HPLC determination of phenolic compounds in different solvent extracts of mulberry leaves and antioxidant capacity of extracts

Zhenjiang Wang\textsuperscript{a,b}, Cuming Tang\textsuperscript{a,b}, Fanwei Dai\textsuperscript{a,b}, Gengsheng Xiao\textsuperscript{a}, and Guoqing Luo\textsuperscript{a}

\textsuperscript{a}Sericultural & Agri-Food Research Institute Guangdong Academy of Agricultural Sciences, Guangzhou, China; \textsuperscript{b}Key Laboratory of Urban Agriculture in South China, Ministry of Agriculture and Rural Affairs, Guangzhou, China

ABSTRACT
The antioxidant activities and phenolic contents of mulberry leaf extracts obtained by aqueous mixture of acetone, ethanol and methanol were determined. Both alkaline hydrolysis and acid hydrolysis were used to extract bound phenolics. HPLC was used to determine the profile of the extracted phenolics. Three experimental assays, namely, Fe\textsuperscript{2+} reducing antioxidant power (FRAP), ABTS- and DPPH-radical scavenging activity, were employed to characterize their antioxidant activity. This indicates that acetone was the most effective solvent for extracting free phenolics from mulberry leaves, and the acetone extract exhibited the highest antioxidant capacity. In addition, alkaline hydrolysis was found to be the most efficient method for extracting bound phenolics and produced an extract with better antioxidant properties compared to acid hydrolysis.

INTRODUCTION
White mulberry (\textit{Morus alba} L.), a member of the Moraceae family, has a long history of cultivation in Asian countries, and its foliage is a preferable food for silkworms. Mulberry leaves are famous medicine and food plants in China. They are rich in various biological active substances such as polyphenols, alkaloids, polysaccharides and vitamins.\textsuperscript{[1]} Mulberry leaves, containing calcium, carbohydrates, iron, proteins, vitamin B1, vitamin D and \(\beta\)-carotene, are also considered to be a nutritious, palatable and nontoxic food or food additive with medicinal properties.\textsuperscript{[2]} Mulberry leaves are commonly used as anti-diabetic, hypolipidemic, antihypertensive, anti-atherosclerotic and anticonvulsant agents.\textsuperscript{[3]} The pharmacological effects of mulberry leaves are due to the existence of phenolic components, which have been reported to involve rutin, quercetin, isoquercetin and astragalin.\textsuperscript{[4]}

The phenolic compounds can serve as an antioxidant via different mechanistic reaction pathways, such as inhibition of lipid peroxidation, metal chelation, quenching of singlet oxygen and radical scavenging. Various solvent extraction systems have been adopted for extracting polyphenols from plantsubstances,\textsuperscript{[5]} and the types of extraction solvents and methods have been shown to exert an important effect on extraction yields.\textsuperscript{[6]} The method of extraction should enable maximum extraction of the required components and prevent their chemical alteration.\textsuperscript{[7]} Aqueous mixtures containing acetone, ethanol and methanol, as well as water are widely applied for the extraction of plant materials.\textsuperscript{[8]} It has been reported that methanol is the most effective solvent for extracting antioxidants from mulberry leaves.\textsuperscript{[9]} The methanolic extract displays the strongest antioxidant capacity, and it remained stable upon storage at appropriate pH and temperature. The optimal extraction of highly polar active polyphenols from \textit{Medicago sativa} demands an alcohol-water binary solvent system, with a methanol fraction of approximately 60\%,\textsuperscript{[10]} Thabti et al.\textsuperscript{[11]} have used 60\% (w/v) aqueous ethanol as the extraction solvent for black (\textit{Morus nigra} L.), red (\textit{Morus rubra} L.), and white (\textit{Morus alba} L.)
mulberry leaves. Apart from methanolic and ethanolic extracts, acetone is widely regarded as a common solvent for extracting phenolic compounds from plant materials. To facilitate the extraction of bound phenolics, both alkaline hydrolysis and acid hydrolysis can be applied. The present study investigated the extraction of white mulberry leaves using different solvents and extraction approaches, to evaluate the optimal extraction solvent and method. The total quantity of phenolic compounds and antioxidant capacity of the mulberry leaf extract (MLE) were also determined.

**MATERIALS AND METHODS**

*Mulberry leaf sample collection and preparation*

Mulberry cultivar JP5 (*Morus atropurpurea* Roxb.) is a typical plant that grows in the southern part of China. The mulberry leaves were sampled in September 2016 at the South China Branch of the National Mulberry Germplasm Resource Garden in Guangzhou, as governed by the Sericulture & Agri-Food Research Institute of the Guangdong Academy of Agricultural Sciences. The samples were air-dried for 6–8 h at 55°C in a thermostatic hot air-drying oven. The dried leaves were ground into fine powder using a high-speed pulverizer and then kept at 2°C until used.

*Chemicals and reagents*

Rutin, isoquercetin, ferulic acid, resveratrol, epicatechin, syringic acid, scopoletin, caffeic acid, catechuic acid, chlorogenic acid, quercitrin, quercetin, gallic acid, vanillic acid, protocatechuic acid, astragalin, 2,4,6-tris(1-pyridyl)-s-triazine (TPTZ), 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were supplied by the National Institutes for Food and Drug Control (Beijing, China). All chemicals and reagents used in this study were of analytical grade. Other chemicals and reagents used were obtained from Guangzhou Chemical Reagent Factory (Guangzhou, China).

*Extraction of free phenolics*

The extraction method used was a modification of procedures reported by Sun et al., Li et al. and Kim et al. Firstly, lipid was removed from the mulberry leaves using the following steps. Dried mulberry leaves (10 g) were homogenized with n-hexane (250 mL) for 10 min in an ice bath and then centrifuged (4390 × g, 5 min). Subsequently, the supernatant was removed, and the residue was added to a precooled mixture of alcohol and water (8:2, v/v; 250 mL). The mixture was extracted separately through homogenization (5000 rpm, 5 min) and centrifugation (4390 × g, 5 min). The two resulting supernatants were mixed and evaporated to dryness at 50°C using an RE-52AA rotary evaporator. The dried residue from each extraction was dissolved by a mixture of methanol and water (8:2, v/v), filtered through membrane filters (0.22 μm; 25 mm Luer syringe filter) and kept at −80°C until analysis. Each extraction was conducted in duplicate. The residues were retained for future use. To determine the optimal extraction solvent, different organic solvents (e.g. acetone, ethanol and methanol) were used in the organic solvent-water binary mixture at a ratio of 8:2 (v/v).

*Extraction of bound phenolics*

After extracting free phenolics with 80% acetone, the mulberry leaf residues were used for the hydrolysis of the bound phenolics. Half of the sample was subjected to alkaline hydrolysis, while the other half was subjected to acid hydrolysis. Alkaline hydrolysis was performed using the procedures modified from those reported by Nuutila et al., Madhujith et al. and Krygier et al. Briefly, the
residue was hydrolyzed for 1.5 h using 2 M NaOH (100 mL), under continuous stirring with nitrogen gas flow. The mixture was acidified to pH 2 with HCl (6 M) and then extracted 6 times with ethyl acetate. The combined ethyl acetate extract was evaporated to dryness at 45°C. The dried extracts were redissolved in 80% methanol, filtered through membrane filters (0.22 μm), and kept at −80°C until used.

Acid hydrolysis was carried out using the procedure modified from that reported by Dongxiaol et al.[13] Briefly, after free phenolic extraction, the mulberry leaf residues were hydrolyzed at 85°C for 1.5 h using a mixture of methanol and concentrated sulfuric acid (90:10, v/v; 100 mL) under continuous shaking with nitrogen gas flow. The mixture was neutralized to pH 2 with NaOH (10 M) and then extracted 6 times with ethyl acetate. The combined ethyl acetate extract was evaporated to dryness at 45°C. The dried extracts were redissolved in 80% methanol, filtered through membrane filters (0.22 μm), and kept at −80°C until used.

**Determination of Total Polyphenol Content (TPC)**

The TPC of mulberry juice was analyzed using the Folin–Ciocalteu colorimetric method described previously by Fu.[18]

**HPLC determination of phenolics**

Identification and quantification of common phenolic compounds were conducted by HPLC, using an ADME column (5 μm; 250 × 4.6 mm) for the separation. The mobile phase was a mixture of solvent A (phosphoric acid: water [0.2:100]) and solvent B (acetonitrile). The gradient of solvents was programmed as follows: 0–20 min, 10% B; 20–30 min, 16% B; 30–40 min, 16% B; 40–60 min, 20% B; 60–70 min, 30% B; 70–71 min, 35% B; 71–75 min, 80% B; 75–76 min, 80% B; and 76–90 min, 10% B. The flow rate of solvents was set at 1.0 mL/min, and the column temperature was maintained at 25°C. HPLC chromatograms of MLEs were recorded at 280 nm and 350 nm. The detection method used was modified from the procedures reported by Hyun et al.,[19] Kotásková et al.[20] and Gundogdu et al.[21]

**FRAP determination**

FRAP was assessed through a redox-linked reduction of Fe³⁺-TPTZ to a blue-colored Fe²⁺ complex, using the methods modified from those reported by Zou et al.,[22] Alothman et al.[23] and Benzie et al.[24] The FRAP reagents were pre-warmed at 37°C and prepared freshly each time. The MLEs were incubated with FRAP reagents at 25°C for 4 min. Absorbance measurement was conducted using an UV2300II spectrophotometer at 593 nm. Distilled water was used as the blank sample. FRAP data were presented as μmol Fe²⁺ equivalents per g of dry weight (μmol Fe²⁺/g DW).

**ABTS evaluation**

To evaluate the total antioxidant capacities of the MLEs, ABTS radical scavenging assay was performed based on the method of Re et al.[25] with minor modifications introduced by Sumczynski et al.[26] and Wang et al.[27] Briefly, a stock solution of the radical ABTS reagent was prepared by incubating ABTS and potassium persulfate in darkness for 16 h at room temperature. The prepared reagent was used within 24 h. The stock solution was diluted with methanol until its absorbance (734 nm) achieved 0.70 ± 0.02. After dilution, ABTS working solution (3.8 mL) was added into 100 μL aliquot of the diluted extract sample. The absorbance (734 nm) was measured exactly after 6 min at room temperature. The antioxidant activities were displayed as μmol of Trolox (a standard antioxidant) equivalent antioxidant capacity (TEAC) per g of dry weight (μmol TEAC/g DW).
**DPPH assessment**

DPPH radical scavenging assay was used to examine the antioxidant activities of the samples, using the modified methods proposed by Yang et al.,[28] Thabti et al.[11] and Anwar et al.[29] MLE and DPPH solutions were diluted to concentrations such that the spectral values were within an appropriate range of the standard curve, and methanol was used to prepare control standard (ascorbic acid) in order to establish a standard curve (R² = 0.993). Aliquots (1 mL) of each diluted MLE were mixed with DPPH solution (5 mL), and the mixture was incubated for 50 min in the dark at room temperature. The absorbance was measured at 520 nm using an UV2300II spectrophotometer, and the data were presented as μmol of ascorbic acid equivalent antioxidant capacity (AEAC) per g of dry weight (μmol AEAC/g DW).

**Statistical analyses**

At least three replicate measurements were recorded for each MLE sample, and all data were presented as mean ± SD. SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) was employed to conduct ANOVA and Tukey’s tests. P values of less than 0.05 were regarded as statistically significant.

**RESULTS AND DISCUSSION**

**TPC and free phenolic profile**

The study found that there was no significant difference in total phenolic content in mulberry leaves of different mulberry varieties by Hao et al.[30] And then, The total phenolic content (TPC) and free phenolic profiles of different MLEs are shown in (Table 1). The total amount of free phenolics ranged from 5957.53 μg/g DW (ethanol extraction) to 6848.43 μg/g DW (acetone extraction), indicating that acetone is a better extraction solvent compared to methanol and ethanol. In addition, the HPLC data (Table 1) revealed that more individual phenolics were extracted by acetone (16) than by methanol (12) and ethanol (15). As a whole, the MLEs obtained from acetone extraction solvent contained high amounts of almost every individual phenolic compound listed in (Table 1). Thus, acetone was the

| Phenolic         | 80% acetone        | 80% methanol      | 80% ethanol       |
|------------------|--------------------|--------------------|--------------------|
| Ferulic acid     | 9.73 ± 0.4a        | ND                 | 10.91 ± 0.62a      |
| Resveratrol      | 3.68 ± 0.24a       | ND                 | 4.10 ± 0.21a       |
| Epicatechin      | 278.85 ± 13.57a    | 232.45 ± 12.49b    | 254.09 ± 8.09b     |
| Syringic acid    | 21.93 ± 1.99a      | 17.99 ± 1.75b      | 15.29 ± 1.17b      |
| Scopoletin       | 69.87 ± 7.6a       | 44.53 ± 5.01b      | 46.80 ± 4.45b      |
| Catechuic acid   | 24.58 ± 2.1a       | 24.48 ± 2.01a      | ND                 |
| Quercitin        | 24.74 ± 2.02a      | ND                 | 24.33 ± 1.82a      |
| Quercetin        | 12.35 ± 1.37a      | ND                 | 12.19 ± 1.1a       |
| Caffeic acid     | 53.13 ± 3.01a      | 44.23 ± 2.75b      | 47.76 ± 1.45b      |
| Rutin            | 669.80 ± 38.83a    | 545.52 ± 45.88b    | 598.99 ± 26.1b     |
| Chlorogenic acid | 4620.90 ± 341.98a  | 4210.27 ± 301.82a  | 3961.03 ± 251.4a   |
| Gallic acid      | 5.80 ± 0.54a       | 5.91 ± 0.5a        | 5.80 ± 0.39a       |
| Vanillic acid    | 19.45 ± 2.55a      | 19.11 ± 2.15a      | 19.35 ± 1.61a      |
| Isoquercitrin    | 619.56 ± 31.3a     | 510.20 ± 30.49b    | 561.06 ± 28.8ab    |
| Protocatechuic acid | 45.21 ± 3.46a   | 38.92 ± 3.1b       | 33.26 ± 2.78b      |
| Astragalin       | 368.84 ± 26.49a    | 319.11 ± 20.95a    | 362.56 ± 14.8a     |
| TPC              | 6848.43 ± 225.26a  | 6012.72 ± 191.02b  | 5957.53 ± 161.82b  |

ND – not detected. Values within a row that have no letters in common are significantly different (p < 0.05).

Different lowercase letters in the same column indicate significant difference between the measurement results (P < 0.05), and the same lowercase letters indicate no significant difference between the measurement results (P > 0.05).
most effective solvent for extracting antioxidants, such as rutin, chlorogenic acid, and isoquercitrin, from mulberry leaves. It is worth noting that acetone can be the most suitable solvent for extracting large quantity and high diversity of phenolic compounds. Taking into account numerous factors, Saeedeh et al.\(^\text{[9]}\) have concluded that methanol is the best extraction solvent. In the present study, it was noted that acetone extraction yielded 16 individual free phenolics (Table 1), with the largest proportions of chlorogenic acid (67.5%), rutin (9.8%), isoquercitrin (9.0%) and astragalin (5.4%).

**TPC and bound phenolic profile**

The TPC and bound phenolic profile of mulberry leaves after acid and alkaline hydrolysis are shown in (Table 2). The total amount of bound phenolics obtained after alkaline hydrolysis (286.43 μg/g DW) was markedly greater than that obtained after acid hydrolysis (41.38 μg/g DW). More individual phenolics were also obtained after alkaline hydrolysis (11) than after acid hydrolysis (6). Extraction after alkaline hydrolysis yielded 48.3% caffeic acid and 17.8% isoquercitrin, but these phenolics were not found in the extract obtained after acid hydrolysis. Notably, acid hydrolysis yielded larger amounts of epicatechin. However, from a practical point of view, alkaline hydrolysis is simpler and less hazardous to be performed as compared to acid hydrolysis, which requires the handling of concentrated sulfuric acid. Taken together, alkaline hydrolysis is obviously the most ideal method for extracting bound phenolics.

The total amount of free phenolics was remarkably greater than that of bound phenolics (Table 1 versus Table 2), and indeed, the amount of the most abundant free phenolic (chlorogenic acid, 4620.9 μg/g DW) far exceeded the total amount of bound phenolics. Epicatechin, rutin, chlorogenic acid, isoquercitrin and astragalin were all far more abundant as free phenolics, with very small amount of chlorogenic acid detected in the extracts of bound phenolics. Resveratrol, scopoletin and catechuic acid were also only detected in the extracts of free phenolics.

**Antioxidant activity and its correlation with phenolic content**

The antioxidant activities of plant samples are widely acknowledged to be affected by numerous factors, including extraction method, extraction solvent and assay system. Different assessments of

### Table 2. Assessments of individual bound phenolics in JPS by HPLC.

| Phenolic            | Bound phenolics (μg/g DW) |
|---------------------|---------------------------|
|                     | Alkaline extract | Acid extract |
| Ferulic acid        | 7.45 ± 0.5        | ND           |
| Epicatechin         | 6.48 ± 0.63\(^a\)  | 22.08 ± 1.84\(^b\) |
| Syringic acid       | 8.29 ± 0.76\(^a\)  | 2.22 ± 0.35\(^b\) |
| Quercitrin          | 11.72 ± 1.15      | ND           |
| Quercetin           | 6.62 ± 0.6\(^a\)  | 11.37 ± 1.27\(^b\) |
| Caffeic acid        | 138.21 ± 11.45    | ND           |
| Rutin               | 15.05 ± 1.25      | ND           |
| Chlorogenic acid    | ND               | 0.80 ± 0.09   |
| Gallic acid         | ND               | 2.32 ± 0.34   |
| Vanillic acid       | 9.65 ± 0.81       | ND           |
| Isoquercitrin       | 51.10 ± 6.5       | ND           |
| Protocatechuic acid | 12.17 ± 1.83\(^a\) | 2.59 ± 0.37\(^b\) |
| Astragalin          | 19.68 ± 2.41      | ND           |
| TPC                 | 286.43 ± 8.04\(^a\) | 41.38 ± 3.56\(^b\) |
| Types               | 11               | 6            |

ND – not detected. Values within a row that have no letters in common are significantly different (\(p<0.05\)).

Different lowercase letters in the same column indicate significant difference between the measurement results (\(P < 0.05\)), and the same lowercase letters indicate no significant difference between the measurement results (\(P > 0.05\)).
Table 3. FRAP, ABTS and DPPH analyses of free phenolics in JP5.

| Solvents   | Free phenolics (μmol/g DW) | FRAP (μmol Fe²⁺/g DW) | ABTS (μmol TEAC/g DW) | DPPH (μmol AEAC/g DW) |
|------------|---------------------------|--------------------|---------------------|---------------------|
| 80% acetone| 6848.43 ± 225.26a         | 64.03 ± 3.56a      | 28.83 ± 1.67a       | 20.28 ± 0.11b       |
| 80% ethanol| 5957.53 ± 161.82b         | 50.04 ± 0.99c      | 25.57 ± 2.19b       | 20.45 ± 0.3a        |
| 80% methanol| 6012.72 ± 191.02b        | 57.83 ± 0.45b      | 26.50 ± 1.26a       | 19.82 ± 0.44b       |

Values within a column that have no letters in common are significantly different (P<0.05). Different lowercase letters in the same column indicate significant difference between the measurement results (P<0.05), and the same lowercase letters indicate no significant difference between the measurement results (P>0.05).

Table 4. FRAP, ABTS and DPPH analyses of bound phenolics in JP5.

| Method         | Bound phenolics (μg/g DW) | FRAP (μmol Fe²⁺/g DW) | ABTS (μmol TEAC/g DW) | DPPH (μmol AEAC/g DW) |
|----------------|--------------------------|-----------------------|----------------------|-----------------------|
| Alkaline extract| 286.43 ± 8.04a           | 17.92 ± 0.39a        | 7.82 ± 0.12a         | 4.11 ± 0.07a          |
| Acid extract    | 41.38 ± 3.56b            | 12.68 ± 1.9b         | 4.09 ± 1.06b         | 6.79 ± 1.52b          |

Values within a column that have no letters in common are significantly different (P<0.05). Different lowercase letters in the same column indicate significant difference between the measurement results (P<0.05), and the same lowercase letters indicate no significant difference between the measurement results (P>0.05).

antioxidant activity are, therefore, needed to be considered due to the different types of antioxidant actions. The FRAP, ABTS and DPPH assay data of different MLEs are shown in (Tables 3 and 4). Notably, the FRAP values of free phenolics in MLEs ranged from 50.04 (ethanol) to 64.03 (acetone) μmol Fe²⁺/g DW, while those of bound phenolics in MLEs ranged from 12.68 (acid) to 17.92 (alkaline) μmol Fe²⁺/g DW. The results obtained from FRAP and ABTS assays were in good agreement with each other but differed a little from those obtained from DPPH assay. For ABTS and FRAP assays, the antioxidant activities of the extracts decreased in the order of 80% acetone > 80% methanol > 80% ethanol, whereas for DPPH assay, the antioxidant activities decreased in the order of 80% ethanol (20.45 μmol TEAC/g DW) > 80% acetone (20.28 μmol TEAC/g DW) > 80% methanol (19.82 μmol TEAC/g DW). The FRAP and ABTS data of the free phenolic MLEs indicated that the ethanol extracts displayed the least antioxidant activity. From the comparison of the three extraction solvents, it was noted that the acetone extracts displayed the greatest antioxidant capacity. Based on the results of the three assays, the alkaline extracts of bound phenolics demonstrated greater antioxidant capacity compared to the acid extracts, indicating that the FRAP, ABTS and DPPH assays were equally appropriate for determining the antioxidant capacity of bound phenolic extracts. Thus, it is essential to use three different assessments of antioxidant activities to minimize variation in results caused by methodological errors.

The linear relationship between the content of free phenol extracted by different solvents and the data of FRAP, ABTS and DPPH is shown in (Table 5). Due to the difference in the effect of extracting free phenol with different solvents in the same sample, the correlation of the data in different determinations is also different. Compared with FRAP and DPPH, no matter what kind of solvent is used, the free phenol content value has the strongest relative relationship with ABTS. The average value of R² is 0.9937, and the average

Table 5. Correlation coefficients (R²) for relationships between free phenol content in extracts of acetone, ethanol and methanol, and FRAP, ABTS, and DPPH assays.

|          | acetone | ethanol | methanol | FRAP | ABTS | DPPH |
|----------|---------|---------|----------|------|------|------|
| acetone  | 1       | 0.9280  | 0.9569   | 0.9769| 0.9950| 0.8764|
| ethanol  | -       | 1       | 0.9962   | 0.9884| 0.9942| 0.7925|
| methanol | -       | -       | 1        | 0.9818| 0.9920| 0.9732|
| FRAP     | -       | -       | -        | 1    | 0.7913| -0.1690|
| ABTS     | -       | -       | -        | -    | 1    | 0.2748|
| DPPH     | -       | -       | -        | -    | -    | 1    |
The total amount of free and bound phenolics in MLEs were evaluated using different extraction solvents and methods. In order to ensure the optimal phenolics extraction capacity from the mulberry leaves, the best extraction time, extraction temperature and number of extractions have been used in this work. The highest amount of free phenolics was obtained by acetone extraction, and the highest amount of bound phenolics was obtained after alkaline hydrolysis. These results were supported by the HPLC profiles of individual phenolic compounds. The total amount of free phenolics was markedly greater than that of bound phenolics. Epicatechin, rutin, chlorogenic acid, isoquercitrin and astragalin were much more abundant in the MLE of free phenolics than that of bound phenolics. An excessively small amount of chlorogenic acid was detected in the MLE of bound phenolics, while resveratrol, scopoletin and catechuic acid were found only in that of free phenolics. MLEs obtained using different solvents exhibited varying levels of antioxidant activities, as determined by the three assay systems. The highest antioxidant activity of free phenolics was found in the acetone MLEs, whereas the highest antioxidant activity of bound phenolic was found in the MLEs obtained after alkaline hydrolysis. The ability to extract of ketones and alcohols may be related to the polarity of the solvent. The toxicity of acetone is relatively large, and defensive measures such as ventilation should be taken during the process of extract. However, since acetone is still a low-toxic solvent and the effect of extract is better. Therefore, we recommend that acetone extraction and alkaline hydrolysis should be used in future studies for the determination of polyphenols.

**Acknowledgments**

The present study was supported by the National Modern Agriculture Industry Technology System, China (CARS-18), Modern Agricultural Industry Technical System Construction Project of Guangdong province, China (No. 2018M1086), Science and Technology Project of Guangdong province, China (No. 2017B020201002), National Key R & D Plan, China (2019YFD1001202), and the Science and Technology Project of Guangzhou city, China (No. 201710010129; No. 201704020026).
Disclosure statement

The authors declare no conflicts of interest.

References

[1] Wei, F.; Chen, C.; Li, C.; Fu, X. Antioxidant and Hypoglycemic Activities of Mulberry Leaf Extract, Tea Polyphenols and Their Compounds. Food Ind Technol. 2018, (21), 299–305.

[2] Bose, P. C.; Genetic Resources of Mulberry and Utilization; CSR and TI: Mysore, India, 1989; pp 183–190.

[3] Baranwal, V. K.; Negi, N.; Khurana, P. Auxin Response Factor Genes Repertoire in Mulberry: Identification and Structural, Functional and Evolutionary Analyses. Genes. 2017, 8, 202.

[4] Doi, K.; Kojima, T.; Makino, M.; Kimura, Y.; Fujimoto, Y. Studies on the Constituents of the Leaves of Morus Alba L. Chem. Pharm. Bull. 2001, 49, 151–153. DOI: 10.1248/cpb.49.151.

[5] Chavan, U. D.; Shahidi, F.; Naczk, M. Extraction of Condensed Tannins from Beach Pea (Lathyrus Maritimus L.) As Affected by Different Solvents. Food Chem. 2001, 75(4), 509–512. DOI: 10.1016/S0308-8146(01)00234-5.

[6] Goli, A. H.; Barzegar, M.; Sahari, M. A. Antioxidant Activity and Total Phenolic Compounds of Pistachio (Pistachia Vera) Hullextracts. Food Chem. 2004, 92, 521–525. DOI: 10.1016/j.foodchem.2004.08.020.

[7] Zuo, Y.; Chen, H.; Deng, Y. Simultaneous Determination of Catechins, Caffeine and Gallic Acids in Geen, Oolong, Black and Pu-erh teas Using HPLC with a Photodiode Array Detector. Talanta. 2002, 57, 307–316. DOI: 10.1016/S0039-9140(02)00039-9.

[8] Sun, T.; Ho, C. Antioxidant Activities of Buckwheat Extracts. Food Chem. 2005, 90, 743–749. DOI: 10.1016/j.foodchem.2004.04.035.

[9] Arabshahi-Delouee, S.; Urooj, A. Antioxidant Properties of Various Solvent Extracts of Mulberry (Morus Indica L.) Leaves. Food Chem. 2007, 102(4), 1233–1240. DOI: 10.1016/j.foodchem.2006.07.013.

[10] Kim, J. M.; Chang, S. M.; Kim, I. H.; Kim, Y. E.; Hwang, J. H.; Kim, K.S.; Kim, W.S. Design of Optimal Solvent for Extraction of Bio-active Ingredients from Mulberry Leaves. Biochem. Eng. J. 2007, 37(3), 271–278.

[11] Inès, T.; Nidhal, M.; Walid, E.; Ali, F. Antioxidant Composition and Antioxidant Activity of White (Morus Alba L.), Black (Morus Nigra L.) And Red (Morus Rubra L.) Mulberry Leaves. Acta Bot. Gallica. 2011, 158(2), 205–214.

[12] Nuutila, A. M.; Kammiovirta, K.; Oksman Caldentey, K. M. Comparison of Methods for the Hydrolysis of Flavonoids and Phenolic Acids from Onion and Spinach for HPLC Analysis. Food Chem. 2002, 76(4), 519–525.

[13] Su, D.; Zhang, R.; Hou, F.; Zhang, M.; Guo, J.; Huang, F.; Deng, Y.; Wei, Z. Comparison of the Free and Bound Phenolic Profiles and Cellular Antioxidant Activities of Litchi Pulp Extracts from Different Solvents. BMC Complementary Altern. Med. 2014, 14(1), 9.

[14] Sun, J.; Chu, Y. F.; Wu, X.; Liu, R. H. Antioxidant and Antiproliferative Activities of Common Fruits. J. Agric. Food Chem. 2002, 50(7), 7449–7454. DOI: 10.1021/jf0207530.

[15] Li, Y.; Liu, M. S.; Wan, H. X.; Sun, H. Y.; Cong, Y. L.; Xu, B. Q.; Liu, D. Determination of Free and Bound Phenol of Corn by High Performance Liquid Chromatography (HPLC). J. Chin. Cereals. Oils Assoc. 2015, 30(9), 108–111.

[16] Madhuijith, T.; Shahidi, F. Antioxidant Potential of Barley as Affected by Alkaline Hydrolysis and Release of Insoluble-bound Phenolics. Food Chem. 2009, 117, 615–620. DOI: 10.1016/j.foodchem.2009.04.055.

[17] Krygier, K.; Sosulski, F.; Hogge, L. Free, Esterified, and Insoluble-bound Phenolic Acids. I. Extraction and Purification Procedure. J. Agric. Food Chem. 1982, 30, 330–334. DOI: 10.1021/jf00110a028.

[18] Fu, L.; (2010) Antioxidant Activities and Total Phenolic Contents of Fruits, Herbal Teas, Tea Drinks and Wild Fruits. Master Dissertation, Sun Yat-Sen University.

[19] H., E. J.; Vu Thi, P.; Cai, L.; Yan, Z.; Li, H.; Yang, S.; Kim, Y.; Kim, S.; Cho, H.; Bao, H.; et al. 2017. Development of HPLC Method for Differentiation of Three Parts of Mulberry Tree. Anal Sci Technol. 30, NO. 3. 130–137.

[20] Eva, K.; Daniela, S.; Jir´ı´, M.; Pavel, V. Determination of Free and Bound Phenolics Using HPLC-DAD, Antioxidant Activity and in Vitro Digestibility of Eragrostis Tef. J. Food Compost. Anal. 2016, 46, 15–21. DOI: 10.1016/j.jfca.2015.11.001.

[21] Gundogdu, M.; Canan, I.; Gecer, M. K.; Kan, T.; Ercisli, S. Phenolic Compounds, Bioactive Content and Antioxidant Capacity of the Fruits of Mulberry (Morus Spp.) Germslap in Turkey. Folia Hortic. 2017, 29(2), 251–262. DOI: 10.1515/fHORT-2017-0023.

[22] Zou, Y. X.; Liao, S. T.; Shen, W. Z.; Liu, F.; Tang, C. M.; Chen, C.-Y.; Sun; Oliver, Y.; Sun, Y. M. Phenolics and Antioxidant Activity of Mulberry Leaves Depend on Cultivar and Harvest Month in Southern China. Int. J. Mol. Sci. 2012, 13, 16544–16553. DOI: 10.3390/ijms131216544.

[23] Alothan, M.; Rajeev, B.; Karim, A. A. Antioxidant Capacity and Phenolic Content of Selected Tropical Fruits from Malaysia, Extracted with Different Solvents. Food Chem. 2009, 115, 785–788. DOI: 10.1016/j.foodchem.2008.12.005.
[24] Benzie, I. F. F.; Strain, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. Anal. Biochem. 1996, 239, 70–76. DOI: 10.1006/abio.1996.0292.

[25] Re, R.; Pellegrini, N.; Proteggente, A.; Panalla, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radic. Biol. Med. 1999, 26, 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.

[26] Sumczynski, D.; Bubelova´, Z.; Sneyd, J.; Erb-Weber, S.; Mlcˇek, J. Total Phenolics, Flavonoids, Antioxidant Activity, Crude Fibre and Digestibility Innon-traditional Wheat Flakes and Muesli. Food Chem. 2015, 174, 319–325. DOI: 10.1016/j.foodchem.2014.11.065.

[27] Wang, X. Q.; Li, C. Y.; Liang, D.; Zou, Y. J.; Li, P. M.; Ma, F. W. Phenolic Compounds and Antioxidant Activity Inred-fleshed Apples. J. Funct. Foods. 2015, 18, 1086–1094. DOI: 10.1016/j.jff.2014.06.013.

[28] Yang, J. F.; Ou, X. Q.; Zhang, X. X.; Zhou, Z. Y.; Ma, L. Y. Effect of Different Solvents on the Measurement of Phenolics and the Antioxidant Activity of Mulberry (Morus Atropurpurea Roxb.)with Accelerated Solvent Extraction. J. Food Sci. 2017, 82, 605–612. DOI: 10.1111/1750-3841.13638.

[29] Farooq, A.; Shamsa, K.; Ghulam, S.; Khalid, M. A.; Anwar, H. G. Antioxidant and Antimicrobial Attributes of Different Solvent Extracts from Leaves of Four Species of Mulberry. Int J Pharmacol. 2015, 11(7), 757–765. DOI: 10.3923/ijp.2015.757.765.

[30] Hao, J.; Zhang, D.; Wan, Y.; Zhao, W.; Yao, X.; Li, L. Comparison of the Content and Activity of Main Active Constituents of Mulberry Leaves of Five Main Mulberry Varieties in Shaanxi Province. Sericulture Sci. 2019, (1), 100–107.

[31] Deng, G. F.; Xu, X. R.; Guo, Y. J.; Xia, E.-Q.; Li, S.; Wu, S.; Chen, F.; Ling, W.-H.; Li, H.-B. Determination of Antioxidant Property and Their Lipophilic and Hydrophilic Phenolic Contents in Cereal Grains. J. Funct. Foods. 2012, 4, 906–914. DOI: 10.1016/j.jff.2012.06.008.