Hindawi
Journal of Chemistry
Volume 2020, Article ID 5976090, 5 pages
https://doi.org/10.1155/2020/5976090

*Research Article*

# Characterization of Phenolic Constituents from *Prunus cerasifera* Ldb Leaves

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Received 3 August 2019; Revised 11 December 2019; Accepted 23 December 2019; Published 11 January 2020

Academic Editor: Alvin Holder



To elucidate the chemical compositions of *Prunus cerasifera* Ldb leaves, the methanol extracts were firstly fractionated by ethyl acetate and *n*-butanol, respectively. The phenolic acid-rich fractions (ethyl acetate extracts) were further isolated by various chromatographic columns (CC) including MCI macroporous resin, normal-phase silica gel, Sephadex gel LH-20, octadecyl silane (ODS), and preparative HPLC to yield the phenolic compounds. The isolated compounds were analyzed by $^{1}$H-nuclear magnetic resonance ($^{1}$H-NMR), $^{13}$C-NMR, and electrospray ionization mass spectral (ESI-MS) spectroscopy. Eleven phenolic acids were identified as *p*-coumaric acid (**1**), caffeic acid (**2**), ferulic acid (**3**), chlorogenic acid (**4**), 3-*O*-caffeoylquinic acid (**5**), 5-*O*-coumaroylquinic acid (**6**), 3-*O*-caffeoylquinic acid methyl ester (**7**), chlorogenic acid methyl ester (**8**), 3-*O*-caffeoyl-5-*O*-coumaroylquinic acid or 3-*O*-coumaroyl-5-*O*-caffeoylquinic acid (**9**), gallic acid (**10**), and protocatechuic acid (**11**). The current study pioneers to identify and report all the phenolic constituents from *P. cerasifera* Ldb leaves.

## 1. Introduction

Plums (genus *Prunus* of family Rosaceae) are a famous juicy and nutritious fruit cultivated throughout the world [1]. Various plum species are grown worldwide including *Prunus domestica* L., *P. salicina* Lindl., *P. americana* Marsh., *P. cerasifera* Ehrh., *P. insititia* L., and *P. spinosa* L. [2] and regionally utilized in different industries. In China, *P. cerasifera* Ehrh. (vernacular name mirabalan) is native to the Tianshan area of the Xinjiang province and well liked by the community due to its higher nutrition values [1, 3]. The genus *Prunus* have extensively been studied for their phytochemical screening and reported to contain different polyphenols and their derivatives [4–11]. Moreover, *P. cerasifera* is an endangered fruit species grown wild in Southern slopes of the Huicheng area in the Xianjiang province of China [12]. *P. cerasifera* var. atropurpurea is being planted as an ornamental tree due to its year-round purple leaves which are full of natural edible pigments and anthocyanins [3, 13, 14]. Huge literature reported its biological significance, and it bears strong antioxidant potential along with biologically active ingredients particularly the flavonoids, saponins, and various phenolic acids [15, 16].

Fruits are essential in diet to meet the health needs mainly covered by the various vitamins, flavonoids, and phenolic compounds [17]. *Prunus* species have less antioxidant properties compared to other fruits, but are an important part of daily diet [18]. Phenolic compounds are the products of plant metabolism and constitute a diverse

class of the plant-based compounds and mainly bears strong antioxidant capacity, vitamins, and carotenoids [19]. The phenolic compounds are also called polyphenols composed of multiple hydroxylated aromatic rings, which are the main source of certain biological and antioxidant activities [20]. Fruits of *P. cerasifera* are rich in health-promoting antioxidants and polyphenols, hence prevents the onset of several diseases and is given much focus in the recent decade [21].

Except colour-stable anthocyanins, various organic acids, the primary metabolites including pectin, mineral elements, and almost all essential amino acids have been also isolated and reported in *P. cerasifera* fruits [22]. There are hardly any studies which have focused on the polyphenolics in *P. cerasifera*. Hence, the current study was designed to elucidate the complete chemical profile of the leaves of the *P. cerasifera* plant to identify and report specific polyphenols.

## 2. Materials and Methods

*2.1. General Experimental Procedures.* $^1$H-NMR and $^{13}$C-NMR were tested by a Varian 500 MHz instrument (Varian, Palo Alto, CA, USA) and tetramethylsilane (TMS) as internal standard materials. Electrospray ionization mass spectral (ESI-MS) data were tested by an AB-QTRAP 4500 mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA). High-performance liquid chromatography (HPLC) were performed on a Hitachi Elite LaChrom system consisting of a L2130 pump, L-2200 autosampler, and a L-2455 diode array detector, which were all operated by the EZChrom Elite software. The UV spectral data were acquired by the HPLC analysis. All solvents were of either the ACS or HPLC grade.

*2.2. Plant Material Extraction and Isolation.* The leaves of *P. cerasifera* Ldb were collected locally from the Daxigou region, Huocheng city (Kazakh Autonomous Prefecture of Ili, Xinjiang province, China). The leaves of *P. cerasifera* (2.0 Kg, dry weight) were extracted thoroughly with MeOH (3 × 10 L) at room temperature and combined those extracts to finally get a dried MeOH extract (210 g). A part of the extract (200 g) was resuspended in $H_2O$ (4.0 L) and successively partitioned with EtOAc (3 × 4.0 L) and *n*-butanol to yield dried EtOAc (55 g) and *n*-butanol (68 g) extracts, respectively.

The EtOAc fraction (50 g) was firstly chromatographed on an MCI column (3.6 × 18 cm) and eluted by a gradient system mixture of MeOH/$H_2O$ (0 : 1 to 9 : 1, *v/v*) to get 4 subfractions (EPA-EPD). Fraction EPB was separated by the silica gel chromatography column eluted with a mixture of $CHCl_3$/MeOH (100 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 8 : 1, 6 : 1, 4 : 1, and 2 : 1, *v/v*) to give 7 subfractions ($EPB_1$–$EPB_7$). Subfractions $EPB_3$ was isolated by an ODS column eluting with a gradient system mixture of MeOH/$H_2O$ (1 : 9 to 6 : 4, v/v) to get 4 subfractions ($EPB_3A$–$EPB_3D$). Subfraction $EPB_3B$ was separated by the Sephadex gel LH-20 (2.5 × 70 cm) eluted with isocratic MeOH to give 2 main subfractions, which were finally purified by preparative HPLC eluted with a gradient system mixture of MeOH/$H_2O$ to yield compounds **4, 5, 6, 7, 8,** and **9**. Subfraction $EPB_3C$ was separated by the Sephadex gel LH-20 (2.5 × 70 cm) eluted with isocratic MeOH to give 7 main subfractions ($EPB_3C_1$–$EPB_3C_7$), which were finally purified by preparative HPLC eluted with a gradient system mixture of MeOH/$H_2O$ to yield compounds **1, 2**, and **3**. Subfraction $EPB_3D$ was chromatographed on an ODS column (2.5 × 18 cm) eluting with a gradient system mixture of MeOH/$H_2O$ (1 : 9 to 4 : 6, v/v) to afford 4 subfractions, which were finally purified by preparative HPLC eluted with a gradient system mixture of MeOH/$H_2O$ to yield compounds **10** and **11**.

## 3. Results and Discussion

*3.1. Identified Phenolic Acids.* Eleven phenolic acids (Figure 1) were identified as *p*-coumaric acid (**1**), caffeic acid (**2**), ferulic acid (**3**), chlorogenic acid (**4**), 3-*O*-caffeoylquinic acid (**5**), 5-*O*-coumaroylquinic acid (**6**), 3-*O*-caffeoylquinic acid methyl ester (**7**), chlorogenic acid methyl ester (**8**), 3-*O*-caffeoyl-5-*O*-coumaroylquinic acid or 3-*O*-coumaroyl-5-*O*-caffeoylquinic acid (**9**), gallic acid (**10**), and protocatechuic acid (**11**).

*3.2. Spectroscopic and Spectrometric Data*

*p*-Coumaric acid (**1**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 307, 296 (sh), and 241 nm; (−) ESIMS, *m/z* 163.15 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, and J/Hz), see Table 1.

Caffeic acid (**2**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 327, 297 (sh), and 242 nm; (−) ESIMS, *m/z* 179.09 $[M-H]^-$. $^1$H-NMR (500 MHz, CD3OD, $\delta$, ppm, and J/Hz), see Table 1.

Ferulic acid (**3**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 328, 298 (sh), and 242 nm; (−) ESIMS, *m/z* 193.12 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, and J/Hz), see Table 1.

Chlorogenic acid (**4**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 327, 298 (sh), and 242 nm; (−) ESIMS, *m/z* 353.03 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, and J/Hz), see Table 2. $^{13}$C-NMR (125 MHz, $CD_3OD$) 74.8 (C-1), 36.8 (C-2), 70.0 (C-3), 72.1 (C-4), 70.5 (C-5), 37.4 (C-6), 175.6 (C-7), 126.4 (C-1′), 113.8 (C-2′), 145.3 (C-3′), 148.1 (C-4′), 115.1 (C-5′), 121.6 (C-6′), 145.7 (C-7′), 113.8 (C-8′), and 167.3 (C-9′).

3-*O*-Caffeoylquinic acid (**5**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 327, 298 (sh), and 242 nm; (−) ESIMS, *m/z* 353.21 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, and J/Hz), see Table 2. $^{13}$C-NMR (125 MHz, $CD_3OD$) 72.0 (C-1), 36.8 (C-2), 70.1 (C-3), 70.6 (C-4), 69.9 (C-5), 37.4 (C-6), 175.6 (C-7), 126.4 (C-1′), 113.8 (C-2′), 145.4 (C-3′), 148.1 (C-4′), 115.0 (C-5′), 121.5 (C-6′), 145.7 (C-7′), 113.8 (C-8′), and 167.2 (C-9′).

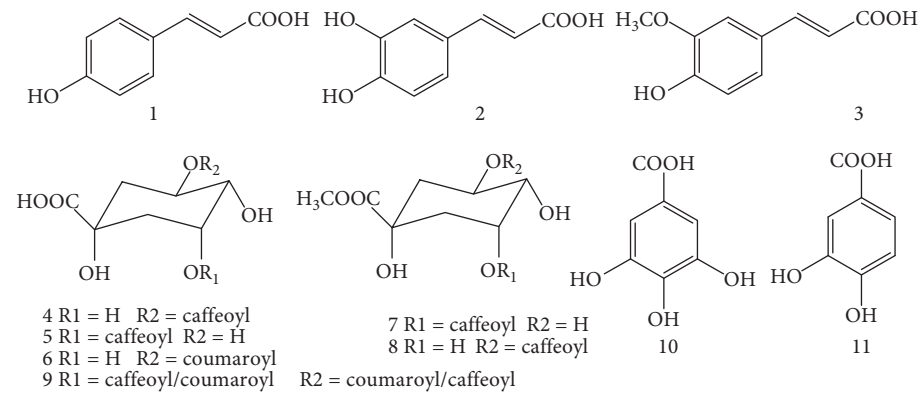


Figure 1: The chemical structures of the compounds **1**–**11** isolated from *P. cerasifera* Ldb leaves.

Table 1: $^1$H-NMR (500 MHz, $CD_3OD$) data of compounds **1**–**3**.

| No. | 1<br>δH (J Hz) | 2<br>δH (J Hz) | 3<br>δH (J Hz) |
|---|---|---|---|
| 2 | 7.40 (1H, d, 8.6) | 7.03 (1H, d, 1.8) | 7.18 (1H, d, 1.8) |
| 3 | 6.80 (1H, d, 8.6) | | |
| 5 | 6.80 (1H, d, 8.6) | 6.76 (1H, d, 8.4) | 6.80 (1H, d, 8.4) |
| 6 | 7.40 (1H, d, 8.6) | 6.91 (1H, dd, 8.4, 1.8) | 7.05 (1H, dd, 8.4, 1.8) |
| 7 | 7.58 (1H, d, 15.9) | 7.52 (1H, d, 15.9) | 7.60 (1H, d, 15.9) |
| 8 | 6.27 (1H, d, 15.9) | 6.22 (1H, d, 15.9) | 6.32 (1H, d, 15.9) |
| $OCH_3$ | | | 3.89 (3H, s) |

Table 2: $^1$H-NMR (500 MHz, $CD_3OD$) data of compounds **4**–**9**.

| No. | 4<br>δH (J Hz) | 5<br>δH (J Hz) | 6<br>δH (J Hz) | 7<br>δH (J Hz) | 8<br>δH (J Hz) | 9<br>δH (J Hz) |
|---|---|---|---|---|---|---|
| 2 | 1.96–2.17 (2H, m) | 1.94–2.14 (2H, m) | 1.90–2.12 (2H, m) | 1.90–2.13 (2H, m) | 1.90–2.12 (2H, m) | 2.12–2.32 (2H, m) |
| 3 | 4.08 (1H, ddd, 1.8, 4.9, 4.9) | 5.23 (1H, d, 4.1) | 4.07 (1H, s) | 5.18 (1H, ddd, 1.8, 4.9, 4.9) | 4.07 (1H, s) | 5.36 (1H, m) |
| 4 | 3.65 (1H, dd, 3.1, 8.8) | 3.63 (1H, dd, 3.1, 8.5) | 3.76 (1H, dd, 3.0, 8.1) | 3.63 (1H, dd, 3.1, 7.5) | 3.76 (1H, dd, 4.1, 7.8) | 3.96 (1H, dd, 3.0, 8.0) |
| 5 | 5.25 (1H, ddd, 4.5, 9.4, 9.4) | 4.07 (1H, ddd, 3.6, 8.5, 8.5) | 5.25 (1H, ddd, 4.5, 9.4, 9.4) | 4.05 (1H, ddd, 9.0, 9.0, 4.5) | 5.24 (1H, ddd, 4.5, 9.4, 9.4) | 5.42 (1H, m) |
| 6 | 1.96–2.17 (2H, m) | 1.94–2.14 (2H, m) | 1.90–2.12 (2H, m) | 1.90–2.13 (2H, m) | 1.90–2.12 (2H, m) | 2.12–2.32 (2H, m) |
| 2′/2″ | 6.96 (1H, d, 2.1) | 6.95 (1H, d, 2.1) | 7.37 (1H, d, 8.3) | 6.94 (1H, d, 2.0) | 7.09 (1H, d, 1.9) | 7.04 (1H, s)/7.46 (1H, d, 10.5) |
| 3′/3″ | | | 6.70 (1H, d, 8.3) | | | 6.80 (1H, d, 10.5) |
| 5′/5″ | 6.68 (1H, d, 8.2) | 6.68 (1H, d, 8.2) | 6.70 (1H, d, 8.3) | 6.68 (1H, d, 8.2) | 6.70 (1H, d, 7.9) | 6.76 (1H, d, 8.4)/6.80 (1H, d, 10.5) |
| 6′/6″ | 6.86 (1H, dd, 2.1, 8.2) | 6.86 (1H, dd, 2.1, 8.2) | 7.37 (1H, d, 8.3) | 6.85 (1H, dd, 2.0, 8.2) | 6.96 (1H, dd, 1.9, 7.9) | 6.95 (1H, d, 8.4)/7.46 (1H, d, 10.5) |
| 7′ | 7.48 (1H, d, 15.9) | 7.47 (1H, d, 15.9) | 7.52 (1H, d, 15.9) | 7.42 (1H, d, 15.9) | 7.52 (1H, d, 15.9) | 7.66/7.55 (1H, d, 15.9) |
| 8′ | 6.19 (1H, d, 15.9) | 6.18 (1H, d, 15.9) | 6.22 (1H, d, 15.9) | 6.13 (1H, d, 15.9) | 6.25 (1H, d, 15.9) | 6.39/6.24 (1H, d, 15.9) |
| $OCH_3$ | | | | 3.60 (3H, s) | 3.88 (3H, s) | |

5-*O*-Coumaroylquinic acid (**6**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 307, 298 (sh), and 242 nm; (−) ESIMS, *m*/*z* 337.21 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, δ, ppm, and J/Hz), see Table 2.

3-*O*-Caffeoylquinic acid methyl ester (**7**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 327, 298 (sh), and 242 nm; (−) ESIMS, *m*/*z* 367.23$[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, δ, ppm, and J/Hz), see Table 2.

Chlorogenic acid methyl ester (**8**) white powder: UV-Vis (MeOH) $\lambda_{max}$ = 327, 298 (sh), and 242 nm; (−) ESIMS, *m*/*z* 367.04 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, δ, ppm, and J/Hz), see Table 2.

3-*O*-Caffeoyl-5-*O*-coumaroylquinic acid or 3-*O*-coumaroyl-5-*O*-caffeoylquinic acid (**9**) white powder: UV-Vis (MeOH) $\lambda_{max} = 327$, 298 (sh), and 242 nm; (−) ESIMS, *m*/*z* 367.04 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, J/Hz), see Table 2.

Gallic acid (**10**) white powder: UV (MeOH) $\lambda_{max}$: 276 nm; (−) ESI-MS, *m*/*z* 169.01 $[M-H]^-$; $^1$H-NMR (500 MHz, $CD_3OD$, $\delta$, ppm, and J/Hz): 7.06 (2H, s, H-galloy l-2, 6); $^{13}$C-NMR (125 MHz, $CD_3OD$): 120.5 (C-1), 108.9 (C-2, 6), 145.0 (C-3, 5), 138.1 (C-4), and 168.9 (C-7).

Protocatechuic acid (**11**) white powder: UV-Vis (MeOH) $\lambda_{max} = 279$ nm; (−) ESIMS, *m*/*z* 153.09 $[M-H]^-$. $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$ 7.34 (1H, s, H-2), 7.32 (1H, dd, $J = 2.1$, H-6), and 6.69 (1H, d, $J = 7.9$, H-5). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$ 121.7 (C-1), 114.3 (C-2), 144.6 (C-3), 150.1 (C-4), 116.3 (C-5), 122.5 (C-6), and 168.7 (C-7).

*3.3. Procedures of Constituent Identification.* The bioactive compounds and antioxidant contents of the *Prunus* fruit are varied widely and mainly depends on the flesh colour and nectarine and so on. [6]. Previously, major focus has been paid on the evaluation of phytochemicals from the fruits [12, 23]. A great variation in the total phenolic content among different myrobalan plum fruits (1.34 to 6.11 g/kg FW) was reported [12, 23]. Furthermore, there was a qualitative and quantitative variation in the phenolic content of these plants having variable genetic backgrounds (between and within species and clones) and between different physiological and developmental stages [24, 25].

All of the compounds in the present study were obtained as white powers. The UV spectrum of compounds **1**–**3** showed $\lambda_{max}$ at 327/307, 298 (shoulder), and 242 nm, which indicated that compounds **1**–**3** were hydroxycinnamic acid derivatives. The $^1$H-NMR spectrum of compounds **1**–**3** showed trans-ene double-bond signals at $\delta$ 7.52–7.60 (1H, d, $J = 15.9$ Hz, H-7) and 6.22–6.32 (1H, d, $J = 15.9$ Hz, H-8). While an AA'BB' system at 7.40 (2H, d, $J = 8.6$ Hz, H-2, 6) and 6.80 (2H, d, $J = 8.6$ Hz, H-3, 5) of compound **1** was easily identified as *p*-coumaric acid. An ABX system of compounds **2** and **3** was found; furthermore, a methoxy signal of compound **3** appeared at 3.89 (3H, s, -$OCH_3$), and so compounds **2** and **3** were identified as caffeic acid and ferulic acid, respectively.

Compounds **4**–**6** showed a similar UV spectrum as compounds **1**–**3**; the $^1$H-NMR spectrum of compounds **4**–**5** was similar with compound **2**, showing trans-ene double-bond signals at $\delta$ 7.47–7.48 (1H, d, $J = 15.9$ Hz, H-7) and 6.18–6.19 (1H, d, $J = 15.9$ Hz, H-8), An ABX system signals at $\delta$ 6.95–6.96 (1H, d, 2.1), 6.86 (1H, dd, 2.1, 8.2), 6.68 (1H, d, 8.2), and quinic acid signals at [**4**: $\delta$ 4.08 (1H, ddd, $J = 1.8$, 4.9, 4.9, H-3), 3.65 (1H, dd, $J = 8.8$, 3.1, H-4), 5.25 (1H, ddd, $J = 9.4$, 9.4, 4.5, H-5), 1.96–2.17 (4H, m, H-2, H-6); **5**: $\delta$ 5.23 (1H, brd, $J = 4.1$, H-3), 3.63 (1H, dd, $J = 8.5$, 3.1, H-4), 4.07 (1H, ddd, $J = 8.5$, 8.5, 3.6, H-5), and 1.94–2.14 (4H, m, H-2, H-6)] indicated that compounds **4**–**5** were caffeoyl-substituted quinic acid derivatives. As described previously [26, 27], the position of caffeoyl substitution can be determined by the analysis of the chemical shift and coupling constants of the oxygenated methine protons of the quinic acid core, in which the H-5 signal showed a ddd type peak with coupling constants at 8.0–9.0 Hz, 8.0–9.0 Hz, and 3.0–5.0 Hz, while the H-3 signal had a small coupling constant and showed a brd or brs type peak. Compounds **4** and **5** were identified as chlorogenic acid (**4**) and 3-*O*-caffeoylquinic acid (**5**). The $^1$H-NMR spectrum of compound **6** was similar with compound **1**, while quinic acid signals appeared of compound **6**. As described ahead, the oxygenated methine protons of the quinic acid core of compound **6** was similar with compound **4**, so compound **6** was identified as 5-*O*-coumaroylquinic acid (**6**). The UV spectrum and $^1$H-NMR spectrum of compounds **7**-**8** were similar with compounds **4**-**5**, but due to the $OCH_3$ signals of compounds **7**–**8**, they were identified as 3-*O*-caffeoylquinic acid methyl ester (**7**) and chlorogenic acid methyl ester (**8**), respectively. The compound **9** showed similar UV spectrum and $^1$H-NMR spectrum with compounds **4**-**5**, while another AA'BB' system appeared in compound **9**, which indicated coumaroyl and caffeoyl acylated of the quinic acid. The downshifts of H-3 and H-5 signals indicated compound **9** was tentatively identified as 3-*O*-caffeoyl-5-*O*-coumaroyl-quinic acid or 3-*O*-coumaroyl-5-*O*-caffeoylquinic acid (**9**), and the final structure should further be analyzed by 2D-NMR including HMBC, HSQC, and so on.

Recently, a study developed a green two-dimensional HPLC-DAD/ESI-MS method for analysing anthocyanins from *P. cerasifera* var. atropurpurea leaves and improved their stability in energy drinks by the addition of phenolic acids. Different mobile phases (ethanol and tartaric acid) were used for one-dimensional HPLC-DAD for quantitative analysis of anthocyanins, and method validation analyses showed that the developed method was accurate, stable, and reliable for the analysis of *P. cerasifera* anthocyanins [4]. Many studies reported that phenolic compounds having strong antioxidant potential depends upon maturity, cultivars, environment conditions, growing season, storage condition, and pre- and postharvest practices. But similar compounds isolated in the present study related to the leaves of *P. cerasifera* deemed to have similar biological effects.

## 4. Conclusion

Taken together, the present study described the first time of isolation of the phenolic constituents from *P. cerasifera* Ldb leaves. Eleven phenolic acids including *p*-coumaric acid (**1**), caffeic acid (**2**), ferulic acid (**3**), chlorogenic acid (**4**), 3-*O*-caffeoylquinic acid (**5**), 5-*O*-coumaroylquinic acid (**6**), 3-*O*-caffeoylquinic acid methyl ester (**7**), chlorogenic acid methyl ester (**8**), 3-*O*-caffeoyl-5-*O*-coumaroylquinic acid or 3-*O*-coumaroyl-5-*O*-caffeoylquinic acid (**9**), gallic acid (**10**), and protocatechuic acid (**11**) were identified. The current study pioneers to identify and report all the phenolic constituents from *P. cerasifera* Ldb leaves.

## Data Availability

No data were used to support this study. Samples of the compounds 1–11 are available from the authors.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## Acknowledgments

This research was financially supported by the Foundation of Yili Normal University (2017YSYY07).

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