# Screening, Optimization and Bioavailability of Natural Deep Eutectic Solvent Extracts from *Radix Pueraria*

*Yan Huang[#,1], Jiehong Yang[#,1], Yu Zhao[1], Li Yu[1], Yu He[1], Haitong Wan*[1], Chang Li*[1]*

[1] Zhejiang Chinese Medical University, Hangzhou, 310057, PR China

[#] These authors contributed equally to this work.

[*] Corresponding Authors

[*] Chang Li, lichang@zju.edu.cn; Tel./Fax: +86 571 86613716

[*] Haitong Wan, whtong@126.com; Tel./Fax: +86 571 86613716

## *Supporting Information*

## Table of contents <span>Page</span>

Table S1. Different ratios of composition of NaDESs

| No. | NaDES | Type of HBA | Type of HBD | HBA/HBD ratio(water) |
|---|---|---|---|---|
| 1 | ChCl-Ur | | Urea | 1:2 |
| 2 | ChCl-Am | | Acetamide | 1:1:(1) |
| 3 | ChCl-Glu | | D-Glucose | 1:1:(2) |
| 4 | ChCl-Ma | | Maltose | 2:1:(4) |
| 5 | ChCl-Xyl | Choline Chloride | Xylitol | 5:2:(5) |
| 6 | ChCl-Gly | | Glycerol | 1:2 |
| 7 | ChCl-Lac | | Lactic Acid | 1:1 |
| 8 | ChCl-Maa | | DL-Malic Acid | 1:1 |
| 9 | Bet-Ur | | Urea | 1:1:(2) |
| 10 | Bet-Ma | | Maltose | 5:2:(6) |
| 11 | Bet-Suc | | Sucrose | 2:1:(7) |
| 12 | Bet-Xyl | Betaine | Xylitol | 1:1:(1) |
| 13 | Bet-Gly | | Glycerol | 1:1 |
| 14 | Bet-Lac | | Lactic Acid | 1:1:(1) |
| 15 | Bet-Maa | | DL-Malic Acid | 1:1:(1) |
| 16 | L-Pro-Ur | | Urea | 1:1:(3) |
| 17 | L-Pro-Am | | Acetamide | 1:1:(2) |
| 18 | L-Pro-Glu | | D-Glucose | 1:1:(5) |
| 19 | L-Pro-Suc | | Sucrose | 2:1:(10) |
| 20 | L-Pro-Sor | L-proline | D-Sorbitol | 1:2:(4) |
| 21 | L-Pro-Gly | | Glycerol | 2:5 |
| 22 | L-Pro-Ca | | Citric Acid | 1:1:(2) |
| 23 | L-Pro-Lac | | Lactic Acid | 1:3 |
| 24 | L-Pro-Maa | | DL-Malic Acid | 1:1:(1) |
| 25 | L-Pro-Laa | | L-Ascorbic Acid | 1:1 |

Table S2. Extraction yields of PUE, 3-MPR and PRX from RP using NaDESs and traditional solvents (values are expressed as mean ± SD)

| Entry | Solvent | PUE (mg/g) | 3-MPR (mg/g) | PRX (mg/g) |
|---|---|---|---|---|
| 1 | water | 44.9±1.7 | 5.7±0.3 | 2.9±0.5 |
| 2 | methanol | 45.5±3.9 | 5.8±0.7 | 3.8±0.7 |
| 3 | ChCl-Ur | 51.0±2.8 | 7.4±0.2 | 4.4±0.6 |
| 4 | ChCl-Am | 53.9±2.9 | 6.8±0.4 | 4.3±0.6 |
| 5 | ChCl-Glu | 57.4±0.7 | 6.6±0.2 | 4.0±0.6 |
| 6 | ChCl-Ma | 53.1±1.5 | 7.2±1.3 | 4.5±1.1 |
| 7 | ChCl-Xyl | 52.3±5.0 | 6.4±1.3 | 4.0±1.0 |
| 8 | ChCl-Gly | 54.6±8.9 | 6.0±1.4 | 5.2±0.8 |
| 9 | ChCl-Lac | 51.4±6.8 | 6.6±0.5 | 4.6±0.5 |
| 10 | ChCl-Maa | 66.2±0.4 | 6.4±0.4 | 3.6±0.2 |
| 11 | Bet-Ur | 50.4±2.8 | 7.8±0.3 | 5.0±0.7 |
| 12 | Bet-Ma | 60.7±1.0 | 7.2±0.5 | 4.4±0.7 |
| 13 | Bet-Suc | 55.9±1.9 | 7.4±0.6 | 4.5±0.7 |
| 14 | Bet-Xyl | 55.7±3.9 | 7.0±0.3 | 4.5±0.4 |
| 15 | Bet-Gly | 54.0±2.3 | 7.6±1.6 | 4.8±1.4 |
| 16 | Bet-Lac | 52.9±2.3 | 8.8±1.1 | 5.0±0.7 |
| 17 | Bet-Maa | 70.4±1.6 | 6.6±0.5 | 4.1±0.7 |
| 18 | L-Pro-Ur | 53.1±2.9 | 8.1±1.0 | 4.9±0.6 |
| 19 | L-Pro-Am | 59.1±3.9 | 7.3±1.0 | 4.2±0.3 |
| 20 | L-Pro-Glu | 56.2±1.0 | 6.7±0.7 | 3.5±0.3 |
| 21 | L-Pro-Suc | 56.9±8.5 | 7.7±0.3 | 4.4±0.5 |
| 22 | L-Pro-Sor | 55.2±1.0 | 8.3±1.6 | 5.3±0.4 |
| 23 | L-Pro-Gly | 62.4±2.1 | 8.3±1.3 | 5.1±0.4 |
| 24 | L-Pro-Ca | 67.7±3.1 | 9.7±0.8 | 5.5±0.5 |
| 25 | L-Pro-Lac | 59.5±3.0 | 6.9±0.6 | 4.5±0.9 |
| 26 | L-Pro-Maa | 74.0±2.8 | 6.9±0.4 | 4.0±0.1 |
| 27 | L-Pro-Laa | 54.0±2.5 | 6.8±0.4 | 3.8±0.1 |

Table S3. Extraction yields of PUE, 3-MPR and PRX from RP using L-Pro-Maa under different conditions ( values are expressed as mean±SD)

| Extraction Condition | | PUE (mg/g) | 3-MPR (mg/g) | PRX (mg/g) |
|---|---|---|---|---|
| A. S/L Ratio (mg/mL) | 25 | 77.1 | 10.4 | 7.1 |
| | 50 | 47.0 | 6.6 | 4.5 |
| | 100 | 32.4 | 5.0 | 3.6 |
| | 150 | 26.1 | 5.2 | 3.8 |
| | 200 | 21.5 | 5.3 | 3.7 |
| B. DES Content (%) | 15 | 76.4 | 10.8 | 7.0 |
| | 30 | 82.7 | 10.5 | 7.2 |
| | 60 | 86.3 | 14.2 | 9.4 |
| | 75 | 77.1 | 10.4 | 7.1 |
| C. Extraction Time (min) | 15 | 38.3 | 4.9 | 3.4 |
| | 30 | 77.1 | 10.4 | 7.1 |
| | 45 | 75.0 | 10.4 | 6.8 |
| D. Extraction Temperature(°C) | 30 | 75.0 | 10.3 | 6.7 |
| | 40 | 93.5 | 16.7 | 11.2 |
| | 50 | 77.1 | 10.4 | 7.1 |
| | 60 | 75.9 | 11.6 | 8.4 |
| | 70 | 58.6 | 7.9 | 5.4 |

Table S4. Calibration curves, linear ranges, LOD and LOQ for analytes by HPLC

| No. | Wavelength (nm) | Calibration Curve | $R^2$ | Linear Range (mg/L) | LOD (mg/L) | LOQ (mg/L) |
|---|---|---|---|---|---|---|
| PUE | | y = 43724x + 94419 | 0.9992 | 100-1000 | 3.9 | 12.0 |
| 3-MPR | 254 | y=65.556x+139.88 | 0.9998 | 5-200 | 1.6 | 4.7 |
| PRX | | y=75.047x+65.267 | 0.9991 | 1-100 | 0.3 | 1.0 |

* A calibration curve was obtained by injecting standard solutions of PUE at different concentrations, 1000, 750, 500, 250 and 100 mg/L. The calibration curve as follow: y = 43,724x + 94,419 ($R^2$=0.9992).

* A calibration curve was obtained by injecting standard solutions of 3-MPR at different concentrations, 200, 100, 50, 25 and 5 mg/L. The calibration curve as follow: y = 65.556x + 139.88 ($R^2$=0.9998).

* A calibration curve was obtained by injecting standard solutions of PRX at different concentrations, respectively 100, 50, 25, 10 and 1 mg/L. The calibration curve as follow: y = 75.047x+65.267 ($R^2$=0.9991).

* LOD and LOQ was determined following references:

Reference:

[1] Mannino G, Di Stefano V, Lauria A, et al. Vaccinium macrocarpon (cranberry)-based dietary supplements: Variation in mass uniformity, proanthocyanidin dosage and anthocyanin profile demonstrates quality control standard needed[J]. Nutrients, 2020, 12(4).

[2] Sarkar M, Khandavilli S, Panchagnula R. Development and validation of rp-hplc and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2006, 830(2): 349-354.

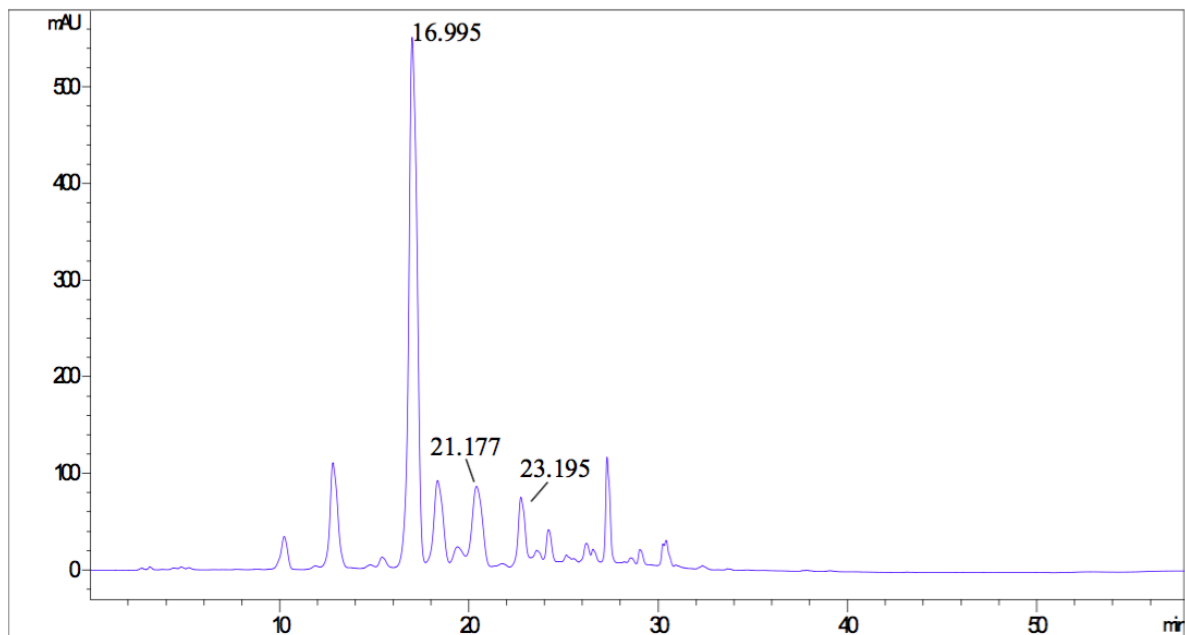Figure S1. HPLC chromatogram of aqueous extract of RP (wavelength=254nm)



Figure S2. HPLC chromatogram of L-Pro-Maa extract of RP (wavelength=254nm)
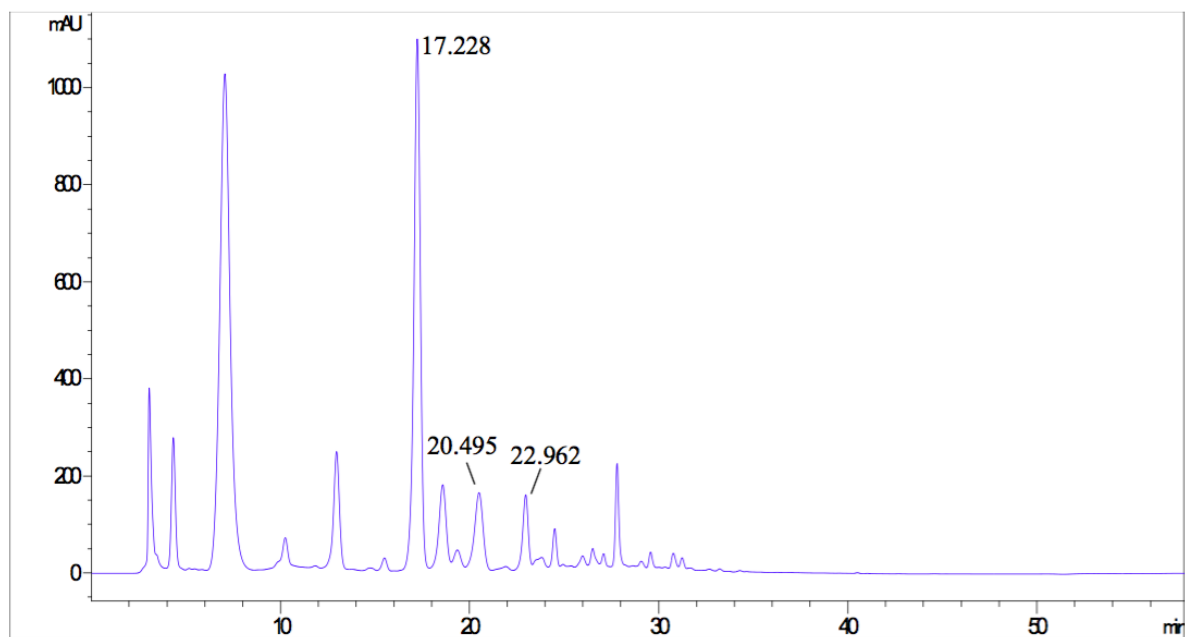
Figure S3. HPLC chromatogram of methanol extract of RP（wavelength=254nm）