Food as medicine: UHPLC-PDA-HRMS characterization of active compounds in cinnamon extract before and after simulated digestion

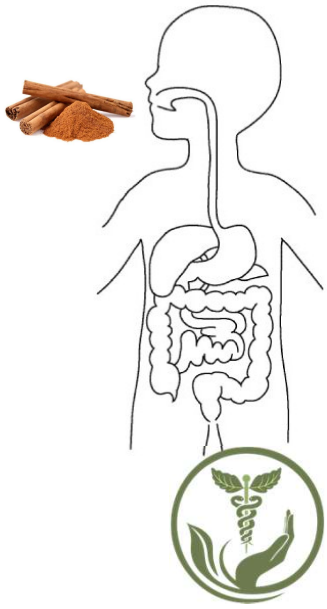
Authors - F. Romaniello[1], P. Fusari[1], S. Pagliari[2], A. Bulbarelli[3], P. Rovellini[1]

[1] Innovhub stazioni sperimentali per le industrie s.r.l., Milan, Italy
[2] Zooplantlab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy
[3] School of Medicine and Surgery, University of Milano-Bicocca, 20126 Monza, Italy

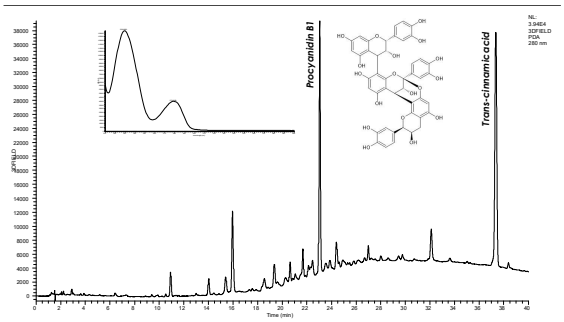
INTRODUCTION

Herbs and spices are rich in natural bioactive substances with antioxidants and anti-inflammatory properties. **Cinnamon** with its 250 species is a wide-spread spice. Moreover, the **anti-inflammatory** power of cinnamon extract has been demonstrated in various models of inflammatory diseases such as colitis and arthritis [1]. In literature, the **anti-oxidant** and **anti-inflammatory** activity studies and the chemical characterization refer only to the spice extracts but not to the product of **digestion**, typical of oral assumptions. For that reason, in the present study it has been chosen to carry out an **UHPLC-PDA-HRMS untargeted characterization** of the cinnamon extracts not only before but also after gastrointestinal digestion.

EXPERIMENTAL

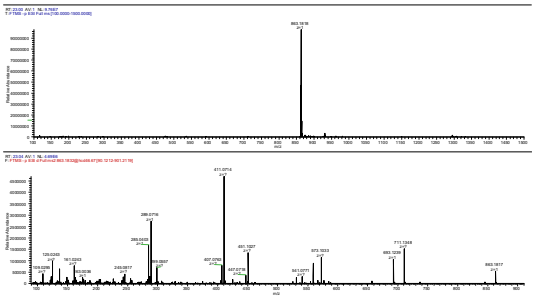


The pulverized Cinnamomum verum bark were extracted according to Cheng et al. work [2]. Briefly 2 grams of powders were extracted with 40 mL of milliQ water (drug-solvent ratio 1:20) for one hour using a rotavapor with heating bath temperature set at 60°C and rotation speed of 100 rpm. Then 75% ethanol was added in 1:1 ratio so the polysaccharides contained in the extract can be precipitated. Finally the solvent was evaporated with rotavapor and the dry fraction was resuspended in 5 mL of water.
A full characterization of cinnamon extract's active compounds was achieved. The chromatographic separation was conducted on a **Luna Omega Polar C18** (150mm x 2.1 mm, 3µm) (Phenomenex, Castelmaggiore, Italy) in reverse phase mode. The identification was carried out using a Thermo Vanquish UHPLC system coupled with a **Thermo Orbitrap Exploris 120 mass spectrometer** by **HESI** source and a Vanquish Diode Array Detector (Thermo Scientific, Rodano, Italy). The source parameters were as follows: spray voltage 3500 V, ion transfer tube temperature 320 °C, vaporizer temperature 300 °C; sheath and auxiliary gas flow were 45 and 10 AU respectively.
Spectra were recorded in **full-mass mode** within a range of **100-1500 *m/z*** in **positive** and **negative ionization**. The Orbitrap **resolution** was set at **120000** and the RF Lens (%) was 80. **Data-dependent MS** experiments were performed in stepped collision energy mode, the normalized collision energy were **20, 40 and 80%, HCD** (higher energy collisional dissociation) fragmentation was achieved.



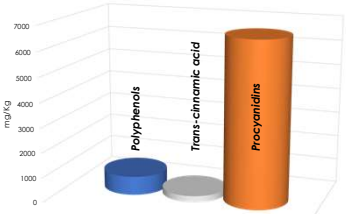
*Chromatographic separation (280 nm) of cinnamon extract **before** digestion*

Phenolic compound were characterized according to the corresponding spectral characteristics (**UV** and **MS/MS spectra**), **accurate mass**, characteristic **MS fragmentation** and **libraries** comparison in semi-automatic way through Thermo Scientific **Compound Discoverer Software®**.



Full mass and MS/MS spectra of procyanidin B1 (catechin/epicatechin trimer)

77 MOLECULES INDIVIDUATES and IDENTIFIED by HRMS, UV and MS/MS spectra

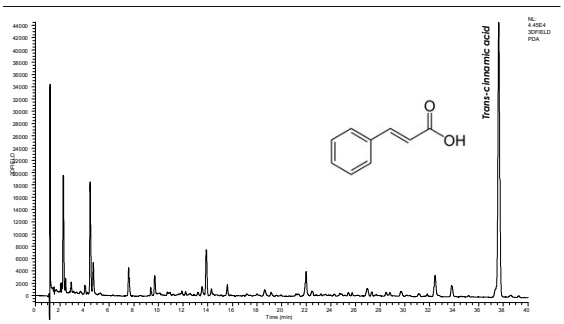


*Quantification of natural compounds in cinnamon extract (mg/Kg) **before digestion***

The chromatographic trace is characterized by two very intense peaks attributed to **cinnamtannin B1** and **trans-cinnamic acid**, and by a broad peak starting at 18 minutes and ending at 40 minutes. This wide-ranging peak is ascribable to the procyanidins with different degree of polymerisation

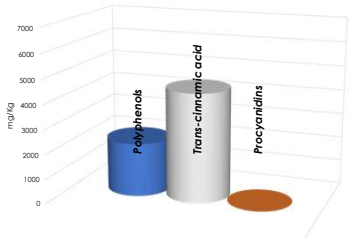
DIGESTION

The **gastrointestinal digestion simulation** was **performed** using the indications given by Minekeus et al. [3]. The process was simulated in the 3 phases: **oral phase**, **gastric phase** and **intestinal phase.** At the end of the three phases of the digestive process, the resulting extract was characterized under the same conditions reported above by UPLC-PDA-HRMS.



*Chromatographic separation (280 nm) of cinnamon extract **after** digestion*

After digestion we can observe the complete disappearance of all catechin polymers, and an **increasing** of **trans-cinnamic acid**, from 250 to 4500 mg/kg and other phenolic compounds such as **2-hydoxybenzoic acid**, **coumarin** and **hydroxybenzaldehyde** which saw a tenfold increase in content.



*Quantification of natural compounds in cinnamon extract (mg/Kg) **after digestion***

CONCLUSIONS

The aqueous extract of **Cinnamomum verum bark** exhibit virtuous **anti-oxidant activity**, confirmed by literature and scavenging tests. Through UHPLC-PDA-HRMS analysis the bioactive compounds were identified and quantified. However, after the digestion the molecules pattern undergoes a radical change with the disappearance of procyanidins and the increase and the appearance of small and hydrophilic molecules with proved **antioxidant and anti-inflammatory activity**[4].
Biological tests on antioxidant and anti-inflammatory activity of digested cinnamon extract are also being conducted which seem to confirm the chemical data.

Have your daily dose of cinnamon

References

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