



Identification of natural bioactive compounds to promote the biodiversity of French Riviera

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What is biodiversity?


- Neologism proposed at UN Earth Summit of Rio de Janeiro in 1992



<http://www.mangerdumiel.com/?p=967>

- **Biodiversity** (also called **biological diversity**) is the degree of variation of life forms within a given ecosystem, biome, or an entire planet

Biodiversity, a great chance of development for the région Provence-Alpes-Côte d'Azur (PACA)



- The Mediterranean Basin is one of the 34 biodiversity « **hot spots** ».
- The PACA region is situated at a phytogeographical corner between the Alps and the Mediterranean Sea.



<http://villageampus83.blog.lemonde.fr/2009/10/29/paca-ou-un-sautre-norm/>

- ✓ The PACA region contains **2/3 of the biodiversity of Metropolitan France.**
- ✓ In particular, the Maritime Alps are **the most important endemic center** of the whole Alps.

✓ Main research topics

- Analysis of natural extracts (historically great expertise in the field of Flavors and Fragrances)
- Discovery of new molecules from natural extracts (plants, flowers, fruits, roots,...)
- Focused properties:
 - organoleptic (odor, flavor)
 - bioactive (antioxidant, antimicrobial)

✓ Our work strategy is always divided into three steps

1. Extraction
2. Fractionation (purification)
3. Characterization (identification)

✓ Presentation of two current projects



Solidago

NATUBAVAL
Naturel Bactéricide Valorisation

1. Experimental methodology to isolate bioactive compounds from natural matrices

1. Extraction

= enrichment

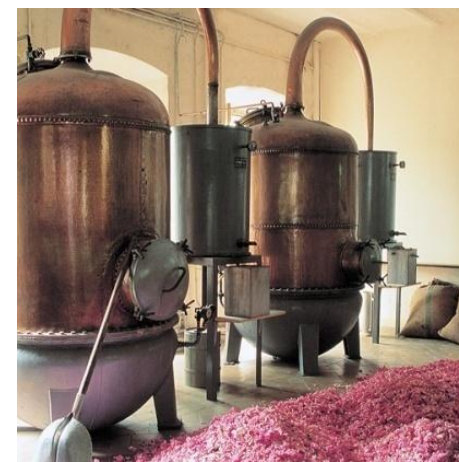
- Decoction in various solvents (water, alcohol...)
- Soxhlet
- Hydrodistillation
- ...

Infusion



<http://olharfeliz.typepad.com/cuisine/2007/08/index.html>

Distillation



<http://fabricationdesparfums.com/?p=51>

1. Experimental methodology to isolate bioactive compounds from natural matrices

1. Extraction

= enrichment

- Decoction in various solvents (water, alcohol...)
- Soxhlet
- Hydrodistillation
- ...

2. Fractionation = isolation

- Ultrafiltration
- Ion-exchange resins
- SPE
- High Performance Liquid Chromatography (HPLC)

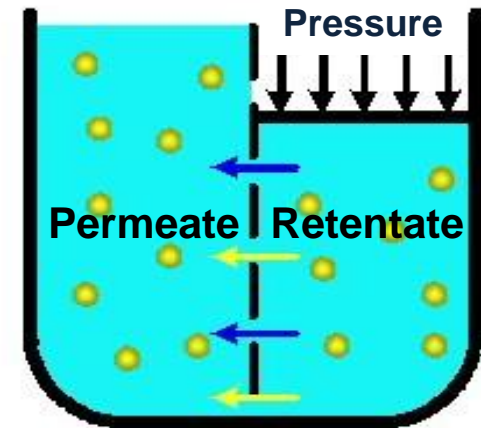
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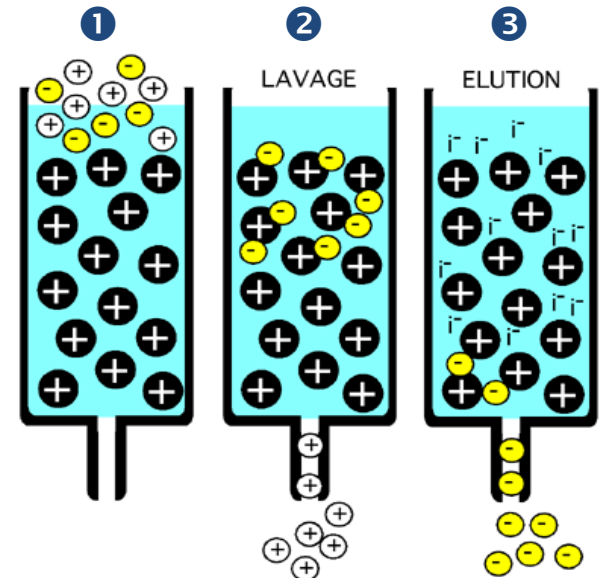
Ultrafiltration

Separation based on size



Membrane

Ion-exchange
Separation based on electrical charge



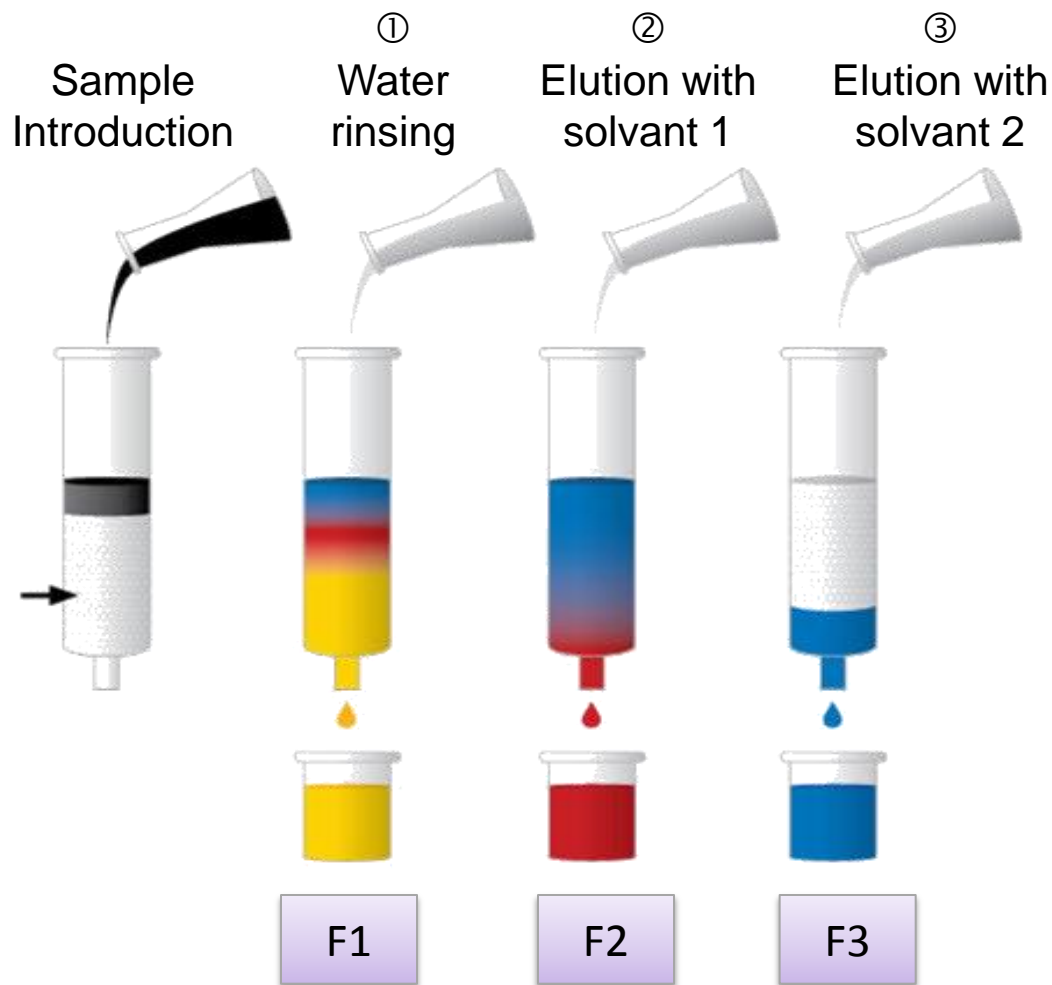
1. Experimental methodology to isolate bioactive compounds from natural matrices

2. Fractionation = isolation

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Solid-Phase Extraction (SPE)

Separation based on chemical affinity



1. Experimental methodology to isolate bioactive compounds from natural matrices

1. Extraction

= enrichment

- Decoction in various solvents (water, alcohol...)
- Soxhlet
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- ...

2. Fractionation

= isolation

- Ultrafiltration
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- High Performance Liquid Chromatography (HPLC)

3. Identification

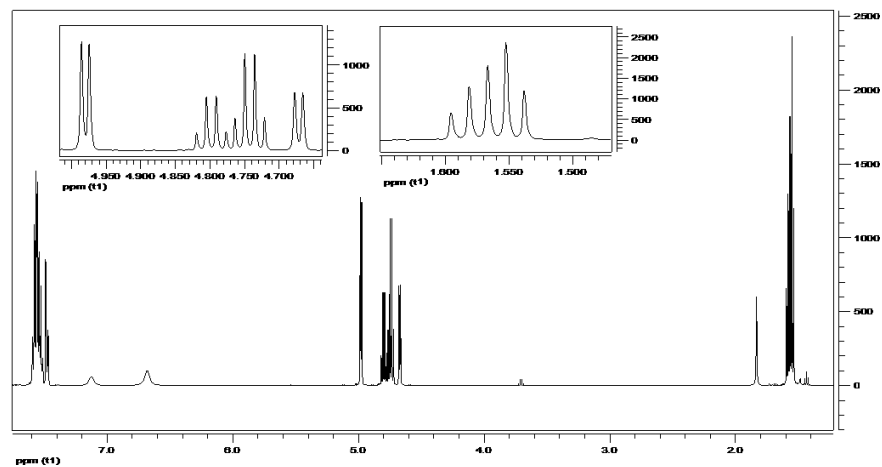
- Nuclear Magnetic Resonance (NMR)
- Infrared
- Mass spectroscopy

1. Experimental methodology to isolate bioactive compounds from natural matrices

3. Identification

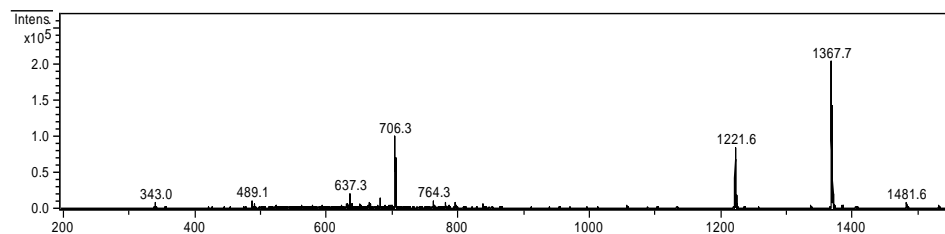
- Nuclear Magnetic Resonance (NMR)
- Infrared
- Mass spectroscopy

NMR: Atom linking and environment



Mass spectrometry (MS):

Molecular mass of the compound and raw formula



Presentation of 2 current projects aiming at promoting local biodiversity

Solidago

- Identification of new bioactive compounds dedicated to oral care.
- Financial support from the Local Council of Maritimes Alpes



- Collaborations:
 - Laboratory of Oral Microbiology (LOM)
 - Solidages



NATUBAVAL

Naturel Bactéricide Valorisation

- Development of original and natural preservatives for cosmetics and nutraceuticals
- Labelled by the PASS Cluster (APRF 2009)

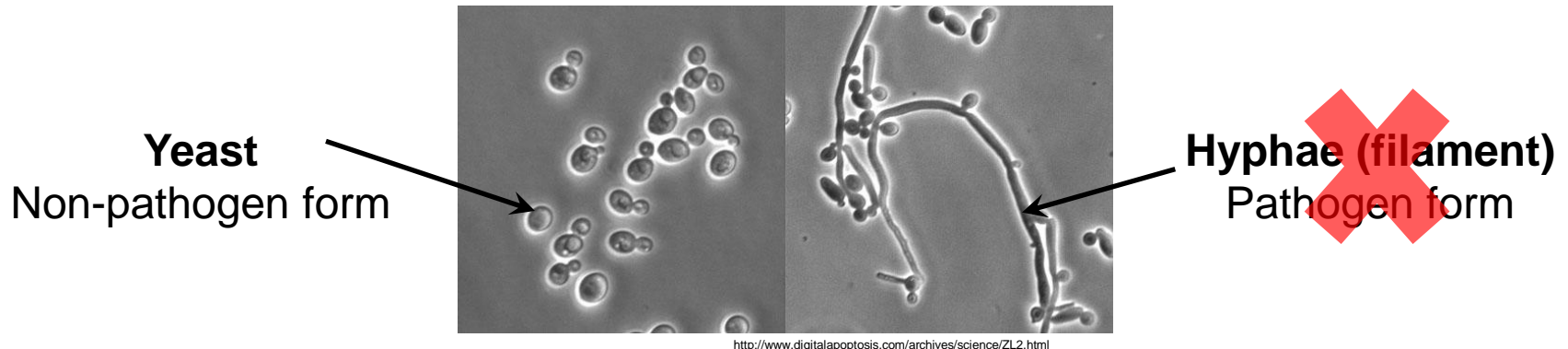


- Collaborations :
 - Sofia Cosmétiques
 - NATUREX



2. Solidago Project: Introduction

- ✓ Project launched in January 2010 (PhD thesis of Lise Laurençon)
 - ✓ Development of a mouthwash to prevent senior and medicated patients from candidosis
- ⇒ **Objective:** Inhibiting the pathogen form (hyphae) of *Candida albicans* while preserving its commensal form (yeast-like), as well as the other bacteria of the oral biofilm.



- ⇒ A local plant ***Solidago virgaurea*** (Asteraceae) was shown to present interesting results and was selected to be further investigated.

2. Solidago Project: *Solidago virgaurea* subspecies



Solidago virgaurea virgaurea

- Height: up to 1m
- Ramified floral scape
- Europe (0 to 1500 m)



Various publications

+ Phytotherapy patents:

Antiviral (H1N1),
coagulative, fungicide,
natural latex (cosmetics) ...



Solidago virgaurea alpestris

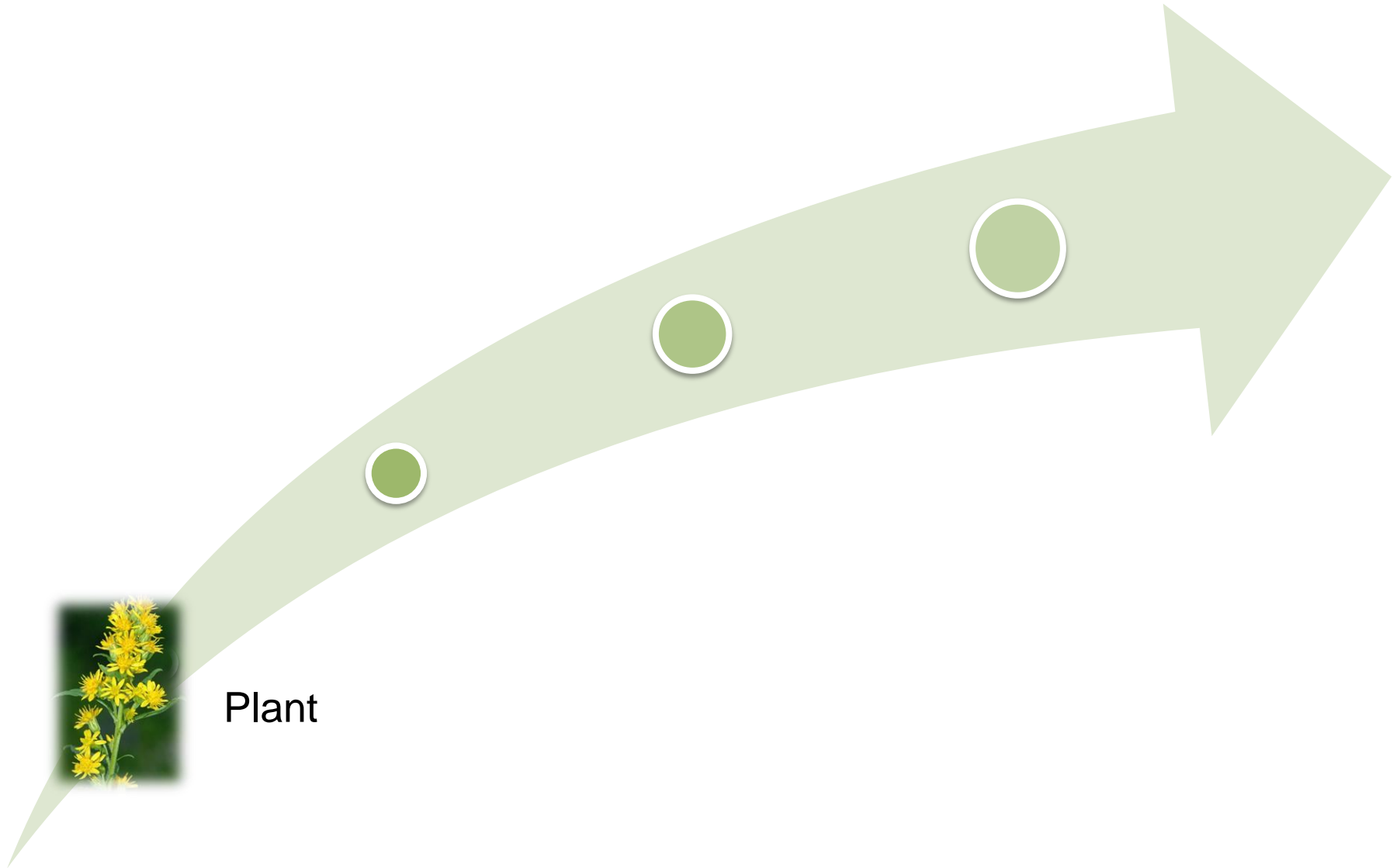
- Height: up to 40cm
- No ramification
- Alpine areas (1600 to 2700 m)



**No phytochemical study
But plant available in the
Maritim Alps**

2. Solidago Project: Experimental Protocol Performed

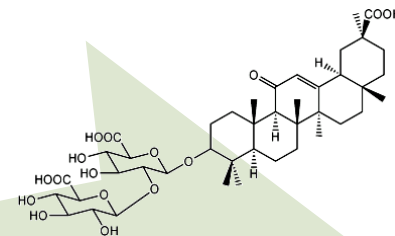
⇒ Fractionation step-by-step to isolate the active molecules



2. Solidago Project: Experimental Protocol Performed

⇒ Fractionation step-by-step to isolate the active molecules

Characterization of the chemical composition of the fractions using HPLC (High Performance Liquid Chromatography)



Decoction



Raw aqueous extract

Fractionation



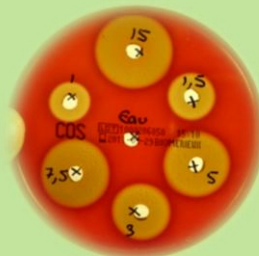
Active fraction

HPLC prep



Pure active compounds

Plant



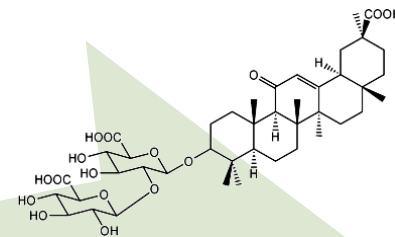
Bioassays after 24h and 48h incubation:

- Hemolysis in agarose gel
- Counting of *Candida* colonies
- Study of the percentage of *Candida* filament formed

2. Solidago Project: Experimental Protocol Performed

⇒ Fractionation step-by-step to isolate the active molecules

Characterization of the chemical composition of the fractions using HPLC (High Performance Liquid Chromatography)



Decoction



Raw aqueous extract

Fractionation



Active fraction

HPLC prep



Pure active compounds

Identification in progress



Optimization of the extraction process

Plant





✓ Project launched in January 2011
(PhD Theses of Florence Merck and Audrey Kerdudo)

✓ Inventory of the medicinal and aromatic plants of the PACA region and screening for bioactive plants
(preservative: antimicrobial, and/or antioxydant)

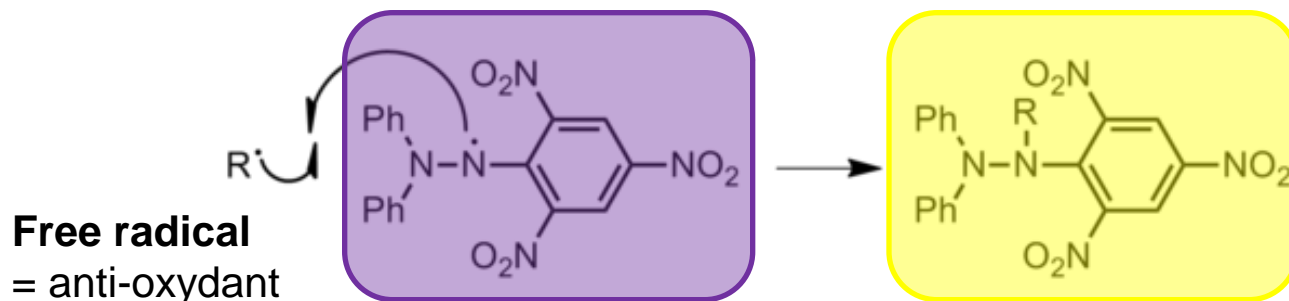
⇒ **Objective:** identify original natural bioactive compounds in order to propose new preservatives for cosmetics and nutraceuticals.

⇒ **Two activity tests:**

- chemical : focused on **antioxydant activity**
- microbiological: **antimicrobial activity**

3. NATUBAVAL Project: Screening for antioxidant compounds

Colorimetric test using **DPPH** (2,2-diphenyl-1-picrylhydrazyl)



Experimental strategy:

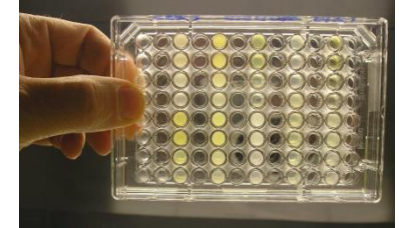
1. Screening for anti-oxydant extracts using DPPH test
2. Fractionation guided by DPPH test
3. Isolation of the active compounds



3. NATUBAVAL Project: Screening for antimicrobial compounds

✓ 4 microorganisms selected from European Pharmacopoeia

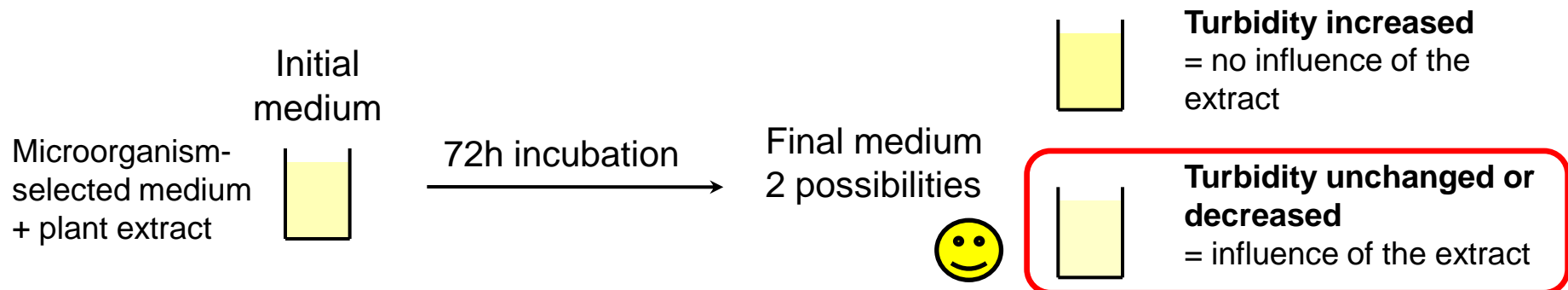
- *Aspergillus niger* (fungus)
- *Candida albicans* (yeast)
- *Staphylococcus aureus* (bacteria Gram +)
- *Pseudomonas aeruginosa* (bacteria Gram -)



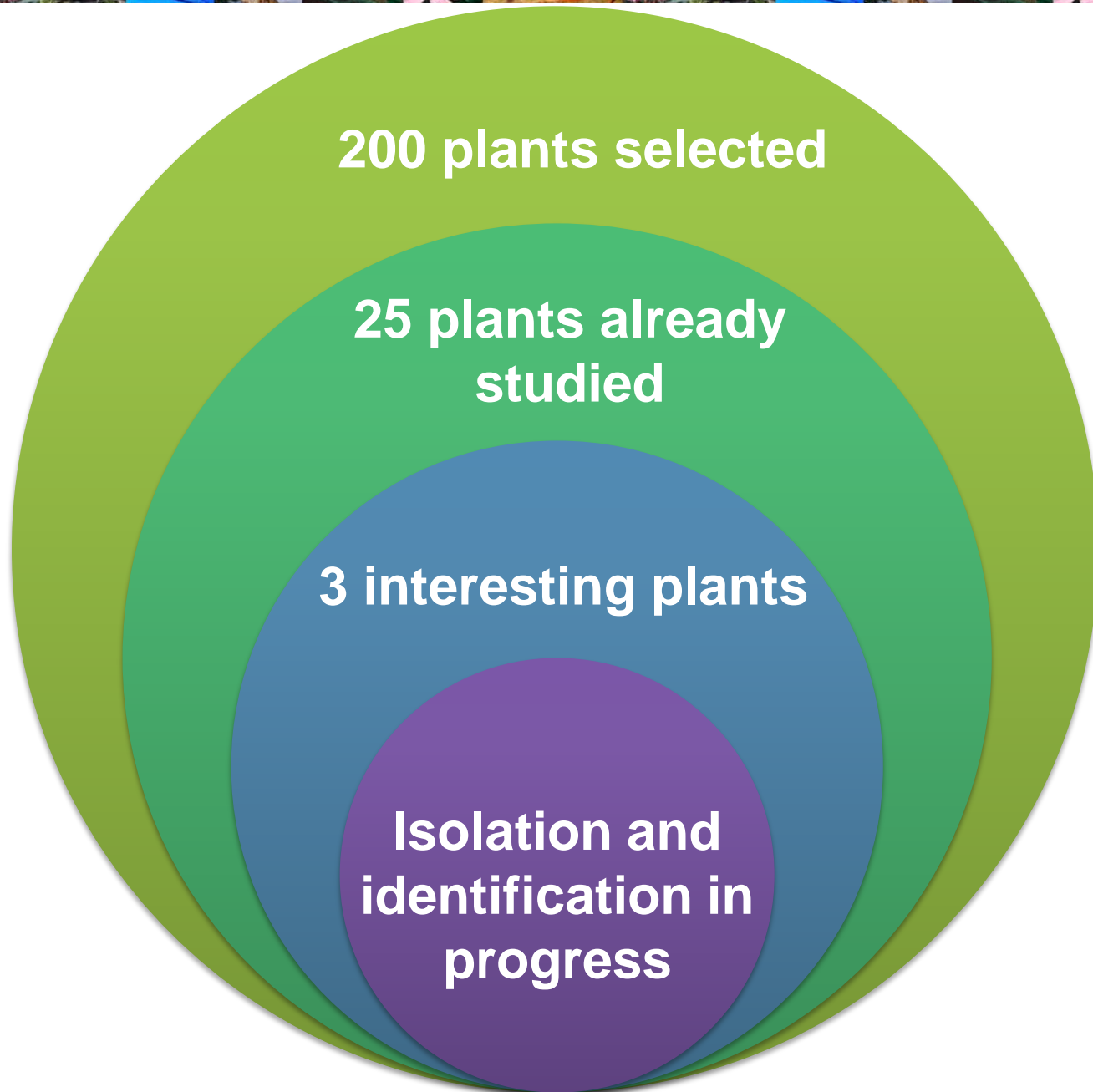
Picture from BioPreserv

✓ Dilution method:

- Homogenous dispersion of the plant extract in a microorganism-selected medium using 96-well plates.
- Turbidity taken as an indicator of bacterial density.
- Turbidity measured after 24h, 48h and 72h of incubation
- Grade of microbial inhibition related to the turbidity of the medium and measured by spectrophotometry



3. NATUBAVAL Project: work in progress





*Nature is still a great source
of inspiration for the
development of new bioactive
ingredients*



Aknowledgements

- Université de Nice Sophia Antipolis
- CNRS



Public
Institutions





- SOFIA Cosmétiques
- Naturex  Ultimate Botanical Benefits
- Acphytaroma 
- Biopreserv 
- Nixe

Natubaval
Project

Local
Authorities


Solidago
Project



- Pole PASS
- Conseil Général des Alpes Maritimes 
- Conseil Régional PACA 

CONSEIL GENERAL
DES ALPES-MARITIMES



- Solidages
- Laboratoire de Microbiologie Orale
- Biophyto 



THANK YOU!

ΕΥΧΑΡΙΣΤΟ!

