Bioactivities of 7’-ethoxy-trans-feruloyltyramine from *Portulaca oleracea* L. and its metabolism in rats using ultra-high-performance liquid chromatography electrospray coupled with quadrupole time-of-flight mass spectrometry

Zheming Ying, Mingyue Jiang¹, Lina Wang¹, Xixiang Ying¹, Guanlin Yang

**Abstract:**
This research aims to study the antioxidation and anticholinesterase activities of 7’-ethoxy-trans-feruloyltyramine (ETFT), which was an alkaloid isolated from *Portulaca oleracea* for the first time. Furthermore, its main metabolites and metabolic pathways in rats were also explored. The antioxidation and anticholinesterase effects of ETFT were, respectively, examined using 1,1-diphenyl-2-picrylhydrazyl assay and modified Ellman’s method. The results showed that ETFT exhibited both the good antioxidant and anticholinesterase effects. Its main metabolites in rats were implemented, and nine metabolites were finally found in the rat's plasma and urine, including the oxidation, reduction, hydrolysis, glucuronidation, sulfation, and glutathionylation process.

**Keywords:** 7’-ethoxy-trans-feruloyltyramine, bioactivity, metabolites, ultra-high-performance liquid chromatography electrospray coupled with quadrupole time-of-flight mass spectrometry

**Introduction**

*Portulaca oleracea* L. is distributed in the temperate and tropical regions of the world, which has not only high nutritional value but also resistive effect of inflammatory,[1](#) neuroprotective,[2](#) antitumor,[3](#) and antioxidation.[4](#) This is mainly due to the fact that it contains many bioactive substances such as alkaloids[5-8](#) and flavonoids,[9](#) in which the alkaloids presented remarkable anti-inflammatory.[10-12](#)

In the study, the 7’-ethoxy-trans-feruloyltyramine (ETFT) [Figure 1] was obtained from purslane for the first time. Its structure was identified by comparing with literature.[13](#) The related spectrums are shown in Supplementary Figures 7-15, and its detailed ¹H and ¹³C NMR data is shown in Supplementary Table 1.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Ying Z, Jiang M, Wang L, Ying X, Yang G. Bioactivities of 7’-ethoxy-trans-feruloyltyramine from *Portulaca oleracea* L. and its metabolism in rats using ultra-high-performance liquid chromatography electrospray coupled with quadrupole time-of-flight mass spectrometry. Indian J Pharmacol 2020;52:130-3.
The antioxidation and anticholinesterase effects of ETFT were studied; in addition, the metabolism of ETFT in the rat’s plasma, urine, as well as feces after intravenously administrated was also investigated by the ultra-high-performance liquid chromatography electrospray coupled with quadrupole time-of-flight mass spectrometry.

### Experimental Method

#### Antioxidation assay

Different concentrations (0.25, 0.20, 0.15, 0.10, and 0.05 mg/mL) of ETFT and the 50 μg/mL 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution were prepared. Vitamin C and butylated hydroxyl anisole were used as positive controls, and the methanol was used as the blank control. The absorbance was detected by a U-3010 spectrophotometer at 517 nm.

#### Anticholinesterase assay

The concentrations of ETFT (0.30, 0.25, 0.20, 0.15, and 0.10 mg/mL) and 0.2 U/mL AChE, 15 mmol/L ATCI, and 15 mmol/L DTNB were prepared. For the positive control group, eserine was selected, and for the blank group, methanol was applied. The absorbance was detected at 405 nm using a 96-well microplate reader (HBS-1096A).

#### Instrumentation and condition

Agilent 1290 UHPLC (Agilent, Waldbronn, Germany) with ODS column (3.0 mm × 150 mm, particle size, 1.8 µm) was applied with the mobile phase of 0.1% formic acid (A) and acetonitrile (B). The program system was 15%–70% (B) at 0–10 min, with 0.3 mL/min, and the oven temperature was at 60°C. An Agilent 6520 Q-TOF/MS (Agilent, Waldbronn, Germany) was coupled to the UHPLC system through an ESI interface. The MS parameters in the positive mode: drying gas (nitrogen); 10 L/min at 330°C; nebulizer pressure (45 psi); fragmentation voltage 150 V; ESI Vcap of 3500 V; and collision energy (30 eV). Full-scan information acquisition in the positive ionization mode was m/z 100–800.

#### Animal experiments

Male Sprague–Dawley rats (200 ± 20 g) were used for the animal assay, and this experiment was approved by the Committee of Ethics of Animal Experimentation of Liaoning University of Traditional Chinese Medicine with the approval number 2019YS(DW)-009-01.

After intravenous administration of ETFT (4 mg/kg), 300 μL blood sample was gathered from the orbital venous at 3, 10, and 30 min. Meanwhile, the urine as well as feces samples were respectively collected within 0–24 h.

#### Sample preparation

100 μL sample and 500 μL methanol were added into EP tubes, vortex mixing for 60 s, centrifuging for 15 min (3000 rpm) to remove the protein. The residue was reconstituted in a 100 μL initial mobile phase, and then centrifugating it for 3 min (10,000 rpm). Finally, a 5 μL aliquot was injected for determination.

### Results

#### Antioxidation assay

DPPH scavenging rate of ETFT is shown in Supplementary Figure 1, indicating that the oxidation effect increased along with the increase of concentration, with IC₅₀ to be 0.058 mg/mL.

#### Anticholinesterase assay

The result of the anticholinesterase assay is shown in Supplementary Figure 2, suggesting that the ETFT exerted a dose-dependent inhibitory effect against the AChE with IC₅₀ values of 0.106 mg/mL.

#### Metabolism study

**Analysis of plasma, urine, and feces samples**

The chromatograms of the plasma, urine, and feces samples in positive ion mode are shown in Supplementary Figures 3 and 4. All of the fragment ions are shown in Supplementary Figures 5 and 6. The MS data of nine metabolic products are listed in Supplementary Table 2.

**Identification of the metabolites**

All metabolites and the metabolic pathway are shown in Figure 2.

#### Oxidation

M-5 (m/z 346.3313) was in the same value with M-6. M-5 was from ETFT’s olefine reduced and methoxy oxidization. M-6 was from ETFT in which both N and ethoxyl were oxidized to hydroxyl.

#### Glucuronide conjugates

M-2 (m/z 309.1808) was formed by ferulic acid, the hydrolysate of M-6, via the serial process of glucuronidation (370.1362 Da) and losing a H₂O and a CO₂. M-3 (m/z 367.1857) was formed from the fragmentation ion (138.0778), which experienced glucuronidation (491.2049 Da) and then losing two H₂O and two CO₂.
Sulfate conjugates
M-1 (m/z 199.1240) was formed by -SO₃ (80.0642 Da) combining with the fragmentation ion (120.0626 Da) that was obtained when M-5 happened oxidation and reduction reaction, then losing H₂O. M-4 (m/z 337.1750) was formed by two -SO₃ (80.0642 Da) combining with the fragmentation ion (176.0523 Da) of M-6. M-8 (m/z 337.1750) was the combination of -SO₃ (80.0642 Da) and the fragmentation ion (107.0547 Da).

Glutathione conjugates
M-7 (m/z 271.0609) was the combination of C₉H₁₁O₂ (151.0857) and + C₃H₅NO₂S (119.111 Da).

Reduction
M-9, the fragment ion at m/z 348.2179, was the reduction of M-6 in olefins.

Discussion
The bioactive assays indicated that the ETFT has a remarkably antioxidation effect compared with that of BHA, although its antioxidation effect has no more than that of Vitamin C. Moreover, it also exhibited an anticholinesterase effect with dose-dependent.

In the metabolism experiment, no ETFT was detected after intravenous administration, but a peak consisting of two molecules (M-5 and M-6) was found at 3 min and gradually enhanced at 10 and 30 min, which means that ETFT has converted into M-5 and M-6. And furthermore, other metabolites were formed from M-5 and M-6. Among nine metabolites, six metabolites (M-1 to M-6) were found in the plasma, and three metabolites (M-7 to M-9) were found in the urine samples. Any metabolites were not found in the feces, suggesting that ETFT was not metabolized via bile.

Acknowledgment
This work was funded by a project of the National Natural Science Foundation of China (No. 81573546).

Financial support and sponsorship
The National Natural Science Foundation of China (No. 81573546).
Conflicts of interest
There are no conflicts of interest

References

1. Yang X, Yan Y, Li J, Tang Z, Sun J, Zhang H, et al. Protective effects of ethanol extract from Portulaca oleracea L. on dextran sulphate sodium-induced mice ulcerative colitis involving anti-inflammatory and antioxidant. Am J Transl Res 2016;8:2138-48.

2. Sumanthi T, Christinal J. Neuroprotective effect of Portulaca oleracea ethanolic extract ameliorates methylmercury induced cognitive dysfunction and oxidative stress in cerebellum and cortex of rat brain. Biol Trace Elem Res 2016;172:155-65.

3. Zhao R, Gao X, Cai YP, Shao XY, Jia GY, Huang YL, et al. Antitumor activity of Portulaca oleracea L. polysaccharides against cervical carcinoma in vitro and in vivo. Carbohydr Polym 2013;96:376-83.

4. Jiao ZZ, Yue S, Sun HX, Jin TY, Wang HN, Zhu RX, et al. Indoline amide glucosides from Portulaca oleracea: Isolation, structure, and DPPH radical scavenging activity. J Nat Prod 2015;78:2588-97.

5. Zhao CC, Ying ZM, Tao XJ, Jiang MY, Ying XX, Yang GL. A new lactam alkaloid from Portulaca oleracea L. and its cytotoxicity. Nat Prod Res 2018;32:1548-53.

6. Ying ZM, Li CY, Gao MZ, Ying XX, Yang GL. Pharmacokinetics and metabolism of olerciamide a from Portulaca oleracea L. in rats by UHPLC-UV and UHPLC-ESI-Q-TOF/MS. Biomed Chromatogr 2018;2:14061.

7. Li CY, Ying ZM, Gao MZ, Wei WJ, Hao D, Xu L, et al. Two new similar alkaloids from Portulaca oleracea L. Nat Prod Res 2017;31:1792-8.

8. Xu L, Ying ZM, Wei WJ, Hao D, Wang HB, Zhang WJ, et al. A novel alkaloid from Portulaca oleracea L. Nat Prod Res 2016;31:902-8.

9. Xu XQ, Yu LS, Chen GN. Determination of flavonoids in Portulaca oleracea L. by capillary electrophoresis with electrochemical detection. J Pharm Biomed Anal 2006;41:493-9.

10. Meng YH, Ying ZM, Xiang Z, Hao D, Zhang WJ, Zheng Y, et al. The anti-inflammation and pharmacokinetics of a novel alkaloid from Portulaca oleracea L. J Pharm Pharmacol 2016;68:397-405.

11. Li CY, Meng YH, Ying ZM, Xu N, Hao D, Gao MZ, et al. Three novel alkaloids from Portulaca oleracea L. and their anti-inflammatory effects. J Agric Food Chem 2016;64:5837-44.

12. Li CY, Meng YH, Ying ZM, Xu N, Hao D, Gao MZ, et al. Correction to three novel alkaloids from Portulaca oleracea L. and their anti-inflammatory effects. J Agric Food Chem 2017;65:993-4.

13. Maciel IJ, Chaves OS, Brito Filho SG, Teles VC, Fernandes MG, Assis TS, et al. New alcamide and anti-oxidant activity of Pilosocereus gounellei A. Weber ex K. Schum. Bly. ex Rowl. (Cactaceae). Molecules 2015;21:11-2.
Supplementary Figure 1: Antioxidation effect of 7'-ethoxy-trans-feruloyltyramine

Supplementary Figure 2: Anticholinesterase effect of 7'-ethoxy-trans-feruloyltyramine

Supplementary Figure 3: The extracted ion chromatograms of metabolites from 7'-ethoxy-trans-feruloyltyramine in the rat plasma. (a) Blank rat plasma; rat plasma collected at (b) 3 min, (c) 10 min, and (d) 30 min after intravenous administration

Supplementary Figure 4: The extracted ion chromatograms of metabolites from 7'-ethoxy-trans-feruloyltyramine in the rat urine and feces. (a) Blank rat urine; (b) rat urine after intravenous administration of 7'-ethoxy-trans-feruloyltyramine; (c) blank rat feces; (d) rat feces collected after intravenous administration of 7'-ethoxy-trans-feruloyltyramine
Supplementary Figure 5: MS/MS spectra of M-5 and M-6 in rat plasma collected at 3, 10, and 30 min after intravenous administration of 7’-ethoxy-trans-feruloyltyramine.

Supplementary Figure 6: MS/MS spectra that phase I and phase II metabolism in rat urine and plasma collected at 30 min after intravenous administration of 7’-ethoxy-trans-feruloyltyramine.
Supplementary Figure 7: Ultraviolet spectrum of 7'-ethoxy-trans-feruloyltyramine in methanol

Supplementary Figure 8: Infrared spectrum of 7'-ethoxy-trans-feruloyltyramine

Supplementary Figure 9: $^1$H NMR (500 MHz) spectrum of 7'-ethoxy-trans-feruloyltyramine
Supplementary Table 1: \(^1\)H NMR (500 MHz) and \(^13\)C NMR (125 MHz) data of 7’-ethoxy-trans-feruloyltyramine in MeOD

| Position | δ_c (ppm) | Type | δ_h (J in Hz) |
|----------|-----------|------|---------------|
| 1        | 128.32    | C    |               |
| 2        | 111.58    | CH   | 7.13 (1H, d, 1.85) |
| 3        | 149.33    | C    |               |
| 4        | 149.91    | C    |               |
| 5        | 116.49    | CH   | 6.79 (1H, m)  |
| 6        | 123.32    | CH   | 7.03 (1H, dd, 1.90; 8.25) |
| 7        | 142.21    | CH   | 7.44 (1H, d, 15.7) |
| 8        | 118.71    | CH   | 6.47 (1H, d, 15.75) |
| 9        | 169.24    | C    |               |
| 3-OCH₃   | 56.42     | CH₂  | 3.88 (3H, s)  |
| 1’       | 132.20    | C    |               |
| 2’/6’    | 129.08    | CH   | 7.18 (1H, m)  |
| 3’/5’    | 116.29    | CH   | 6.79 (1H, m)  |
| 4’       | 158.41    | C    |               |
| 7’       | 81.35     | CH   | 4.37 (1H, q, 6.48) |
| 8’       | 47.20     | CH₂  | H_a=3.40; H_b=3.49 (2H, m) |
| 1’’      | 65.13     | CH₂  | 3.37 (2H, m)  |
| 2’’      | 15.57     | CH₃  | 1.16 (3H, t, 7.05) |

Supplementary Table 2: Ultra-high-performance liquid chromatography electrospray coupled with quadrupole time-of-flight mass spectrometry data of nine metabolites of 7'-ethoxy-trans-feruloyltyramine in rats

| No. | Retention time (min) | Experimental (m/z) | Theoretical (m/z) | Formula | Metabolic process | Specimen |
|-----|----------------------|--------------------|-------------------|---------|------------------|----------|
| M-1 | 4.4                  | 199.1240           | 199.1189          | C₇H₆O₄S | Oxidation, reduction, dehydration, sulfation | Plasma   |
| M-2 | 5.0                  | 309.1808           | 309.1301          | C₉H₆O₇ | Hydrolysis, glucuronidation | Plasma   |
| M-3 | 5.2                  | 367.1857           | 367.1769          | C₉H₂O₆ | Glucuronidation, dehydration, decarbonation | Plasma   |
| M-4 | 6.5                  | 337.1750           | 337.1750          | C₇H₆O₇S₂ | Oxidation, sulfation | Plasma   |
| M-5 | 9.4                  | 346.3313           | 346.1933          | C₁₀H₁₀NO₆ | Oxidation | Plasma   |
| M-6 | 9.4                  | 346.3313           | 346.1611          | C₁₀H₁₀NO₆ | Oxidation | Plasma   |
| M-7 | 7.4                  | 271.0609           | 271.2046          | C₁₀H₁₈NO₆S | Glutathionylation | Urine    |
| M-8 | 7.8                  | 188.0708           | 188.1268          | C₁₀H₆O₄S | Sulfation | Urine    |
| M-9 | 9.7                  | 348.2179           | 348.1769          | C₁₀H₁₂NO₆ | Reduction | Urine    |
Supplementary Figure 10: $^{13}$C NMR (125 MHz) spectrum of 7'-ethoxy-trans-feruloyltyramine

Supplementary Figure 11: DEPT spectrum of 7'-ethoxy-trans-feruloyltyramine
Supplementary Figure 12: $^1$H–$^1$H COSY spectrum of $7'$-ethoxy-trans-feruloyltyramine

Supplementary Figure 13: HMBC spectrum of $7'$-ethoxy-trans-feruloyltyramine

Supplementary Figure 14: HSQC spectrum of $7'$-ethoxy-trans-feruloyltyramine

Supplementary Figure 15: NOESY spectrum of $7'$-ethoxy-trans-feruloyltyramine