# *Castanea sativa* bud-derivatives: an innovative green extraction and re-use strategy to valorize food supplement by-products

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## Introduction

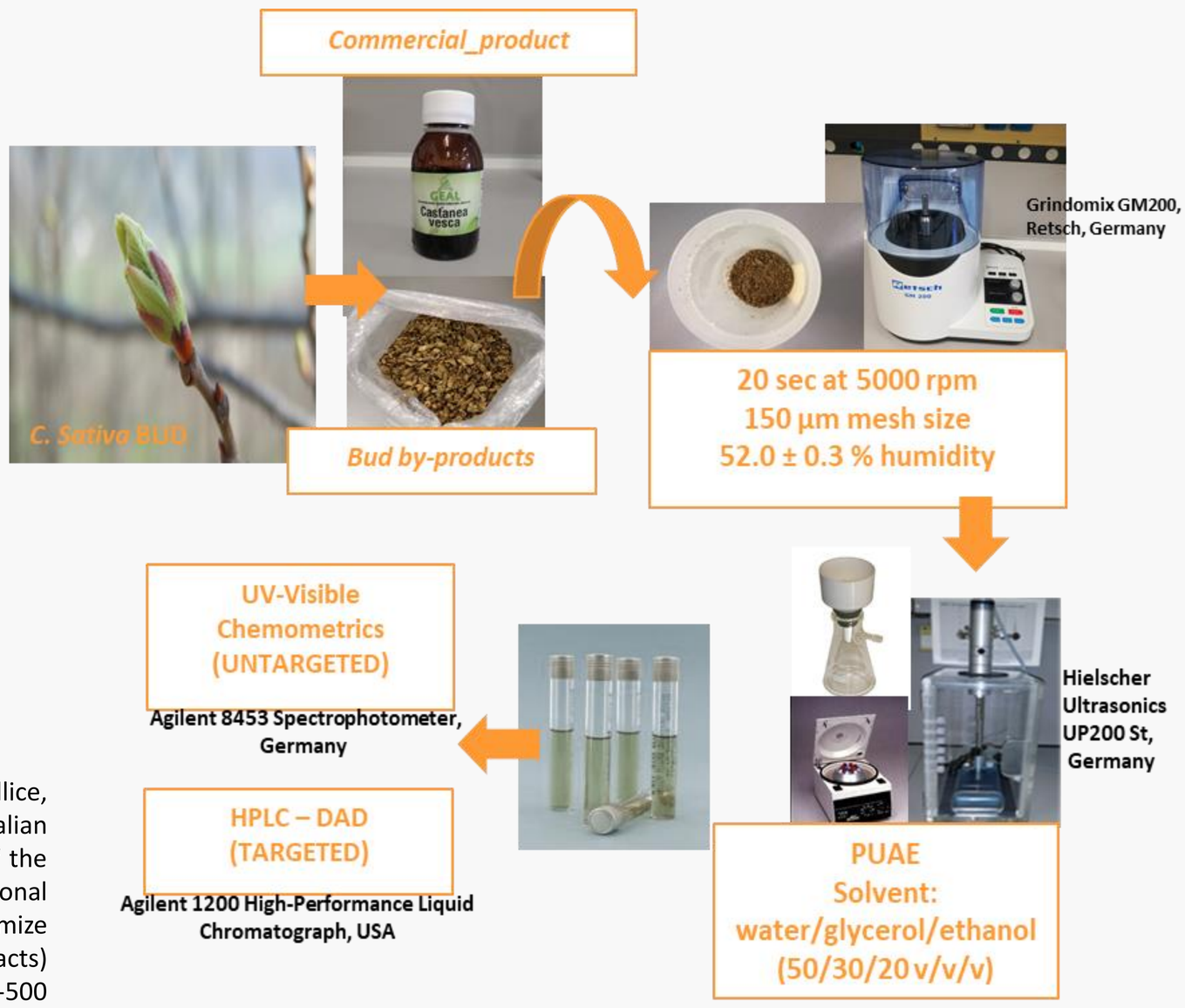
Bud-derivatives, obtained macerating meristematic tissues of trees and plants, represented a category of natural products commercialized in the Europe Community as plant food supplements. They are very expensive products compared to other botanicals, since the collection period of their raw materials is extremely limited over time[1]. In this study, a re-use procedure based on an innovative green extraction to valorize *C. sativa* bud by-products (CBs) is presented.

Pulsed Ultrasound-Assisted Extraction (PUAE)[2] has been employed to extract further valuable material from CBs, using the same solvent of the corresponding commercial Glyceric Macerate (GM). Design of Experiment (DOE) and Untargeted spectroscopic fingerprints were employed to screen the best extraction conditions. Targeted chromatographic fingerprints have been used to compare the most promising extracts with the corresponding commercial GM.

This study takes place in the context of an EU Interreg Alcotra (France-Italy) cooperation project called **FINNOVER (2017-2020)[3]**.

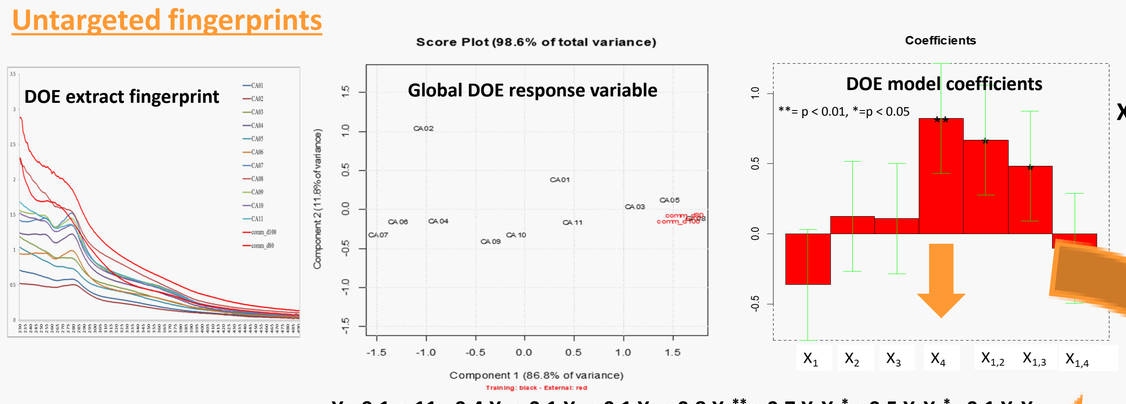
## Materials and methods

CBs were collected from plants spontaneously grown in the valleys of Chisone, Pellice, Germanasca, Bronda, and Varaita (Turin, Italy, March 2017) and were used by an Italian company of food supplements (Geal Pharma, Bricherasio, Turin) for the production of the corresponding GMs according to the European Pharmacopeia 8th edition. A $2^{4-1}$ fractional factorial design (8 experiments plus 3 central point experiments) was carried out to optimize the PUAE process conditions. A data matrix $\mathbf{A}_{11,271}$(training set) of 11 rows (DOE extracts) and 271 columns (**untargeted spectroscopic fingerprints**: UV-Vis absorbances at 230-500 nm) was prepared.




As "global" response variable (Y), the score on the first principal component (PC1), obtained by the PCA (Principal Component Analysis) was taken into account. Analogously the *C. sativa* Commercial product was tested at two different dilutions (external test set: commercial product_d80, commercial product_d100). Both the most promising extracts and the commercial product were furtherly investigated by HPLC[1] (**targeted chromatographic fingerprints**).

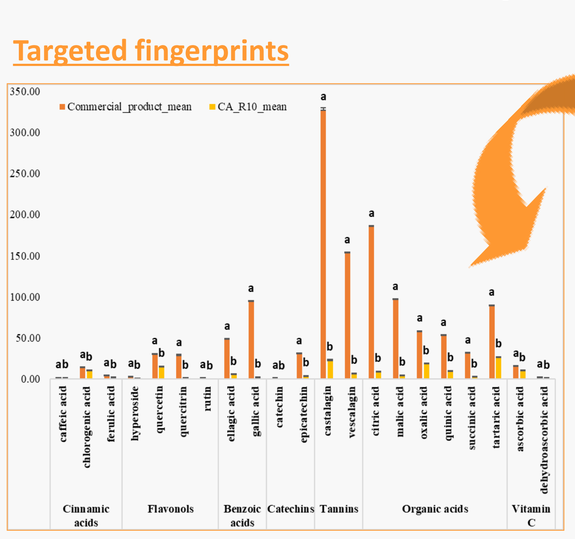
### Untargeted fingerprints

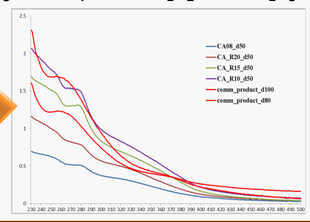



$X_1$ amplitude 50 %
$X_2$ duty cycle 80%
$X_3$ extraction time 15 min
$X_4$ solvent/ratio 1/10

$$Y=-9.1\ e{-}11 - 0.4\ X_1 + 0.1\ X_2 + 0.1\ X_3 + 0.8\ X_4^{**} + 0.7\ X_1X_2^{*} + 0.5\ X_1X_3^{*} - 0.1\ X_1X_4$$

### Targeted fingerprints




| | | Commercial_product_mean | CA_R10_mean |
|---|---|---|---|
| Cinnamic acids | (mg/100 $g_{FW}$) | 20.18 ± 0.01[a] | 14.51 ± 0.01[b] |
| Flavonols | | 64.22 + 0.04[a] | 18.10 ± 0.02[b] |
| Benzoic acids | | 143.66 + 0.01[a] | 8.23 ± 0.01[b] |
| Catechins | | 32.30 ± 0.01[a] | 4.53 ± 0.01[b] |
| Tannins | | 481.22 ± 0.07[a] | 30.27 ± 0.05[b] |
| Organic acids | | 516.51 ± 0.01[a] | 73.06 ± 0.01[b] |
| Vitamin C | | 18.08 ± 0.01[a] | 11.71 ± 0.01[b] |
| TBCC | | 1276.17 ± 0.13[a] | 160.42 ± 0.09[b] |

## Results

The PC1 scores of the test set, were close to those ones of the "best" DOE extract (**CA 08**) for both the considered dilutions. Since $X_4$ resulted the most important variable in building the model, further experiments were planned setting it to 1/20, 1/15 and 1/10 (experiments: CA_R20, CA_R15, CA_R10) hoping both to improve the extraction yield and to save extraction solvent. The extract CA_R10 resulted the most promising, since it seemed even more similar to the commercial product and thus deserving of further HPLC compositional investigation. Their targeted phytochemical fingerprints are reported and compared with that one of the corresponding commercial GM. Flavonols, phenolic acids expressed as benzoic and cinnamic acids, catechins, tannins as polyphenolic markers, as well as organic acids and vitamin C, were considerer into account. Qualitatively, the chromatographic profiles of both **CA_R10** and commercial GM are almost identical, showing that PUAE is able to extract something still useful and valuable from the bud bagasse. In particular, CA_R10 extract has a content in secondary metabolites of 160.42 mg/g of fresh weight marcs, which represents **about the 12%** of the corresponding commercial GM (1276.17 mg/g of fresh weight buds).

## Conclusions

The valorisation of bud marcs remaining after the production of GMs, in this case study of *C. sativa*, could have a significant economic impact for the commercial producers, representing an important innovation in this sector.

A green and relatively low-cost re-use strategy has been presented, which is also applicable for different herbal preparations, to obtain value-added products from food supplement by-products in alternative to incineration or composting.

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[2] Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, *13*, 8615–8627. 10.3390/ijms13078615
[3] ALCOTRA project 2017-2020 Italia-Francia, Finnover n° 1198

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