# In Vitro Tyrosinase Inhibitory and Antioxidant Activities of Extracts and Constituents of Paeonia lactiflora Pall. Flowers

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# *In vitro* tyrosinase inhibitory and antioxidant activities of extracts and constituents of *Paeonia lactiflora* Pall. flowers

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**Abstract:** The aim of this study was to investigate the tyrosinase inhibitory and antioxidant activities of the extracts and constituents of *Paeonia lactiflora* Pall flowers 'Madame de Verneville'. The 70% ethanol extract was purified by combination of chromatographic methods to afford twenty-six known compounds. Their structures were elucidated based on 1D and 2D nuclear magnetic resonance (NMR) spectra and mass spectrometry. High performance liquid chromatography (HPLC) analysis showed that the dominant compounds were 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranoside (**16**) and 6-*O*-*m*-digalloyl-1,2,3,4-tetra-*O*-galloyl-*β*-D-glucopyranoside (**19**). The *in vitro* fungal tyrosinase inhibition potential and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the extract and isolated compounds were evaluated. 70% EtOH extracthas a high tyrosinase inhibitory and good antioxidant activities ($IC_{50}$ = 0.35 and 2.3 µg·mL$^{-1}$, respectively). Compound **15** (1,2,3,6-*tetra*-*O*-galloyl-*β*-D-glucopyranoside) showed high tyrosinase inhibitory and antioxidant activities ($IC_{50}$ = 0.23 and 3.1 µg·mL$^{-1}$, respectively).

**Keywords:** antioxidant activity, tyrosinase inhibitory activity, Paeoniaceae.

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## 1. INTRODUCTION

Oxidative stress, a deleterious process that can damage all cell structures is generated by reactive oxygen species (ROS) accumulation in the cell, either from excessive production or insufficient neutralization [1,2]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ such as dismutase, peroxidase, and catalase enzymes, as well as glutathione and cytochrome, or externally supplied through foods [3,4]. In view of the significant damage occasioned by oxidative stress, in recent years researchers have undertaken the search for antioxidant compounds that can delay or inhibit the initiation or propagation of oxidative chain reaction and thus prevent or repair oxidative damage done to the body's cells by oxygen [5,6]. Currently, a variety of synthetic antioxidant supplements are available. However, antioxidants derived from natural sources have attracted many interests for use in foods or pharmaceutical preparations [7].

Various dermatological disorders such as freckling, age spots and sites of actinic damage are caused by the accumulation of dermal melanin pigment, which is synthesized in melanocytes via the action of tyrosinase, [8-10]. Therefore, there is a large demand for developing antityrosinase and antioxidant products to treat and protect against hyperpigmentation and ageing of the skin caused by ultraviolet rays, ROS, free radicals and other insults.

*Paeonia lactiflora* Pall. (Paeoniaceae) is an important ornamental and medicinal plant worldwide [11,12]. The roots of *P. lactiflora* are one of the most important sources of bioactive materials in traditional Chinese medicine, with claims of anti-inflammatory, antibacterial, antiviral, antioxidant, antispasmodic, tonic, astringent and analgesic properties [13-15]. The flowers of *P. lactiflora* are used in aromatherapy. Many papers report the chemical constituents, mainly monoterpenoid glycosides and tannins, in roots of *P. lactiflora* [15-18]. In contrast, the chemical constituents of *P. lactiflora* flowers have not been studied in detail. Recent study indicated that the extract of *P. lactiflora* flowers was rich of polyphenols and eight compounds were identified [19]. A pentagalloylglucose and four flavonoids isolated from the methanol extract of *P. lactiflora* flowers showed significant inhibitory effect on $Cu^{2+}$-induced low-density lipoprotein [20]. Another study showed that the ethyl ether extract from *P. lactiflora* flowers had antioxidant and anti-inflammatory activities [21]. To our knowledge, the antioxidant and tyrosinase inhibitory activities of compounds isolated from *P. lactiflora* flowers have not yet been reported.

Here we report the isolation and structural elucidation of 26 chemical constituents from the ethanol extract of *P. lactiflora* flowers. Antioxidant activities of the isolated compounds were determined by using DPPH scavenging radical assay and their tyrosinase inhibitory effects were evaluated according to *in vitro* fungal tyrosinase assay.

## 2. MATERIALS AND METHOD

### *2.1. Plant Material and Reagents*

Flowers of *Paeonia laciflora* Pall. 'Madame de Verneville' were collected in April 2013 by Dr. Laure Pasquier from LVMH research department at Saint Jean de Braye, France. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, kojic acid, tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), deuterated methanol (MeOD-$d_4$) were purchased from Sigma-Aldrich (Saint-Quentin, France). Acetonitrile ($CH_3CN$), methanol (MeOH), trichloromethane ($CHCl_3$), ethyl acetate (EtOAc), ethanol (EtOH), dimethyl sulfoxide (DMSO) and trifluoroacetic acid (TFA) were purchased from Carlo Erba Reactifs SDS (Val de Reuil, France).

### *2.2. Extraction and Isolation*

The lyophilized flowers of *P. lactiflora* (50 g) were macerated with EtOH: $H_2O$ (7:3) (1 L x 3, each 24h) at room temperature. After removal of the solvent, the residue was dissolved in water (200 mL) and then extracted with EtOAc (3 × 100 mL each) to yield the EtOAc (8.7 g) and the $H_2O$ (19.2 g) extracts. The EtOAc extract was subjected to vacuum liquid chromatography (VLC) over silica gel eluting with $CHCl_3$-MeOH-$H_2O$ (98:2:0-60:40:7) to afford seven fractions EA1-EA7. Fraction EA3 (0.24 g), eluted with $CHCl_3$-MeOH (9:1), was purified by semi-prep HPLC (conditions: 20-25% of acetonitrile ($CH_3CN$) in 15 min) to afford compounds **7** (*rt* 6.2 min, 111 mg) and **11** (*Rt* 10.6 min, 49 mg). Fraction EA4 (1.44 g), eluted with $CHCl_3$-MeOH (8:2), was purified by preparative HPLC (20-35% $CH_3CN$ in 60 min) yielding 40 subfractions. Subfraction [21] was purified by semi-prep HPLC (17-21% of $CH_3CN$ in 20 min) to give compounds **17** (*Rt* 22.5 min, 24 mg) and **18** (*Rt* 24.2 min, 22 mg). Subfractions [30-34] was further purified by semi-prep HPLC (isocratic elution with 23% $CH_3CN$) to afford compounds **25** (*Rt* 19.2 min, 7 mg) and **26** (*Rt* 21.3 min, 23 mg). Fraction EA5 (2.6 g), eluted with $CHCl_3$-MeOH (7:3), was purified by prep. HPLC (20-30% $CH_3CN$ in 60 min) to give compounds **15** (*Rt* 20 min, 10 mg), **16** (*Rt* 23 min, 165 mg), and **19** (*Rt* 24.2 min, 19 mg). The $H_2O$ extract (18 g) was subjected to VLC over RP18 (9 x 6 cm) eluted with MeOH-$H_2O$ (0:10-10:0) to give seven fractions (W1-W7). Fractions W2 (0.55 g) and W3 (0.20 g), eluted with MeOH-$H_2O$ (1:9), were combined and purified by preparative HPLC (5-20% $CH_3CN$ in 60 min) to give compounds **4** (52 mg) and **2** (12.6 mg). Subfraction [4] was further purified by semi-prep HPLC (0-5% $CH_3CN$ in 15 min) to afford compounds **3** (*Rt* 5.9 min, 4 mg) and **1** (*Rt* 10.3 min, 23 mg), whereas purification of subfraction [12] in the same conditions yielded compound **5** (*Rt* 15.3 min, 19 mg). Fraction W4 (0.35 g), eluted with MeOH-$H_2O$ (2:8), was purified by preparative HPLC (5-20% $CH_3CN$ in 60 min) to give compound **24** (60 mg). Subfraction [6] was further purified by semi-prep HPLC (10-17% $CH_3CN$ in 20 min) to afford compound **9** (*Rt* 11.3 min, 17 mg). Fraction W5 (1.17 g), eluted with MeOH-$H_2O$ (4:6), was purified by preparative HPLC (20-60% $CH_3CN$ in 60 min) to give compound **8** (19 mg). Subfraction [11] was further purified by semi-prep HPLC (10-15% $CH_3CN$ in 17 min) to afford compound **13** (*Rt* 16.3 min, 2 mg). Subfraction [15] was further purified by semi-prep HPLC (isocratic elution 17% of $CH_3CN$) to afford compounds **22** (*Rt* 10.3 min, 2 mg) and **10** (*Rt* 14.2 min, 4 mg). Subfraction [16] was purified by semi-prep HPLC (15-20% $CH_3CN$ in 17 min) to afford compound **12** (*Rt* 13.8 min, 12 mg). Subfractions [20-21] were purified by semi-prep HPLC (15-20% $CH_3CN$ in 15 min) to afford compound **14** (*Rt* 13.8 min, 35 mg). Subfractions [24-28] were purified by semi-prep HPLC (19% $CH_3CN$) to afford compound **20** (*Rt* 11.2 min, 23 mg). Fraction W6 (0.28 g), eluted with MeOH-$H_2O$ (6:4), was purified by prep. HPLC (20-60% $CH_3CN$ in 60 min) to give compounds **6** (6 mg), **21** (24 mg), and **23** (6 mg). Subfraction [11] was purified by semi-prep HPLC (10-15% $CH_3CN$ in 17 min) to afford compound **13** (*Rt* 16.3 min, 2 mg).

### *2.3. HPLC Analysis of the 70% EtOH Extract*

The mobile phase compositions, gradient elution procedure and detection wave length were optimized to get the most useful chemical information and best separation on the HPLC chromatograms of *P. lactiflora*. To improve the resolution and to limit the ionization of target compounds, TFA was added to the binary mixture of $CH_3CN$-water as recommended by He et al. [22]. A binary mixture of $CH_3OH$-water with different concentrations of TFA were also investigated. Finally, the mobile phase consisting of $CH_3CN$-$H_2O$/0.0025% TFA solution (v/v, pH 2.28) was chosen for the determination of *P. lactiflora* fingerprint chromatogram with large number of peaks on the chromatogram achieving within 60 min. The chromatographic separation was achieved using the gradient program: 5% (for 5 min), to 20% (in 15 min), 20% (for 10 min), to 25% (in 20 min) and to 35% (in 10 min) $CH_3CN$ in 0.0025% TFA. The chromatogram was monitored at a wavelength of 205 nm during the experiment. The column temperature was maintained at 30 °C and the injection volume of sample solution (5 mg/mL) was 20 µL. The analysis was performed in triplicates. The $H_2O$ used for mobile phase was prepared daily, filtered (0.22 µm membrane filter) and then degassed.

### *2.3. Tyrosinase Enzyme Assay*

The tyrosinase inhibitory activity was determined according to the method described previously [23]. L-DOPA was used as the substrates in this experiment. Samples were prepared at concentrations of 400, 100, 50, 25 and 12.5 $\mu g \cdot mL^{-1}$ in 10% DMSO in aqueous solution and 100 µL of each concentration were added to 96-well plate and then 100 µL of 135 U/mL fungal tyrosinase in phosphate buffer solution (PBS, pH 6.8) were added. After pre-incubation at 25 °C for 10 min, 100 µL of L-DOPA (0.5 mM, PBS pH 6.8) were added into 96-well plate. The reaction mixture was incubated for another 5 min at 25 °C. The amount of dopachrome in the mixture was determined by the measurement of the absorbance of each well at 475 nm. Kojic acid was used as positive control agent. The inhibitory percentage of tyrosinase was calculated according to the following equation: % inhibition = $\{[(A-B) - (C-D)]/(A-B)\}\times 100$ (*A*: Ab at 475 nm without test substance; *B*: Ab at 475 nm without test substance and tyrosinase; *C*: Ab at 475 nm with test substance; *D*: Ab at 475 nm with test substance, but without tyrosinase).

### *2.4. DPPH Radical Scavenging Activity*

The radical scavenging activity of crude extracts, fractions and purified compounds was measured using the DPPH method [23]. 5 µL of different concentrations of the samples were added to 95 µL of a DPPH solution (158 µM, dissolved

in EtOH 50%). The reaction proceeded for 30 min at 37 °C on a 96-well microplate and the absorbance was then read at 515 nm. The DPPH inhibition percentage was calculated as followed: % inhibition $[(Ab_{control}-Ab_{sample})/Ab_{control}]\times100$. A DPPH solution in EtOH 50% was used as a control. The curve of the % scavenging activity against the concentration of sample was prepared by MSExcel based program to obtain the $IC_{50}$. Samples were prepared at concentrations of 100, 50, 25, 6.2 and 3.1 $\mu g \cdot mL^{-1}$. Ascorbic acid was used as a positive control.

## 3. EXPERIMENTAL:

### *3.1. General Experimental Procedures*

Absorbance (Ab) values in the DPPH free radical scavenging assay and tyrosinase enzyme assay were read on a Fluostar omega microplate reader (BMG labtech). NMR spectra were carried in MeOH-$d_4$ on Bruker Avance DRX III 500 instruments ($^1$H at 500 MHz and $^{13}$C at 125 MHz). Standard pulse sequences and parameters were used to obtain 1D ($^1$H and $^{13}$C *J*-mod) and 2D ($^1$H-$^1$H COSY, ROESY, HSQC, and HMBC) spectra. ESI-MS were measured on a Micromass Q-TOF micro instrument. Silica gel 60 (Merck, 63-200 µm) or LiChroprep RP18 (Merck, 40-63 µm) were used for VLC. An Armen instrument equipped with an AP 250/500 pump, ACC 250/500 sampler, and a Merck UV-detector K-2501 was used for preparative HPLC. A Lichrospher RP18 prepacked column (Merck 250×50mm, 12µm) was used with binary gradient eluent ($H_2O$ and $CH_3CN$) and a flow rate of 50 $mL \cdot min^{-1}$; the chromatogram was monitored at 250 nm. Analytical and semi-preparative HPLC were performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and Chromeleon software. RP18 column (Phenomenex 250 × 15 mm, Luna 5µ) was used for semi-prep HPLC with binary gradient eluent ($H_2O$ (pH 2.4 with TFA); $CH_3CN$) and a flow rate of 5 $mL \cdot min^{-1}$; the chromatogram was monitored at 205, 210, 250, and 360 nm whereas an RP18 column (Phenomenex 250×4.6 mm, Luna 5µ) was used for analytical HPLC with binary gradient eluent ($H_2O$ (pH 2.4 with TFA); $CH_3CN$) and a flow rate of 1 $mL \cdot min^{-1}$.

### *3.2. Statistical Analyses*

The data obtained in this study were expressed as mean ± standard deviation (SD). All the tests were conducted in triplicate and concentrations yielding 50% inhibition ($IC_{50}$) were determined by interpolation of concentration % inhibition curve obtained by MSExcel 2010 software.

## 4. RESULTS and DISCUSSIONS:

### *4.1. Structure Identification of P. lactiflora Compounds*

Structural elucidation of compounds **1**-**26** was performed by NMR analysis ($^1$H, $^{13}$C *J*-mod, $^1$H-$^1$H COSY, ROESY, HSQC, and HMBC) and mass spectrometry. Their spectroscopic data were in perfect agreement with those reported in the literature.

*Compounds isolated from the $H_2O$ extract*

Compound **1**: Positive ESI-MS: *m/z* 193.12 $[M+Na]^+$; $^1$H NMR: $\delta_H$ 7.07 (s, H-2,H-6); $^{13}$C NMR : $\delta_C$ 122.8 (C-1), 108.8 (C-2,C-6), 144.8 (C-3,C-5), 137.4 (C-4), 170.8 (C-7). Comparing the results with Ref. [24], compound **1** was identified as gallic acid.

Compound **2**: Positive ESI-MS: *m/z* 517.1 $[M+Na]^+$; $^1$H NMR: $\delta_H$ 3.3-4.10 (11 H, m, sugar H), 4.41, 4.25 (each 1H, d, *J*=12 Hz, H-1′), 6.95 (s, H-2″,H-6″); $^{13}$C NMR: $\delta_C$ 95.5 (C-1), 72.3 (C-2), 74.0 (C-3), 70.8 (C-4), 74.5 (C-5), 61.8 (C-6), 63.8 (C-1′), 104.4 (C-2′), 78.5 (C-3′), 74.0 (C-4′), 83.0 (C-5′), 62.2 (C-6′), 119.8 (C-1″), 109.1 (C-2″,C-6″), 146.0 (C-3″,C-5″),139.1 (C-4″), 166.3 (C-7″). Comparing the results with Ref. [25], compound **2** was identified as 1′-*O*-galloylsucrose.

Compound **3**: Positive ESI-MS: *m/z* 517.2 $[M+Na]^+$; $^1$H NMR : $\delta_H$ 3.3-4.2 (11 H, m, sugar H), 4.4, 4.27 (each 1H, m, H-6′), 5.42 (d, *J*=4 Hz, H-1), 7.08 (s, H-2″,H-6″); $^{13}$C NMR: $\delta_C$ 92.7 (C-1), 72.3 (C-2), 73.8 (C-3), 70.5 (C-4), 75.8 (C-5), 61.4 (C-6), 62.9 (C-1′), 104.8 (C-2′), 77.9 (C-3′), 75.8 (C-4′), 79.9 (C-5′), 66.9 (C-6′), 120.7 (C-1″), 110.2 (C-2″,C--6″), 145.8 (C-3″,C-5″), 139.2 (C-4″), 168.0 (C-7″). Comparing the results with Ref. [25], compound **3** was identified as 6′-*O*-galloylsucrose.

Compound **4**: Positive ESI-MS: *m/z* 355.3 $[M+Na]^+$; $^1$H NMR: $\delta_H$ 5.52 (d, *J*=7.9 Hz, H-1), 3.23 (t, *J*=8 Hz, H-2), 3.28 (t, *J*=8 Hz, H-3), 3.16 (t, *J*=8.2 Hz, H-4), 3.25 (m, H-5), 3.67 (dd, *J*=11.5, 2.1, H-6a), 3.47 (dd, *J*=11.5, 5.1, H-6b), 5.42 (d, *J*=4 Hz, H-1′), 7.02 (s, H-2′,H-6′); $^{13}$C NMR: $\delta_C$ 94.9 (C-1), 73.1 (C-2), 77.1 (C-3), 70.0 (C-4), 78.3 (C-5), 61.0 (C-6), 119.0 (C-1′), 109.4 (C-2′,C-6′), 146.0 (C-3′,C-5′), 139.4 (C-4′), 165.1 (C-7′). Comparing the results with Ref. [26], compound **4** was identified as 1-*O*-galloyl-*β*-D-glucopyranoside.

Compound **5**: Positive ESI-MS: *m/z* 355.5 $[M+Na]^+$; $^1$H NMR: $\delta_C$ 5.10 (d, *J*=3.4 Hz, H-1α), 4.55 (d, *J*=7.9 Hz, H-1β), 4.55 (4H, m, H-6αβ), 3.10-4.5 (8 H, m, α-glc and β-glc-H), 7.10 (s, H-2′,H-6′); $^{13}$C NMR : $\delta_C$ 96.9 (C-1β), 74.8 (C-2β), 76.5 (C-3β), 70.3 (C-4β), 74.2 (C-5β), 63.5 (C-6), 92.6 (C-1α), 72.4 (C-2α), 73.4 (C-3α), 70.5 (C-4α), 69.5 (C-5α), 120.0 (C-1′), 109.0 (C-2′,C-6′), 145.0 (C-3′,C-5′), 138.4 (C-4′), 167.0 (C-7′). Comparing the results with Ref. [27], compound **5** was identified as 6-*O*-galloyl-D-glucopyranoside.

Compound **6**: Positive ESI-MS: *m/z* 378.3 $[M+Na]^+$; $^1$H NMR: $\delta_C$ 5.45 (d, *J*=7.2 Hz, H-1), 3.25 (m, H-2), 3.27 (t, *J*=8.4 Hz, H-3), 3.32 (t, *J*=8.4 Hz, H-4), 3.29 (m, H-5), 3.55 (m, H-6a), 3.85 (dd, *J*=11.7, 1.8 Hz, H-6b), 2.31, 2.58 (each d, *J*=13.4 Hz, H-2′), 1.68 (m, H-4′), 2.24 (m, H-5′), 7.50 (t, *J*=6.9 Hz, H-6′), 1.64 (s, H-8′), 1.69 (s, H-9′), 1.35 (s, H-10′); $^{13}$C-NMR: $\delta_C$ 103.7 (C-1), 73.6 (C-2), 77.5 (C-3), 70.1 (C-4), 77.8 (C-5), 61.2 (C-6), 179.7 (C-1′), 47.2 (C-2′), 79.6 (C-3′), 23.7 (C-4′), 41.5 (C-5′), 125.3 (C-6′), 138.1 (C-7′), 25.9 (C-8′), 17.9 (C-9′), 23.9 (C-10′). Comparing the results with Ref. [28], compound **6** was identified as 3-hydroxy-citronellic acid 3-*O*-*β*-D-glucopyranoside.

Compound **8**: Positive ESI-MS: *m/z* 503 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 1.84 (d, *J*=12.6 Hz, H-3a), 2.22 (d, *J*=12.6 Hz, H-3b), 2.62 (dd, *J*=6.6, 1.0 Hz, H-5), 1.98 (dd, *J*=11.0, 1.0 Hz, H-7a), 2.52 (dd, *J*=11.0, 6.9 Hz, H-7b), 4.79 (d, *J*=12.6 Hz, H-8a), 4.74 (d, *J*=12.6 Hz, H-8b), 5.46 (*s*, H-9), 1.0 (s, $H_3$-10),

4.55 (d, *J*=7.6 Hz, H-1′), 3.24 (t, *J*=8.4 Hz, H-2′), 3.26 (t, *J*=8.5 Hz, H-3′), 3.25 (m, H-5), 3.65 (dd, *J*=11.8, 5.1 Hz, H-6′a), 3.88 (dd, *J*=11.8, 2.1 Hz, H-6′b), 8.08 (t, *J*=8.3 Hz, H-2″,H-6″), 7.63 (t, *J*=7.5 Hz, H-4″), 7.50 (t, *J*=7.5 Hz, H-3″,H-5″); $^{13}$C-NMR: $\delta_C$ 98.4 (C-1), 87.3 (C-2), 44.6 (C-3), 106.4 (C-4), 44.0 (C-5), 72.3 (C-6), 23.4 (C-7), 61.7 (C-8), 102.3 (C-9), 19.6 (C-10), 100.2 (C-1′), 72.3 (C-2′), 78.1 (C-3′), 71.8 (C-4′), 78.0 (C-5′), 62.9 (C-6′), 131.3 (C-1″), 129.7 (C-2″,C-6″), 134.4 (C-4″), 130.8 (C-3″,C-5″), 167.8 (C-7″). Comparing the results with Ref. [29], compound **8** was identified as paeoniflorin.

Compound **9** : Positive ESI-MS: *m/z* 519.3 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 1.83 (d, *J*=12.6 Hz, H-3a), 2.22 (d, *J*=12.6 Hz, H-3b), 2.62 (dd, *J*=6.6, 1.0 Hz, H-5), 1.99 (dd, *J*=11.1, 1.1 Hz, H-7a), 2.51 (dd, *J*=11.0, 6.9 Hz, H-7b), 4.77 (d, *J*=3.5 Hz, $H_2$-8), 5.45 (*s*, H-9), 1.0 (s, $H_3$-10), 4.55 (d, *J*=7.6 Hz, H-1′), 3.23 (t, *J*=8.5 Hz, H-2′), 3.25 (t, *J*=8.5 Hz, H-3′), 3.25 (m, H-5′), 3.30 (t, *J*=8.4 Hz, H-4′), 3.66 (dd, *J*=11.9, 5.1 Hz, H-6′a), 3.88 (dd, *J*=11.9, 2.1 Hz, H-6′b), 8.21 (d, *J*=8.8 Hz, H-2″,H-6″), 7.12 (d, *J*=8.8 Hz, H-3″,H-5″); $^{13}$C-NMR: $\delta_C$ 98.4 (C-1), 87.3 (C-2), 44.6 (C-3), 106.4 (C-4), 44.0 (C-5), 72.3 (C-6), 23.4 (C-7), 61.7 (C-8), 102.3 (C-9), 19.6 (C-10), 100.2 (C-1′), 72.3 (C-2′), 78.1 (C-3′), 71.8 (C-4′), 78.0 (C-5′), 62.9 (C-6′), 121.6 (C-1″), 132.4 (C-2″,C-6″), 114.6 (C-3″,C-5″), 163.6 (C-4″), 166.9 (C-7″). Comparing the results with Ref. [30], compound **9** was identified as oxypaeoniflorin.

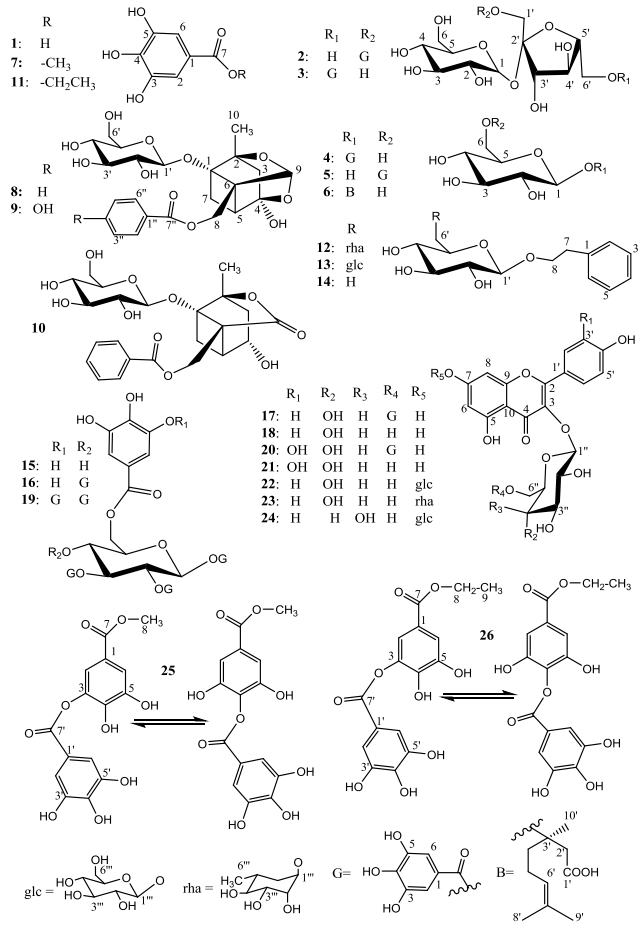


Figure 1: Semi structural formulae of compounds 1-26 isolated from *P. lactiflora* flowers.

Compound **10**: Positive ESI-MS: *m/z* 503.6 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 2.01 (dd, *J*=15.6, 1.6 Hz, H-3a), 2.40 (dd, *J*=15.6, 6.8 Hz, H-3b), 4.26 (dd, *J*=6.4, 4.8 Hz, H-4), 2.91 (dd, *J*=7.5, 5.1 Hz, H-5), 2.04 (d, *J*=11.2 Hz, H-7a), 2.79 (dd, *J*=11.2, 7.9 Hz, H-7b), 4.67 (d, *J*=11.2 Hz, H-8a), 4.80 (d, *J*=11.2 Hz, H-8b), 1.52 (s, $H_3$-10), 4.52 (d, *J*=7.9 Hz, H-1′), 3.22 (t, *J*=8.3 Hz, H-2′), 3.24 (t, *J*=8.4 Hz, H-3′), 3.35 (t, *J*=8.4 Hz, H-4′), 3.30 (m, H-5′), 3.63 (dd, *J*=11.7, 4.9 Hz, H-6′a), 3.82 (dd, *J*=11.7, 2.0 Hz, H-6′b), 8.07 (dd, *J*=8.0, 1.0 Hz, H-2″, H-6″), 7.63 (t, *J*=7.5 Hz, H-4″), 7.49 (t, *J*=7.5 Hz, H-3″, H-5″); $^{13}$C-NMR: $\delta_C$ 86.9 (C-1), 93.5 (C-2), 41.6 (C-3), 68.4 (C-4), 41.5 (C-5), 56.9 (C-6), 28.5 (C-7), 62.0 (C-8), 178.0 (C-9), 20.5 (C-10), 100.1 (C-1′), 72.4 (C-2′), 78.1 (C-3′), 71.5 (C-4′), 78.0 (C-5′), 62.8 (C-6′), 131.3 (C-1″), 129.9 (C-2″,C-6″), 134.3 (C-4″), 130.7 (C-3″,C-5″), 167.9 (C-7″). Comparing the results with Ref. [30], compound **10** was identified as albiflorin.

Compound **12**: Positive ESI-MS: *m/z* 441.5 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 7.09 (d, *J*=7.4 Hz, H-2, H-6), 7.50 (t, *J*=7.4 Hz, H-3, H-5), 7.62 (t, *J*=7.4 Hz, H-4),2.97 (dt, *J*=7.2, 4.2 Hz, H-7), 3.78 (dq, *J*=9.7, 7.2 Hz, H-8a), 4.06 (dq, *J*=9.6, 7.2 Hz, H-8b), 4.32 (d, *J*=7.8 Hz, H-1′), 3.20 (t, *J*=8.8 Hz, H-2′), 3.36 (t, *J*=8.4 Hz, H-3′), 3.30 (t, *J*=8.4 Hz, H-4′), 3.41 (m, H-5′), 3.64 (dd, *J*=11.2, 5.8 Hz, H-6′a), 4.00 (dd, *J*=11.2, 1.1 Hz, H-6′b), 4.70 (s, H-1″), 3.86 (dd, *J*=3.2, 1.1 Hz, H-2″), 3.70 (dd, *J*=8.4, 3.3 Hz, H-3″), 3.39 (t, *J*=8.5 Hz, H-4″), 3.70 (m, H-5″), 1.28 (d, *J*=6.2 Hz, H-6″); $^{13}$C-NMR: $\delta_C$ 140.1 (C-1), 130.1 (C-2,C-6), 127.2 (C-4), 139.4 (C-3,C-5), 37.3 (C-7), 72.2 (C-8), 104.5 (C-1′), 75.0 (C-2′), 78.1 (C-3′), 71.4 (C-4′), 77.2 (C-5′), 68.1 (C-6′), 102.2 (C-1″), 72.1 (C-2″), 72.3 (C-3″), 74.1 (C-4″), 69.8 (C-5″), 18.0 (C-6″). Comparing the results with Ref. [31], compound **12** was identified as 2-phenylethyl-[$\alpha$-L-rhamnopyranosyl-(1→6)]-$\beta$-D-glucopyranoside.

Compound **13**: Positive ESI-MS: *m/z* 457.5 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 7.30 (m, H-2-H-6), 2.95 (t, *J*=7.2 Hz, H-7), 3.79 (dq, *J*=9.7, 7.8 Hz, H-8a), 4.10 (dq, *J*=9.6, 7.7 Hz, H-8b), 4.34 (d, *J*=7.8 Hz, H-1′), 3.22 (t, *J*=8.3 Hz, H-2′), 3.39 (t, *J*=8.4 Hz, H-3′), 3.32 (t, *J*=8.4 Hz, H-4′), 3.49 (m, H-5′), 3.81 (dd, *J*=11.7, 5.3 Hz, H-6′a), 4.18 (dd, *J*=11.7, 1.8 Hz, H-6′b), 4.40 (d, *J*=7.8 Hz, H-1″), 3.22 (t, *J*=8.2 Hz, H-2″), 3.37 (t, *J*=8.4 Hz, H-3″), 3.30 (t, *J*=8.5 Hz, H-4″), 3.29 (m, H-5″), 3.68 (dd, *J*=11.9, 5.1 Hz, H-6″a), 3.88 (dd, *J*=11.9, 1.5 Hz, H-6″b); $^{13}$C-NMR: $\delta_C$ 140.1 (C-1), 130.1 (C-2, C-6), 127.2 (C-4), 139.4 (C-3, C-5), 37.2 (C-7), 71.9 (C-8), 104.4 (C-1′), 75.0 (C-2′), 78.0 (C-3′), 71.4 (C-4′), 77.2 (C-5′), 69.8 (C-6′), 104.8 (C-1″), 75.1 (C-2″), 78.1 (C-3″), 71.6 (C-4″), 77.9 (C-5″), 62.7 (C-6″). Comparing the results with Ref. [32], compound **13** was identified as 2-phenylethyl)]-$\beta$-D-glucopyranosyl-(1→6)]-$\beta$-D-glucopyranoside.

Compound **14**: Positive ESI-MS: *m/z* 307.6 $[M+Na]^+$; $^1$H-NMR: $\delta_H$ 7.30 (m, H-2-H-6), 2.95 (td, *J*=7.1,1.5 Hz, H-7), 3.79 (dq, *J*=9.6, 7.2 Hz, H-8a), 4.11 (dq, *J*=9.6, 7.7 Hz, H-8b), 4.32 (d, *J*=7.8 Hz, H-1′), 3.22 (t, *J*=8.4 Hz, H-2′), 3.38 (t, *J*=8.4 Hz, H-3′), 3.35 (t, *J*=8.4 Hz, H-4′), 3.32 (m, H-5′), 3.71 (dd, *J*=11.7, 4.9 Hz, H-6′a), 3.88 (dd, *J*=11.7, 2.0 Hz, H-6′b); $^{13}$C-NMR: $\delta_C$ 140.0 (C-1), 129.3 (C-2, C-6), 127.2 (C-4), 130.0 (C-3, C-5), 37.2 (C-7), 71.7 (C-8), 104.3 (C-1′), 75.1 (C-2′), 78.1 (C-3′), 71.6 (C-4′), 77.9 (C-5′), 62.7 (C-6′). Comparing the results with Ref. [19], compound **14** was identified as 2-phenylethyl-$\beta$-D-glucopyranoside.

Compound **20**: Positive ESI-MS: *m/z* 639.0 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.20 (d, *J*=2.0 Hz, H-6), 6.38 (d, *J*=2.0 Hz, H-8), 7.55 (d, *J*=2.1 Hz, H-2′), 6.73 (d, *J*=8.5 Hz, H-5′), 7.59 (dd, *J*=8.8, 2.1 Hz, H-6′), 5.20 (d, *J*=7.5 Hz, H-1″), 3.50 (t, *J*=8.5 Hz, H-2″), 3.48 (t, *J*=8.5 Hz, H-3″), 3.36 (t, *J*=9 Hz, H-4″), 3.25 (m, H-5″), 4.30 (dd, *J*=11.5, 1.9 Hz, H-6″a), 4.36 (dd, *J*=11.5, 5.0 Hz, H-6″b); 6.95 (s, H-2‴,H-6‴); $^{13}C$ NMR: $\delta_C$ 158.0 (C-2), 133.7 (C-3), 177.8 (C-4), 161.6 (C-5), 99.1 (C-6), 164.6 (C-7), 93.9 (C-8), 156.7 (C-9), 104.3 (C-10), 121.3 (C-1′), 115.7 (C-2′), 145.2 (C-3′), 148.8 (C-4′), 116.1 (C-5′), 121.3 (C-6′), 102.8 (C-1″), 74.4 (C-2″), 76.6 (C-3″), 70.1 (C-4″), 75.6 (C-5″), 62.9 (C-6″) 119.8 (C-1‴), 109.1 (C-2‴,C-6‴), 145.8 (C-3‴,C-5‴), 138.8 (C-4‴), 166.8 (C-7‴). Comparing the results with Ref. [33], compound **20** was identified as quercetin-3-*O*-(6″-*O*-galloyl)-glucoside.

Compound **21**: ESI-MS: *m/z* 487.2 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.62 (d, *J*=1.8 Hz, H-6), 6.40 (d, *J*=1.8 Hz, H-8), 7.75 (d, *J*=1.5 Hz, H-2′), 6.90 (d, *J*=8.4, Hz, H-5′), 7.60 (dd, *J*=8.4, 1.5 Hz, H-6′), 5.25 (d, *J*=7.4 Hz, H-1″), 3.52 (t, *J*=8.5 Hz, H-2″), 3.45 (t, *J*=8.5 Hz, H-3″), 3.40 (t, *J*=8.5 Hz, H-4″), 3.25 (m, H-5″), 3.61 (dd, *J*=11.8, 5.3 Hz, H-6″a), 3.74 (dd, *J*=11.8, 2.0 Hz, H-6″b); $^{13}C$ NMR: $\delta_C$ 158.4 (C-2), 133.6 (C-3), 179.4 (C-4), 162.9 (C-5), 100.1 (C-6), 166.5 (C-7), 94.8 (C-8), 158.9 (C-9), 105.5 (C-10), 123.0 (C-1′), 116.0 (C-2′), 145.9 (C-3′), 149.8 (C-4′), 117.6 (C-5′), 123.3 (C-6′), 104.4 (C-1″), 75.7 (C-2″), 78.3 (C-3″), 71.1 (C-4″), 78.6 (C-5″), 62.5 (C-6″). Comparing the results with reference data [19], compound **21**was identified as quercetin 3-*O*-*β*-D-glucopyranoside.

Compound **22**: positive ESI-MS: *m/z* 633.2 $[M+Na]^+$;$^1H$ NMR: $\delta_H$ 6.55 (d, *J*=2.0 Hz, H-6), 6.80 (d, *J*=2.0 Hz, H-8), 8.11 (d, *J*=8.0 Hz, H-2′,H-6′), 6.91 (d, *J*=9.0 Hz, H-3′,H-5′), 5.35 (d, *J*=7.9 Hz, H-1″), 3.46 (t, *J*=8.1 Hz, H-2″), 3.32 (t, *J*=8.5 Hz, H-3″), 3.32(m, H-4″), 3.45 (m, H-5″), 3.55 (dd, *J*=11.8, 5.2 Hz, H-6″a), 3.73 (dd, *J*=11.8, 2.1 Hz, H-6″b), 5.08 (d, *J*=7.7 Hz, H-1‴), 3.53 (m, H-2‴), 3.52 (m, H-3‴), 3.42 (t, *J*=8.4 Hz, H-4‴), 3.24 (m, H-5‴), 3.75 (dd, *J*=11.9, 5.1 Hz, H-6‴a), 3.97 (dd, *J*=11.9, 2.1 Hz, H-6‴b); $^{13}C$ NMR: $\delta_C$ 155.5 (C-2), 134.1 (C-3), 178.1 (C-4), 161.4 (C-5), 100.3 (C-6), 163.2 (C-7), 94.7 (C-8), 156.3 (C-9), 105.5 (C-10), 121.3 (C-1′), 131.0 (C-2′,C-6′), 114.7 (C-3′,C-5′), 160.3 (C-4′), 102.3 (C-1″), 74.3 (C-2″), 76.4 (C-3″), 70.0 (C-4″), 76.9 (C-5″), 61.2 (C-6″), 100.2 (C-1‴), 73.3 (C-2‴), 77.2 (C-3‴), 69.9 (C-4‴), 76.8 (C-5‴), 62.2 (C-6‴). Comparing the results with reference data [34], compound **22** was identified as kaempferol- 3, 7-*di*-*O*-*β*-D-glucopyranoside.

Compound **23**: Positive ESI-MS: *m/z* 617.3 $[M+Na]^+$; $^1H$-NMR: $\delta_H$ 6.50 (d, *J*=1.7 Hz, H-6), 6.79 (d, *J*=1.7 Hz, H-8), 8.11 (d, *J*=8.4 Hz, H-2′, H-6′), 6.92 (d, *J*=8.4 Hz, H-3′, H-5′), 5.33 (d, *J*=7.2 Hz, H-1″), 3.46 (t, *J*=8.2 Hz, H-2″), 3.44 (t, *J*=8.2 Hz, H-3″), 3.33 (m, H-4″), 3.35 (m, H-5″), 3.55 (dd, *J*=11.9, 5.0 Hz, H-6″a), 3.72 (dd, *J*=11.9, 1.9 Hz, H-6″b), 5.60 (s, H-1‴), 4.05 (dd, *J*=3.3, 1.0 Hz, H-2‴), 3.86 (dd, *J*=8.3, 3.3 Hz, H-3‴), 3.50 (t, *J*=8.5 Hz, H-4‴), 3.61 (m, H-5‴), 1.17 (d, *J*=6.2 Hz, H-6‴);$^{13}C$ NMR: $\delta_C$ 159.6 (C-2), 135.6 (C-3), 179.7 (C-4), 161.9 (C-5), 100.6 (C-6), 163.6 (C-7), 95.5 (C-8), 158.1 (C-9), 107.4 (C-10), 122.6 (C-1′), 132.4 (C-2′,C-6′), 116.2 (C-3′,C-5′), 161.8 (C-4′), 103.7 (C-1″), 75.7 (C-2″), 78.1 (C-3″), 71.4 (C-4″), 77.5 (C-5″), 62.6 (C-6″), 99.8 (C-1‴), 71.7 (C-2‴), 72.2 (C-3‴), 73.5 (C-4‴), 71.2 (C-5‴), 18.1 (C-6‴). Comparing the results with reference data [35], compound **23** was identified as kaempferol 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside.

Compound **24**: positive ESI-MS: *m/z* 633.1 $[M+Na]^+$;$^1H$ NMR: $\delta_H$ 6.47 (d, *J*=2.0 Hz, H-6), 6.81 (d, *J*=2.0 Hz, H-8), 8.09 (d, *J*=8.9 Hz, H-2′, H-6′), 6.91 (d, *J*=8.9 Hz, H-3′, H-5′), 5.50 (d, *J*=7.4 Hz, H-1″), 3.20 (t, *J*=8.0 Hz, H-2″), 3.25 (dd, *J*=8.0, 3.3 Hz, H-3″), 3.09 (d, *J*=3.3, H-4″), 3.11 (m, H-5″), 3.35 (m, H-6″a), 3.57 (dd, *J*=11.8, 1.1 Hz, H-6″b), 5.10 (d, *J*=7.4 Hz, H-1‴), 3.27 (m, H-2‴), 3.34 (t, *J*=8.0 Hz, H-3‴), 3.20 (t, *J*=8.4 Hz, H-4‴), 3.46 (m, H-5‴), 3.47 (m, H-6‴a), 3.71 (dd, *J*=11.8, 1.1 Hz, H-6‴b); $^{13}C$ NMR: $\delta_C$ 157.3 (C-2), 133.9 (C-3), 178.1 (C-4), 161.3 (C-5), 99.8 (C-6), 163.3 (C-7), 94.9 (C-8), 156.5 (C-9), 106.1 (C-10), 121.2 (C-1′), 131.5 (C-2′,C-6′), 115.6 (C-3′,C-5′), 160.7 (C-4′), 101.3 (C-1″), 74.7 (C-2″), 76.9 (C-3″), 70.3 (C-4″), 78.0 (C-5″), 61.3 (C-6″), 101.2 (C-1‴), 73.6 (C-2‴), 77.0 (C-3‴), 70.1 (C-4‴), 77.6 (C-5‴), 62.1 (C-6‴). Comparing the results with reference data [33], compound **24** was identified as kaempferol 3-*O*-*β*-D-galactopyranosyl-7-*O*-*β*-D-glucopyranoside.

*Compounds isolated from the EtOAc extract*

Compound **7**: Positive ESI-MS: *m/z* 207.5 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 7.08 (2H, s, H-2′,H-6′), 3.82 (3H, s, $OCH_3$), $^{13}C$ NMR: $\delta_C$ 120.3 (C-1), 109.3 (C-2, C-6), 144.8 (C-3,C-5), 138.2 (C-4), 168.0 (C-7), 52.3 ($OCH_3$). Comparing the results with Ref. [32], compound **7** was identified as methyl gallate.

Compound **11**: Positive ESI-MS: *m/z* 221.4 $[M+Na]^+$;$^1H$ NMR (500 MHz, MeOH-$d_4$) $\delta$: 7.10 (2H, s, H-2′,H-6′), 3.82 (3H, s, $OCH_3$), 1.40 (3H, t, *J*=7.1 Hz, $CH_3$), 4.35 (2H, q, *J*=7.1 Hz, $OCH_2$); $^{13}C$ NMR (125 MHz, MeOH-$d_4$) $\delta$: 121.8 (C-1), 110.0 (C-2, C-6), 146.5 (C-3,C-5), 139.7 (C-4), 168.5 (C-7), 14.6 ($CH_3$), 61.7 ($OCH_2$). Comparing the results with Ref. [32], compound **11** was identified as ethyl gallate.

Compound **15**: Positive ESI-MS: *m/z* 811.5 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.24 (d, *J*=8.0 Hz, H-1), 5.48 (t, *J*=8.3 Hz, H-2), 5.52 (t, *J*=8.5 Hz, H-3), 3.90 (t, *J*=8.5 Hz, H-4), 4.10 (m, H-5), 4.52 (m, H-6a), 4.60 (dd, *J*=12.0, 1.0 Hz, H-6b), 7.60, 7.20, 7.01, 6.98 (each s, galloyl H-2′, H-6′); $^{13}C$ NMR: $\delta_C$ 93.6 (C-1), 71.9 (C-2), 76.1 (C-3), 69.5 (C-4), 76.1 (C-5), 63.4 (C-6), 118.0, 118.1, 119.0, 119.2 (4 × C-1′), 107.9, 109.0, 109.2, 109.2 (4 × C-2′, 4 × C-6′), 144.9, 145.0, 145.1, 145.3 (4 × C-3′, 4 × C-5′), 138.2, 138.5, 138.9, 139.1 (4 × C-4′), 164.8, 165.1, 165.5, 166.5 (4 ×C-7′). Comparing the results with Ref. [36], compound **15** was identified as 1,2,3,6-*tetra*-*O*-galloyl-*β*-D-glucopyranoside.

Compound **16**: Positive ESI-MS: *m/z* 963.5 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.28 (d, *J*=7.9 Hz, H-1), 5.61 (t, *J*=8.4 Hz, H-2), 5.93 (t, *J*=8.5 Hz, H-3), 5.65 (t, *J*=8.5 Hz, H-4), 4.42 (m, H-5), 4.41 (m, H-6a), 4.55 (dd, *J*=12.0, 1.0 Hz, H-6b), 7.14, 7.07, 7.01, 6.98, 6.87 (each s, galloyl H-2′, H-6′); $^{13}C$ NMR: $\delta_C$ 92.4 (C-1), 70.8 (C-2), 72.7 (C-3), 68.4 (C-4), 73.0 (C-5), 61.7 (C-6), 118.3, 118.8, 118.9, 119.0, 119.4 (5×C-1′), 108.9, 109.0, 109.1, 109.2, 109.3 (5×C-2′, 5×C-6′), 144.9, 145.0, 145.1, 145.2, 145.3 (5×C-3′, 5×C-5′), 138.6, 138.7, 138.8, 138.9, 139.4 (5×C-4′), 164.8, 165.5, 165.6, 165.9, 166.5 (5×C-7′). Comparing the results with Ref. [37],

compound **16** was identified as 1,2,3,4,6-*penta*-*O*-galloyl-*β*-D-glucopyranoside.

Compound **17**: ESI-MS: *m*/*z* 623.0 $[M+H]^+$; $^1H$ NMR: $\delta_H$ 6.18 (d, *J*=2.0 Hz, H-6), 6.39 (d, *J*=2.0 Hz, H-8), 7.92 (d, *J*=8.4 Hz, H-2′,H-6′), 6.76 (d, *J*=8.4 Hz, H-3′,H-5′), 5.42 (d, *J*=7.2 Hz, H-1″), 4.17 (dd, *J*=2.0 Hz, H-6″), 3.51 (t, *J*=8.4 Hz, H-2″), 3.49 (t, *J*=8.4 Hz, H-3″), 3.34 (t, *J*=9.1 Hz, H-4″), 3.24 (m, H-5″), 4.32 (dd, *J*=11.8, 1.8 Hz, H-6″a), 4.34 (dd, *J*=11.8, 5.2 Hz, H-6″b), 6.89 (s, H-2‴,H-6‴); $^{13}C$ NMR: $\delta_C$ 157.5 (C-2), 133.5 (C-3), 177.4 (C-4), 161.1 (C-5), 99.6 (C-6), 165.4 (C-7), 94.6 (C-8), 156.9 (C-9), 104.5 (C-10), 121.5 (C-1′), 131.2 (C-2′, C-6′), 115.3 (C-3′, C-5′), 161.5 (C-4′), 101.6 (C-1″), 74.4 (C-2″), 76.4 (C-3″), 69.8 (C-4″), 74.4 (C-5″), 63.0 (C-6″), 119.5 (C-1‴), 108.2 (C-2‴, C-6‴), 145.6 (C-3‴, C-5‴), 138.8 (C-4‴), 167.3 (C-7‴). Comparing the results with reference data [19], compound **17** was identified as kaempferol 3-*O*-(6-*O*-galloyl)-*β*-D-glucopyranoside.

Compound **18**: ESI-MS: *m*/*z* 471.2 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.23 (d, *J*=2.0 Hz, H-6), 6.42 (d, *J*=2.0 Hz, H-8), 8.05 (d, *J*=8.9 Hz, H-2′, H-6′), 6.93 (d, *J*=8.9 Hz, H-3′, H-5′), 5.28 (d, *J*=7.4 Hz, H-1″), 3.49 (t, *J*=8.5 Hz, H-2″), 3.48 (t, *J*=8.5 Hz, H-3″), 3.36 (t, *J*=9 Hz, H-4″), 3.25 (m, H-5″), 3.56 (dd, *J*=12.0,5.3 Hz, H-6″a), 3.70 (dd, *J*=12.0, 2.0 Hz, H-6″b);$^{13}C$ NMR: $\delta_C$ 157.9 (C-2), 134.0 (C-3), 178.0 (C-4), 161.0 (C-5), 98.7 (C-6), 164.5 (C-7), 93.7 (C-8), 157.0 (C-9), 104.3 (C-10), 121.4 (C-1′), 130.9 (C-2′,C-6′), 114.8 (C-3′,C-5′), 160.0 (C-4′), 102.7 (C-1″), 74.2 (C-2″), 76.4 (C-3″), 69.8 (C-4″), 76.8 (C-5″), 61.1 (C-6″). Comparing the results with reference data [19], compound **18** was identified as astragalin.

Compound **19**: Positive ESI-MS: *m*/*z* 1115.5 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ 6.27 (d, *J*=8.3 Hz, H-1), 5.63 (t, *J*=9.2 Hz, H-2), 5.94 (t, *J*=9.3 Hz, H-3), 5.62 (t, *J*=8.5 Hz, H-4), 4.45 (m, H-5), 4.46 (m, H-6a), 4.56 (dd, *J*=12.2, 4.4 Hz, H-6b), 7.25, 7.06, 7.01, 6.98, 6.87 (each s, galloyl H-2′,H-6′ and H-2″,H-6″), 7.48, 7.32 (each d, *J*=2.0 Hz, H-2′, H-6′ of galloyl at C-6); $^{13}C$ NMR: $\delta_C$ 93.8 (C-1), 72.2 (C-2), 74.0 (C-3), 70.0 (C-4), 74.3 (C-5), 63.5 (C-6), 121.1-119.7 (6×galloyl C-1), 111.0-110.1 (6×galloyl C-2, 6×galloyl C-6), 144.9-147.9 (12×galloyl C-3, C-5), 140.1-140.7 (6×galloyl C-4), 166.2-167.3 (6×galloyl C-7). Comparing the results with Ref. [39], compound **19** was identified as 6-*O*-*m*-digalloyl-1,2,3,4-*tetra*-*O*-galloyl-*β*-D-glucopyranoside.

Compound **25**: Positive ESI-MS: *m*/*z* 359.2 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ (*m*-isomer) 7.39 (d, *J*=1.7 Hz, H-2), 7.25 (d, *J*=1.7 Hz, H-6), 3.48 (s, H-8), 7.23 (d, *J*=2.1 Hz, H-2′), 7.10 (d, *J*=2.1 Hz, H-6′), , (*p*-isomer) 7.20 (s, H-2, H-6), 3.86 (s, H-8), 7.21 (s, H-2′, H-6′); $^{13}C$ NMR: $\delta_C$ (*m*-isomer) 120.6 (C-1), 117.3 (C-2), 147.6 (C-3), 139.7 (C-4), 146.6 (C-5), 114.7 (C-6), 166.3 (C-7), 52.5 (C-8), 121.4 (C-1′), 110.9 (C-2′, C-6′), 146.4 (C-3′, C-5′), 140.0 (C-4′), 168.2 (C-7′), (*p*-isomer) 128.4 (C-1), 109.9 (C-2, C-6), 151.7 (C-3, C-5), 132.5 (C-4), 166.2 (C-7), 52.7 (C-8), 120.5 (C-1′), 110.2 (C-2′,C-6′), 146.3 (C-3′,C-5′), 140.1 (C-4′), 168.2 (C-7′). Comparing the results with Ref. [39], compound **25** was identified as an equilibrium mixture of methyl *m*-digallate and methyl *p*-digallate.

Compound **26**: Positive ESI-MS: *m*/*z* 373.3 $[M+Na]^+$; $^1H$ NMR: $\delta_H$ (*m*-isomer) 7.41 (d, *J*=2.1 Hz, H-2), 7.28 (d, *J*=2.1 Hz, H-6), 4.35 (q, *J*=7.1 Hz, H-8), 1.39 (t, *J*=7.1 Hz, H-9), 7.13 (s, H-2′,H-6′), (*p*-isomer) 7.26 (d, *J*=2.1 Hz, H-2), 7.41 (d, *J*=2.1 Hz, H-6), 4.38 (q, *J*=7.1 Hz, H-8), 1.38 (t, *J*=7.1 Hz, H-9), 7.24 (s, H-2′,H-6′); $^{13}C$ NMR: $\delta_C$ (*m*-isomer) 120.4 (C-1), 117.2 (C-2), 147.6 (C-3), 139.6 (C-4), 146.6 (C-5), 114.5 (C-6), 166.3 (C-7), 60.5 (C-8), 13.2 (C-9), 121.5 (C-1′), 109.3 (C-2′,C-6′), 146.4 (C-3′,C-5′), 140.0 (C-4′), 168.2 (C-7′), (*p*-isomer) 128.4 (C-1), 109.9 (C-2,C-6), 151.7 (C-3,C-5), 132.5 (C-4), 166.2 (C-7), 52.7 (C-8), 13.3 (C-9), 120.5 (C-1′), 110.2 (C-2′,C-6′), 146.3 (C-3′,C-5′), 140.1 (C-4′), 168.2 (C-7′). Comparing the results with Ref. [40], compound **26** was identified as equilibrium mixture of ethyl *m*-digallate and ethyl *p*-digallate.

The major components purified from the EtOAc part were compounds **16** (1.94%), **7** (1.30 %), and **4** (0.58 %), whereas in the $H_2O$ part, the major components were compounds **24** (0.33%), and **4** (0.29 %).

Compounds **1**, **7**, **8**, **9**, **16**, **17**, **18**, **20**, **21**, and **22** were previously identified in the methanol extract of *P. lactiflora* flowers and compounds **1**, **4**, **8**, **9**, **14**, **16**, **18** and **21** in the methanol extract of *P. suffruticosa* flowers and in these cases, compounds **7**, **8** and **16** were the major compounds [18]. Compounds **1**, **4**, **8**, **9**, **11**, **15**, **16** and **19** were previously identified in the roots of *P. lactiflora* [15, 26, 35]. To our knowledge, the other compounds were identified for the first time in *P. lactiflora* flowers.

### *4.2. HPLC Analysis of the 70% EtOH Extract*

On the ultraviolet spectra of HPLC-UV chromatograms of compounds detected in *P. lactiflora*, maximum absorbance values were observed around 205, 232, 278, and 340 nm. Hence characteristic chromatographic patterns were obtained by using 205 nm as the detection wavelength. There were 16 characteristic peaks found in the chromatogram, which covered more than 90% of the total area (Fig. 2). Sixteen components were identified by comparing their retention time and UV spectrum with those of isolated pure compounds (**2-5**, **7**, **8**, **11**, **13-19**, **25**, and **26**) which were eluted in parallel under the same conditions. Major components of 70% EtOH extract (peack area at 205 nm) were composed of **16**, **17**, **18**, **19**, **25**, and **26** (Fig. 2).

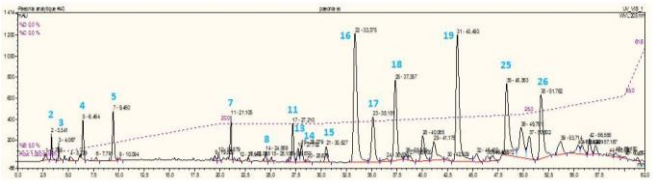

**Figure 2:** HPLC chromatographic profile of the 70% EtOH extract of *P. lactiflora* flowers.

### *4.3. DPPH Free Radical Scavenging Activity*

The 70% EtOH from *P. lactiflora* exhibited significant scavenging effect on DPPH radicals ($IC_{50}$ 2.3 $\mu g \cdot mL^{-1}$) compared to ascorbic acid used as positive control ($IC_{50}$ 11.3 $\mu g \cdot mL^{-1}$, respectively) (Table 1). To identify the compounds responsible for the observed activity, this extract was dissolved in $H_2O$ and partitioned with EtOAc. The organic

phase was about 10 times more active than the crude extract ($IC_{50}$ = 0.3 µg·mL$^{-1}$).

Compounds **7**, **15**, **16**, **25**, and **26** isolated from the EtOAc phase exhibited good scavenging effect on DPPH radicals with $IC_{50}$ ranging from 0.48 to 12.8 µg·mL$^{-1}$ (Table 1). Compound **7** was the most potent ($IC_{50}$ 0.48 µ·mL$^{-1}$) However, the fact that the $IC_{50}$ values of the nine phenolic compounds isolated from EtOAc extract were higher than that of the crude EtOAc extract (0.3 µg·mL$^{-1}$), could be related to the combination effects or to more potent components in the crude EtOAc extract yet to be discovered as lead natural antioxidants by further chemical research. On the other hand, the hydrophilic phase had a relatively lower scavenging effect than the 70% EtOH extract ($IC_{50}$ = 86.7 µg·mL$^{-1}$) and the most active compounds isolated from this phase were **11**, **15**, **20**, and **21** with $IC_{50}$ ranging from 2.37 to 5.2 µg·mL$^{-1}$ (Table 1).

**Table 1.** $IC_{50}$ values of mushroom tyrosinase inhibition and antioxidant effects of *P. lactiflora* flowers extracts and compounds. Data were expressed as a mean value of three independent experiments.

| | $DPPH^+$ scavenging $IC_{50}$ (µg·mL$^{-1}$) | Tyrosinase $IC_{50}$ (µg·mL$^{-1}$) |
|---|---|---|
| **1** | 1.80 | > 200 |
| **2** | > 200 | > 200 |
| **3** | 7.07 | 48 |
| **4** | 4.23 | 45 |
| **5** | 4.07 | 47 |
| **6** | > 200 | > 200 |
| **7** | 0.48 | > 200 |
| **8** | > 200 | > 200 |
| **9** | > 200 | > 200 |
| **10** | > 200 | > 200 |
| **11** | 2.37 | 41.0 |
| **12** | > 200 | > 200 |
| **13** | > 200 | > 200 |
| **14** | > 200 | > 200 |
| **15** | 3.10 | 0.23 |
| **16** | 4.52 | 0.38 |
| **17** | 7.83 | 0.35 |
| **18** | > 200 | > 200 |
| **19** | 12.0 | 37 |
| **20** | 4.53 | > 200 |
| **21** | 5.20 | 46 |
| **22** | > 200 | > 200 |
| **23** | > 200 | > 200 |
| **24** | 12.8 | 34 |
| **25** | 1.63 | > 200 |
| **26** | 2.03 | > 200 |
| EtOH extract | 2.3 | 0.35 |
| EtOAc extract | 0.3 | 0.35 |
| $H_2O$ extract | 86.7 | 0.35 |
| Kojic acid [b] | - | 6.4 |
| Ascorbic acid [b] | 11.3 | - |

[a]NA: 50% inhibition not achieved at the concentration of 200 µg·mL$^{-1}$.
[b]Used as a positive control.

*4.4. Fungal Tyrosinase Inhibition*

The whitening activity of 70% EtOH, $H_2O$ and EtOAc extracts was evaluated, in addition to compounds **1**-**26** by using the fungal tyrosinase inhibitory assay. We found that 70% EtOH, $H_2O$ and EtOAc extracts possess good tyrosinase inhibitory activity with $IC_{50}$ 0.35 µg·mL$^{-1}$ for each one compared to kojic acid used as positive control ($IC_{50}$ 6.4 µg·mL$^{-1}$). Compounds **15**-**17** isolated from the EtOAc extract reduce significantly fungal tyrosinase activity with $IC_{50}$ ranging from 0.23 to 0.35 µg·mL$^{-1}$ (Table 1). Only compound **24** isolated from the hydrophilic phase exhibited moderate tyrosinase inhibitory activity ($IC_{50}$ 34 µg·mL$^{-1}$). Compound **15** was the most active ($IC_{50}$ 0.23 µg·mL$^{-1}$) compared to kojic acid ($IC_{50}$ 6.4 µg·mL$^{-1}$) used as positive control (Table 1).

## CONCLUSION

In summary, 26 known compounds were isolated from the flowers of *Paeonia lactiflora*. A simple, accurate, and reliable method was developed to evaluate the quality of *P. lactiflora* extracts by using the established HPLC fingerprint and the determination of sixteen compounds. This method might find its applicability to the determination of regional variation of the active compounds and to a cultivation management for *P. lactiflora*. The antioxidant assays revealed that 70% EtOH extract and EtOAc phase had significant antioxidant abilities in DPPH scavenging activity. The most active compound was identified as methyl gallate (**7**). Through the *in vitro* fungal tyrosinase inhibition screening, we found, that 70% EtOH, EtOAc, and $H_2O$ extracts and compounds **15, 16** and **17** inhibit the target protein activities with good $IC_{50}$ values. It is interesting that the tetra- and penta-galloylglucoses (**15** and **16**) and the flavonoid **17** have bi-functionality, not just have antioxidative abilities, but also had the inhibitory effects on fungal tyrosinase. These constituents from *P. lactiflora* exhibited potential applications in medical cosmetology and food supplementation, simultaneously.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.


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