



# CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF FRENCH BFA PROPOLIS EXTRACTS

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Ms Catherine FLURIN



# INTRODUCTION

## PROPOLIS

- **Complex resinous product** collected by honeybees from **buds**, leaves and exudates from **various trees** and plants mixed with beeswax and salivary secretions
- Two major classes\* of propolis:
  - **European-type** (poplar type): rich in flavonoids as well as phenolic acids and their esters (Europe, China and other countries)
  - **Tropical-type** (Brazilian-type): rich in terpenic and prenylated derivatives
- Used inside the hive:
  - to seal the walls, to reduce the entry of the hive
  - as a « chemical weapon » against microorganisms
- Used in folk medicine as:  
**antioxidant**, antifungal, antibacterial, antiviral, anti-inflammatory...



© Sonas



© Ballot-Flurin

### Ballot-Flurin Apiculteurs (BFA)

- Company (**SME**) located in the South-West of France
- Pionner in France in **organic beekeeping**
- **Expert in natural health**
- Inventor of the **Apiculture douce®** (Gentle beekeeping) based on the language of bees and a deep respect for nature
- **More than 60 effective products** (Apitherapy and Apicosmetic products) of the **highest quality with NO side effects:**
  - 100% harmless, proved under medical control
  - Efficient
  - With 0% contaminant, proved by analysis

➡ **Collaboration with the faculty of pharmacy of Angers, France:**

- **PhD** : Chemical characterization and biological activities of propolis extracts

## I. Chemical composition

- 1) Propolis sample
- 2) Extractions
- 3) Identification
- 4) Quantification

## II. Antioxidant activity

- 1) Total polyphenol content
- 2) DPPH assay
- 3) ORAC assay

Conclusion

# I. CHEMICAL COMPOSITION

# I. CHEMICAL COMPOSITION

## 1) PROPOLIS SAMPLE

### Mixture of 24 propolis samples from:

- different regions of France, predominantly in the **South-West**
- 2010 and 2011



Collection sites of the French BFA propolis samples

# I. CHEMICAL COMPOSITION

## 2) EXTRACTIONS

Five extractions with different solvents:

### Protocol n° 1

Water

H<sub>2</sub>O,  
15min,  
100° C

**Aqueous  
extract**

7% yield

Residue  
+ waxes

65-68%  
yield

### Protocol n° 2

70% EtOH, MeOH

70% EtOH  
or MeOH  
3x2h, 25° C

**Alcoholic  
extract +  
waxes**

Residue

-18° C over  
night

**70% EtOH  
or MeOH  
extract**

Waxes

### Protocol n° 3

DCM, Mixed solvents\*

Cyclohexane  
3x2h, 25° C

Cyclohexanic  
ext.: waxes

**Residue**

DCM or mixed  
solvents  
3x2h, 25° C

**DCM or Mixed  
solvents  
extracts**

Residue

50-59% yield

7

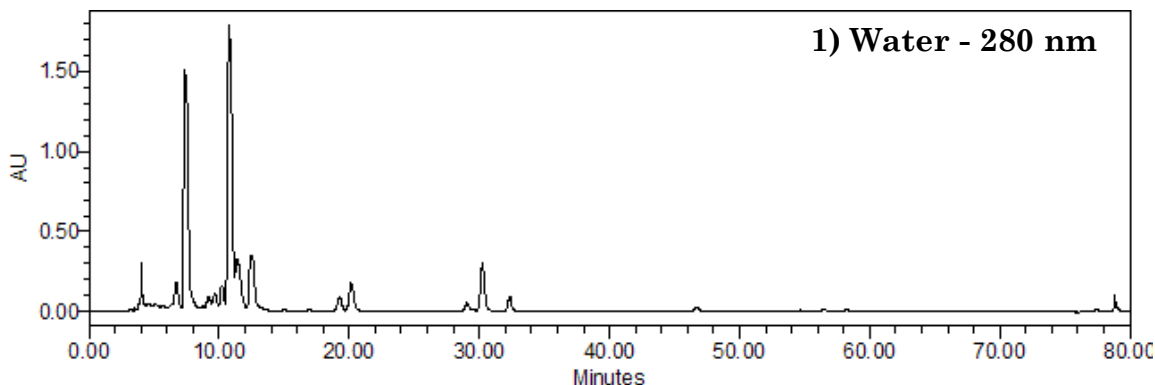
\*DCM: dichloromethane; Mixed solvents: DCM/MeOH/H<sub>2</sub>O 31/19/4

# I. CHEMICAL COMPOSITION

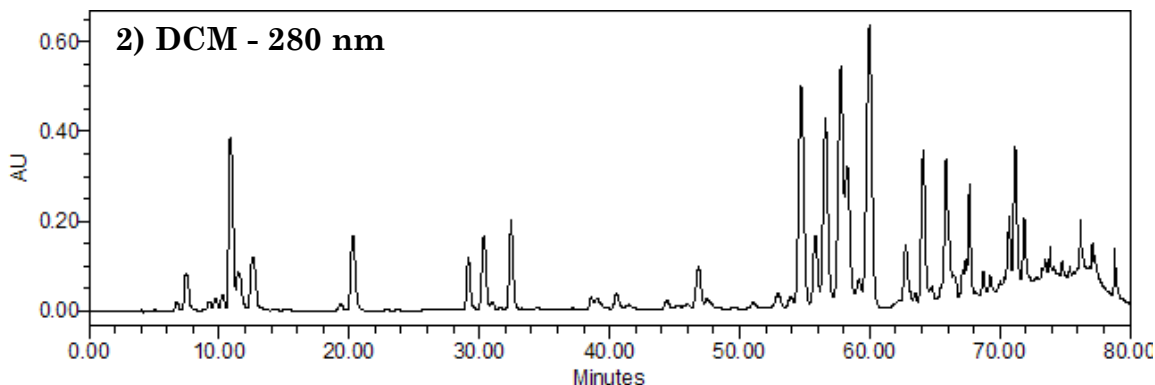
## 3) HPLC/DAD PROFILES

### Two types of HPLC/DAD profiles:

➤ **Aqueous type**



➤ **Alcoholic, DCM and mixed solvents type**



**Conditions:** HPLC system: Waters 2695 with DAD detector 2996, Phenomenex Luna C18 (150x4.6 mm) 3 $\mu$ m column, injection 10 mg/mL, flow rate 0.4 mL/min, mobile phase: H<sub>2</sub>O + 0.1% formic acid (A) et MeOH + 0.1% formic acid, gradient elution: 40%B (0-10min), 40-50%B (10-25min), 50-60%B (25-55min), 60-90%B (55-70min), 90%B (70-80min)



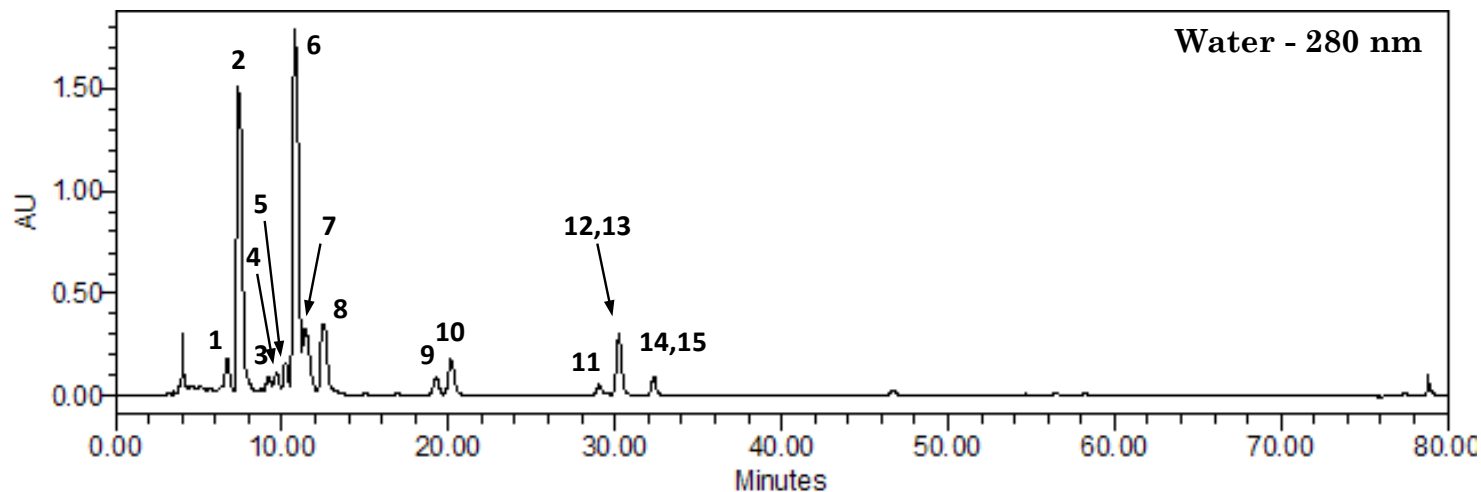
# I. CHEMICAL COMPOSITION

## 3) IDENTIFICATION

### Chemical characterization

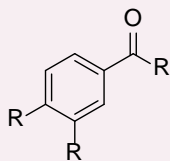
- **Fractionation by Flash chromatography**
- **HPLC/UV**
- **HPLC/MS**
- **Scientific literature**
- **Commercial standards**
- **$^1\text{H}$  and  $^{13}\text{C}$  NMR (1 and 2 dimensions)**

### 1) Aqueous extract



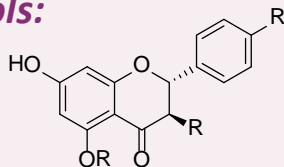
#### ○ *Benzaldehyde and benzoic acid derivatives:*

- 1 3,4-dihydroxybenzaldehyde
- 3 4-hydroxybenzaldehyde
- 4 Vanilline
- 5 4-Hydroxyacetophenone
- 9 Benzoic acid



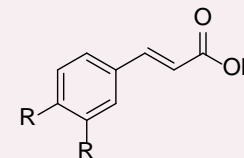
#### ○ *Flavanones/Dihydroflavonols:*

- 11 Pinobanksin-5-methyl ether
- 14 Pinobanksin
- 15 Naringenin

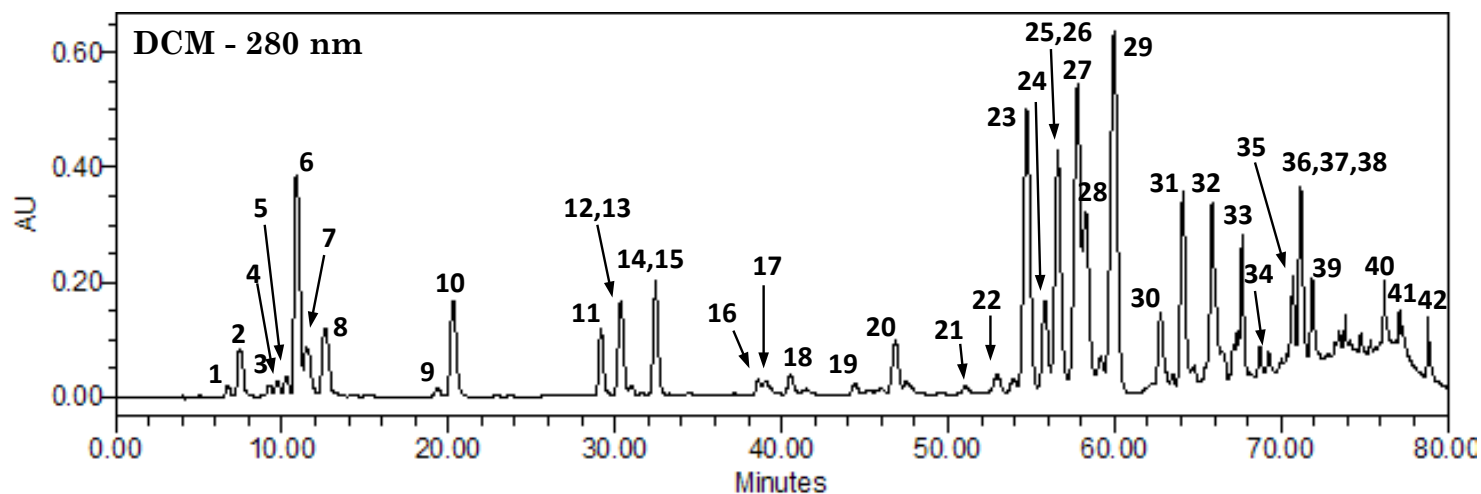


#### ○ *Cinnamic acid derivatives:*

- 2 Caffeic acid
- 6 *p*-coumaric acid
- 7 Ferulic acid
- 8 Isoferulic acid
- 10 3,4-dimethoxycinnamic acid
- 12 Cinnamic acid
- 13 4-methoxycinnamic acid

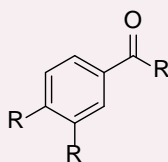


### 2) Alcoholic, DCM and mixed solvents extracts



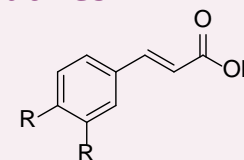
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- 5 4-Hydroxyacetophenone
- 9 Benzoic acid

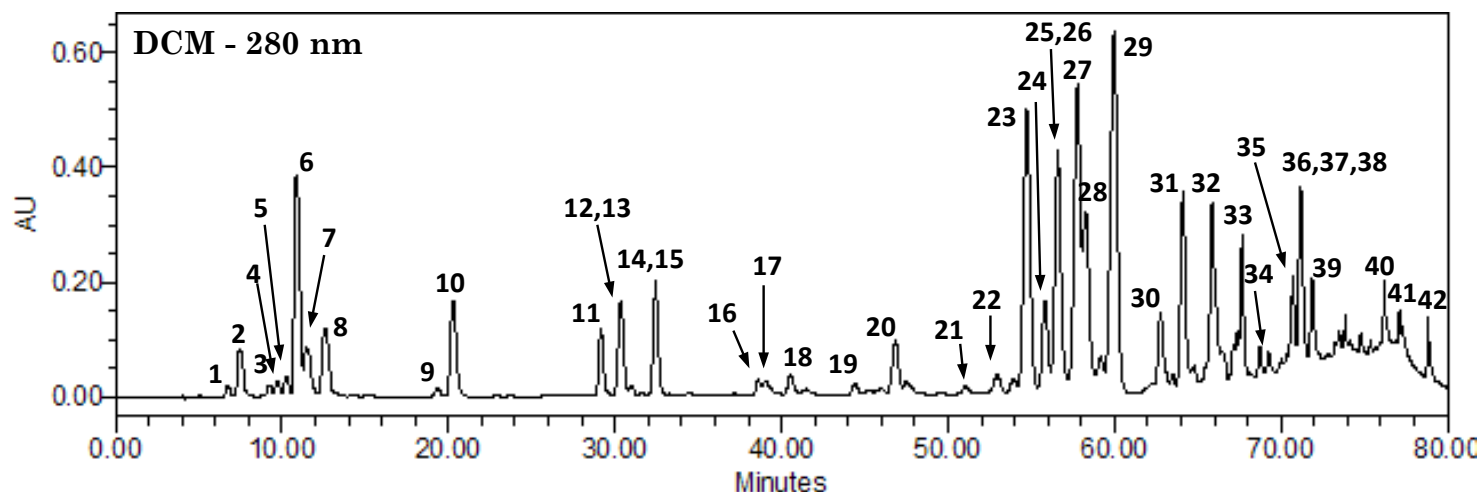


#### ○ *Cinnamic acid derivatives:*

- 2 Caffeic acid
- 6 *p*-coumaric acid
- 7 Ferulic acid
- 8 Isoferulic acid
- 10 3,4-dimethoxycinnamic acid
- 12 Cinnamic acid
- 13 4-methoxycinnamic acid
- 20 Cinnamylidene acetic acid

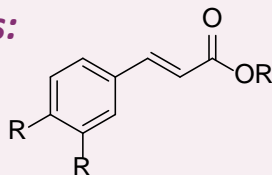


### 2) Alcoholic, DCM and mixed solvents extracts



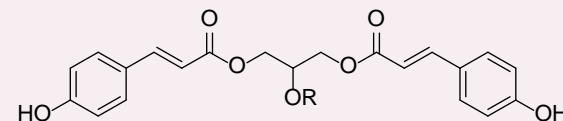
#### ○ *Cinnamic ester derivatives:*

- 24 Isopent-3-enyl caffeate
- 25 Benzyl caffeate
- 28 Prenyl caffeate
- 30 Caffeic acid phenylethyl ester (CAPE)
- 32 Benzyl *p*-coumarate
- 33 Cinnamyl caffeate
- 36 Cinnamyl isoferulate
- 37 Cinnamyl *p*-coumarate
- 40 Benzyl cinnamate
- 41 Cinnamyl cinnamate
- 42 Cinnamyl cinnamylidene acetate



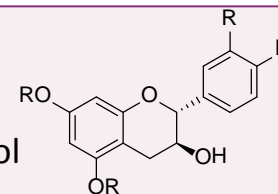
#### ○ *Glycerol derivatives:*

- 16 1,3-dicoumaroylglycerol
- 26 2-acetyl-1,3-dicoumaroylglycerol

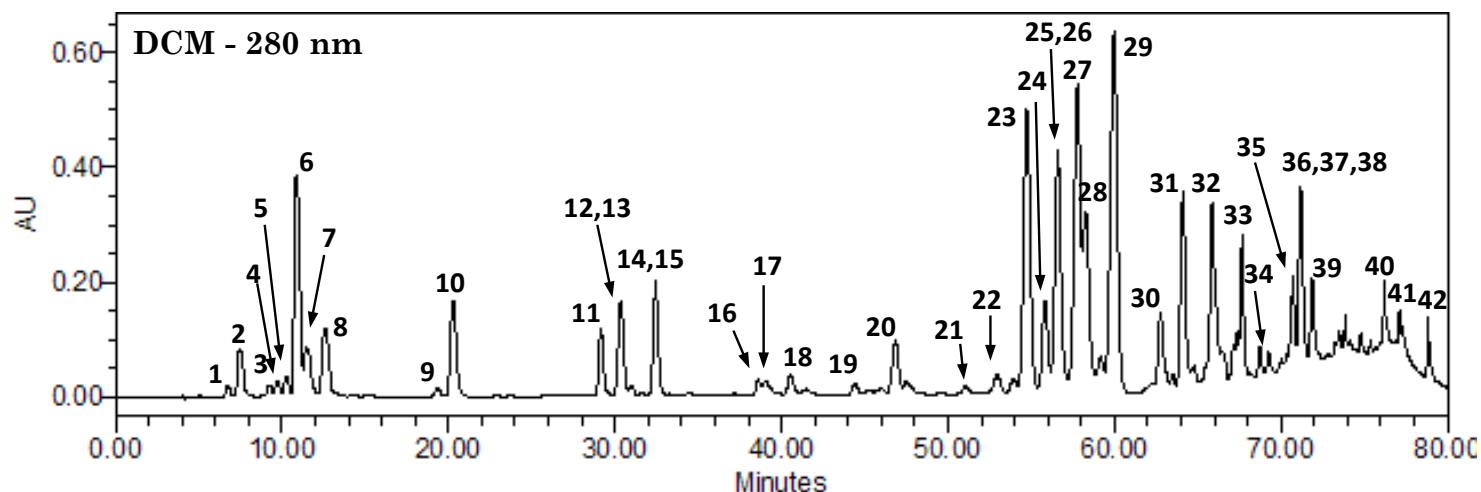


#### ○ *Flavan-3-ol:*

- 34 New flavan-3-ol

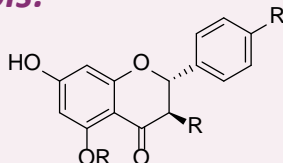


### 2) Alcoholic, DCM and mixed solvents extracts



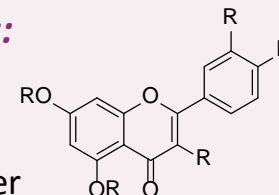
#### ○ *Flavanones/Dihydroflavonols:*

- 11** Pinobanksin-5-methyl ether
- 14** Pinobanksin
- 15** Naringenin
- 22** Pinocembrin-5-methyl ether
- 23** Pinocembrin
- 27** Pinobanksin-3-acetate
- 35** Pinostrobin
- 38** Alpinone-3-acetate

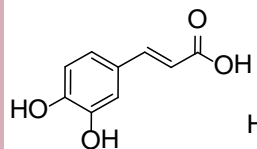
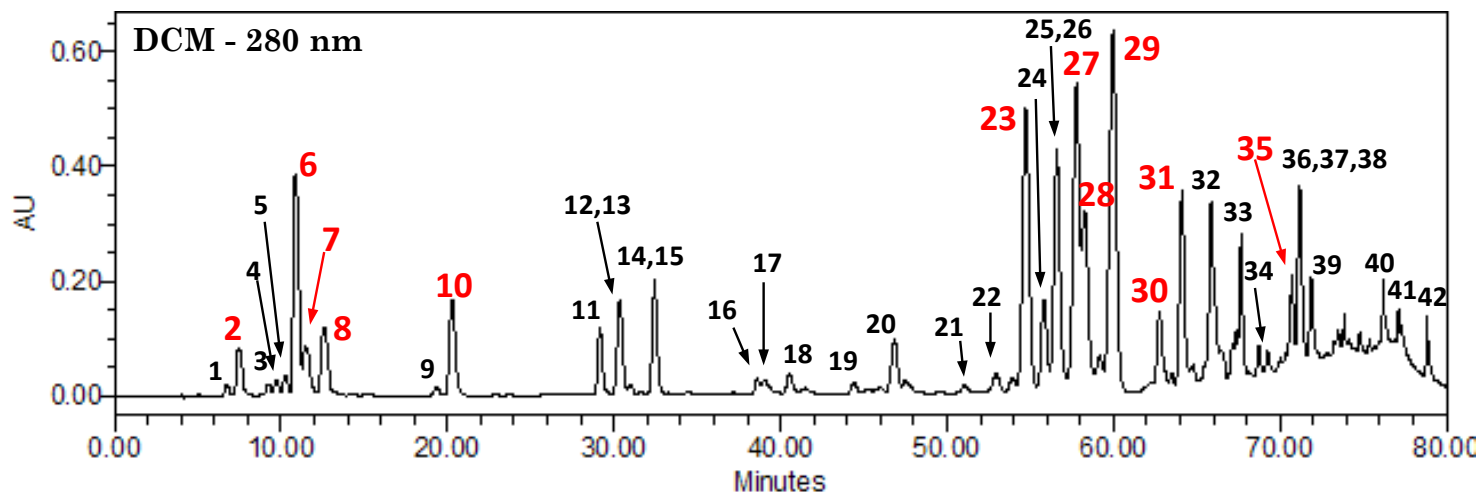


#### ○ *Flavones/Flavonols:*

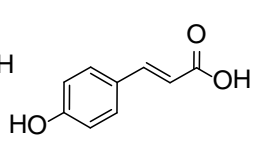
- 17** Kaempferol
- 18** Apigenin
- 19** Luteolin methyl ether
- 21** Quercetin-7-methyl ether
- 29** Chrysin
- 31** Galangin
- 39** Tectochrysin



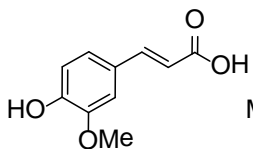
### Quantification of 12 major components (HPLC/DAD)



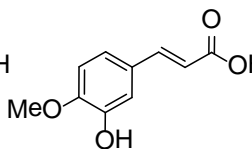
2 Caffeic acid



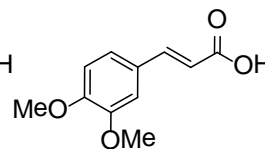
6 *p*-coumaric acid



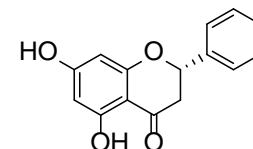
7 Ferulic acid



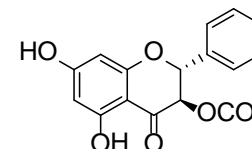
8 Isoferulic acid



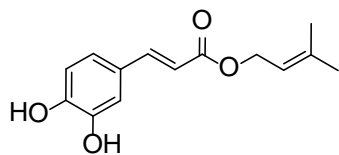
10 3,4-dimethoxycinnamic acid



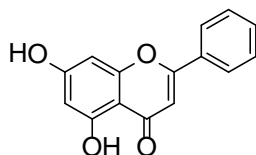
23 Pinocebrin



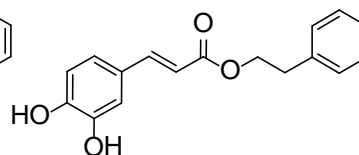
27 Pinobanksin-3-acetate



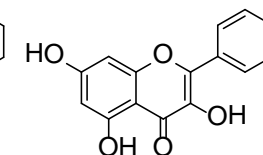
28 Prenyl caffeate



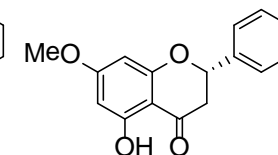
29 Chrysin



30 CAPE

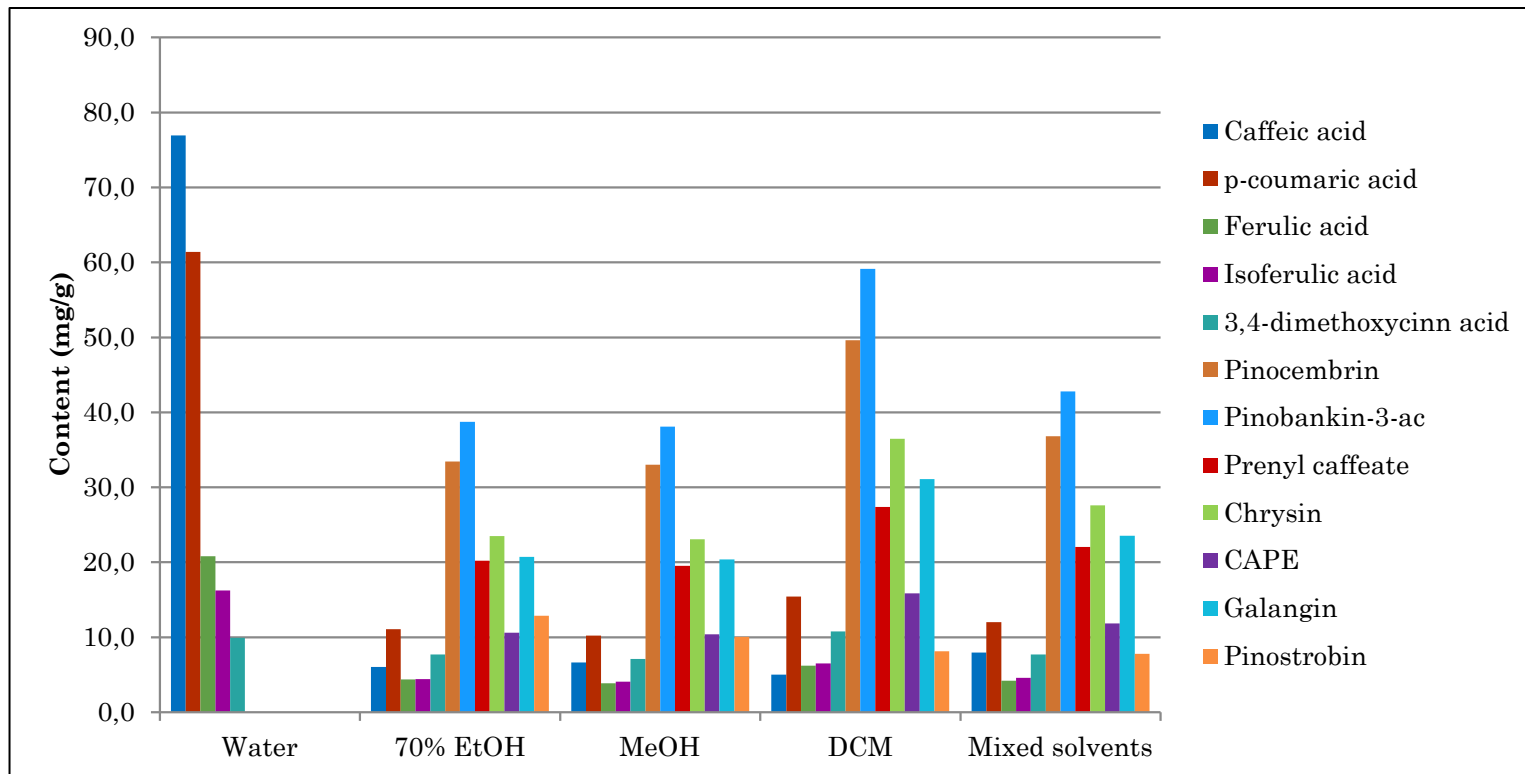


31 Galangin



35 Pinostrobin

### Quantification of 12 major components (HPLC/DAD)



- **Aqueous extract:** predominantly caffeic and p-coumaric acids (total 185 mg/g)
- **Other extracts:** same profiles (pinobanksin-3-acetate, pinocembrin, chrysin, galangin...)
- **Alcoholic extracts:** Similar values for the 12 components (total 194 mg/g)
- **DCM extract:** best contents (total 272 mg/g)

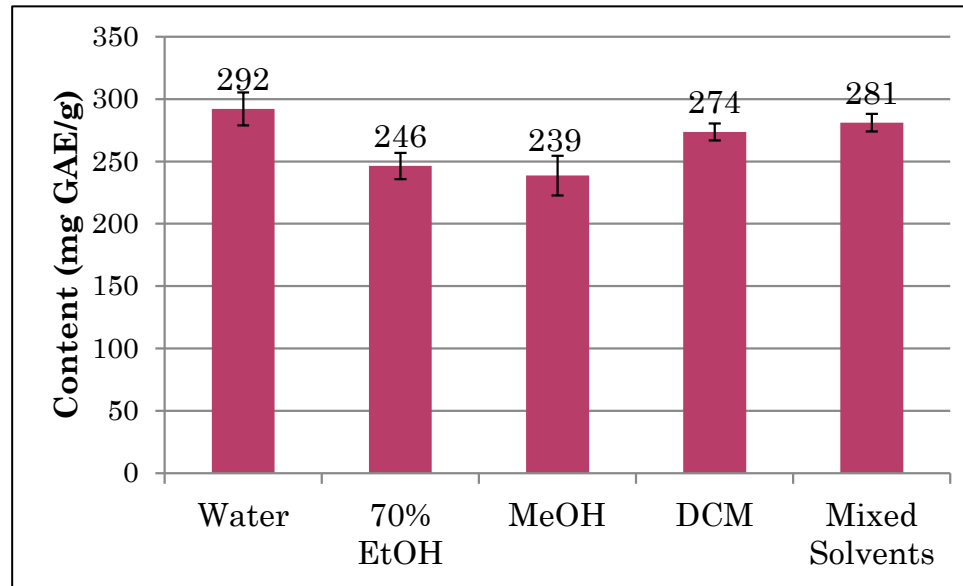
## II. ANTIOXIDANT ACTIVITY



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### 1) POLYPHENOLIC CONTENT

### Total polyphenol content (Folin-Ciocalteu method)



- Polyphenol contents between **239 and 292 mg GAE/g<sup>1</sup>** (European type propolis: 200-300 mg GAE/g)<sup>2</sup>
- Contents of aqueous, DCM and mixed solvents extracts slightly higher than alcoholic ones

<sup>1</sup> GAE/g: milligram of Gallic Acid Equivalent per gram

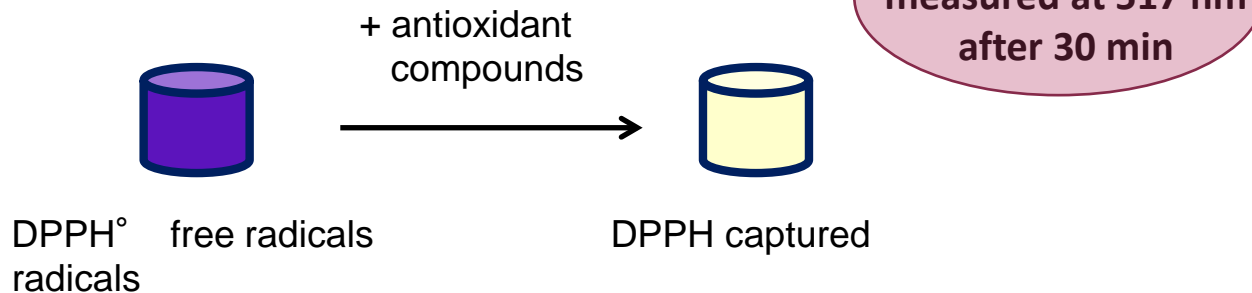
<sup>2</sup> Laskar et al. 2010, Kumazawa et al. 2004

## II. ANTIOXIDANT ACTIVITY

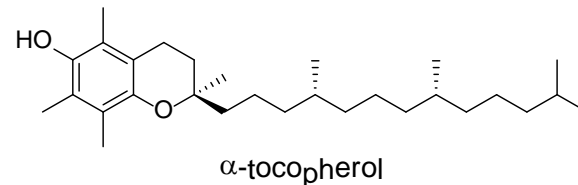
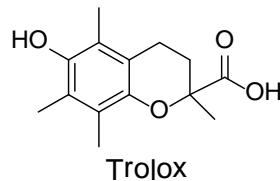
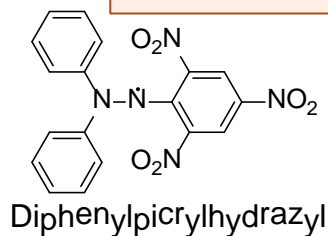
### 2) DPPH ASSAY

### DPPH (diphenylpicrylhydrazyl) colorimetric assay

*Principle:*



- Method based on **singulet electron transfer (SET)**
- Standard: **Trolox**, a water-soluble analog of  $\alpha$ -tocopherol (vitamin E)
- Results expressed as micromole of trolox equivalent per gram of extract ( $\mu\text{mol TE/g}$ )

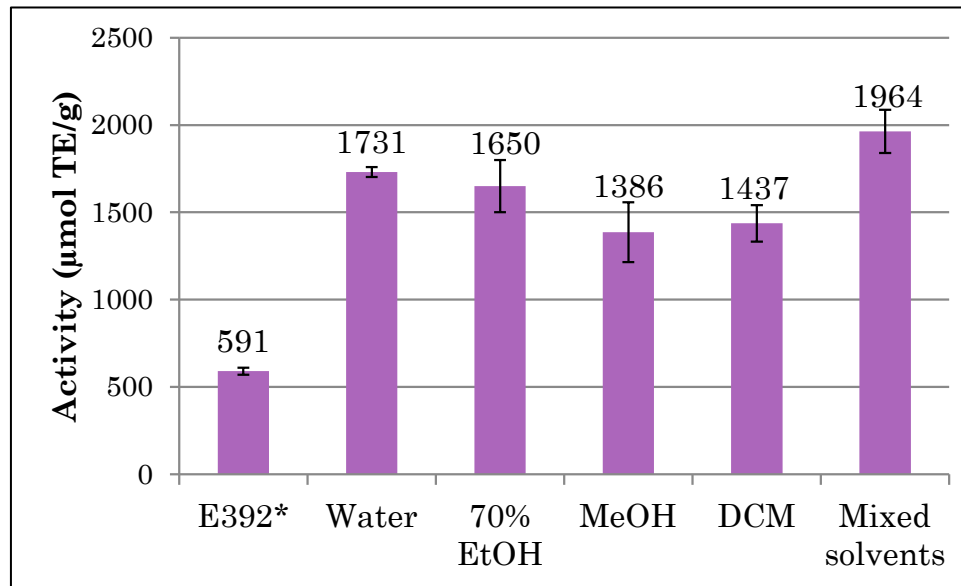


## II. ANTIOXIDANT ACTIVITY

### 2) DPPH ASSAY

### DPPH (diphenylpicrylhydrazyl) colorimetric assay

#### Results:



- Activity of propolis extracts 2 to 4 times higher than E392
- Higher activities observed for mixed solvents, aqueous and 70% EtOH extracts

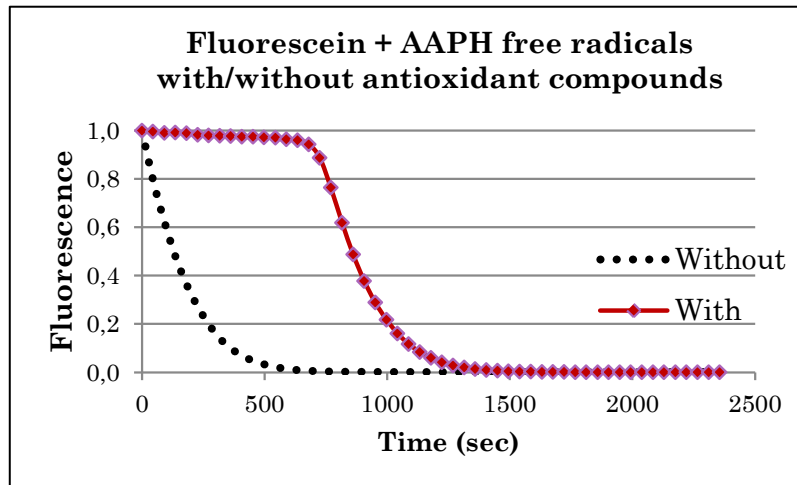
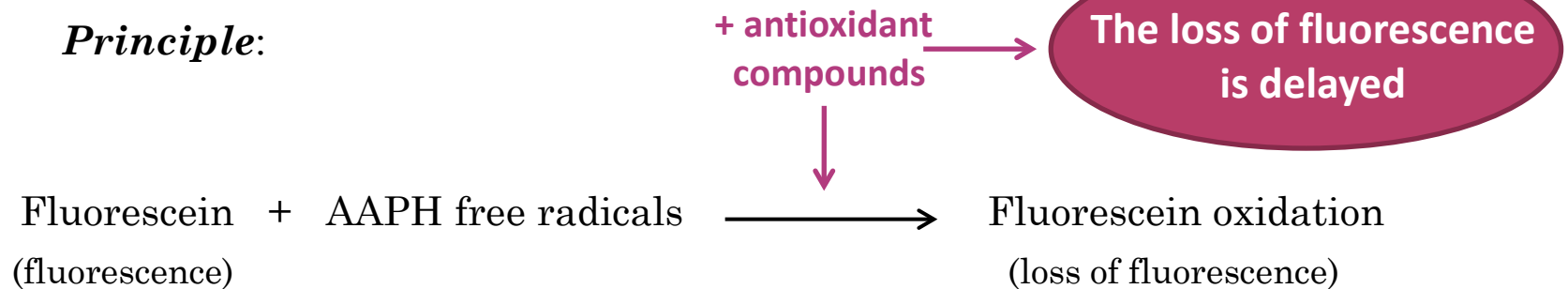
\*E392 = **rosemary extract** used as antioxidant food additive in Europe  
[Official Journal of European Union (Directive 2010/67/UE – L277/17)]

## II. ANTIOXIDANT ACTIVITY

### 3) ORAC ASSAY

## ORAC (Oxygen Radical Absorbance Capacity) fluorimetric assay

### Principle:



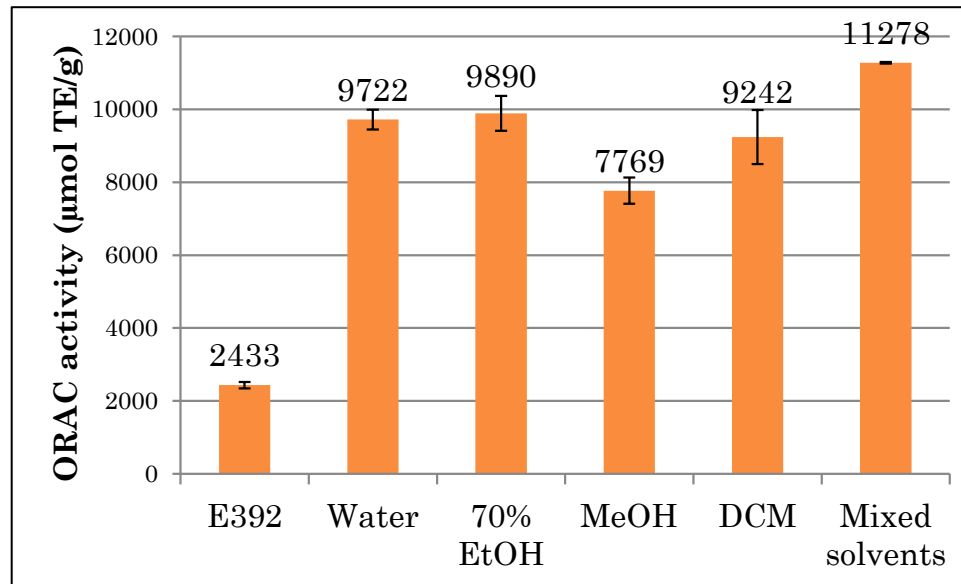
- Method based on **hydrogen atom transfer (HAT)**
- Same standard: **Trolox**

## II. ANTIOXIDANT ACTIVITY

### 3) ORAC ASSAY

### ORAC (Oxygen Radical Absorbance Capacity) fluorimetric assay

*Results:*



- Activity of propolis extracts 3 to 5 times higher than E392
- Higher activities observed for mixed solvents, aqueous and 70% EtOH extracts

## ➤ Chemical composition:

### ○ *Aqueous extract:*

predominantly cinnamic acid derivatives (caffeic and *p*-coumaric acids...)

### ○ *Alcoholic, DCM and mixed solvents extracts:*

- Cinnamic acid derivatives (caffeic and *p*-coumaric acids...)
- Cinnamic ester derivatives (prenyl caffeate, CAPE...)
- Flavanones/dihydroflavonols (pinobanbsin-3-acetate, pinocembrin...)
- Flavones/flavonols (chrysin, galangin...)

⇒ Poplar-type (*Populus nigra* L.)

# CONCLUSION

## ➤ Antioxidant activity:

- **All extracts showed a very good antioxidant activity associated with a high total polyphenol content**
- Activity slightly higher for mixed solvents, aqueous and 70% EtOH extracts

## Comparison of the five extracts

Extracts	Water	70% EtOH	MeOH	DCM	Mixed solvents
Yield (%)	-	++	+++	+	++
Total content of 12 components(mg/g) <b>TC12</b>	+	+	+	+++	++
Total polyphenol content (mgGAE/g) <b>TPC</b>	+++	+	+	++	++
<b>DPPH</b> (μmol TE/g)	++	++	+	+	+++
<b>ORAC</b> (μmol TE/g)	++	++	+	+	+++
<b>Positive points</b>	Best TPC Good DPPH, and ORAC Green solvent: <b>WATER</b>	Good DPPH, ORAC	Medium TPC, DPPH, ORAC	Best TC12 Good TPC Medium DPPH, ORAC	Good TPC Best DPPH and ORAC
<b>Negative points</b>	Low yield	Alcohol	MeOH: toxic alcohol	DCM: toxic organic solvent	Presence of <b>DCM: toxic organic solvent</b>

Water  
extract



Very interesting in pharmaceutical,  
cosmetic and food additive products



# THANKS

## **Ballot-Flurin Apiculteurs**

All Ballot-Flurin Team

The beekeepers beside us who work everyday to make the bees stronger and harvest the best propolis and bee products

## **University of Angers (SONAS Lab)**

Pr Pascal Richomme  
Dr Anne-Marie Le Ray  
Marie-Christine Aumond

# Thank you for your attention