P 27: Impact of targeted UPLC-MS/MS metabolomics on chemical and biochemical characterisation of MAPs

Stefan Martens[1], Eirini Sarrou[2], Paschalina Chatzopoulou[2]

[1]Fondazione Edmund Mach, Centro Ricerca e Innovazione, Department of Food Quality and Nutrition, Via E. Mach, 1, 38010 San Michele all'Adige (TN), Italy, e-mail: stefan.martens@fmach.it (corresponding author)
[2]Hellenic Agricultural Organization "DEMETER", Institute of Plant Breeding and Genetic Resourses, Thermi 57001, Thessaloniki, Greece.

Abstract

Analysis of natural product pattern (metabolites; metabolomics) and its formation (pathway; biosynthesis) in plants, especially in non-model or crop plants such as medicinal and aromatic plants (MAPs), is a research field with significant potential for breeders, growers and consumers. There is an increasing importance for constant and sustainable quality of MAPs final products. Polyphenols are one of the most important compounds for the antioxidant properties of MAPs and are often, if not identified as active principle, used as lead compounds in quality assessment of herbal drugs and related preparation (herbal tea, alcoholic extracts etc.). Therefore, offering an efficient, robust and reliable fast tool to determine these quality features of MAPs will guarantee the growers, industrial users and the consumers from possible frauds.

Keywords: metabolomics, secondary metabolites, polyphenols, MAPs

Introduction

Cultivation of MAPs is of growing interest to guarantee high quality products to be used in different industrial applications (food, beverages, phyto-pharmaceuticals, nutraceuticals), but also to protect natural habitats from overharvesting, due to increasing demand from consumers and the industry. This increased interest in MAPs is considerably associated with naturally occurring antioxidants and bioactive metabolites, such as polyphenols and various volatile organic compounds (VOCs) mainly of the class of terpenoids. Such metabolites are frequently used in food and pharmaceutical industry because of their potential in health promoting properties and disease prevention capability on one side and consumer acceptability on the other side. Sources of antioxidants (extracts or fractions), such as MAPs, have been extensively studied for their antioxidant activity and many indications/applications are known from traditional medicine, while their content and profil of bioactive metabolites as the main source of known, but also new pharmaceutical activities, is rather poorly described. The aim of the present study was the application of fast screening, targeted UPLC-MS/MS metabolomics, on   chemical and biochemical characterization of several MAPs.

Materials and Methods

Chemicals

All reagents for LC-MS analysis were purchased from Sigma-Aldrich with LC-MS grade. Water used in sample preparation and analysis was purified by a Milli-Q water purification system. Specific standards e.g. hypericin, hyperforin, astilbin, amentoflavone, quercetin 3-O-xyloside, carnosol and carnosic acid were obtained from TransMIT PlantMetaChem (Giessen, Germany) and inserted in the analytical method described below.

Plant material

Aerial parts were collected from different accessions of *Salvia fruticosa*, *Sideritis sp.*, *Mentha sp.*, *Oregano sp.*, *Satureja sp.*, *Hypericum perforatum* and *Stachys iva,* cultivated in the experimental field

of IPB&GR (40°34'35'' N 22°57'19'' E) or from wild grown plants in different geographical areas of Greece. The fresh plant material was dried in a shady and dry place at ambient temperature for fifteen days. Commercial tea drugs were obtained from Alfred Galke GmbH (Bad Grund, Germany) and used as reference sample (Table 1).

Tab. 1 List of analysed plant material

Plant species	tissue	Treatment	Samples
Salvia fruticosa	leaves	different harvesting time	2 accessions; 7 time points
Sideritis sp.	herba	wild and cultivated	7 species; 13 accessions plus commercial tea drug
Mentha spicata, M. piperita	leaves, stem	stress and nutrition experiment	2 species; 5 treatments; 2 developmental stages
Origanum vulgare, O. vulgare spp. hirtum, O. onites	herba	wild and cultivated	3 species; 1, 6 and 1 accessions plus commercial tea drug
Satureja montana, S. thymbra	herba	wild and cultivated	2 species (3 and 2 accessions) plus commercial tea drug
Hypericum perforatum	herba, leaves, flowers	wild	3 accessions plus commercial tea drug
Stachys iva	herba	wild	3 accessions plus commercial tea drug

Sample preparation and extraction

100 mg dried tissue was weighed and transferred into a 15 mL falcon tube. A volume of 4 mL of methanol 80% or 5 ml hot water was added to each sample. The samples and solvent were mixed with the help of an orbital shaker for 3 h at room temperature and the extraction proceeded overnight. The resulting solutions where filtered on a 0.22 μm PFTE membrane into a glass vial and analysed with UPLC-MS/MS as described below. Water extracts were cooled down on ice after 10 minutes of incubation and directly filtered as described. Three biological replicates for each sample were done.

Metabolite analysis

The analysis of natural compounds was performed using advanced targeted UPLC-MS/MS method as described (Vrhovsek et al., 2012). Samples were directly injected after extraction. Chromatography, quantification and mass spectrometry conditions can be found in the literature referred to above. Data processing was performed using the Mass Lynx Target Lynx Application Manager (Waters).

Results

A recently established targeted UPLC-MS/MS-MRM method with a library actually consisting of more than 140 natural compounds of various chemical groups was used for the qualitative and quantitative analysis of methanolic and water extracts of different MAPs (Table 1). To further improve metabolic analyses, known and commercially available compounds found in *H. perforatum* and *Salvia sp.* (see Material and Method) were inserted to the database. Twenty eight (*Sideritis sp.*) and fifty five (*Hypericum perforatum*) metabolites were identified respectively, in different concentration in the methanolic and water extracts. This preliminary study revealed a high diversity in the

content and pattern of the secondary compounds in the different plant species. Beside more common derivatives of groups such as benzoic acids, phenylpropanoids, flavones, flavonols found in all extracts, although in different pattern and concentrations, stilbenes, coumarins, flavan-3-ols (including proanthocyanidins) and anthocyanins were only found in *H. perforatum*, carnosol only in *Salvia sp.* and dihydrochalcones in both of these plants. Additionally, in some extracts also intermediates (including their glycosides) of the general flavonoid pathway such as flavanones and dihydroflavonols were detectable.

These differences might due to factors such as: a) genetics (the species and genotype directly influence the content and composition), b) the type of plant tissue (leaves, stems, flowers) and the stage of development, c) environment (soil conditions, climate, altitude, humidity) and d) agronomic cultivation conditions (fertilizer and water supply, grow media, biotic stress etc). Therefore, a robust, fast and reliable metabolic method is needed to characterise different accessions, origins and treatments of MAPs and tissue specific extracts in scientific studies, but also for quality control for industrial applications. Furthermore, based on metabolite profiling comprehensive pathways schemes for identified metabolites can be proposed and together with transcriptomics approaches key genes for certain bioactive metabolites can be selected, for further genetic and biochemical analysis, but also for establishing molecular marker for fast screening of populations and use in fast breeding strategies.

References

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