panelists were required to rinse their mouths with drinking water thrice to eliminate the previous sample's taste. The room temperature was maintained at $25 \pm 2°C$. During the evaluation period, the room will be kept quiet and free from noise.

2.7. Statistical Analysis. All measurements were performed in triplicate and expressed as mean ± S.D. Statistical analysis of the one-way analysis of variance (ANOVA) procedure was performed using IBM SPSS Statistics software (V22.0, IBM Co., Armonk, NY, USA) for intragroup comparison, followed by Duncan's multiple range test to compare the significant differences between groups, while the significant difference was determined as $p < 0.05$.

3. Results and Discussion

3.1. Effects of Extraction Temperature and Time on the TPC and TFC Contents of LFWE. TPC and TFC are important groups of natural compounds that play significant roles in plant growth and defense mechanisms [1, 3, 14, 17]. Flavonoids serve as the primary component in most plants as primary floral pigments, which are recognized as an essential role in various nutritional, pharmaceutical, medicinal, and cosmetic applications [3, 17, 28, 35]. In addition, Wu et al. [12] reported satisfactory cough suppressant activity of the ethanolic extract of loquat leaves, possibly related to its higher flavonoid content.

In this study, the TPC and TFC contents of LFWE were determined after heating and measuring the samples at different temperatures (60, 70, 80, and 90°C) and extraction times (15, 30, 45, 60, and 75 min). The results of TPC content determination showed that the maximum content (116.78 ± 0.19 mg GAE/g DW) was extracted at 90°C for 60 min, compared to other extraction conditions, which was significantly different ($p < 0.05$) (Figure 1(a)). It is worth mentioning that the variation of TPC content was positively correlated with heating time, but a decline occurred at 80°C and 90°C for 75 min extraction. This was attributed to the decrease in phenolic content due to the start of cleavage after prolonged heating at high temperatures. At the same time, the results of this study were consistent with previous studies [36]. Regarding the TFC content, the maximum quantity (30.03 ± 0.25 mg RE/g DW) was found at 90°C with 60 min heated, which was significantly different compared to other extraction conditions ($p < 0.05$) (Figure 1(b)). Interestingly, the results of the TFC and TPC changes in this study showed

the same trend. Shen et al. [19] reported two LF extraction ways with an average extraction rate above 86%, while the maximum TFC and TPC (phenolic compounds) were extracted at 80°C, consistent with this study. Moreover, the maximum value at 80°C may be explained by a shift of some polyphenols and flavonoids from bound to free state at high temperatures, which decreased in content upon rising heating time and temperature [23]. In addition, LF's bioactive compounds (including TPC, TFC, and DPPH radical scavenging ability) decrease as the extraction time increases, with the best extraction time of 30 min [19]. On the contrary, the Wu et al. [37] study showed that the optimal extraction process for LF crude flavonoids was 100°C, with a 2.5 h decoction time, 1 : 20 solid-liquid ratio, and three decoctions. However, LF extraction at 60-70°C required more than 60 min for the flavonoid initiation. This implies that a prolonged time for TFC extraction at low temperatures was required, consistent with previous studies [11, 19, 38]. In contrast, the optimum extraction conditions with 50% EtOH are 60°C and 10 min [22]. It is worth mentioning that the results of this study were higher for LF compared to the TFC (16.3-38.7 μg RE per g FW) and TPC (140-252 μg GAE per g FW) reported for loquat fruits by Ercisli et al. [17].

3.2. Effects of Extraction Temperature and Time on the Phenolic Compounds of LFWE. The phenolic compounds are a group of secondary metabolites found in plants of fruits and vegetables, which play an essential role in providing astringency, bitterness, color, flavor, and antioxidant activity [1, 20, 28]. However, it has been confirmed that the loss of phenolics is reduced at low-temperature storage [26].

The above results show that short-time (15, 30, and 45 minutes) extraction at 60°C cannot effectively extract bioactive components. Therefore, the HPLC analysis of the polyphenolic content of LFWE was carried out primarily at 70°C, 80°C, and 90°C, with 60 and 75 min extraction times. The results of phenolic content variation for each extraction condition showed that the maximum contents of chlorogenic acid and p-coumaric acid were found at 80°C and 90°C for 60 min ($p < 0.05$) (Table 1). Unfortunately, both phenolics showed a decreasing trend at 75 min of extraction, with a significant difference compared to 60 min ($p < 0.05$). Meanwhile, caffeic acid and syringic acid also had the highest amount at 90°C for 60 min but decreased significantly after 75 min of extraction, with significant differences relative to other conditions ($p < 0.05$). In addition, ferulic acid showed a slight increase with different temperature extractions ($p < 0.05$), whereas there was no significant difference in the extraction time. This is hypothesized to be related to the degradation of phenolic acid due to prolonged high-temperature heating [39]. Another possible reason for the decreased free phenol content is that phenolic acid has precipitated with other substances as a polymer [40]. Zar et al. [41] conducted a study on the bioactive contents of the water extract from loquat leaves (boiled at 100°C for 15 min), and the water extract from loquat leaves tea (roasted at 350°C for 30 min) showed a decreasing trend, containing 3-caffeoylquinic acid, 5-caffeoylquinic acid, epicatechin, and procyanidin B2. Hence, this study revealed that the higher
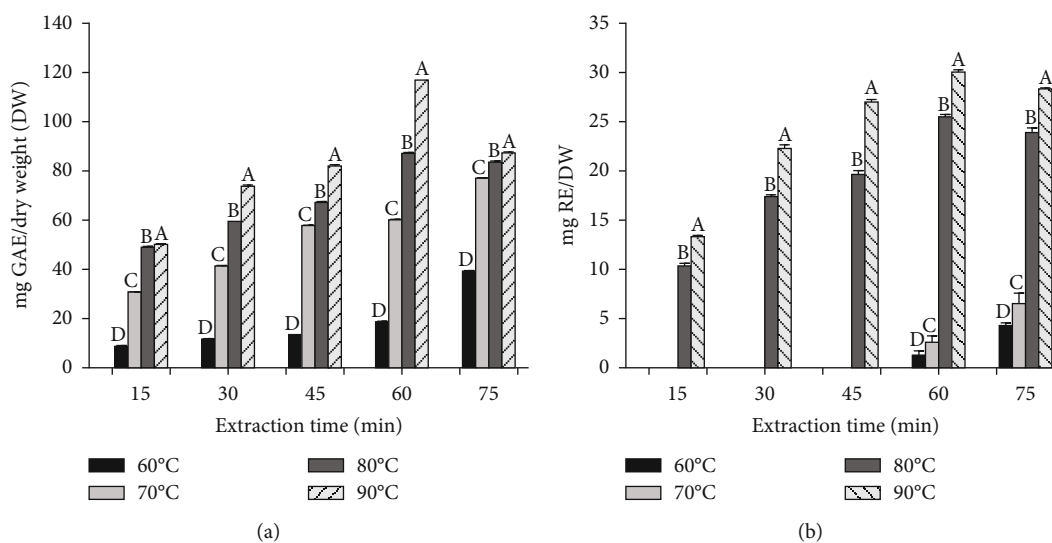
(a)

(b)

FIGURE 1: Effects of different temperatures and times on (a) total polyphenol content (TPC) and (b) total flavonoid content (TFC) of loquat flower extract (LFWE). All values were expressed as mean ± standard deviation ($n = 3$), while different lowercase letters in the figure represent significant differences ($p < 0.05$).

TABLE 1: Effects of different temperatures and times on phenolic acid compounds in loquat flower extract (LFWE).

Phenolic acid compounds (mg/g dry weight DW)	Heating time (min)	Temperature (°C)		
		70	80	90
Chlorogenic acid	60	4.85 ± 0.04^c	9.52 ± 0.10^b	16.84 ± 0.27^a
	75	7.15 ± 0.04^c	8.65 ± 0.05^a	7.39 ± 0.09^b
Ferulic acid	60	1.22 ± 0.01^c	2.32 ± 0.43^b	3.50 ± 0.49^a
	75	1.45 ± 0.04^c	2.65 ± 0.01^b	4.93 ± 0.08^a
Caffeic acid	60	0.15 ± 0.01^c	0.82 ± 0.00^b	2.59 ± 0.03^a
	75	0.44 ± 0.02^c	1.01 ± 0.02^a	0.76 ± 0.02^b
Syringic acid	60	0.24 ± 0.09^c	19.38 ± 1.32^b	73.26 ± 0.84^a
	75	6.56 ± 0.96^c	20.88 ± 0.59^b	39.28 ± 0.73^a
p-Coumaric acid	60	0.31 ± 0.01^c	0.59 ± 0.02^b	0.91 ± 0.05^a
	75	0.50 ± 0.01^b	0.54 ± 0.06^a	0.12 ± 0.01^c

All data are presented as mean ± SD ($n = 3$). The mean values of lowercase letters in the same row differed significantly ($p < 0.05$).

heating temperature (80°C and 90°C) contributed positively to phenolic acid content, especially chlorogenic acid, caffeic acid, syringic acid, and p-coumaric acid. At the same time, the extraction time of 60 min was necessary as a cofactor.

The reducing power results are shown in Figure 2(b), while at 90°C was $50.23 \pm 0.13\%$, $54.13 \pm 0.05\%$, $60.93 \pm 0.33\%$, $82.24 \pm 0.13\%$, and $88.41 \pm 0.18\%$ for low to high extraction times (15-75 min), respectively, with the best performance, which as compared to other extraction conditions and significant differences ($p < 0.05$). Notably, the reducing power rose with prolonged heating time, which revealed similar results to DPPH (Figure 2(a)), whereby previous studies confirmed the association of increased temperatures with the release of bioactive compounds such as TPC and TFC [37, 42–44]. Unfortunately, this study's TPC and TFC

decreased after more than 60 min extraction time. Hence, future applications should avoid prolonged heating to promote the cleavage of these bioactive substances.

3.3. Effects of Different Extraction Temperatures and Times for LFWE on Antioxidant Ability. The results of the DPPH radical scavenging ability of LFWE with different extraction temperatures and time exhibited positive results with increasing extraction temperature and time ($p < 0.05$) (Figure 2(a)). It is worth mentioning the similar effects of TPC variation as discussed above. In this case, LFWE extracted at 90°C for 75 min showed the best DPPH radical scavenging ($44.15 \pm 2.62\%$), and the worst performance was at 60°C ($27.47 \pm 1.94\%$), which was attributed to the antioxidant capacity of LFWE associated with the contents
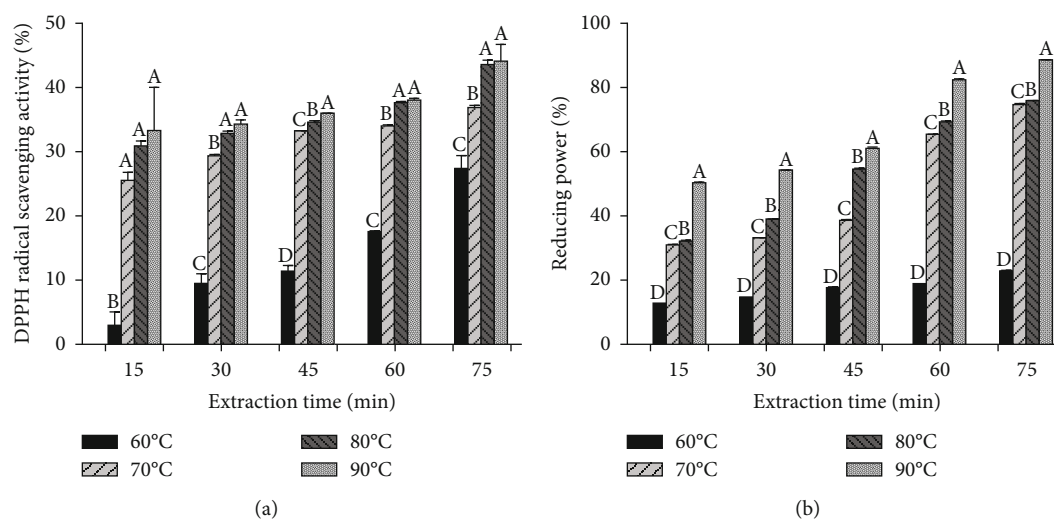
FIGURE 2: Effects of different temperatures and times on (a) DPPH free radical scavenging rate and (b) reducing power (reduction degree, %) of loquat flower extract (LFWE). All values were expressed as mean ± standard deviation ($n = 3$), while different lowercase letters in the figure represent significant differences ($p < 0.05$).

of bioactive components (TPC or TFC). In addition, it has been noted that extracts prepared with absolute methanol or ethanol showed the highest antioxidant activity, followed by 50% ethanol; thus, the higher the ethanol content, the better the antioxidant activity, which implies that these metabolites have less solubility in water than ethanol [28]. In contrast, Wu et al. [37] reported that as the alcohol concentration rose, the TPC polarity in LF would be degraded, and the categories were altered.

3.4. Correlation Analysis of Bioactive Components and Antioxidant Capacity of LFWE.

The correlation between the bioactive component content and antioxidant ability of LFWE showed a positive correlation between TPC and TFC ($r = 0.923651$). In contrast, individual phenolic acids showed a positive correlation with TPC for chlorogenic acid ($r = 0.99482$), ferulic acid ($r = 0.999985$), caffeic acid ($r = 0.9734$), and syringic acid ($r = 0.970527$) (Table 2); caffeic acid ($r = 0.9734$) and syringic acid ($r = 0.970527$) showed a significant positive correlation, but significant negative correlation ($p < 0.05$) with p-coumaric acid ($r = -0.56592$). In addition, there were positive correlations between TPC, TFC, and DPPH ($r = 0.89923$ and 0.998231), and reducing power ($r = 0.962994$ and 0.78618), respectively, while this study's results were consistent, as reported by Huang et al. [22] and Lü et al. [24].

3.5. Effects of Various Concentrations of LFWE for Mouse Macrophage RAW264.7 Call Lines' Survival Rate.

Inflammation is a process strongly influenced by nonpharmacological interventions (such as diet). Its nature of the response to eliminate damaging factors and repair damaged tissues in a temporary or self-limiting manner of the immune system is one of the multiple responses used to protect the body from harm [1]. Interestingly, where a systematic review and meta-analysis reported that plant-based index was not

related to body composition, at the same time, most studies of total and central fat did not find any association with plant-based index because the plant-based index must be considered in the context of food processing and not all vegetable foods are healthy [45].

However, persistence results in chronic inflammation, which includes diabetes, coronary artery disease, and asthma [46, 47], whereas less attention has been paid to the anti-inflammatory effects of floral sources consumed in dietary patterns [1]. In this study, an MTT assay was used to evaluate the toxicity of LFWE at various concentrations (1, 2.5, 5, and 10 mg/mL) on the RAW264.7 cell lines while preventing apoptosis due to high concentrations. This study found that the survival activities of RAW264.7 cell lines treated with all concentrations were over 95% with no significant difference. Therefore, it was suggested that LFWE had low cytotoxic effects on RAW264.7 call lines' viability (Figure 3(a)). However, the cell viability was slightly lower at 10 mg/mL LFWE, which marginally affected the cell proliferation of RAW264.7 cells but within the permissible range. Despite no significant statistical difference, the cell viability at 2.5 and 5 mg/mL LFWE exceeded that of the control group. Therefore, LFWE contains bioactive compounds that promote cell proliferation. Previous studies have shown the potential of phenolic compounds in fruits and vegetables to stimulate cell proliferation and encourage immunomodulatory performance [48]. In contrast, according to the cell morphology observation, an LFWE concentration of 10 mg/mL showed cells' initial deformation and apoptosis (Figure 3(c)). At the same time, the changes in other groups were not significant, which was simultaneously consistent with the cell viability results described above. This also implies that LFWE at concentrations of 2.5 and 5 mg/mL were not toxic to cells, but bioactive compounds enhanced cell proliferation and viability. According to previous studies, the stimulation of cell proliferation exhibiting

TABLE 2: Correlation analysis between bioactive components and antioxidant capacity of loquat flower extract (LFWE).

Variable	TPC	TFC	Chlorogenic acid	Ferulic acid	Caffeic acid	Syringic acid	p-Coumaric acid	DPPH	Reducing power
TPC	1								
TFC	0.923651								
Chlorogenic acid	0.99482	0.87991							
Ferulic acid	0.999985	0.925745	0.994246						
Caffeic acid	0.9734	0.811278	0.991648	0.972125					
Syringic acid	0.970527	0.804072	0.989997	0.969187	0.999925				
p-Coumaric acid	-0.56592	-0.83868	-0.47918	-0.57045	-0.36197	-0.35055			
DPPH	0.89923	0.998231	0.850102	0.901623	0.775079	0.767299	-0.86957		
Reducing power	0.962994	0.78618	0.985403	0.961497	0.999129	0.999565	-0.32277	0.748043	1

immunomodulatory potential has been associated with phenolic compounds in fruits and vegetables [48]. Phenolic compounds have a strong antioxidant capacity, scavenging intracellular DPPH radicals and improving the activity of cellular SOD, GPx, or other antioxidant enzymes [49, 50], thus contributing positively to cell viability.

3.6. Effects of Various Concentrations of LFWE-Treated LPS-Induced Mouse Macrophage RAW264.7 Cell Line for PGE_2 Content.

PGE_2 is a primary downstream product of COX-2-mediated arachidonic acid metabolism in many cells (such as epithelial cells, fibroblasts, and inflammatory cells), which serves a key role in many early inflammatory events, facilitating the recruitment of leukocytes into inflammatory sites [51]. In parallel, it has increased blood flow, vascular permeability, and nociception, leading to inflammatory symptoms, redness, swelling, fever, and pain [51]. In addition, it also regulates the activation of endogenous stem cells, angiogenesis and other processes, and also plays a vital role in the normal reproductive function of women [52, 53]. In this study, the PGE_2 productions in various concentrations (1, 5, and 10 mg/mL) of LFWE-treated RAW264.7 cell lines were 65.9%, 77.17%, and 72.47%, respectively, which were significantly lower than the control and induction groups. There were significant differences ($p < 0.05$) (Figure 3(b)). The best performance was achieved at 1 mg/mL, suggesting that it was the most effective for suppressing inflammatory responses. However, the cytokine PGE_2 expression and LFWE were not dose-dependent.

Additionally, in terms of the cell morphological observation, it was observed that the number of cells in the induced group was significantly reduced (with more gaps) (Figure 3(d)), which differed compared to the LFWE-treated groups. However, the 5 and 10 mg/mL groups (Figures 3(d) and 3(e)) showed significantly lower cell aggregation and proliferation than the 1 mg/mL group, with substantially duller coloration. Interestingly, the cell viability profile of the 1 mg/mL LFWE group compared to the control group matched the earlier described results. Moreover,

Choi et al. [54] have revealed that the higher flavonoid content reduces PGE_2 production, which leads to anti-inflammatory effects. In addition, hot water extracts have shown antioxidant, free radical scavenging, and anti-inflammatory activities comparable to those of organic solvent extracts with the potential to modulate the gut microbiota attributed to the presence of bioactive components such as TPC and TFC [38, 55].

3.7. Physicochemical Characteristics and Sensory Evaluation.

The results of the physicochemical characterization are shown in Table 3. Since this pilot study of sugar-free LF tea without food additives, the samples' soluble solids (°Brix) revealed no significant differences between all groups. Notably, pH was negatively correlated with extraction time, significantly different for each group ($p < 0.05$). It was attributed to the release of phenolic acids from LF with increased extraction time. However, the results were also consistent with TPC and TFC. The chromatic results showed that the L^* values gradually decreased with more extraction time ($p < 0.05$); a^* values showed no difference in each group. However, the b^* and ΔE values progressively increased with prolonged extraction time ($p < 0.05$). Meanwhile, it means that the visual color variation occurred more obviously among the groups as the color of the sample gradually darkened, and brightness decreased with the increased extraction time.

The sensory evaluation results showed that extraction for 15 min was the least significant of all evaluation items (Table 3), which may be attributed to the short extraction time as the flavor-related components were not fully released. However, the extracted 45 min was the highest performance among all evaluation items, with a significant difference from other groups ($p < 0.05$). Moreover, in the above results, the maximum bioactive compounds were obtained at 60-75 min of extraction, which may be related to the loss of readily volatile aromatic compounds (pleasant aroma) [56], evaporated over 45 min of extraction (thermal degradation), and disappeared.
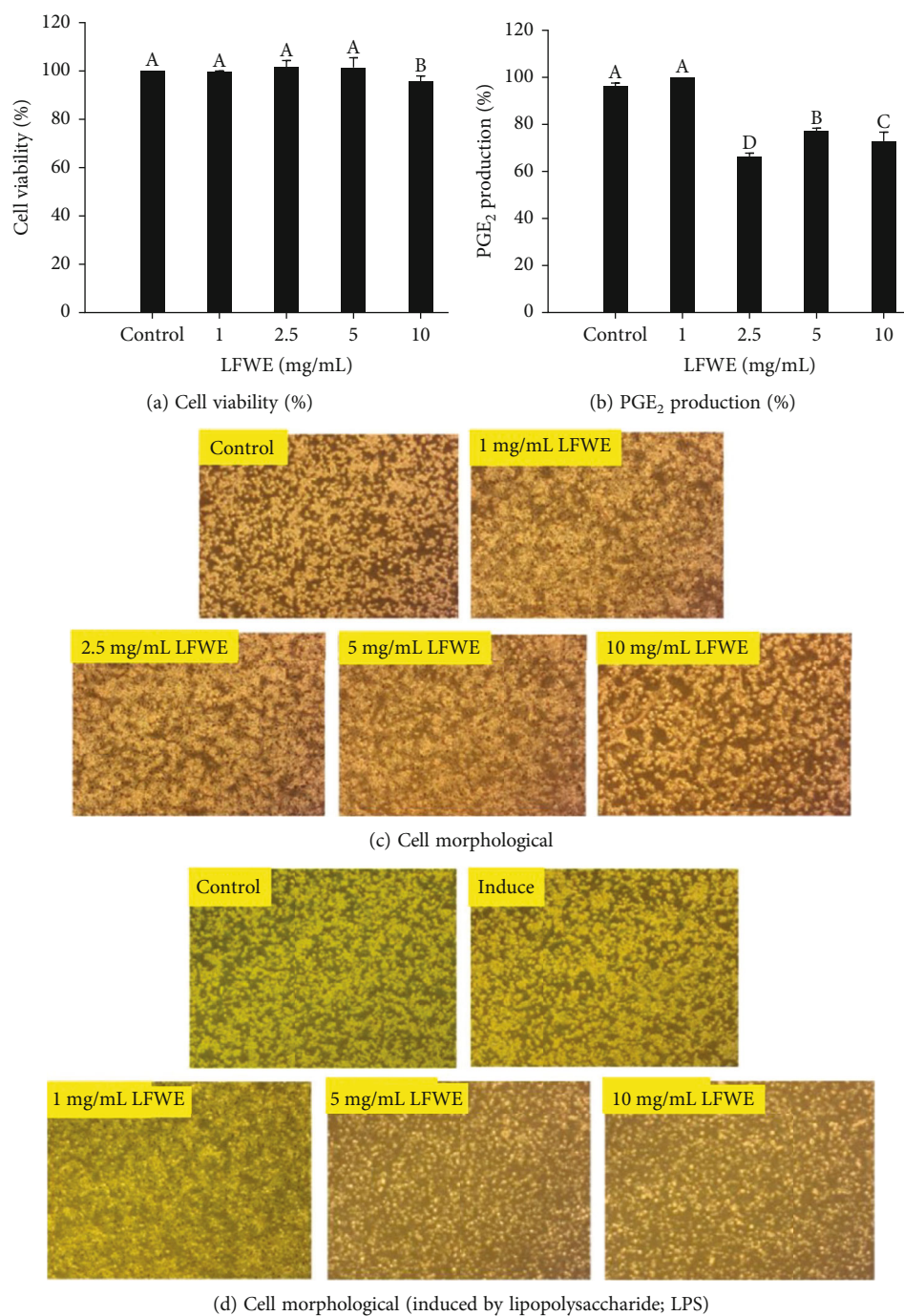
(a) Cell viability (%)

(b) PGE$_2$ production (%)

(c) Cell morphological

(d) Cell morphological (induced by lipopolysaccharide; LPS)

FIGURE 3: Effects of different concentrations of LFWE on the (a) cell viability, (b) PGE$_2$ production, and (c, d) cell morphological of mouse macrophage RAW264.7 cell lines. All values were expressed as mean ± standard deviation ($n = 3$), while different lowercase letters in the figure represent significant differences ($p < 0.05$).

TABLE 3: Physicochemical analysis and sensory evaluation of loquat flower tea.

Sugar-free loquat flower tea	Heated at 90°C			
	15 min	30 min	45 min	60 min
Physical analysis				
°Brix	0.20 ± 0.00^c	0.20 ± 0.00^c	0.40 ± 0.00^b	0.60 ± 0.00^a
pH	6.31 ± 0.00^b	6.41 ± 0.00^a	5.83 ± 0.00^c	5.49 ± 0.00^d
L^*	16.55 ± 0.00^a	16.28 ± 0.00^b	16.03 ± 0.00^c	15.66 ± 0.01^d
a^*	-0.36 ± 0.04^a	-0.28 ± 0.05^a	-0.27 ± 0.04^a	-0.26 ± 0.00^a
b^*	0.85 ± 0.02^d	1.53 ± 0.02^c	2.13 ± 0.02^b	2.87 ± 0.00^a
$\triangle E$	1.56 ± 0.02^d	2.28 ± 0.02^c	2.93 ± 0.02^b	3.76 ± 0.01^a
Sensory evaluation				
Color	5.85 ± 2.52^a	6.25 ± 2.00^a	7.00 ± 1.92^a	6.60 ± 2.31^a
Smell	4.85 ± 2.15^c	6.05 ± 1.72^b	6.35 ± 1.98^a	6.40 ± 1.91^a
Taste	5.05 ± 1.91^b	6.80 ± 1.40^a	7.00 ± 1.22^a	6.90 ± 1.87^a
Taste persistence	4.75 ± 2.28^b	6.05 ± 1.53^a	6.35 ± 2.06^a	6.85 ± 1.39^a
Overall acceptance	5.00 ± 1.79^e	6.00 ± 1.34^c	7.00 ± 1.20^a	6.00 ± 2.13^b

Data of physical analysis were presented as mean $\pm$ SD ($n = 3$). Sensory evaluation data were presented as mean $\pm$ SD ($n = 20$). The mean values of lowercase letters in the same row differed significantly ($p < 0.05$).

According to the above results, it was found that the extraction time of hot water had a significant effect on the sensory evaluation of sugar-free LF tea. The tea with a short extraction time was relatively brighter in color but lacked smell and taste. On the contrary, the smell and taste of tea extracted for a long time were sufficient. Still, the color was darker, and the flavor would gradually deteriorate as the extraction time was extended. Thus, the most balanced performance (the appearance of color, smell, taste, and persistence) with the highest overall acceptability of the 90°C extraction condition for 45 min leads to the most appropriate for future commercialization of the process.

4. Conclusions

In this study, LFWE showed more bioactive components (TPC and TFC) and satisfactory antioxidant ability with 80-90°C extraction conditions and 60 min, while LFWE without cytotoxicity was also effective in decreasing the cellular inflammatory marker PGE2. In addition, the bioactive components of LFWE's sugar-free LF tea were not damaged by thermal treatment in food processing (45 min of hot water extraction at 90°C). Overall, LFWE showed antioxidant and anti-inflammatory properties, providing high levels of bioactive components with optimal taste and flavor under appropriate conditions. This study was beneficial for enhancing the recyclability of agricultural by-products and might serve as a reference base for future anti-inflammatory product investigations and advance LFWE bioactive components to develop healthier products.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

Highlights. (1) LF is an ornamental, edible, and medicinal high-quality, wholesome food. (2) LFWE's biological activity was affected by the extraction conditions. (3) 1 mg/mL LFWE enhanced RAW264.7 cell proliferation. (4) A positive correlation was observed between TPC, TFC, and antioxidant ability. (5) There was a significant positive correlation between phenolic acid and TPC; only *p*-coumaric acid showed a negative correlation.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Authors' Contributions

Si-Yu Chen and Ping-Hsiu Huang contributed equally to this work. Shu-Ling Hsieh and Chih-Yao Hou contributed equally to be the correspondence authors.

Acknowledgments

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