

Optimized microwave assisted extraction (MAE) of alkaloids and polyphenols from Berberis roots using multiple-component analysis

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Supplementary Table 1: Plackett-Burman design (PBD) for estimation of berberine, palmatine and total phenols (TP) as responses under MAE.

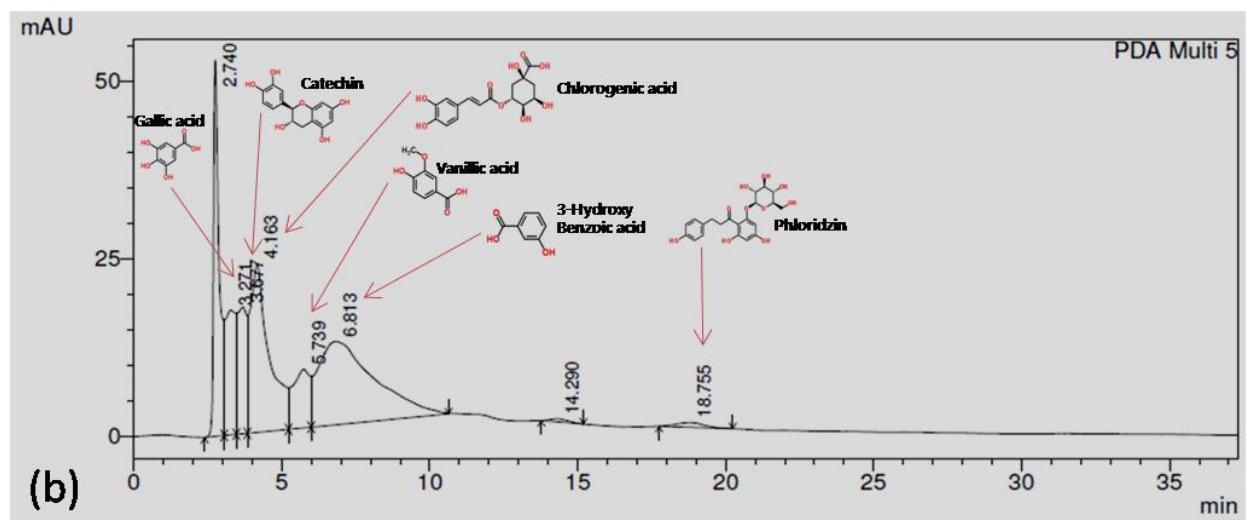
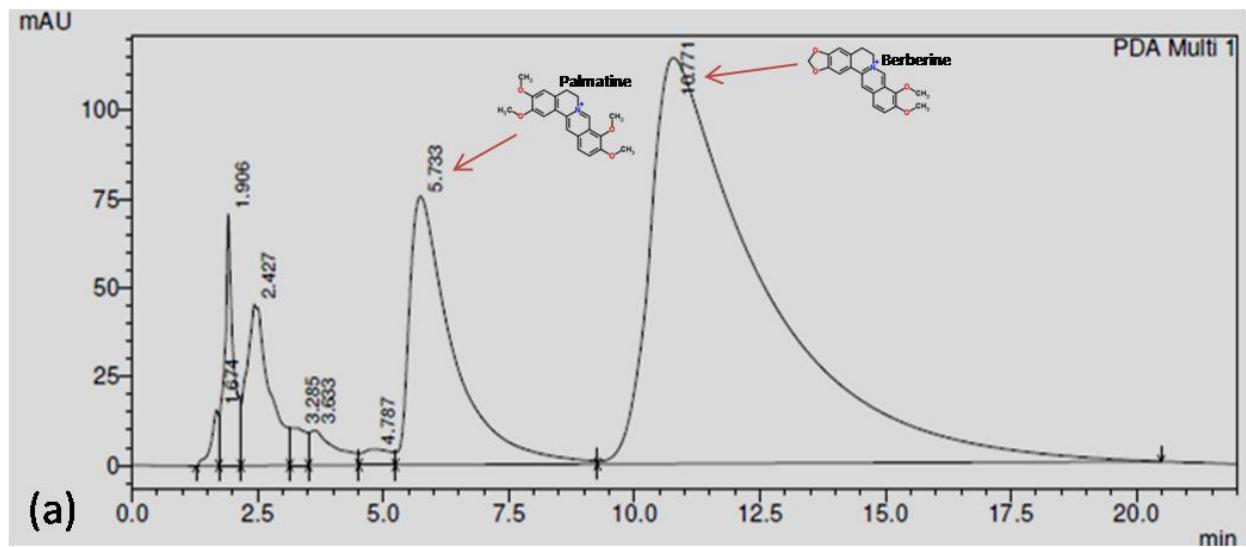
| Run | Factors | | | | | Responses | | |
|----------------------------|----------------|----------------|----------------|----------------|----------------|------------------------------------|------------------------------------|---------------------------------|
| | X ₁ | X ₂ | X ₃ | X ₄ | X ₅ | Berberine (mg g ⁻¹) | Palmatine (mg g ⁻¹) | TP (mg GAE g ⁻¹) |
| 1 | 300 (+1) | 5 (+1) | 50 (+1) | 2.5 (-1) | 20 (-1) | 18.82 | 8.99 | 9.94 |
| 2 | 300 (+1) | 2 (-1) | 50 (+1) | 6.0 (+1) | 20 (-1) | 7.89 | 7.18 | 8.78 |
| 3 | 300 (+1) | 2 (-1) | 20 (-1) | 2.5 (-1) | 80 (+1) | 35.82 | 7.37 | 5.91 |
| 4 | 300 (+1) | 5 (+1) | 20 (-1) | 2.5 (-1) | 20 (-1) | 16.28 | 4.99 | 4.73 |
| 5 | 100 (-1) | 5 (+1) | 50 (+1) | 6.0 (+1) | 20 (-1) | 5.31 | 6.60 | 7.47 |
| 6 | 100 (-1) | 2 (-1) | 20 (-1) | 6.0 (+1) | 20 (-1) | 7.23 | 3.66 | 3.92 |
| 7 | 300 (+1) | 2 (-1) | 50 (+1) | 6.0 (+1) | 80 (+1) | 21.75 | 9.01 | 8.67 |
| 8 | 300 (+1) | 5 (+1) | 20 (-1) | 6.0 (+1) | 80 (+1) | 20.09 | 5.29 | 4.58 |
| 9 | 100 (-1) | 2 (-1) | 50 (+1) | 2.5 (-1) | 80 (+1) | 25.14 | 9.64 | 8.90 |
| 10 | 100 (-1) | 2 (-1) | 20 (-1) | 2.5 (-1) | 20 (-1) | 13.54 | 4.48 | 3.97 |
| 11 | 100 (-1) | 5 (+1) | 20 (-1) | 6.0 (+1) | 80 (+1) | 17.53 | 5.02 | 3.99 |
| 12 | 100 (-1) | 5 (+1) | 50 (+1) | 2.5 (-1) | 80 (+1) | 18.89 | 8.53 | 7.28 |
| $\beta_{\text{Berberine}}$ | 2.75* | - 1.21 | - 1.06 | - 4.06** | 5.85*** | | | |
| $\beta_{\text{Palmatine}}$ | 0.41* | - 0.16 | 1.59*** | - 0.60** | 0.75** | | | |
| β_{TP} | 0.59* | - 0.18 | 2.00*** | - 0.28 | 0.04 | | | |
| <i>Model</i> | | | | | | | | |
| <i>F-value</i> | | | | | | 15.48** | 41.78*** | 27.77*** |
| <i>R</i> ² | | | | | | 0.92 | 0.97 | 0.95 |

X₁ = microwave power (W), X₂ = irradiation time (min), X₃ = sample to solvent ratio (g/ml), X₄ = solvent pH, X₅ = solvent concentration (%), TP = total phenols, β = regression coefficient, R² = coefficient of determination; Level of significance * p<0.05, ** p<0.01, *** p<0.001

Supplementary Table 2: Central Composite Design (CCD) model with responses as alkaloids, polyphenolics and antioxidant activities for different levels of independent variables.

| Run | Independent variables | | | Berberine (mg g ⁻¹) | Palmatine (mg g ⁻¹) | Dependent variables (Response) | | | | |
|-----|-----------------------|----------------|----------------|------------------------------------|------------------------------------|--------------------------------|----|------|------|------|
| | X ₁ | X ₂ | X ₃ | | | X ₄ | TP | DPPH | ABTS | FRAP |
| 1 | 200 (-1) | 30 (-1) | 5.0 (+1) | 100 (+1) | 25 | 10 | 11 | 23 | 11 | 151 |
| 2 | 600 (+1) | 50 (0) | 3.5 (0) | 70 (0) | 14 | 8 | 18 | 39 | 17 | 170 |
| 3 | 600 (+1) | 70 (+1) | 2.0 (-1) | 40 (-1) | 30 | 11 | 15 | 40 | 10 | 150 |
| 4 | 600 (+1) | 30 (-1) | 5.0 (+1) | 40 (-1) | 9 | 5 | 10 | 23 | 9 | 116 |
| 5 | 400 (0) | 50 (0) | 2.0 (-1) | 70 (0) | 20 | 9 | 16 | 33 | 10 | 170 |
| 6 | 400 (0) | 50 (0) | 3.5 (0) | 100 (+1) | 26 | 10 | 11 | 35 | 13 | 159 |
| 7 | 600 (+1) | 30 (-1) | 5.0 (+1) | 100 (+1) | 12 | 5 | 11 | 24 | 12 | 204 |
| 8 | 600 (+1) | 70 (+1) | 2.0 (-1) | 100 (+1) | 50 | 25 | 21 | 55 | 23 | 230 |
| 9 | 600 (+1) | 30 (-1) | 2.0 (-1) | 100 (+1) | 16 | 6 | 14 | 24 | 13 | 225 |
| 10 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 17 | 9 | 13 | 35 | 12 | 135 |
| 11 | 400 (0) | 50 (0) | 5.0 (+1) | 70 (0) | 17 | 9 | 12 | 33 | 10 | 144 |
| 12 | 600 (+1) | 30 (-1) | 2.0 (-1) | 40 (-1) | 15 | 7 | 13 | 20 | 10 | 162 |
| 13 | 600 (+1) | 70 (+1) | 5.0 (+1) | 40 (-1) | 15 | 10 | 14 | 40 | 9 | 100 |
| 14 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 17 | 8 | 12 | 35 | 13 | 136 |
| 15 | 600 (+1) | 70 (+1) | 5.0 (+1) | 100 (+1) | 34 | 15 | 16 | 51 | 18 | 215 |
| 16 | 400 (0) | 30 (-1) | 3.5 (0) | 70 (0) | 15 | 7 | 10 | 24 | 11 | 128 |
| 17 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 18 | 9 | 12 | 35 | 13 | 134 |
| 18 | 200 (-1) | 70 (+1) | 5.0 (+1) | 40 (-1) | 13 | 9 | 13 | 36 | 6 | 102 |
| 19 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 18 | 9 | 14 | 36 | 12 | 134 |
| 20 | 200 (-1) | 70 (+1) | 5.0 (+1) | 100 (+1) | 36 | 12 | 18 | 43 | 13 | 165 |
| 21 | 200 (-1) | 30 (-1) | 2.0 (-1) | 40 (-1) | 14 | 6 | 13 | 19 | 9 | 131 |
| 22 | 200 (-1) | 70 (+1) | 2.0 (-1) | 40 (-1) | 17 | 10 | 14 | 36 | 6 | 114 |
| 23 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 17 | 8 | 13 | 34 | 12 | 137 |
| 24 | 400 (0) | 50 (0) | 3.5 (0) | 70 (0) | 17 | 8 | 13 | 36 | 11 | 133 |
| 25 | 200 (-1) | 30 (-1) | 5.0 (+1) | 40 (-1) | 20 | 5 | 18 | 21 | 9 | 116 |
| 26 | 200 (-1) | 70 (+1) | 2.0 (-1) | 100 (+1) | 41 | 16 | 16 | 44 | 14 | 144 |
| 27 | 400 (0) | 70 (+1) | 3.5 (0) | 70 (0) | 25 | 11 | 14 | 41 | 11 | 120 |
| 28 | 200 (-1) | 50 (0) | 3.5 (0) | 70 (0) | 17 | 8 | 14 | 32 | 10 | 123 |
| 29 | 400 (0) | 50 (0) | 3.5 (0) | 40 (-1) | 16 | 8 | 12 | 32 | 10 | 101 |
| 30 | 200 (-1) | 30 (-1) | 2.0 (-1) | 100 (+1) | 22 | 8 | 9 | 22 | 8 | 130 |

X₁ = microwave power (W), X₂ = sample to solvent ratio (g mL⁻¹), X₃ = solvent pH , X₄ = solvent concentration (%), TP = total phenols (mg GAE g⁻¹), DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability (mM AAE g⁻¹), ABTS = 2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation inhibition (mM AAE g⁻¹), FRAP = Ferric reducing antioxidant power (mM AAE g⁻¹)



Supplementary Figure 1: Chromatograms of alkaloids and polyphenolic antioxidants in *Berberis* roots