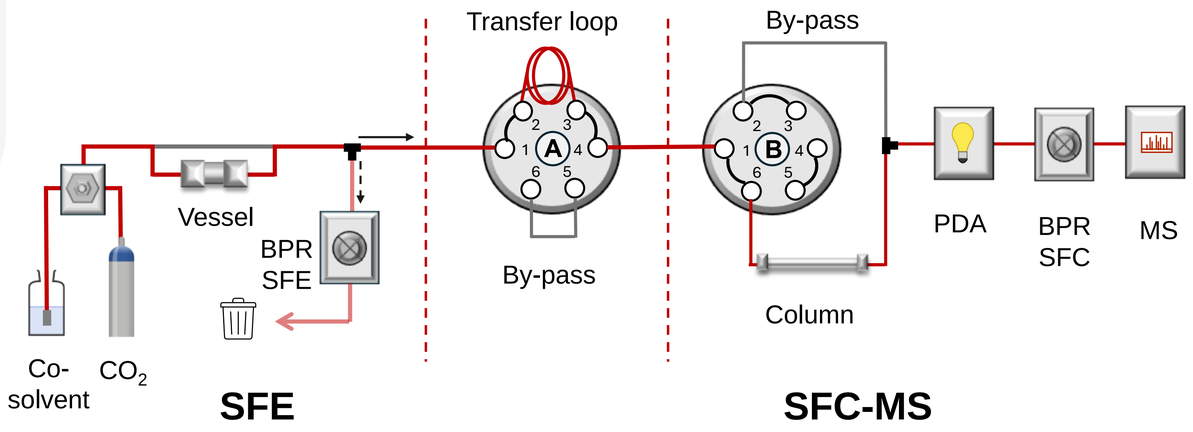
Online combination of supercritical fluid extraction coupled with chromatography and mass spectrometry (SFE-SFC-MS) to analyze plastic additives in medical devices

Benjamin Caux[1,2],Ramy Abou Naccoul[2], Shinnosuke Horie[3], Caroline West[1]

[1]University of Orleans, ICOA, CNRS UMR7311, rue de Chartres, BP 6759, 45067 Orléans cedex 2, France
[2]Shimadzu France, Le luzard 2, Bat A, Bd Salvador Allende Noisiel, 77448 Marne-la-Vallée, France
[3]Shimadzu Europa Gmbh, Albert-Hahn-Straße 6-10, 47269 Duisburg, Germany

The use of **plastic additives** in the formulation of plastic materials is essential to obtain specific physico-chemical properties. However, the formulation is usually not communicated by manufacturers, while the **migration** of certain compounds can be **harmful for health**. Extraction of plastic additives is already described in the literature, but sample preparation steps are highly likely to cause contaminations from laboratory consumables such as syringes or filters. **Online combination** between extraction and analysis reduces the number of sample preparation steps, contamination, use of consumables and risk of sample degradation. In this study, we investigate **supercritical CO_2** to extract and analyze plastic additives in plastic medical devices.

Shimadzu Nexera UC

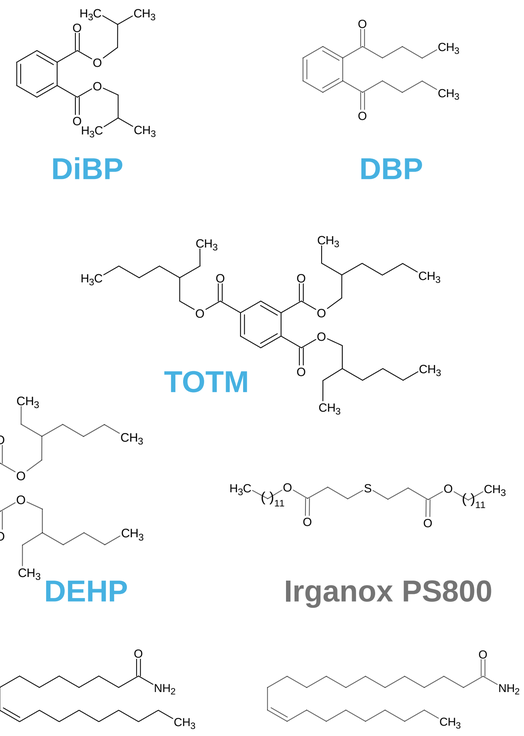


Method optimization

Column screening

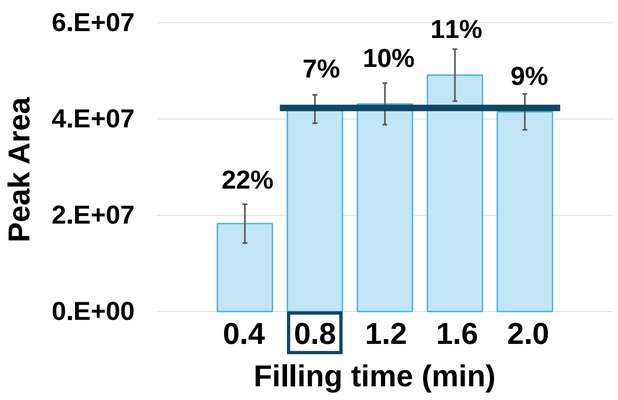
In order to find the best column to achieve the desired separation, **plastic additives** were analyzed on 7 stationary phases from the ShimPack UC series. **Derringer desirability functions** were used to rank them:

- ❖ Number of eluted compounds
- ❖ Retention time distribution
- ❖ Peak width at half height
- ❖ Peak asymmetry
- ❖ Unwanted isomers separation
- ❖ Desirable isobars separation



Plasticizers Lubricants Antioxidants

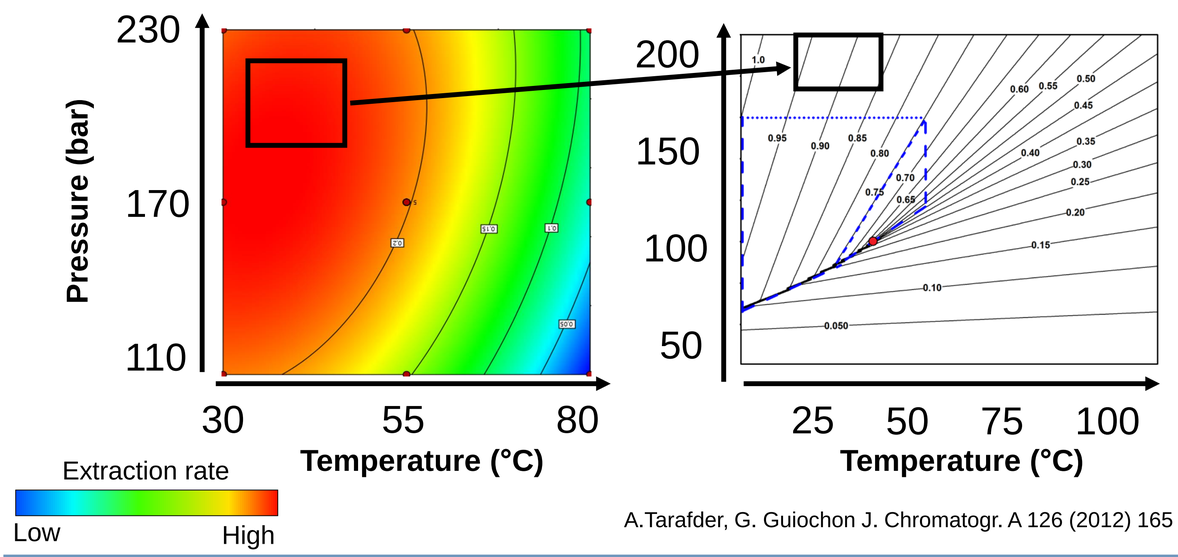
Filling time of the transfer loop



A **transfer loop** was used to hyphenate SFE to SFC-MS, as it **improves repeatability** and offers the possibility to optimize extraction and separation steps independently. However, with a transfer loop, the filling time is an **important parameter** to adjust.

Design of Experiments

To obtain the best extraction yield, a design of experiments (DoE) was carried out, taking **pressure** (from 110 to 230 bar) and **temperature** (from 30 to 80 °C) as variables. A **3-level full factorial design** was used, including 13 experiments (5 replicates at the central point) done directly with the **online SFE-SFC-MS combination**. The results were best fitted with a **quadratic model** and indicated that **the best extraction yield** was obtained at the **highest density of supercritical CO_2.**



A.Tarafder, G. Guiochon J. Chromatogr. A 126 (2012) 165

Extraction method

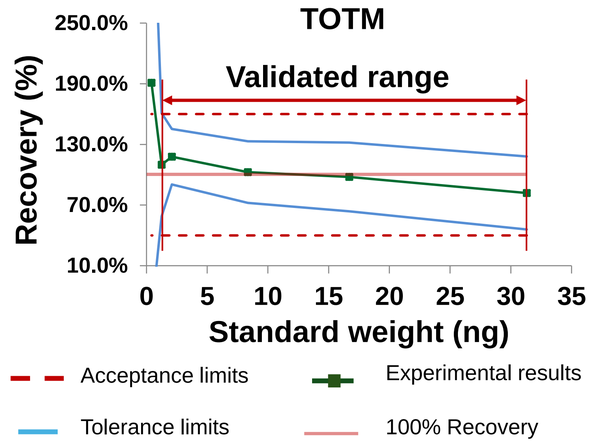
Solvent	Pure CO_2
Temperature	40 °C
Pressure (BPR SFE)	200 bar
Pressure (BPR SFC)	198 bar
Injection Rate (IR)	3%
Static extraction time	20 min
Dynamic extraction time	1.6 min
Transfer loop filling time	0.8 min

Separation method

Co-Solvent	5% methanol (MeOH)
Oven temperature	25 °C
Pressure (BPR SFC)	150 bar
Flow rate	3 mL/min
Column	Shimpack UC Phenyl (250 x 4.6 mm ; 5 µm)
MS detection	ESI (Scan & SIM)
Make-up solvent	98% MeOH ; 2% H_2O + 20 mM ammonium formate in water

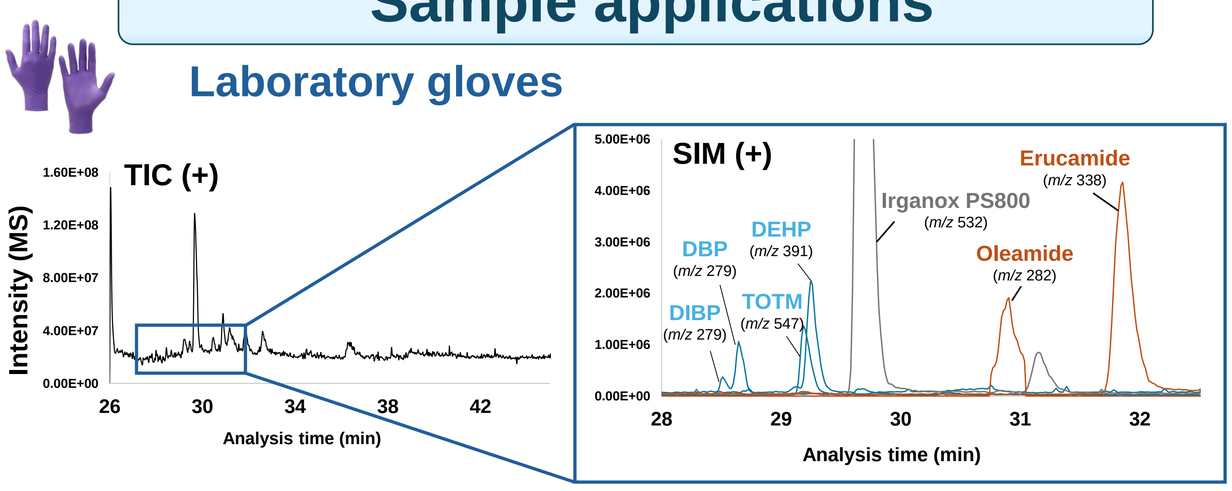
Validation

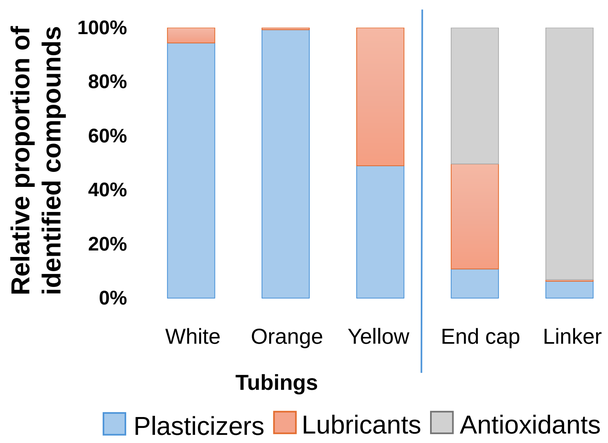
Accuracy profiles were used to validate the quantification method. Two validation series were performed: the **calibration series** used liquid injection while the **validation series** used the extractor. A sample accuracy profile is shown in this figure, indicating the mass range in which the method is validated.



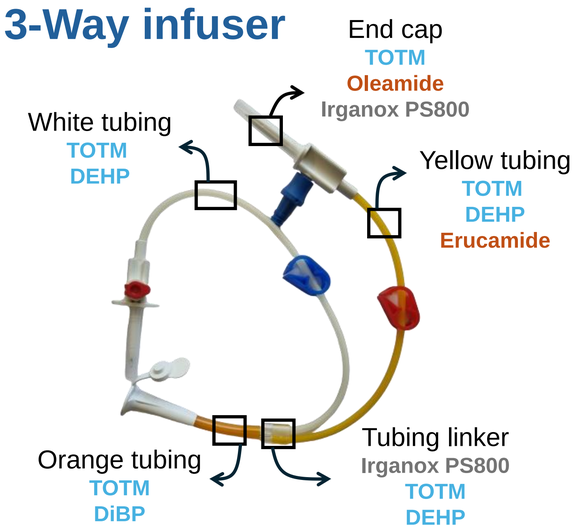
Sample applications

Laboratory gloves





3-Way infuser



To conclude, supercritical CO_2 allows **extracting and analyzing plastic additives in medical devices**. **Online SFE-SFC-MS** avoids laboratory contaminations and allows **accurate and reliable quantitation**.

Special thanks to **Clement De Saint Jores** for his supervision and advice and to **Antoine Leriche** for technical assistance in this project. **Marie Lecœur** and **Catherine Foulon** are acknowledged for advice and the gift of samples. **Shimadzu Corporation** and **French ANRT** (grant number 2022/1054) are acknowledged for financial support.

UNDERSTANDING DIMERIZATION PROCESSES AND BIASED SIGNALING ELICITED BY SEROTONIN 5HT7 RECEPTOR

B. Abdoul Fatahou Kaboré[1], Chayma El Khamlichi[1], Arun K. Shukla[2] and Sévérine Morisset-Lopez[1]

(1) Centre de Biophysique Moléculaire, Team « Neurobiology of Receptors and Therapeutic Innovations », CNRS UPR 4301, rue Charles Sadron, 45071 ORLEANS Cedex 01

(2) Indian Institute of Technology (IIT), Kanpur, India

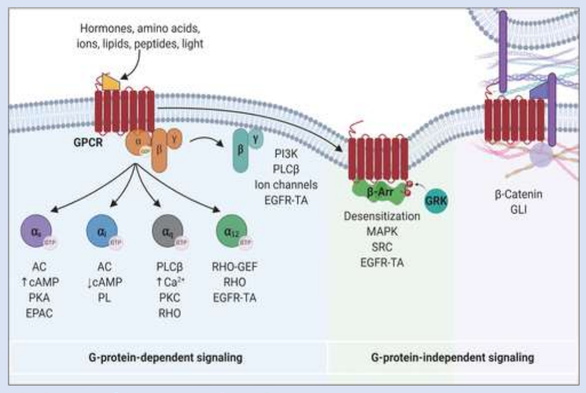
SUMMARY : G Protein-coupled receptors (GPCRs) are involved in regulation of manifold physiological processes. Originally described as requiring coupling to intracellular G-proteins, GPCRs also use G-protein-independent pathways through β-arrestins recruitment. Biased ligands, by favoring one of the multiple bioactive conformations of GPCR, allow selective signaling through either of these pathways. This concept of functional selectivity of a ligand has emerged as an interesting property for the development of new therapeutic molecules. However, at molecular level, the intricate details of $5HT_7R$ activation, transducer-coupling, regulatory mechanisms as well as its downstream signaling and dimerization state are not well understood. Also, dimerization can provide different physiological consequences, enhancing, inhibiting or cross-signaling some receptors initial effects.

Considering the role of 5-HT_7R in neurological disorders, its ability to form constitutive heteromeric complexes reveals a new level of complexity in its signaling and opens the way for new strategies to achieve diverse and nuanced 5-HT_7R regulation. Better understanding the structural and functional bases of biased signaling is an important issue for drug development.

INTRODUCTION

The serotonin 5-HT7 Receptor

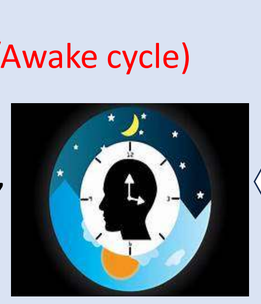
- 5HT7R belongs to the superfamily of G-Protein-coupled receptors (GPCRs)
- Coupled by Serotonin and involved in many physiological processes



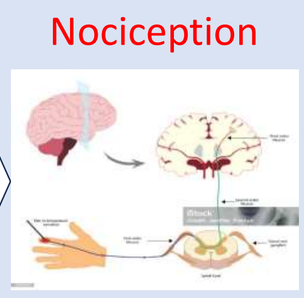
Mood Regulation

Circadian rythmicity (Sleep/Awake cycle)

(Psychiatric disorders, Smooth muscle contraction, Etc.)

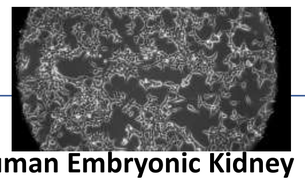


Nociception

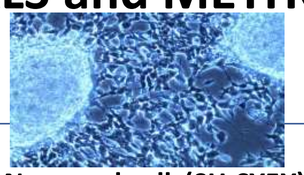


MATERIALS and METHODS

Cell lines



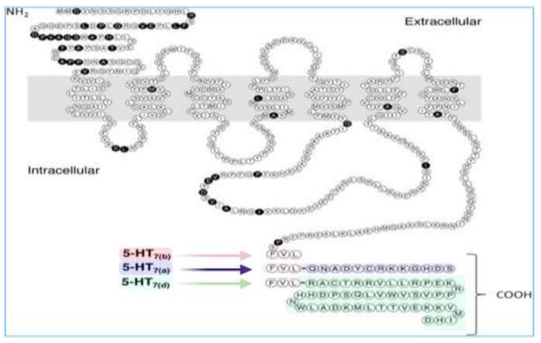
Human Embryonic Kidney
-HEK293 A
-HEK293 A KO β-Arrestin

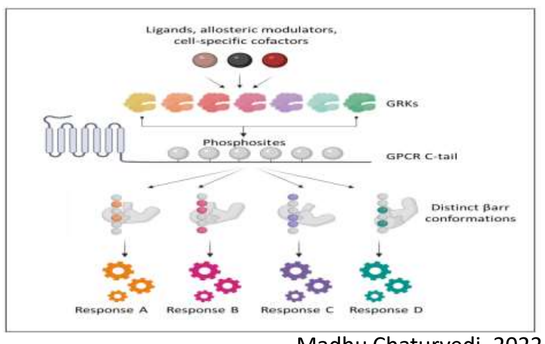


Neuronal cells(SH-SY5Y)

Primary culture of neurons

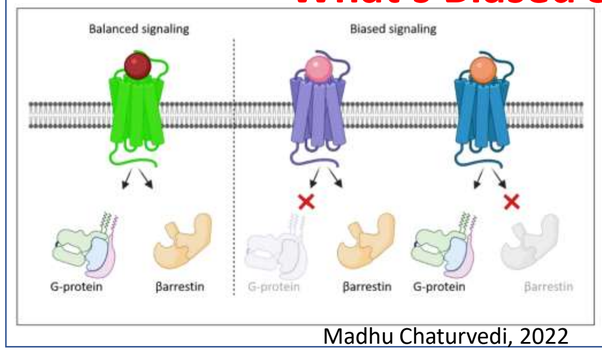
5HT7R Isoforms: A, B & D (different in C-ter sequences)



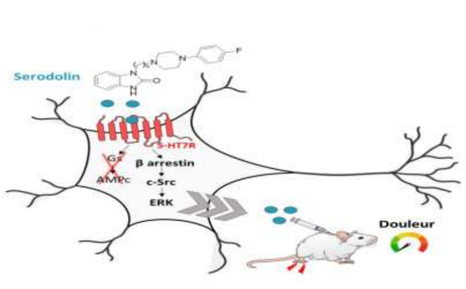


Madhu Chaturvedi, 2022

What's Biased Signaling?



Madhu Chaturvedi, 2022



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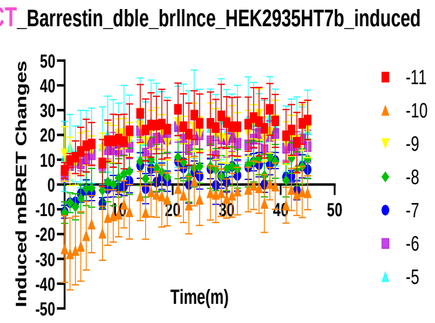
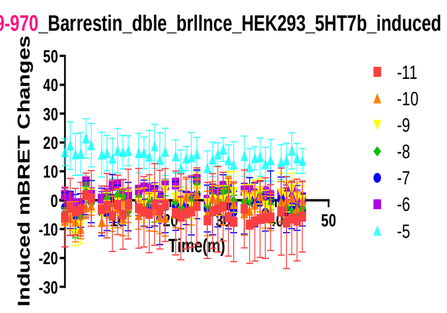
Isoforms biased signaling

BRET assay

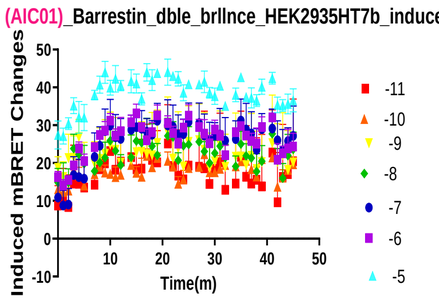
5CT_Barrestin_dble_brIInce_HEK2935HT7b_induced



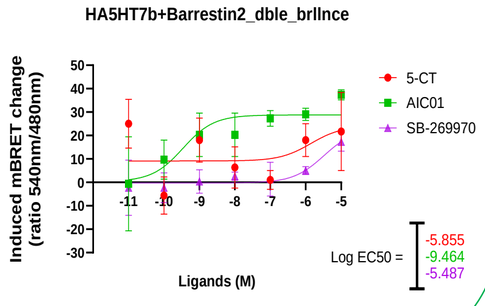
SB269-970_Barrestin_dble_brIInce_HEK293_5HT7b_induced

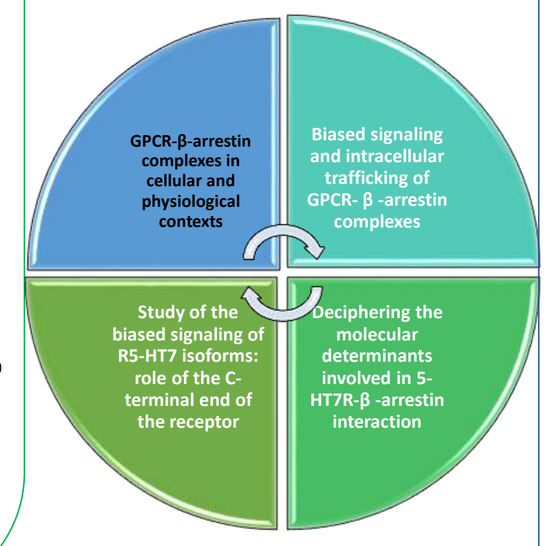


Serodolin (AIC01)_Barrestin_dble_brIInce_HEK2935HT7b_induced



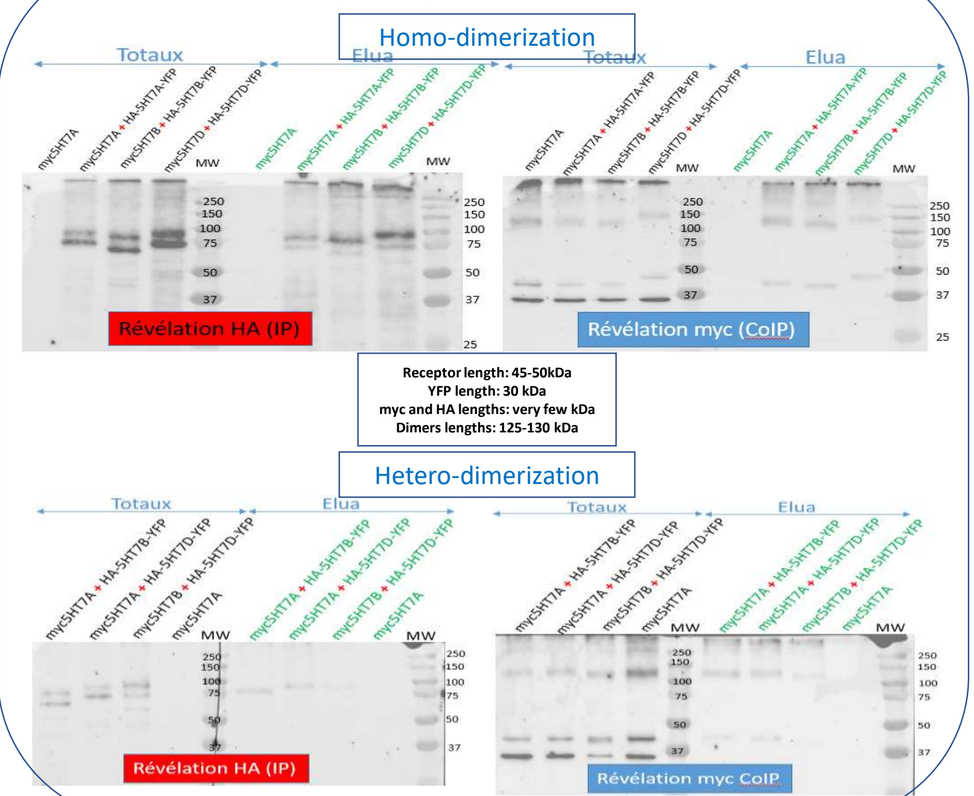
HA5HT7b+Barrestin2_dble_brIInce





Isoforms expression and dimerization

Co-Immunoprecipitation



COLLABORATION

High-resolution structural details of the Serodolin-5-HT7R- β-arrestin complexes (Neg-Stain EM; Cryo-EM)

Perspectives

NanoBiT Complementation assay
Fluorescence microscopy
Flow cytometry

LIGANDS CARACTERISTICS :

Buffer (HBSS)

Classic agonists (5-HT, 5-CT, LP211, E55888)

Antagonists (SB269-970)

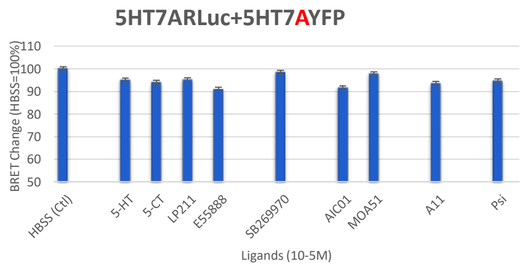
Biased agonists (AIC01, MOA51)

Allosteric modulator, VHH (A11)

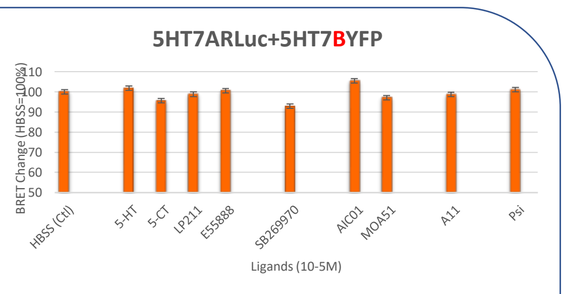
Psychedelic (PSsi

Ligands effects on isoforms dimerization

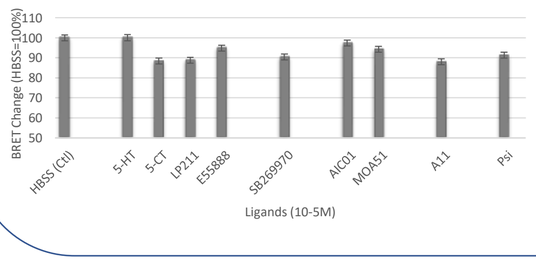
5HT7ARLuc+5HT7AYFP



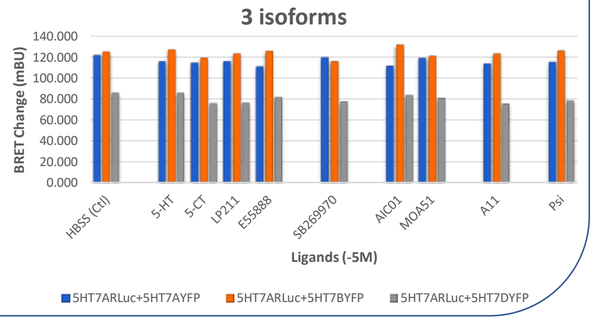
5HT7ARLuc+5HT7BYFP



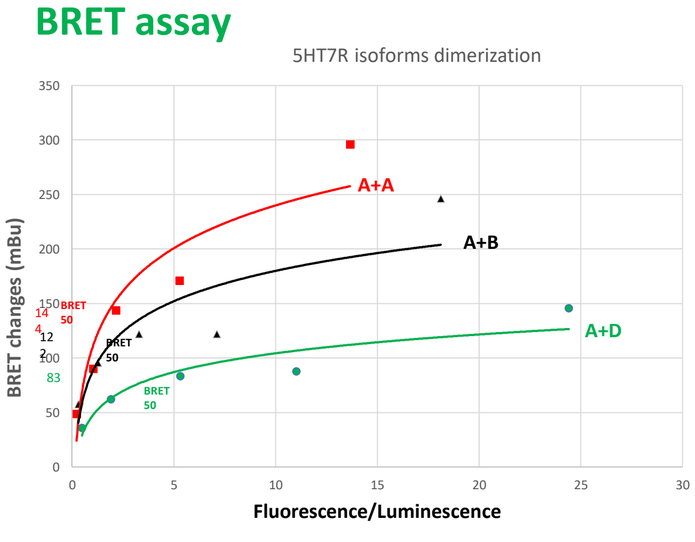
5HT7ARLuc+5HT7DYFP



3 isoforms



BRET assay



Mitigation of copper toxicity with vines coproducts-based biochar. Evaluation by percolation and phytotoxicity test.

Hugo Henaut [1], **Sylvain Bourgerie** [1], **Domenico Morabito** [1]

[1] University of Orleans, P2E, EA 1207, INRAe-USC1328, Orleans, France

Introduction

Background: The use of copper (Cu) in viticulture (especially $CuSO_4$) over decades has led to the accumulation of this toxic metal in soil. Copper can be phytotoxic, harmful to microfauna and microflora, and can eventually reach water resources, where it poses a risk to human health (*Macary, 2023*). Therefore, it has become necessary to limit its toxicity in vineyards, for example by reducing its bioavailability (the unbound fraction, which is accessible to organisms and susceptible to runoff). Biochar is a good candidate to solve this problem.

Biochar is a carbon-rich product, made by pyrolysis from organic biomass, that have high sorption capacities on trace elements (*Cao, 2019*) as well as agronomical benefits (*Bolan, 2024*). Viticulture produces many by-products such as pruning waste or pressing residues that are more or less used and need to be valorised (*Frikha, 2021*). In this study, we evaluate the potential of five different biochars, made from vine by-products to bound copper into the soil matrix (sorption) and limit its phytotoxicity.

Materials and Methods

1. Soil preparation

Vineyard soil collected in Bourgeuil, France

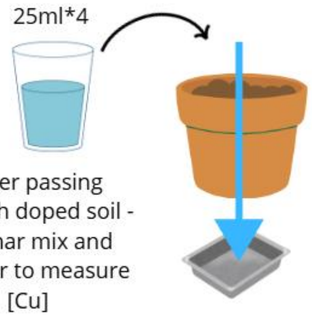
Soil dried, sieved and doped to 2000mgCu/kg, mix with differents biochar

2. Tested modalities

Modalities abbreviation	Mix content
T	Undoped soil (30mgCu/kgSoil)
2K	Doped soil (2000mgCu/kgSoil)
BM	Doped soil + Biochar of vine marc at 2 or 4% (w/w)
BP	Doped soil + Biochar of seeds at 2 or 4% (w/w)
BPE	Doped soil + Biochar of exhausted seeds at 2 or 4% (w/w)
BS1	Doped soil + Biochar of prunnings (1 pyrolysis) at 2 or 4% (w/w)
BS2	Doped soil + Biochar of prunnings (2 pyrolysis) at 2 or 4% (w/w)

3. Percolation experimentation

25ml*4



Water passing through doped soil - biochar mix and recover to measure [Cu]

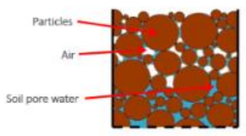
4. Phytotoxicity tests

Phaseolus vulgaris to grow during 20 days.

Organs collected after 20d and [Cu] measured

Aerial part lenght recorded after 20d

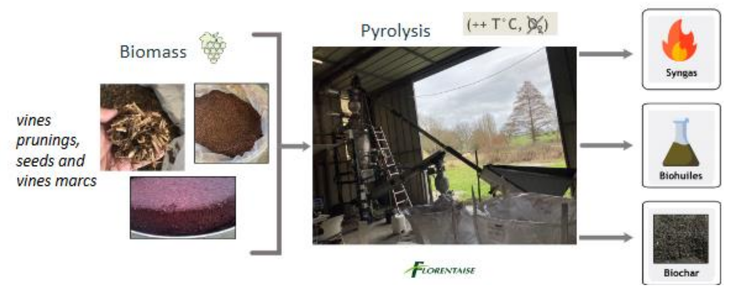
SPW collected after 20d and [Cu] measured



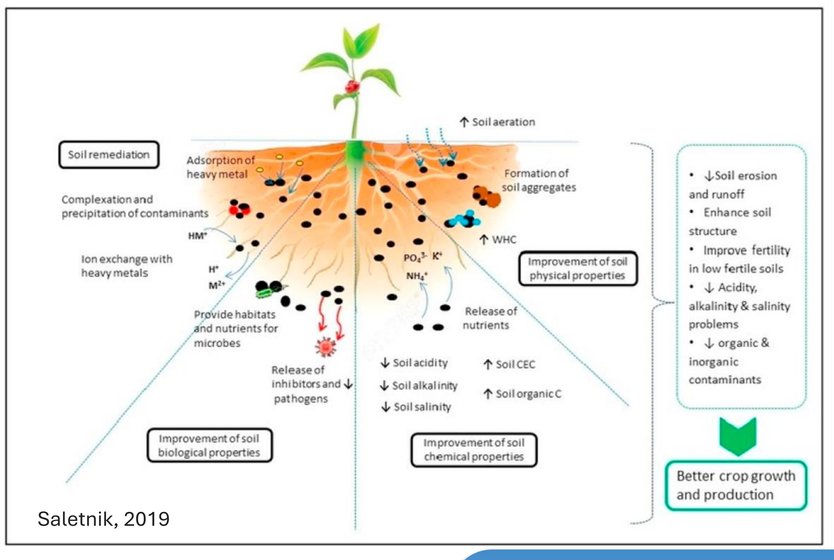
DW = Dry Weight; SPW = Soil Pore Water ; Letters indicates the level of significance *(α=5%)*

Focus on biochar

How is biochar produced ?



What are the main biochar properties when used as soil amendements ?



Saletnik, 2019

Results

1. Percolation experiment.

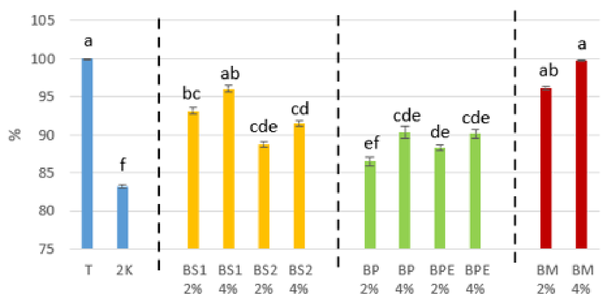


Figure 1: % of copper retained in various biochar amended soils.

T = undoped Bourgueil vineyard soil without biochar ; 2K = doped Bourgueil vineyard soil without biochar ; other traitements correspond to Bourgueil vineyard soil doped and amended with differents biochar at two doses (2 and 4% w/w), n=3.

The aim was to estimate Cu retention capacity of vineyards soils amended with various biochar at two applications doses :

- All modalities containing biochars (except BP at 2%) show significant higher Cu retention than control without biochar (2K).
- BM 2% & 4% and BS1 4% modalities show no significant differences compared with control (T) which demonstrate that almost **all Cu is maintain in the soil**.

Tested biochar help to **retain** Cu into the soil matrix used. These results can be explained by two main properties of biochar : (1) such amendment has the capabilities to increases soil pH and (2) Cu is less bioavailable in **alkaline** pH (*Trentin, 2022*). Biochars also adsorb trace elements such as Cu in their structure (*Nguyen, 2023*).

2. Phytotoxicity test using Phaseolus vulgaris – lenghts of aerial part

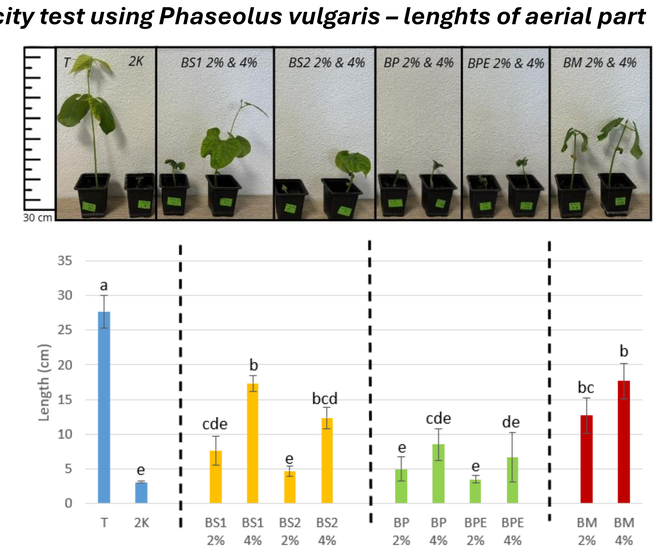


Figure 2: Lenghts (cm) of *Phaseolus vulgaris* aerial parts after growth on various biochar amended soils.

T = undoped Bourgueil vineyard soil without biochar ; 2K = doped Bourgueil vineyard soil without biochar ; other traitements correspond to Bourgueil vineyard soil doped and amended with differents biochar at two doses (2 and 4% w/w), n=3.

Soil amended with different biochars (at 2 doses) were tested for their ability to modulate *P. vulgaris* growth under Cu contaminated soil :

- Cu supply (2K) **decreased** *P. vulgaris* growth up to 6 times
- BS1, BS2, BP, BPE at 2% and BP, BPE at 4% didn't allow a significant better growth *of P. vulgaris* than control without biochar (2K).
- **BM 2% & 4% and BS1, BS2 4%** grow significantly more than control without biochar (2K), emphasizing the fact that these biochar help to mitigate Cu toxicity. Nevertheless, they didn't reach the growth of the undoped control (T).

Photos illustrate differences between modalities

3. Phytotoxicity test using Phaseolus vulgaris – [Cu] SPW

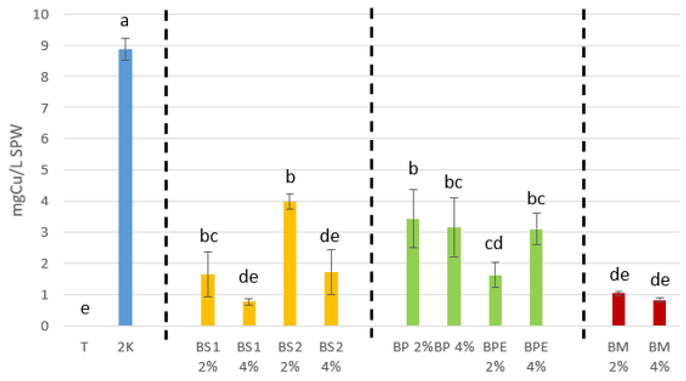


Figure 3: Copper concentration in SPW ($mg.L^{-1}$) from various biochar amended soils

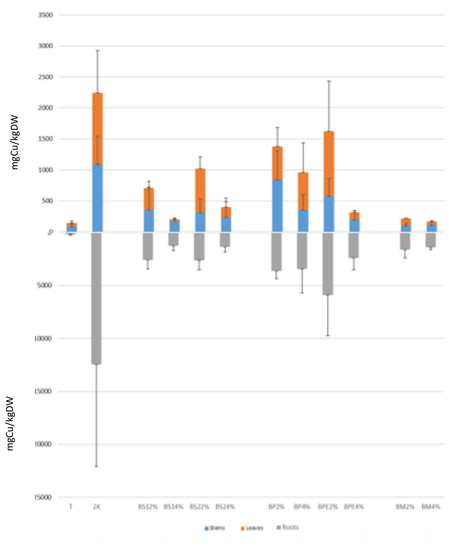
T = undoped Bourgueil vineyard soil without biochar ; 2K = doped Bourgueil vineyard soil without biochar ; other traitements correspond to Bourgueil vineyard soil doped and amended with differents biochar at two doses (2 and 4% w/w), n=3.

The aim was to evaluate biochars capacity to modulate Cu concentrations from **SPW** (fraction of Cu which is available to organisms and susceptible to runoff) :

- All modalities with biochar present a significant **lower [Cu]** than the control without biochar (2K).
- **BM and BS1/BS2 at 4%** show the lowest Cu concentration in the SPW.

Less Cu concentration in SPW suggests that more Cu is **retained** in the soil.

4. Phytotoxicity test using Phaseolus vulgaris – Cu uptake in organs



	Leaves	Stems	Roots
2K	a	a	a
T	d	c	c
BM2%	bcd	bc	b
BM4%	cd	bc	b
BP2%	abc	ab	ab
BP4%	abc	abc	ab
BPE2%	ab	abc	ab
BPE4%	bcd	bc	ab
BS12%	abcd	abc	ab
BS14%	d	bc	b
BS22%	abc	abc	ab
BS24%	bcd	bc	b

Letters indicates the differences between modalities for each organs (α=5%)

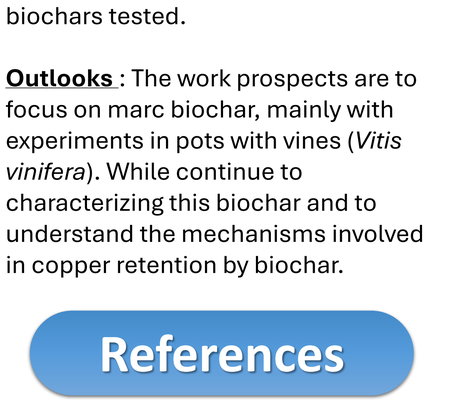
Figure 4: Copper concentration (mg/kgDW) for each *Phaseolus vulgaris* organs having grown on various biochar amended soils.

T = undoped Bourgueil vineyard soil without biochar ; 2K = doped Bourgueil vineyard soil without biochar ; other traitements correspond to Bourgueil vineyard soil doped and amended with differents biochar at two doses (2 and 4% w/w), n=3 .

Cu **uptake** by *P. vulgaris* was measured in aerial and roots parts of plant to evaluate biochars effect on Cu concentrations and distribution in plant organs :

- Cu accumulates mainly in the **roots**, as described in *Wang, 2024*.
- For BP, at 2 and 4% and BS1, BS2 at 2%, Cu accumulation wasn't significantly different in each organ than in control without biochar (2K).
- For each organs, **BM 2% & 4% and BS1 4%** shows the lower Cu accumulation, sometimes reaching control (T) level.

Materials used



Raw biomass (top) and biochar associated (botom) used in this study.

Conclusion

Conclusion : biochars tested allow to maintain copper in the soil with moderate leaching (*Figure 1*) while reducing the fraction of copper present in the pore water of the soil (*Figure 3*). Biochars used also reduce copper toxicity towards P. vulgaris by reducing Cu levels in organs (*Figure 4*) and allowing a better growth (*Figure 2*). Finally, the **biochar of marc** seems to be the best by-products among all biochars tested.

Outlooks : The work prospects are to focus on marc biochar, mainly with experiments in pots with vines (*Vitis vinifera*). While continue to characterizing this biochar and to understand the mechanisms involved in copper retention by biochar.

References

- *Bolan et al. (2024)*
- *Cao et al. (2019)*
- *Frikha et al. (2021)*
- *Macary et al. (2023)*
- *Nguyen et al. (2023*
- *Saletnik et al. (2018)*
- *Trentin et al. (2022)*
- *Wang et al. (2024)*

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VALORIZATION OF THE PROTECTED SPECIES *DROSERA ROTUNDIFOLIA* VIA *IN VITRO* CULTURE AND ENHANCED METABOLITE PRODUCTION FOR COSMETIC APPLICATIONS

Malorie Laffon[1,2], Christophe Hano[3], Franck Michoux[2], Clément Lemoine[2], Arnaud Lanoue[1], Nathalie Giglioli-Guivarc'h[1]

[1]University of Tours, EA2106 Plant Biomolecules and Biotechnology, Faculty of Pharmacy, 31 avenue Monge, 37200 Tours, France
[2]Evonik Advanced Botanicals, 220 rue Henri Potez, 37210 Parçay Meslay, France
[3]University of Orléans, UMR7311 ICOA, Chemistry department, Rue de Chartres, 45100 Orléans, France

*Corresponding author e-mail: malorie.laffon@etu.univ-tours.fr

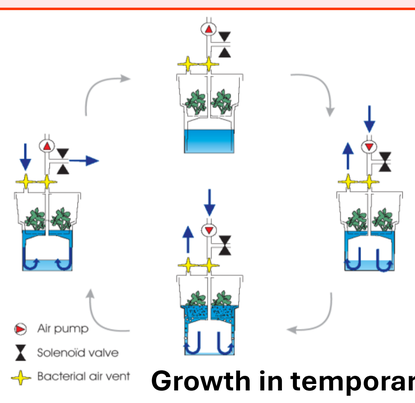
INTRODUCTION

Drosera rotundifolia, the round-leaved sundew, is a carnivorous plant from the *Droseraceae* family. This species is distributed in the northern hemisphere and found in shady areas of acidic peat bogs. Growing on low nitrogen soils, its growth is slow and requires an external intake of nitrogen from insects that are trapped on the mucilages produced at the surface of the glandular trichomes.

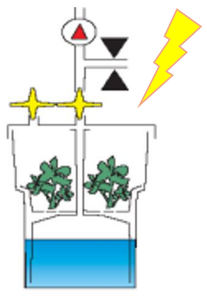
D. rotundifolia is rich in 1,4 naphtoquinones, specialized metabolites known for their antioxidant, anti-inflammatory and antimicrobial activities.
However, it is an endangered species in several French regions, and thus benefits from the status of protected species in France, making its harvest and commercialization forbidden in the country. To overcome these challenges, *in vitro* plant cultures have been developed to ensure sterile environment, controlled conditions and minimal use of space, making it a sustainable option for the study of this species.
This work led to the development of the first *D. rotundifolia* cultures in temporary immersion bioreactors for the valorization of specialized metabolites with industrial purposes.

METHODS

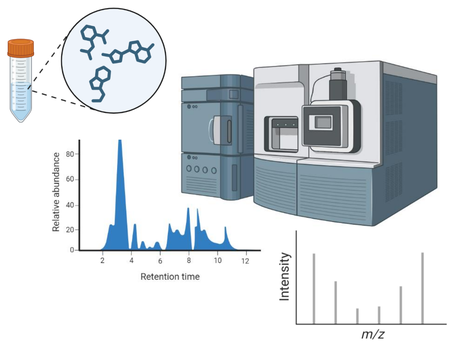
***In vitro* cultures initiation and multiplication in Magenta box**



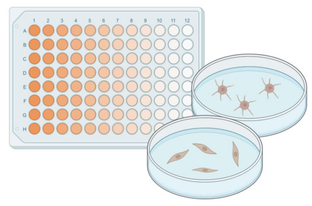
Growth in temporary immersion bioreactors (RITA®)



Elicitation experiments (biotic and abiotic elicitors, signal molecules)



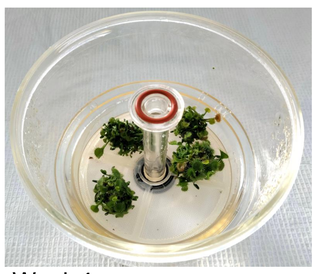
Extraction and metabolic profiling by UPLC-MS



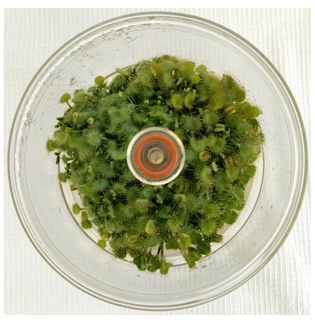
Biological activity tests (keratinocytes, fibroblasts, melanocyres and epithelial cell lines and cell free systems)

GROWTH IN BIOREACTORS

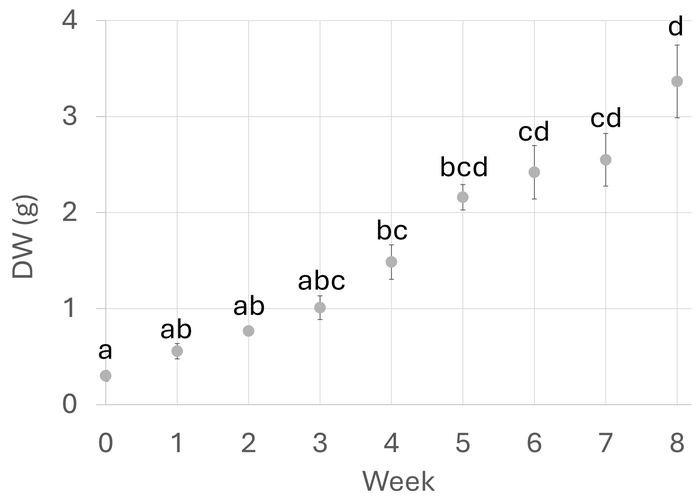
- Evaluation of biomass growth for 8 weeks



Week 1



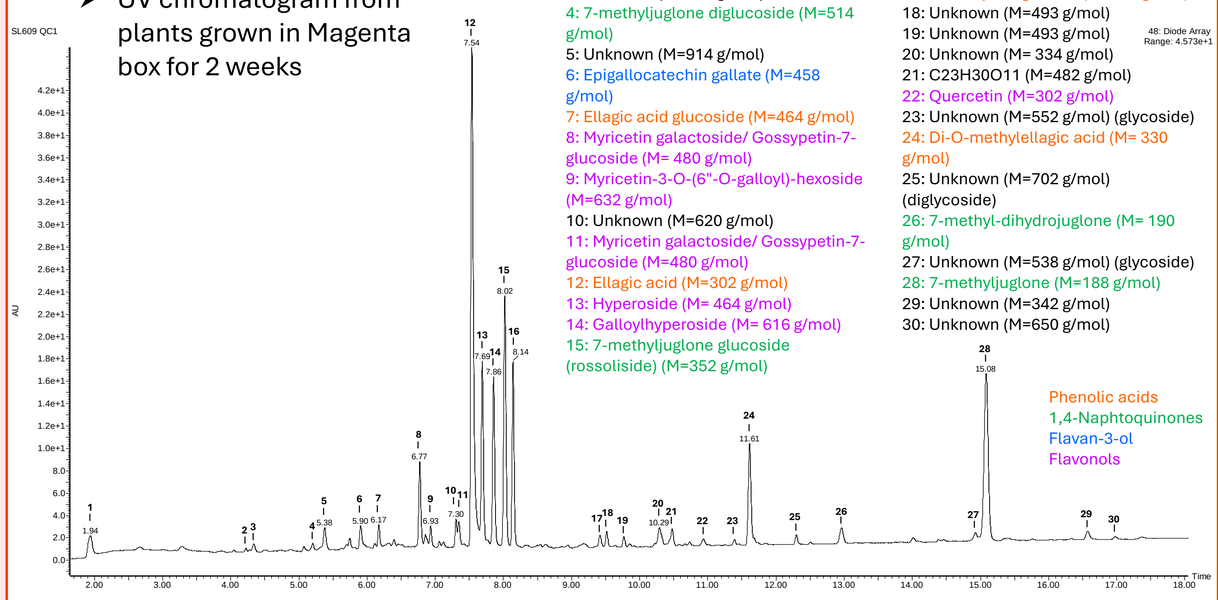
Week 8



→ **11-fold increase in biomass dry weight**

METABOLIC PROFILING

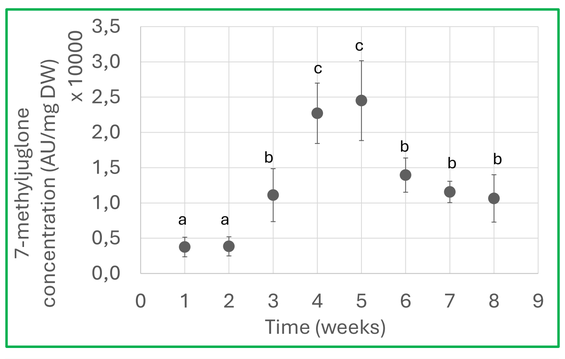
- UPLC-DAD-ESI-TQD analysis
- UV chromatogram from plants grown in Magenta box for 2 weeks

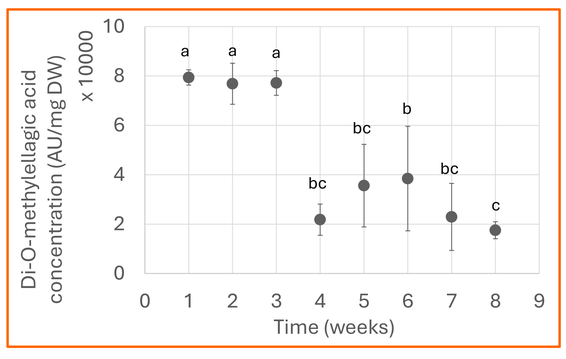


1: Gallic acid (M=170 g/mol)
2: C18H22O10 (M=398 g/mol)
3: Unknown (M=762 g/mol)
4: 7-methyljuglone diglucoside (M=514 g/mol)
5: Unknown (M=914 g/mol)
6: Epigallocatechin gallate (M=458 g/mol)
7: Ellagic acid glucoside (M=464 g/mol)
8: Myricetin galactoside/ Gossypetin-7-glucoside (M= 480 g/mol)
9: Myricetin-3-O-(6"-O-galloyl)-hexoside (M=632 g/mol)
10: Unknown (M=620 g/mol)
11: Myricetin galactoside/ Gossypetin-7-glucoside (M=480 g/mol)
12: Ellagic acid (M=302 g/mol)
13: Hyperoside (M= 464 g/mol)
14: Galloylhyperoside (M= 616 g/mol)
15: 7-methyljuglone glucoside (rossoliside) (M=352 g/mol)
16: Di-O-methylellagic acid glycoside (M= 492 g/mol)
17: 3-Methylellagic acid (M=316 g/mol)
18: Unknown (M=493 g/mol)
19: Unknown (M=493 g/mol)
20: Unknown (M= 334 g/mol)
21: C23H30O11 (M=482 g/mol)
22: Quercetin (M=302 g/mol)
23: Unknown (M=552 g/mol) (glycoside)
24: Di-O-methylellagic acid (M= 330 g/mol)
25: Unknown (M=702 g/mol) (diglycoside)
26: 7-methyl-dihydrojuglone (M= 190 g/mol)
27: Unknown (M=538 g/mol) (glycoside)
28: 7-methyljuglone (M=188 g/mol)
29: Unknown (M=342 g/mol)
30: Unknown (M=650 g/mol)

→ **30 compounds detected and 17 identified**
→ **13 unknown compounds in *Drosera rotundifolia* extracts**

METABOLITE CONTENT DURING GROWTH





→ **Glycosylation of 7-methyljuglone after the 4th week**

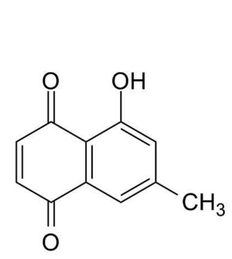
→ **Glycosylation of di-*O*-methylellagic acid after the 4th week**

METABOLITE PRODUCTION : BIOREACTOR VS GREENHOUSE PLANTS

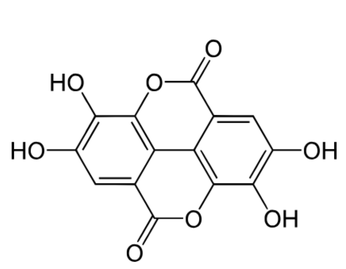
- Quantification in temporary immersion bioreactors after 8 weeks of growth
- Ethanolic extracts

Compound	Plants in temporary immersion bioreactors (mg/g DW)	Greenhouse plants* (mg/g DW)
7-methyljuglone	114,14 ± 32,56	0,63
Ellagic acid	81,79 ± 6,69	4,68
Hyperoside	45,23 ± 4,46	12,62

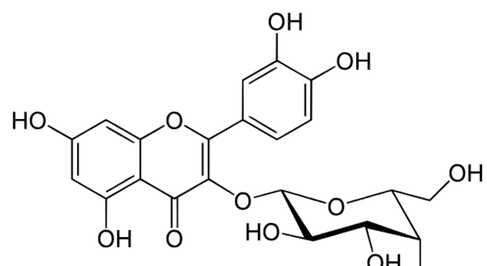
* Krenn et al., 2004



7-methyljuglone



Ellagic acid



Hyperoside

→ **Higher metabolite production for plants grown in bioreactors compared to greenhouse plants**

CONCLUSIONS

→ High growth rate : optimisation of *in vitro* culture (culture medium and subculture technique)
→ First metabolic profiling of temporary immersion cultures of *D. rotundifolia* :
- 17 compounds identified using literature data and pure standards
- 13 unknown compounds in *D. rotundifolia* extracts
- Absence of plumbagin (2-methyljuglone), a major compound in wild plants

→ Kinetic studies of plant growth and metabolites production in bioreactors
→ Highest production of 7-methyljuglone, ellagic acid and hyperoside in bioreactors than in greenhouse
→ No specialized metabolites induction with first elicitation experiments (data not shown)

PERSPECTIVES

→ Quantification of major compounds
→ HRMS analysis for identification of unknown peaks
→ Elicitation experiments
→ Evaluation of biological activity (*in vitro* and *ex vivo* tests with cosmetic targets)
→ Toxicity tests

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