Macedonian pharmaceutical bulletin, 68 (Suppl 2) 27 - 28 (2022)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.008
Short communication

Rapid annotation of acylquinic acids in *Tanacetum balsamita* L. with a practical strategy of diagnostic ions based on Orbitrap mass spectrometry

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Introduction

Tanacetum balsamita L. (costmary) is distributed in South-East of Europe and South-West of Asia but naturalized in most parts of the world (Oberprieler et al., 2009). The plant is commonly grown in the herb garden. It accumulates remarkable amounts of essential oil (Baczek et al. 2017). Fresh and dried leaves of costmary possess a strong lemony-minty flavour and a sweet astringent taste. Costmary has a long traditional usage as aromatic plant in Europe and Asia. Leaves are used as flavourings in soups and meats, sausages and cakes. Costmary has been used for more than several centuries as flavor, carminative and cardiotonic in traditional medicine of Mediterranean and Balkan countries. The herb is well known for antibacterial, antioxidant, digestive and astringed effects.

The study aims to assess the acylquinic acids (AQA) in the costmary leaves, flower heads and roots in non-targeted profiling by ultra high-performance liquid chromatography - quadrupol-Orbitrap high resolution mass spectrometry (UHPLC-HRMS).

Materials and methods

Plant material and sample preparation

Plant material was harvested from an herb garden in the foot of Sredna Gora Mountain. Powdered, air-dried leaves, roots and flower heads were extracted with 80% methanol by sonication, the solvent was evaporated *in vacuo* and extracts were lyophilized.

Ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS)

UHPLC-HRMS analysis was performed on a reversed phase column Waters Cortecs C18 (2.7 µm, 2.1 × 100 mm) column maintained at 40°C. The chromatographic analyses were run using: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), as mobile phase, and the following gradient: 5% B for 1 min, gradually turned to 30% B over 19 min, increased to 50% B over 5min, increased to 70% B over 5 min, and finally increased to 95% over 3 min. The system was then turned to the initial condition of 5% B and equilibrated over 4 min. The flow rate and the injection volume were set to 300 µL/min and 1 µL, respectively.

Mass analyses were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific, Inc.) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific). The tune parameters were as follows: spray voltage −2.5 kV; sheath gas flow rate 38; auxiliary gas flow rate 12; capillary temperature 320 °C; probe heater temperature 320 °C. Acquisition was acquired at Full-scan MS and Data Dependent-MS^2 modes. Full-scan spectra over the *m/z* range 100 to 1500 were acquired in negative ionization mode at a resolution of 70,000. For DD-MS^2 mode, instrument parameters were as follows: microscans 1, resolution 17,500, AGC target 1e5, maximum IT 50ms, MSX count 1, Top5,

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isolation window 2.0 *m/z*, stepped normalized collision energy (NCE) 10, 20, 60 eV. Data acquisition and processing were carried out with Xcalibur 4.0 software (ThermoScientific).

The key points in the acylquinic annotation/dereplication were 1) the accurate masses in Full MS and ddMS^2, 2) MS/MS fragmentation patterns, 3) relative abundance of the precursor and fragment ions, 4) elemental composition, 5) matching with the simulated monoisotopic peak profiles and 6) literature data. Fragmentation key for acylquinic acids was proposed.

Results and discussion

Like the plants from *Tanacetum* genus, costmary contains a large number of acylquinic acids (Gevrenova et al, 2020). The AQA annotation was based on the diagnostict ions for each subclass AQA (Gevrenova et al., 2020). Thus, 5-caffeoyl-, 5-coumaroyl- and 5-feruloylquinic acids were witnessed by the base peak at *m/z* 191.055 [quinic acid-H]$^-$. In addition, 3-caffeoyl- and 3-feruloylquinic acid, together with the respective 4-substituted derivatives were found as well.

Three peaks at *m/z* 515.120 [M-H]$^-$ were consistent with di-caffeoylquinic acid isomers (*di*CQA). Two of them yielded prominent ions at *m/z* 173.044 and 135.044 [caffeic acid-H-CO_2]$^-$ indicating vicinal *di*CQA. The "dehydrated" ion at *m/z* 335.077 [CQA-H-H_2O]$^-$ clearly defined 3,4-*di*CQA, while its negligible abundance in the second compound evidenced 4,5-*di*CQA. A base peak at *m/z* 191.055 accompanied with abundant ions at *m/z* 179.034 and 135.044 witnessed 3,5-*di*CQA (Gevrenova et al., 2020). The assignment of four feruloyl-caffeolylquinic acids (FCQA) ([M-H]$^-$ at *m/z* 529.135) was evidenced by the prominent fragment ions at *m/z* 367.103 [M-H-caffeoyl]$^-$ and 134.036 [ferulic acid-H-CH_3-CO_2]$^-$. Vicinal FCQA were discernible by the prominent ion at *m/z* 173.044 as was seen in *di*CQA. In the same way, the structure of 3F-4CQA was suggested, while the prominent fragment ion at *m/z* 367.103 pointed out on 4,5 isomers, being more abundant in 4F-5CQA compared to 4C-5FQA (Gevrenova et al., 2020). The base peak at *m/z* 193.050 together with the abundant ions at *m/z* 367.103 and 134.036 suggested 3F-5CQA. The assignment of three *p*-coumaroyl-caffeoylquinic acids (*p*-CoCQA) isomers at *m/z* 499.125 was supported by the distinctive fragments at *m/z* 337.093 [M-H-caffeoyl]$^-$, *m/z* 163.039 [*p*-CoA-H]$^-$ and *m/z* 119.049 [*p*-CoA-H-CO_2]$^-$ for *p*-coumaric acid (Gevrenova et al., 2020). 3-*p*-Co-5CQA was evidenced by the base peak at *m/z* 163.039. Vicinal 4C-5-*p*-CoQA was supported by the abundant ions at *m/z* 353.087 and 173.044. Two peaks yielded a precursor ion at *m/z* 533.131 along with prominent fragment ions at *m/z* 371.099 [M-H-caffeoyl]$^-$ and *m/z* 135.044 for hydroxyldihydrocaffeoyl-caffeolylquinic acids (HC-CQA) By analogy with previously described *di*AQA, they were assigned to 3-HC-5CQA and 1C-3HCQA.

Three peaks gave a precursor ion at *m/z* 677.153 together with consequent losses of caffeoyl moieties at *m/z* 515.120 [M-H-caffeoyl]$^-$, 353.089 [M-H-2caffeoyl]$^-$ and 191.055 [M-H-3caffeoyl]$^-$ indicating *tri*CQA. Among them, 1,3,5-*tri*CQA was discernible by the ions at *m/z* 191.055 and 179. 034, while 1,3,4-*tri*CQA was deduced from the ions at *m/z* 173.044 and 179.034. The most hydrophobic 3,4,5- *tri*CQA afforded prominent fragment ions at *m/z* 173.044, 135.044 and 179.034.

Conclusion

Overall, 8 *mono*AQA, 13 *di*AQA and 3 *tri*AQA were evidenced in the assayed *T. balsamita* extracts. Among them, caffeoylquinic, feruloylquinic and coumaroylquinic acids together with dicaffeoylquinic, feruloyl-caffeolylquinic, *p*-coumaroyl-caffeoylquinic, hydroxydihydrocaffeoyl-caffeolylquinic and tricaffeoylquinic acids. This profile was dominated by chlorogenic acid, 3,5 *di*CQA and 4,5 *di*CQA acids. The majority of the compounds are reported for the first time in costmary. The detailed phytochemical investigation highlights costmary as valuable source for possible applications in pharmaceuticals products.

Acknowledgments

The study was supported by Contract КП-06-Rila/5, 15.12.2021, Bulgarian National Science Fund.

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