Electronic Supplementary Material (ESI) for Analytical Methods.


# Determination of phenolic compounds in extracts of Amazonian medicinal plants by liquid chromatography-electrospray tandem mass spectrometry

Henrique Faccin[a,b], Roberta Fabricio Loose[a], Carine Viana[c], Osmar A. Lameira[d], Leandro Machado de Carvalho[a,b,c]*

[a]*Department of Chemistry, Federal University of Santa Maria, Santa Maria, RS, 97105-900, Brazil*

[b]*Graduate Program in Chemistry, Center of Natural Sciences, Federal University of Santa Maria, Santa Maria, RS, 97105-900, Brazil*

[c]*Graduate Program in Pharmaceutical Sciences, Center of Health Sciences, Federal University of Santa Maria, Santa Maria, RS, 97105-900, Brazil*

[d]*Laboratório de Agrobiotecnologia, EMBRAPA Amazônia Oriental, Belém, PA, Brazil.*

*Corresponding author. Tel.: +55-5532208870

*E-mail address:* leandrocarvalho@pq.cnpq.br (L. M. de Carvalho)

# Electronic Supplementary Information (ESI)

**Table S1**

Extraction yields (w/w) of the ethanolic extracts obtained from the six studied plant species.

| Plant species | Extraction yields (%, w/w) | | |
|---|---|---|---|
| | *1st harvest* | *2nd harvest* | *3rd harvest* |
| *Cecropia palmata* | 22.05 | 0.47 | 3.53 |
| *Cecropia obtusa* | 10.44 | 1.21 | 1.32 |
| *Bauhinia variegata* var. *variegata* | 4.60 | 3.44 | 8.55 |
| *Bauhinia variegata* var. *alboflava* | 6.87 | 2.76 | 10.97 |
| *Mansoa alliacea* | 4.85 | 1.57 | 8.39 |
| *Connarus perrottetii* var. *angustifolius* | 8.76 | 9.50 | 6.50 |

**Table S2**

Concentrations of phenolic compounds in *Cecropia palmata* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in μg·g$^{-1}$ of plant followed by standard deviation of the concentration (μg·g$^{-1}$).

| Compound | *Cecropia palmata* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3rd harvest* |
| Gallic acid | - | - | - | - | - | - |
| Chlorogenic acid | 475.0 ± 16.6 | 359.1 ± 19.9 | 60.1 ± 1.2 | 240.2 ± 7.7 | 1.1 ± 0.03 | 49.7 ± 1.9 |
| (+)-catechin | 24.0 ± 1.7 | 22.3 ± 0.9 | 3.7 ± 0.6 | 33.5 ± 2.5 | 0.3 ± 0.01 | 1.1 ± 0.2 |
| Vanillic acid | 11.2 ± 2.3 | 18.5 ± 0.3 | 10.2 ± 0.9 | 5.2 ± 2.0 | 0.3 ± 0.02 | 2.3 ± 0.4 |
| Caffeic acid | 23.0 ± 0.7 | 14.5 ± 0.7 | 19.8 ± 0.7 | 34.4 ± 3.1 | 1.9 ± 0.1 | 5.8 ± 0.3 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 4.2 ± 0.4 | 3.6 ± 0.1 | 4.9 ± 0.2 | 17.5 ± 1.0 | 0.8 ± 0.02 | 0.9 ± 0.02 |
| Ferulic acid | 3.4 ± 0.7 | 4.1 ± 0.6 | 2.2 ± 0.8 | 5.0 ± 1.0 | 0.3 ± 0.02 | 0.8 ± 0.1 |
| Rutin | < ILOQ | < ILOQ | 1.0 ± 0.2 | 153.2 ± 7.3 | < ILOQ | < ILOQ |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | 0.2 ± 0.1 | - | 0.1 ± 0.02 | 23.1 ± 1.1 | - | < ILOQ |
| Myricetin | - | - | - | - | - | - |
| Fisetin | - | - | - | - | - | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | 5.0 ± 0.8 | 2.5 ± 0.3 | 2.9 ± 0.4 | 1.3 ± 0.9 | 0.03 ± 0.01 | 0.5 ± 0.2 |
| Quercetin | < ILOQ | < ILOQ | < ILOQ | 42.8 ± 2.7 | < ILOQ | 0.4 ± 0.2 |
| Luteolin | - | - | - | 5.6 ± 0.4 | 0.02 ± 0.004 | 0.3 ± 0.04 |
| Apigenin | - | - | - | 24.4 ± 1.6 | - | - |
| Kaempferol | - | - | - | < ILOQ | - | - |
| 3.6-dihydroxyflavone | - | - | - | - | - | - |
| Chrysin | - | - | - | < ILOQ | - | - |
| Galangin | - | - | - | - | - | - |

**Table S3**

Concentrations of phenolic compounds in *Cecropia obtusa* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in μg·g$^{-1}$ of plant followed by standard deviation of the concentration (μg·g$^{-1}$).

| Compound | *Cecropia obtusa* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3rd harvest* |
| Gallic acid | - | - | - | - | < ILOQ | - |
| Chlorogenic acid | 568.2 ± 26.6 | 287.5 ± 9.2 | 312.1 ± 8.6 | 455.8 ± 4.3 | 8.3 ± 0.3 | 21.6 ± 0.4 |
| (+)-catechin | 66.9 ± 2.7 | 94.9 ± 4.7 | 16.1 ± 0.9 | 24.4 ± 1.7 | 4.2 ± 0.2 | 2.8 ± 0.1 |
| Vanillic acid | 10.3 ± 1.6 | 12.8 ± 1.8 | 4.2 ± 0.8 | 10.1 ± 2.1 | 1.3 ± 0.1 | 0.3 ± 0.1 |
| Caffeic acid | 31.9 ± 1.3 | 21.0 ± 1.3 | 6.2 ± 0.2 | 13.8 ± 1.9 | 4.9 ± 0.2 | 0.4 ± 0.03 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 2.9 ± 0.2 | 2.3 ± 0.3 | 1.8 ± 0.1 | 1.3 ± 0.4 | 0.7 ± 0.01 | 0.1 ± 0.01 |
| Ferulic acid | 3.2 ± 0.7 | 2.8 ± 0.6 | 2.3 ± 0.3 | 1.6 ± 0.5 | 0.3 ± 0.04 | 0.1 ± 0.03 |
| Rutin | 38.0 ± 1.0 | 45.9 ± 2.0 | 75.7 ± 1.0 | 21.9 ± 2.6 | 1.6 ± 0.05 | 6.9 ± 0.1 |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | 3.6 ± 0.2 | 3.5 ± 0.2 | - | 4.4 ± 0.4 | 0.2 ± 0.01 | < ILOQ |
| Myricetin | < ILOQ | - | - | 38.2 ± 9.5 | - | - |
| Fisetin | - | - | - | - | - | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | 1.8 ± 0.6 | 4.1 ± 0.6 | 4.5 ± 0.5 | 0.5 ± 0.3 | 0.1 ± 0.02 | 0.3 ± 0.1 |
| Quercetin | 2.5 ± 0.5 | < ILOQ | < ILOQ | 32.5 ± 2.4 | 9.9 ± 0.3 | < ILOQ |
| Luteolin | < ILOQ | < ILOQ | - | 4.4 ± 0.7 | 0.7 ± 0.01 | < ILOQ |
| Apigenin | - | - | - | 15.3 ± 1.3 | - | - |
| Kaempferol | - | - | - | - | 0.4 ± 0.1 | - |
| 3.6-dihydroxyflavone | - | - | - | - | - | - |
| Chrysin | - | - | - | - | - | - |
| Galangin | - | - | - | - | - | - |

**Table S4**

Concentrations of phenolic compounds in *Bauhinia variegata* var. *variegata* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in $\mu g \cdot g^{-1}$ of plant followed by standard deviation of the concentration ($\mu g \cdot g^{-1}$).

| Compound | *Bauhinia variegata* var. *variegata* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3 harvest* |
| Gallic acid | - | < ILOQ | < ILOQ | - | - | - |
| Chlorogenic acid | 79.6 ± 6.1 | 288.0 ± 8.3 | 384.9 ± 12.2 | 35.8 ± 2.3 | 29.4 ± 1.3 | 100.1 ± 2.2 |
| (+)-catechin | 12.8 ± 0.9 | 56.3 ± 1.4 | 78.8 ± 2.8 | 6.8 ± 0.4 | 4.9 ± 0.3 | 14.6 ± 0.4 |
| Vanillic acid | 5.2 ± 0.7 | 9.5 ± 0.7 | 16.9 ± 0.8 | 1.6 ± 0.4 | 5.7 ± 0.1 | 3.8 ± 0.3 |
| Caffeic acid | 13.1 ± 1.0 | 34.3 ± 1.7 | 27.4 ± 1.7 | 7.7 ± 0.6 | 121.1 ± 2.8 | 22.9 ± 0.6 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 10.3 ± 0.3 | 14.7 ± 0.4 | 19.8 ± 0.5 | 3.8 ± 0.1 | 18.4 ± 0.6 | 8.6 ± 0.2 |
| Ferulic acid | 7.8 ± 0.9 | 13.0 ± 0.9 | 10.9 ± 0.6 | 2.5 ± 0.3 | 28.7 ± 0.5 | 6.9 ± 0.2 |
| Rutin | 608.1 ± 32.3 | 351.1 ± 7.2 | 2517.3 ± 48.0 | 176.8 ± 4.3 | 129.9 ± 4.1 | 832.9 ± 1.4 |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | 24.5 ± 1.4 | 17.2 ± 0.9 | 158 ± 2.9 | 9.7 ± 0.5 | 3.9 ± 0.2 | 22 ± 0.1 |
| Myricetin | - | - | - | 30.2 ± 5.6 | - | - |
| Fisetin | - | - | - | - | 0.3 ± 0.1 | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | 1.4 ± 0.3 | 1.8 ± 0.3 | 3.1 ± 0.6 | 0.4 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.3 |
| Quercetin | - | < ILOQ | < ILOQ | 10.5 ± 0.6 | 99.8 ± 1.5 | 17.8 ± 0.5 |
| Luteolin | - | < ILOQ | < ILOQ | 0.8 ± 0.1 | 0.8 ± 0.1 | 4.6 ± 0.2 |
| Apigenin | - | - | - | 5.0 ± 0.4 | < ILOQ | < ILOQ |
| Kaempferol | - | - | - | 0.3 ± 0.6 | 9.3 ± 1.6 | - |
| 3.6-dihydroxyflavone | - | - | - | - | - | - |
| Chrysin | - | - | - | < ILOQ | < ILOQ | - |
| Galangin | - | - | - | - | - | - |

**Table S5**

Concentrations of phenolic compounds in *Bauhinia variegata* var. *alboflava* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in $\mu g \cdot g^{-1}$ of plant followed by standard deviation of the concentration ($\mu g \cdot g^{-1}$).

| Compound | *Bauhinia variegata* var. *alboflava* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3rd harvest* |
| Gallic acid | - | - | < ILOQ | - | - | - |
| Chlorogenic acid | 226.7 ± 6.2 | 349.7 ± 13 | 371.5 ± 15.8 | 27.5 ± 2.5 | 31.4 ± 1.1 | 81.3 ± 1.2 |
| (+)-catechin | 81.5 ± 4.6 | 123.1 ± 5.7 | 88.8 ± 3.6 | 8.6 ± 1.3 | 8.0 ± 0.1 | 23.0 ± 0.4 |
| Vanillic acid | 8.3 ± 1.6 | 7.0 ± 1.4 | 16.0 ± 0.5 | < ILOQ | 3.0 ± 0.3 | 5.6 ± 0.5 |
| Caffeic acid | 33.5 ± 2.4 | 48.7 ± 2.3 | 31.8 ± 1.1 | 8.3 ± 1.1 | 39.3 ± 0.8 | 21.9 ± 0.5 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 19.2 ± 0.6 | 27.3 ± 1.2 | 19.3 ± 0.3 | 4.8 ± 0.4 | 10.5 ± 0.2 | 13.4 ± 0.2 |
| Ferulic acid | 12.2 ± 1.0 | 10.1 ± 0.9 | 13.5 ± 0.4 | 2.1 ± 0.9 | 9.3 ± 0.2 | 6.2 ± 0.3 |
| Rutin | 746.2 ± 31.3 | 2182.2 ± 29.2 | 1720.8 ± 40.3 | 67.0 ± 3.3 | 274.1 ± 2.0 | 259.3 ± 1.5 |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | 12.3 ± 0.6 | 14.5 ± 0.5 | 16.6 ± 0.7 | 3.7 ± 0.1 | 2.3 ± 0.1 | 9.1 ± 0.2 |
| Myricetin | < ILOQ | - | - | - | - | - |
| Fisetin | - | - | - | - | < ILOQ | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | 2.9 ± 0.8 | 3.3 ± 0.8 | 4.3 ± 0.6 | 0.5 ± 0.3 | 0.6 ± 0.1 | 1.6 ± 0.3 |
| Quercetin | < ILOQ | < ILOQ | 1.2 ± 0.4 | 18.7 ± 2.3 | 37.1 ± 1.4 | 44.2 ± 1.2 |
| Luteolin | < ILOQ | < ILOQ | < ILOQ | 1.6 ± 0.2 | 1.1 ± 0.04 | 2.9 ± 0.2 |
| Apigenin | - | - | - | 6.7 ± 0.6 | < ILOQ | < ILOQ |
| Kaempferol | - | - | - | < ILOQ | 4.5 ± 0.6 | 5.0 ± 1.1 |
| 3.6-dihydroxyflavone | - | < ILOQ | - | - | - | - |
| Chrysin | - | - | - | < ILOQ | 0.1 ± 0.02 | < ILOQ |
| Galangin | - | - | - | - | - | - |

**Table S6**

Concentrations of phenolic compounds in *Mansoa alliacea* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in μg·g$^{-1}$ of plant followed by standard deviation of the concentration (μg·g$^{-1}$).

| Compound | *Mansoa alliacea* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3rd harvest* |
| Gallic acid | - | < ILOQ | < ILOQ | - | - | - |
| Chlorogenic acid | 99.1 ± 3.5 | 128.8 ± 2.7 | 63.0 ± 2.4 | 9.6 ± 0.4 | 3.3 ± 0.05 | 18.1 ± 0.6 |
| (+)-catechin | - | - | - | - | - | - |
| Vanillic acid | 16.4 ± 1.4 | 10.1 ± 0.7 | 10.1 ± 0.8 | 2.7 ± 0.4 | 1.1 ± 0.1 | 5.4 ± 0.5 |
| Caffeic acid | 5.6 ± 0.5 | 9.4 ± 0.4 | 4.8 ± 0.2 | 1.4 ± 0.2 | 0.9 ± 0.03 | 2.2 ± 0.1 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 34.2 ± 1.9 | 51.4 ± 1.2 | 22.4 ± 0.5 | 16.9 ± 1.1 | 5.9 ± 0.1 | 4.8 ± 0.1 |
| Ferulic acid | 25.8 ± 0.7 | 27.7 ± 0.8 | 32.5 ± 0.9 | 11.0 ± 0.4 | 4.2 ± 0.1 | 12.5 ± 0.2 |
| Rutin | 3.6 ± 0.4 | 1.6 ± 0.2 | 8.2 ± 0.5 | 0.4 ± 0.1 | 0.2 ± 0.02 | 4.2 ± 0.1 |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | - | - | 0.1 ± 0.04 | - | - | 0.1 ± 0.02 |
| Myricetin | - | - | - | - | - | - |
| Fisetin | - | - | < ILOQ | - | - | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | 3.7 ± 0.7 | 10.7 ± 0.8 | 6.9 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | 2.5 ± 0.3 |
| Quercetin | - | - | - | - | - | < ILOQ |
| Luteolin | 0.6 ± 0.2 | 0.4 ± 0.1 | 0.3 ± 0.1 | 10.2 ± 0.7 | 5.6 ± 0.2 | 1.8 ± 0.1 |
| Apigenin | 5.2 ± 0.2 | 4.0 ± 0.2 | 5.2 ± 0.3 | 8.6 ± 0.4 | 1.8 ± 0.03 | 3.8 ± 0.1 |
| Kaempferol | - | - | - | - | - | - |
| 3.6-dihydroxyflavone | - | - | - | - | - | - |
| Chrysin | - | - | - | - | - | - |
| Galangin | - | - | - | - | - | - |

**Table S7**

Concentrations of phenolic compounds in *Connarus perrottetii* var. *angustifolius* determined by UHPLC-ESI-MS/MS using infusions and ethanolic extracts of three harvests. The values are given in $\mu g \cdot g^{-1}$ of plant followed by standard deviation of the concentration ($\mu g \cdot g^{-1}$).

| Compound | *Connarus perrottetii* var. *angustifolius* | | | | | |
|---|---|---|---|---|---|---|
| | Infusion | | | Ethanolic extract | | |
| | *1st harvest* | *2nd harvest* | *3rd harvest* | *1st harvest* | *2nd harvest* | *3rd harvest* |
| Gallic acid | 11.6 ± 0.8 | 109.8 ± 5.0 | 65.6 ± 1.3 | 6.6 ± 1.5 | 49.2 ± 1.9 | 44.3 ± 0.9 |
| Chlorogenic acid | - | 2.0 ± 0.7 | - | - | 0.7 ± 0.1 | 0.7 ± 0.1 |
| (+)-catechin | 1007.8 ± 10.3 | 1773.7 ± 26.9 | 1236.4 ± 30.1 | 442.1 ± 31.7 | 374.1 ± 6.3 | 258.4 ± 3.5 |
| Vanillic acid | 9.2 ± 0.9 | 15.2 ± 0.6 | 18.9 ± 1.2 | 6.2 ± 1.5 | 15.9 ± 0.9 | 14.6 ± 0.4 |
| Caffeic acid | - | - | 0.8 ± 0.2 | < ILOQ | - | 0.3 ± 0.1 |
| 6-hydroxycoumarin | - | - | - | - | - | - |
| *p*-coumaric acid | 1.7 ± 0.1 | 2.4 ± 0.1 | 8.2 ± 0.2 | 2.9 ± 0.2 | 1.7 ± 0.04 | 6.1 ± 0.2 |
| Ferulic acid | 7.3 ± 0.6 | 0.9 ± 0.3 | 2.4 ± 0.5 | 10.7 ± 1.0 | 1.0 ± 0.2 | 1.5 ± 0.2 |
| Rutin | 0.5 ± 0.2 | < ILOQ | < ILOQ | 0.4 ± 0.1 | - | < ILOQ |
| 4-Hydroxycoumarin | - | - | - | - | - | - |
| Rosmarinic acid | - | - | - | - | - | - |
| Quercitrin | - | - | - | - | - | < ILOQ |
| Myricetin | - | - | - | - | 17.8 ± 8.3 | 5.6 ± 2.7 |
| Fisetin | - | - | - | - | - | - |
| Resveratrol | - | - | - | - | - | - |
| 3-acetylcoumarin | - | - | - | - | - | - |
| *trans*-cinnamic acid | - | 0.7 ± 0.2 | < ILOQ | - | < ILOQ | 0.4 ± 0.1 |
| Quercetin | - | < ILOQ | < ILOQ | < ILOQ | 11.8 ± 0.2 | 7.3 ± 0.3 |
| Luteolin | - | < ILOQ | - | < ILOQ | - | - |
| Apigenin | - | - | - | 9.5 ± 0.1 | - | - |
| Kaempferol | - | - | - | - | 15.3 ± 1.8 | 7.6 ± 1.2 |
| 3.6-dihydroxyflavone | - | - | - | - | - | - |
| Chrysin | - | - | - | - | - | - |
| Galangin | - | - | - | - | - | - |