MASTERING NATURE: TARGETED METABOLOMIC AND DNA METABARCODING APPROACHES FOR THE AUTHENTICATION OF *OPHIOPOGON JAPONICUS*

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INTRODUCTION

Ophiopogon japonicus (*Thunb.*) *Ker Gawl.* (*Asparagaceae*) tubers are used in traditional Chinese medicine (TCM) [1] and cosmetics. However, *O. japonicus* tubers may be substituted with other plant species in the *Asparagaceae* family, particularly *Liriope spicata* (*Thunb.*) *Lour.* The whole plants of *O. japonicus* and *Liriope* species may be botanically discriminated. However, it is difficult to reliably distinguish *O. japonicus* tubers from the tubers of *Liriope* species using morphological characteristics [2] (Figure 1). Therefore, other authentication approaches are needed to differentiate *O. japonicus* tubers from *Liriope* tubers, such as chemical analysis, DNA based analysis or molecular biology methods that use genetic profiling. High resolution liquid chromatography-mass spectrometry (LC-MS) was selected as the method to chemically characterize material labelled as *O. japonicus* (*Thunb.*) *Ker Gawl.* Metabarcoding is another complementary approach, based on DNA sequencing, allowing to discriminate *O. japonicus* from other species.

The aim of this study was to develop methods to discriminate *Ophiopogon japonicus* from other species to confirm identification and avoid falsification.

Figure 1. Samples of *Liriope spicata* and *O. japonicus* tubers and powders.

1. TARGETED METABOLOMIC APPROACH

MATERIAL & METHODS

Certified references

The *O. japonicus* (*Thunb.*) *Ker Gawl.* and *Liriope spicata* (*Thunb.*) *Lour.* references were certified by the Kew Garden and by Pr Fourasté respectively. Specific markers relative to these species were highlighted.

Sample preparation

Solubilization of the raw material powder at 100g/L in MeOH 80% and stirring of the solution for 24 hours. Paper filtration of the solution followed by 0.45µm filtration. Additional 0.2µm filtration before vial transfer and injection.

Chromatographic Conditions

- UPLC system: Waters ACQUITY UPLC I-Class
- UPLC column: Luna C18 (2), 150 x 3 mm; 3 µm + precolumn (Phenomenex)
- Column temperature: 35°C / Sample temperature: 10°C / Injection volume: 1 µL
- Mobile phases:
  - A: Water/Acetonitrile (90/10 (V/V)) + 0.1% Formic Acid
  - B: Methanol/Acetonitrile (90/10 (V/V)) + 0.1% Formic Acid
- Flow rate: 0.4 mL/min

- Gradient table:

Time (min)	A	B
0	100%	0%
20	0%	100%
25	0%	100%
26	100%	0%
30	100%	0%

Mass detection conditions

- MS/MS system: Waters Xevo G2-XS QTof; UNIFI software
- Polarity/mode: +ESI; Sensitivity mode
- MS range: 50 - 1200 Da / Capillary voltage: 0.5 kV / Cone voltage: 30 V
- Low collision energy: off (6V) / High collision energy ramp: 15 - 30 V
- Temperatures: Source: 120°C / Desolvatation: 550°C
- Flow: Cone gas: 80 L/h / Desolvatation gas: 1000 L/h

RESULTS

1. Analytical authentication of *O. japonicus* and *Liriope spicata*

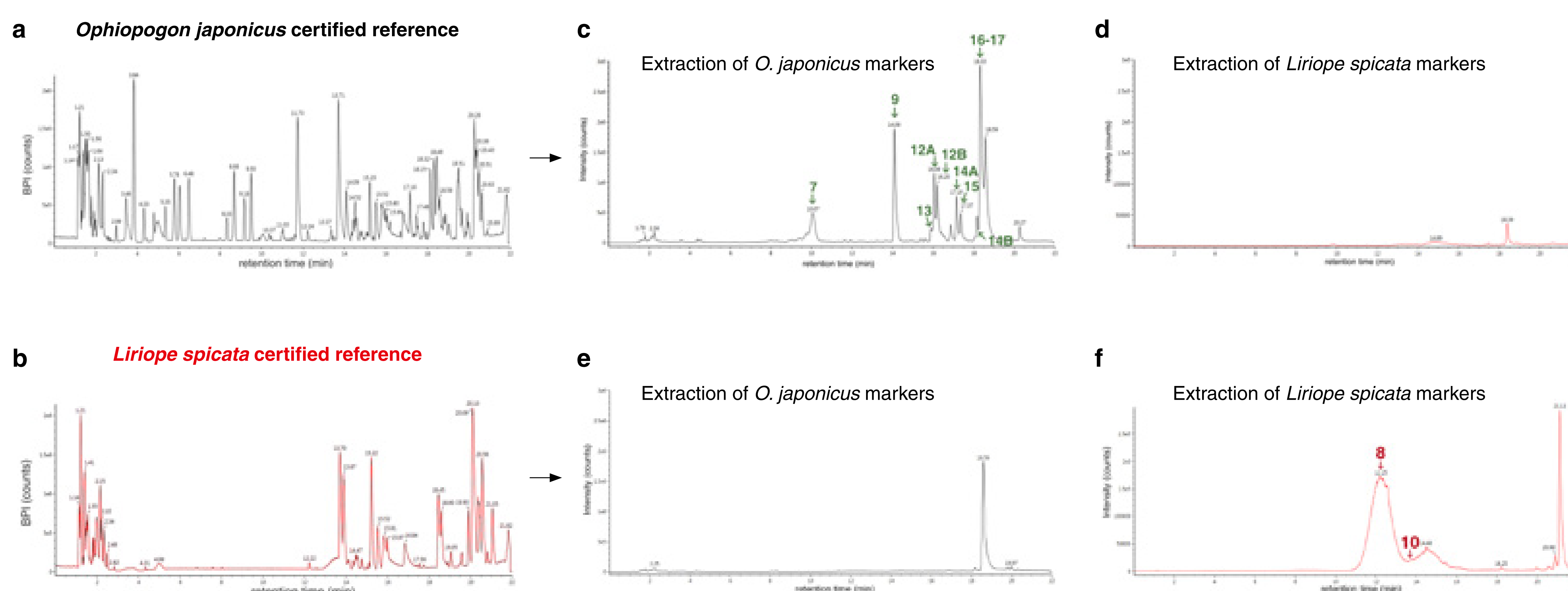


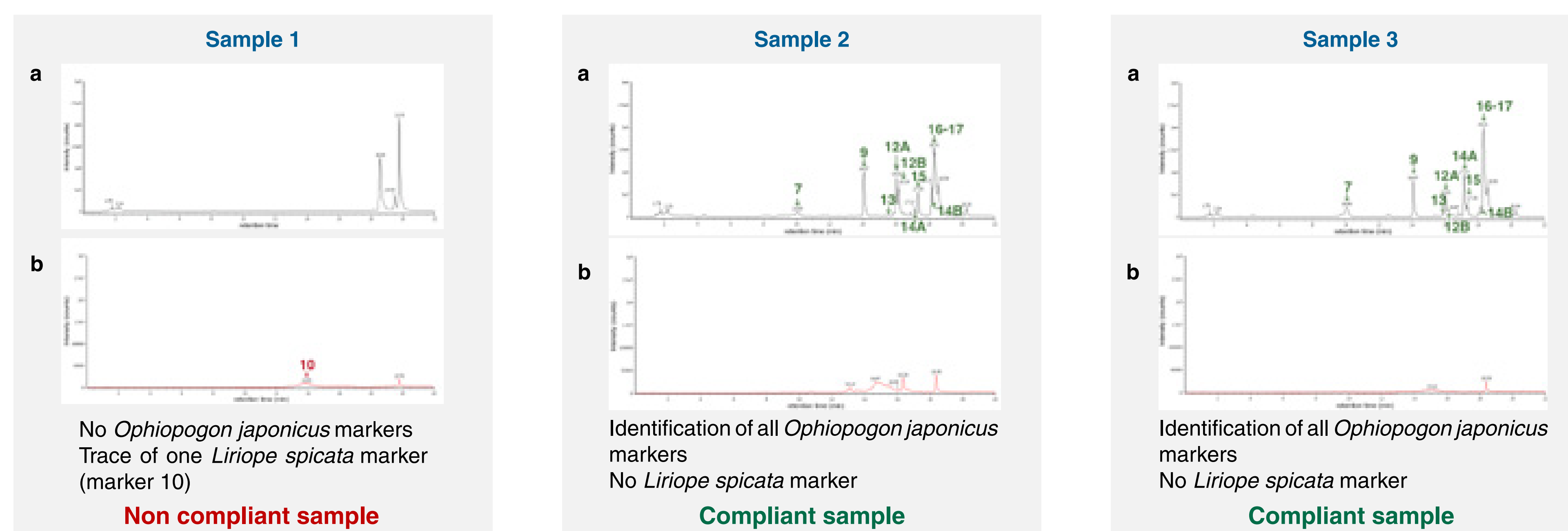
Figure 2. LC-MS chromatograms (positive ESI) of the extracts prepared from the *O. japonicus* (a) and *Liriope spicata* (b) certified references. Extraction of *O. japonicus* (*Thunb.*) *Ker Gawl.* markers (c and e) and *Liriope spicata* (d and f) markers.

Marker	Chemical formula	Assigned coumpound (or isomer)
1	$C_6H_{14}N_4O_2$	Arginine
2	$C_{11}H_{12}N_2O_2$	Tryptophan
3	$C_{16}H_{18}O_8$	*p*-Coumaroyl-quinic acid
4	$C_{22}H_{29}NO_5$	Alkaloid
5 A	$C_{22}H_{33}NO_4$	Alkaloid
5 B		Alkaloid
6 A	$C_{22}H_{31}NO_5$	Alkaloid
6 B		Alkaloid
7	$C_{51}H_{82}O_{23}$	Ophiopogonin I*
8	$C_{45}H_{72}O_{17}$	Spirost-5-ene diol tri-glycoside
9	$C_{20}H_{22}O_7$	7-Hydroxy-3-(2-hydroxy-4-methoxybenzyl)-5-8-dimethoxy-6-methyl-4-chromanone*
10	$C_{52}H_{86}O_{25}$	Steroidal
11 A	$C_{45}H_{72}O_{17}$	Spirost-5-ene diol (or) Spirost-5-en-3-ol tri-glycoside derivative
11 B		
12 A	$C_{19}H_{20}O_7$	5,7-Dihydroxy-3-(2-hydroxy-4-methobenzyl)-8-methoxy-6-methyl-4-chromanone*
12 B		5,7-Dihydroxy-3-(2-hydroxy-4-methobenzyl)-8-methoxy-6-methyl-4-chromanone*
13	$C_{19}H_{18}O_7$	Ophiopogonanone H*
14 A	$C_{20}H_{22}O_5$	O',8-Dimethyl-ophiopogonanone B*
14 B		
15	$C_{18}H_{14}O_6$	Ophiopogonanone A*
16	$C_{19}H_{20}O_5$	8-Methylophiopogonanone B*
17	$C_{45}H_{72}O_{17}$	Spicatoside A
18*	$C_{44}H_{70}O_{16}$	Ophiopogonine D injected reference

Table 1. Identification by LC-MS of *Liriope spicata* markers (in red) and *O. japonicus* (*Thunb.*) *Ker Gawl.* markers (in green). *: reported to occur in Ophiopogon species[2-4].

Chromatograms obtained from referent samples revealed 10 specific markers of *O. japonicus* (*Thunb.*) *Ker Gawl.* and 2 specific markers of *Liriope spicata*. In particular, the range of homoisoflavonoids, detected in the Ophiopogon species, is in accordance with published data that describe *Liriope* species to contain a lower content of homoisoflavonoids relative to those in *O. japonicus*[2-5].

2. Analytical discrimination of 3 different samples of *O. japonicus* supply



Based on our analytical identification data, our results demonstrated that the 1st sample had no *O. japonicus* markers but traces from *Liriope spicata* markers. This raw material supply was not compliant with Ophiopogon supply. The chromatograms obtained for the other samples (from different regions and suppliers) display the specific markers of the certified reference of *O. japonicus* (*Thunb.*) *Ker Gawl.* species.

Figure 3. Extracted LC-MS chromatograms showing the presence or absence of markers of *O. japonicus* and *Liriope spicata* in three samples sold as *O. japonicus*.

2. DNA BARCODING

MATERIAL & METHODS

DNA extraction

DNA from samples was extracted using the DNeasy Plant Mini kit.

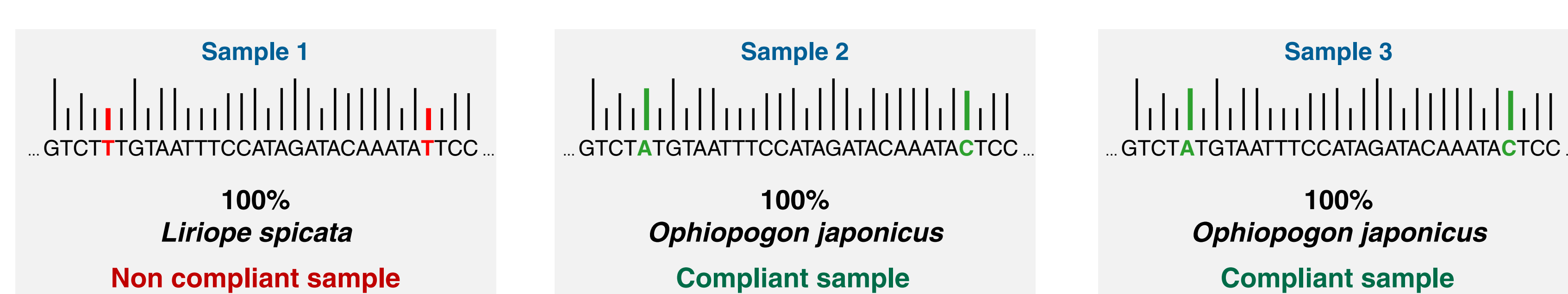
Barcode amplification

DNA barcodes allowing to discriminate the 2 species were amplified using specific primers targeting trnL-trnF barcode of the chloroplast genome.

Metabarcoding (NGS)

Amplicons were sequenced using Illumina MiSeq platform. Sequences were cleaned and blasted against NCBI Genbank database to identify plant species.

RESULTS



Our metabarcoding data showed that only samples 2 and 3 correspond to *O. japonicus* species.

CONCLUSION

This study makes it possible to go beyond the organoleptic, microscopic and macroscopic analyses to discriminate non-differentiable powders. It allows the authentication of raw materials that cannot be discriminated using classical botanically and organoleptically techniques. These reliable methodologies will permit a strict authentication and quality control of our future supplies of this starting raw material, a prerequisite for the development of cosmetic and dermo-cosmetic products.

References:
[1] 1. Pharmacopoeia Commission of the People's Republic of China (2010), China Medical Science press, Beijing.
[2] Wu Y, *et al.*, (2014) Food Res. Int 57:15-25.
[3] Ye M, *et al.*, Am Soc. Mass Spectrom 16:234-243
[4] Combined Chemical Dictionary Online (CCD 17.1) http://ccd.chemnetbase.com/dictionary. Taylor & Francis Group. Retrieved 03/03/2016.
[5] Lin Y, *et al.*, (2010) J. Pharm. Biomed. Anal 52:757-762.

Acknowledgements:
We thank Professor Isabelle Fourasté for its expertise on *Liriope spicata* and to have provided the authenticated sample.