

# **Antioxidant content, free radical scavenging activity and identification of phenolic/flavonoid compounds in pollen of fourteen plants using HPLC / DAD**

Pr Badiaa LYOUSSE

University Sidi Mohamed ben Abdallah

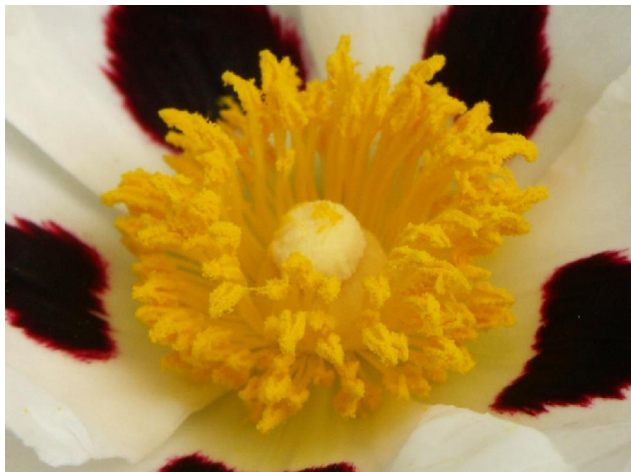
Fez, Morocco



# Each flower produces a large amount of pollen



“ (1) Stamens loaded with pollen before being harvested by bees.



“ (2) Stamens after the anthers have been emptied by bees.

# Bee pollen stocked in beehive: a food source



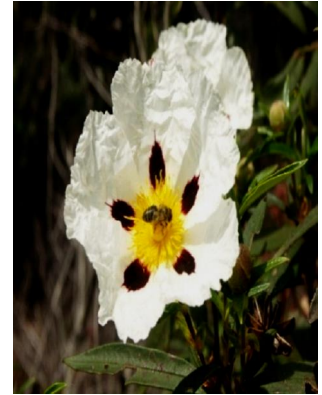
“ More than 30 million years ago bees have developed a method of food preservation that is richer in protein than meat or fish at a temperature of 36°C in an extremely humid atmosphere.

**The role of fermenting agents in bee pollen**

**But bee pollen is also a excellent food for humans**

# Nutritional quality of fresh monofloral bee pollen

- ✓ Energy intake from simple and complex carbohydrates
- ✓ Protein Source
- ✓ Fiber Source
- ✓ Special Lactic bacteria source with « probiotic effect »
- ✓ Vitamins and Minerals source
- ✓ Vegetal antioxydant source (carotenoids, polyphenols)



**Bee pollen is a good food  
for human nutrition  
with many potential effect  
on Health**





## Pollen and Heath claims



In total :

- 7 minerals for Action on : Zinc, Manganese, Copper, Potassium, Calcium, Iron, Magnesium
- 14 fundamental physiological functions based on
- 35 health claims officially licensed - proven and recognized European and international authorities

This shows **pluripotency action pollen flowers all dehydrated.**

Natural action (term regulated by the EEC directive use)



## Floral **origin** – bioactivity compounds



**Anaemia and the hemopoietic system** – bee pollen - Iron



**Antioxidant activity** – *Eucalytus globulus* – flavonoids + ??



**Anti inflammatory activity** - *Pinus densiflora* – flavonoids ??



**Antitumoral activity** – *Brassica napus* – polysaccharide LBPP



**Antiatherosclerotic activity** - fat-soluble pollen extract



**Activity on osteoporosis** – *Cistus ladaniferus* – PM  $\leq$  1000



**Activity in Disorders of the Prostate** – Hidroxamic acid



**Antidiarrhoeal activity** - *Eucalytus globulus*; *Salix atrocinera*



**Antimicrobial activity** – *Ranunculus* sp – herbacetin (flavonoid)

# Summary - Bee Pollen

## ***1. Legislation***

Best Practice Guide for Collection & Preservation  
Quality Control

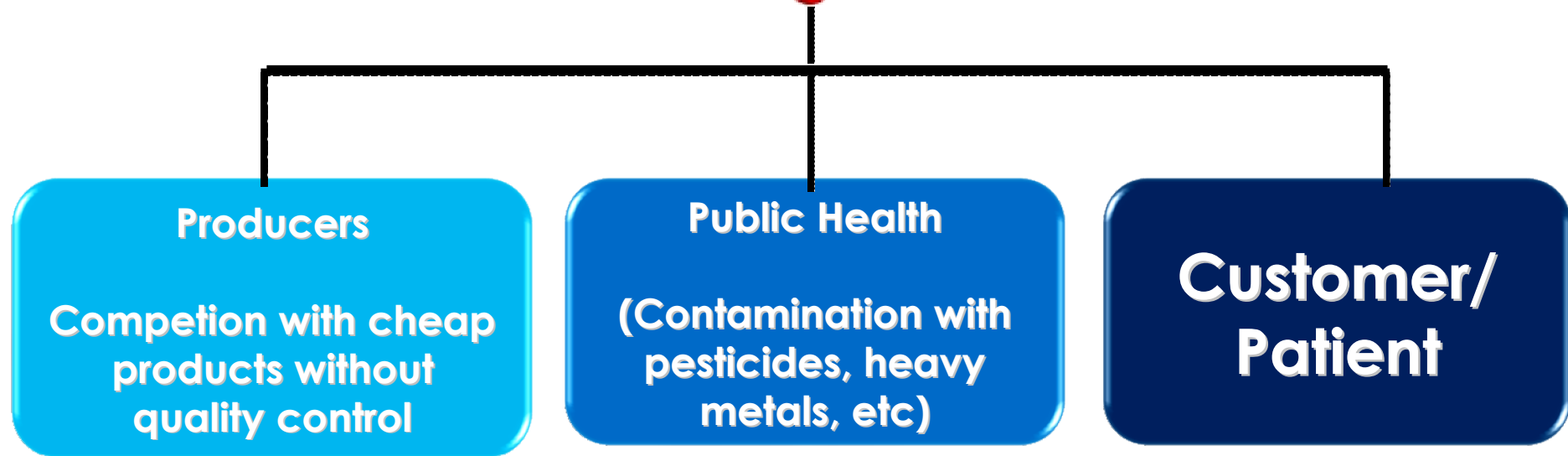
## ***2. Data collected***

Countries involved  
Flora studied around the world

## ***3. Future Challenges***

Publications  
Bee pollen Monographs for Pharmacopea







# Pollen composition and standardisation of analytical methods

CAMPOS, M. G., *et al.* 2008

## Criteria & Standard Methods for Quality Control

*Journal of Apicultural Research and Bee World* 47(2): 156–163 (2008)

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### REVIEW ARTICLE

## Pollen composition and standardisation of analytical methods.



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### Summary

Honey bee pollen is considered to be a food, and national pollen standards exist in different countries such as Brazil, Bulgaria, Poland and Switzerland. It is the aim of the present work to review pollen composition and the analytical methods used for the evaluation of high quality bee pollen. Based on the experience of different countries and on the results of published research, we propose quality criteria for bee pollen, hoping that in the future they will be used as world wide bee pollen standards.

**Keywords:** Standardisation of bee products; bee pollen; quality control

*Campos M.G, Anjos O.: & Amâncio D.*

## Proposed technical regulation for the identity and quality of bee pollen

### Technical regulation for identity

*Objective:* To establish the identity and the minimum quality requirements for bee pollen.

*Target:* The regulation will be applied to bee pollen sold in national and international markets.

#### 1. Description

**1.1.** Definition: Bee pollen is the result of the agglutination of flower pollens, made by worker honey bees, with nectar (and/or honey) and salivary substances, and collected at the hive entrance.

**1.2.** Classification:

**1.2.1.** According to water content:

**1.2.1.1.** Bee pollen: The product collected in the original form, with water content between 20-30 %. Storage of such pollen should be in a freezer to avoid bacterial and mould contamination.

**1.2.1.2.** Desiccated bee pollen: The product submitted to a drying out process in temperatures not higher than 42°C, with water content not higher than 6%.



**1.2.2.** According to the floral source content:

**1.2.2.1** Monofloral bee pollen: the major taxon need to be not less than 80% (different taxa can be used for specific nutritional and therapeutic purposes).

**1.2.2.2** Multifloral bee pollen: include different taxa.

**1.3.** Denomination for sales purposes will include classification according the water and floral source content.



• **FUNCTIONAL FOOD**

• **DIETARY SUPPLEMENT**



• **DRUG**



Samples from  
France, Portugal & New Zealand.  
Brazil,  
Germany,  
India,  
Mexico,  
Poland,  
Romania,  
Slovakia,  
Spain,  
Morocco  
Sultanate of Oman,  
Ukraine, Ethiopia, and Russia have  
recently joined this research group

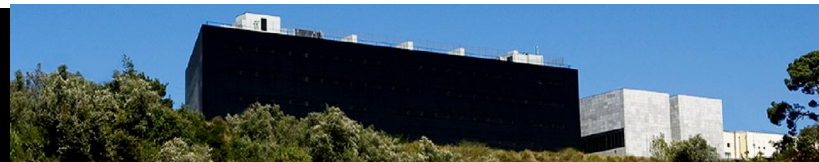
**Members of Bee pollen Working Group  
(Bp WG) IHC Network  
([www.ihc.org](http://www.ihc.org))**



Portugal



UNIVERSIDADE DE COIMBRA



# Identification of Floral Origin

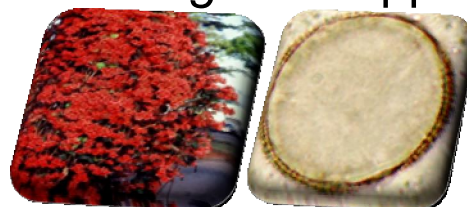


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*Acacia dealbata*



*Bignonia* spp.



*Brassica*



*Camellia sinensis*



*Cocos nucifera* L.



*Coleostephus myconis* L.



*Digitalis purpurea* L.



*Galactites tomentosus* L.



*Ligustrum* sp.



*Lotus* sp.



*Olea europaea* L.



*Tilia* sp..



*Viburnum tinus* L.



*Zea mays* L.



Etc.

Etc.

Espécies com exploração apícola (várias zonas do globo). AMÂNCIO, D. C. - Compostos bioativos do pólen. Tese de mestrado. Faculdade de Farmácia, Universidade de Coimbra (2014) 30-32.

Campos M.G, Anjos O.:  
& Amâncio D.



# Results from various countries

Microscopy

Macro and micro nutrients

Phenolic Content

Minerals

Toxic compounds (Alkaloids,etc)

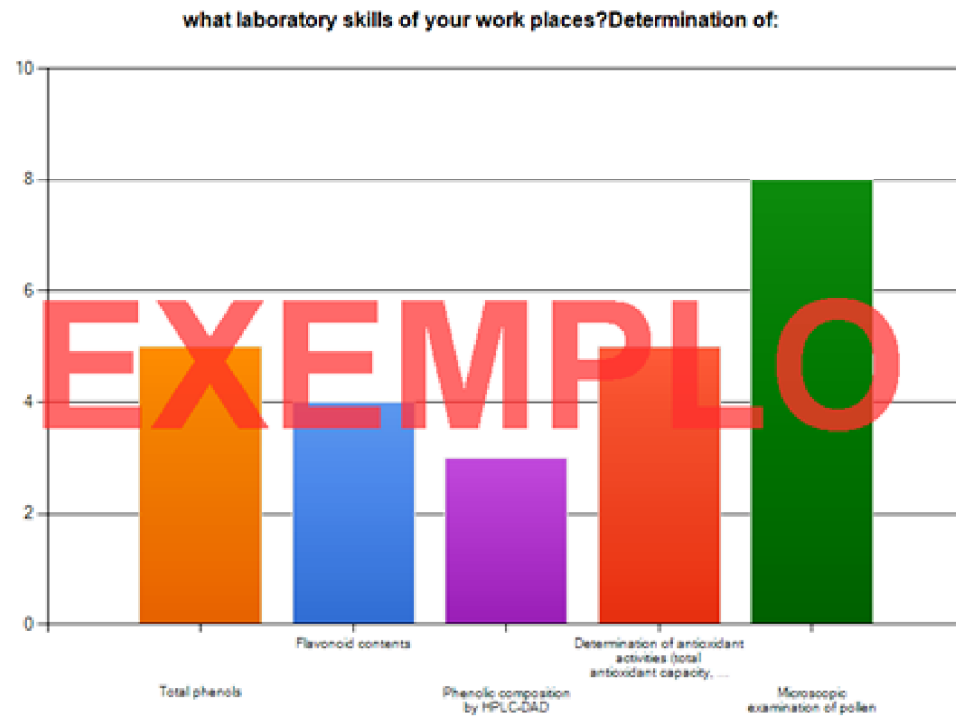
Vitamins

Etc.



**Overview of data from all  
world**

- “ **Palynological analysis**; **physicochemical analyses** of bee pollen (moisture, protein, fat, carbohydrates, ash content, water activity, pH, fatty acids); **microbiological determinations** (commercial quality parameters, indicators of sanitary quality, indicators of safety);
- “ **Determination of biological activities** (antimicrobial, antioxidant, anti-inflammatory and antimutagenic, using diverse methodologies); Determination of bioactive compounds (polyphenols and flavonoids). **Antioxidant activity (Electrochemistry)**.
- “ Experimental protocols to study the efficacy of pollen to manage anemia in animals, antioxidant properties of pollen from moroccan region and quality control
- “ **Microscopic analysis** (Katharina Bieri), **Pyrrolizidine Alkaloids** (QSI, Bremen, Germany)
- “ **Chemical analysis** of Pollen collection gadget
- “ **Macro and microelements** in bee pollen samples - Determination of parameters such as proteins, lipids, ash, **Flavonoid profiles as chemotaxonomic markers**
- “ **Sugar profile** by HPLC **Aminoacids** by HPLC **Colour** (refractometry)



Quality  
Efficacy  
Safety

Brazil  
Bulgaria  
*France*  
Germany  
Greece  
India  
Mexico  
Morocco

Poland  
Portugal  
Romania  
*Spain*  
Slovak  
Sultanate of Oman  
Switzerland



# RISK ASSESSMENT

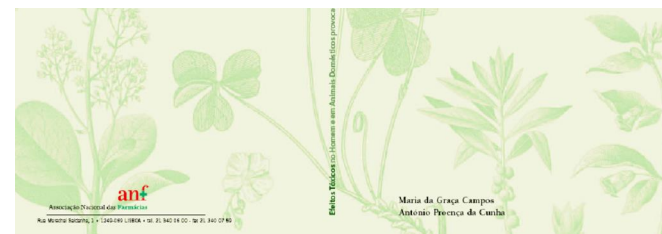
JOURNAL OF  
AGRICULTURAL AND  
FOOD CHEMISTRY

*J. Agric. Food Chem.* XXXX, .

## Hepatotoxic Pyrrolizidine Alkaloids in Pollen and Drying-Related Implications for Commercial Processing of Bee Pollen

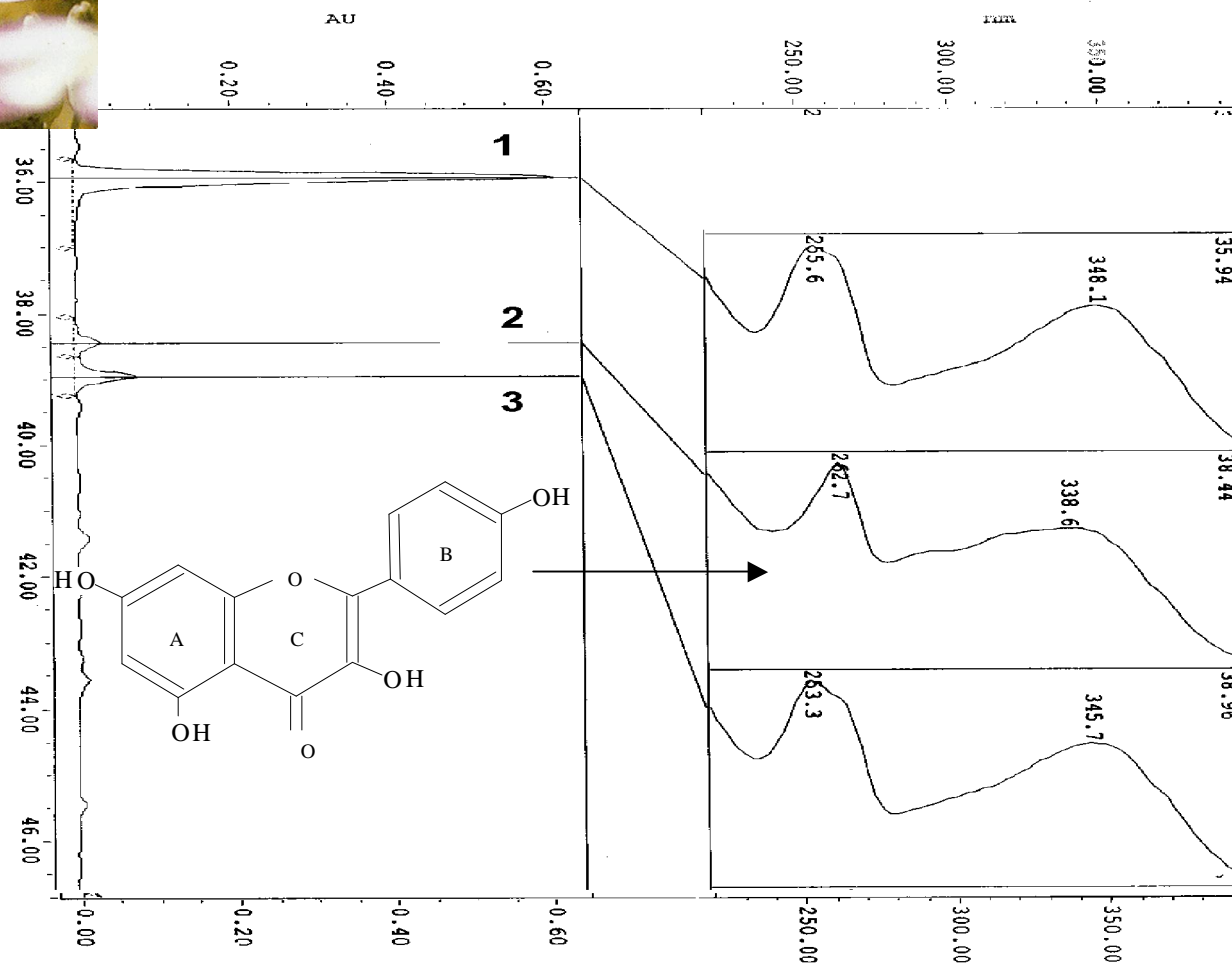
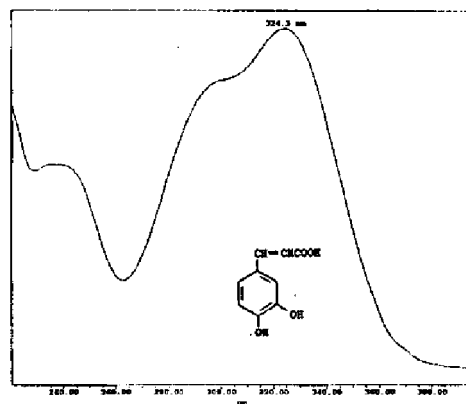
MICHAEL BOPPRÉ,<sup>‡</sup> STEVEN M. COLEGATE,<sup>\*,†</sup> JOHN A. EDGAR,<sup>†</sup> AND  
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D-79085 Freiburg i.Br., Germany





# Bee POLLEN



**Free radical scavenger activity**

# Samples preparation

## Pollen samples

Pollen powder and anthers of the flowers of fourteen plants were removed manually and dried in the shade.

## Pollen extract for antioxidant activity

One gram of dried pollen powder and anthers is macerated in 20 ml of a hydro-ethanolic solution (70%), for one week and after they are sonicated for 5 min centrifuged for 5 min at 2000 g and 20 °C, and the supernatants are removed and kept at 20 °C until use for antioxidant activity.

## Pollen extract for HPLC / DAD analysis

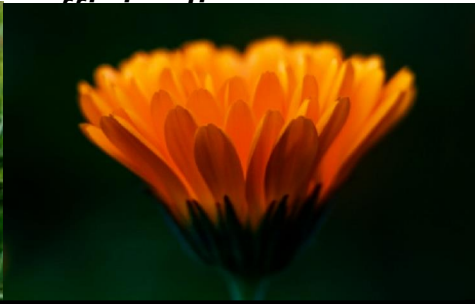
Ten milligrams of dried pollen of each sample were sonicated in an ethanol-water solution (1mL, 50% v/v) for 60 min. The resultant mixtures were centrifuged at 5000 rpm for 10 min and the supernatants were used for HPLC/DAD analysis as previously described (**Campos *et al*, 1997, Campos, 1997**).



*Anethum*



*Calendula*



*Capparis spinosa*



*Papaver rhoeas*



*Acacia pycnantha*



*Malva sylvestris*



*Anacyclus clavatus*



*Anacyclus radiatus*



*Echium creticum*



*Justicia adhatoda*



*Mentha pulegium*



*Opuntia-ficus-indica*



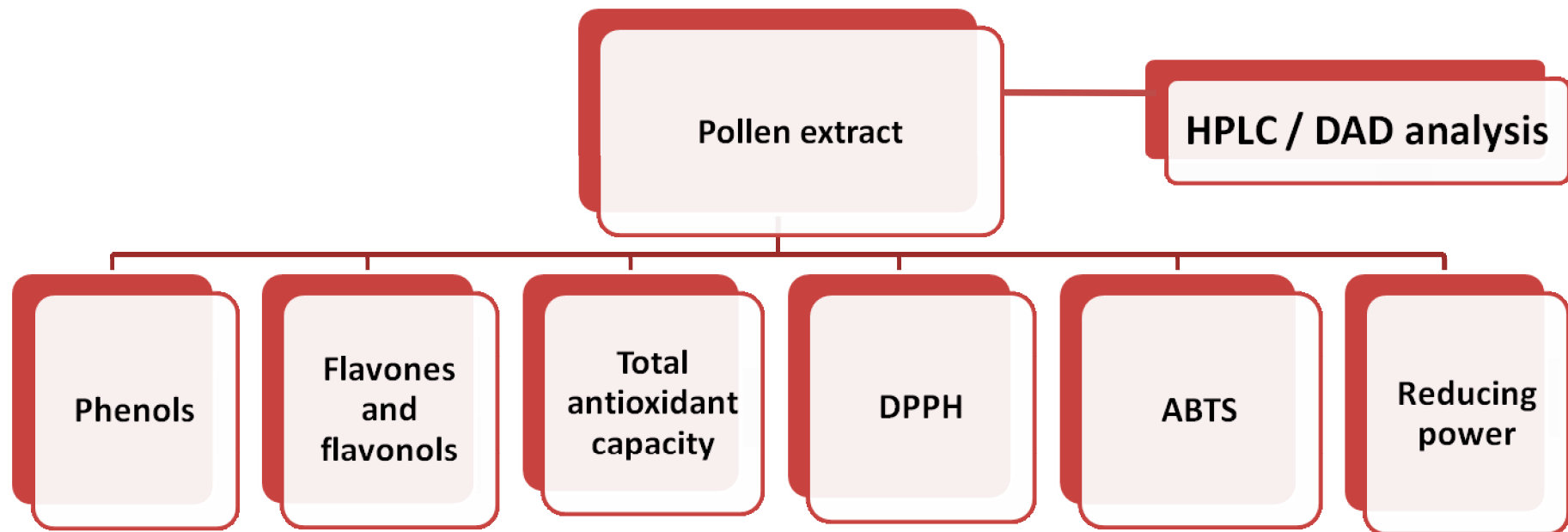
*Scolymus hispanicus*



*Mentha Spicata*



# Analysis carried out

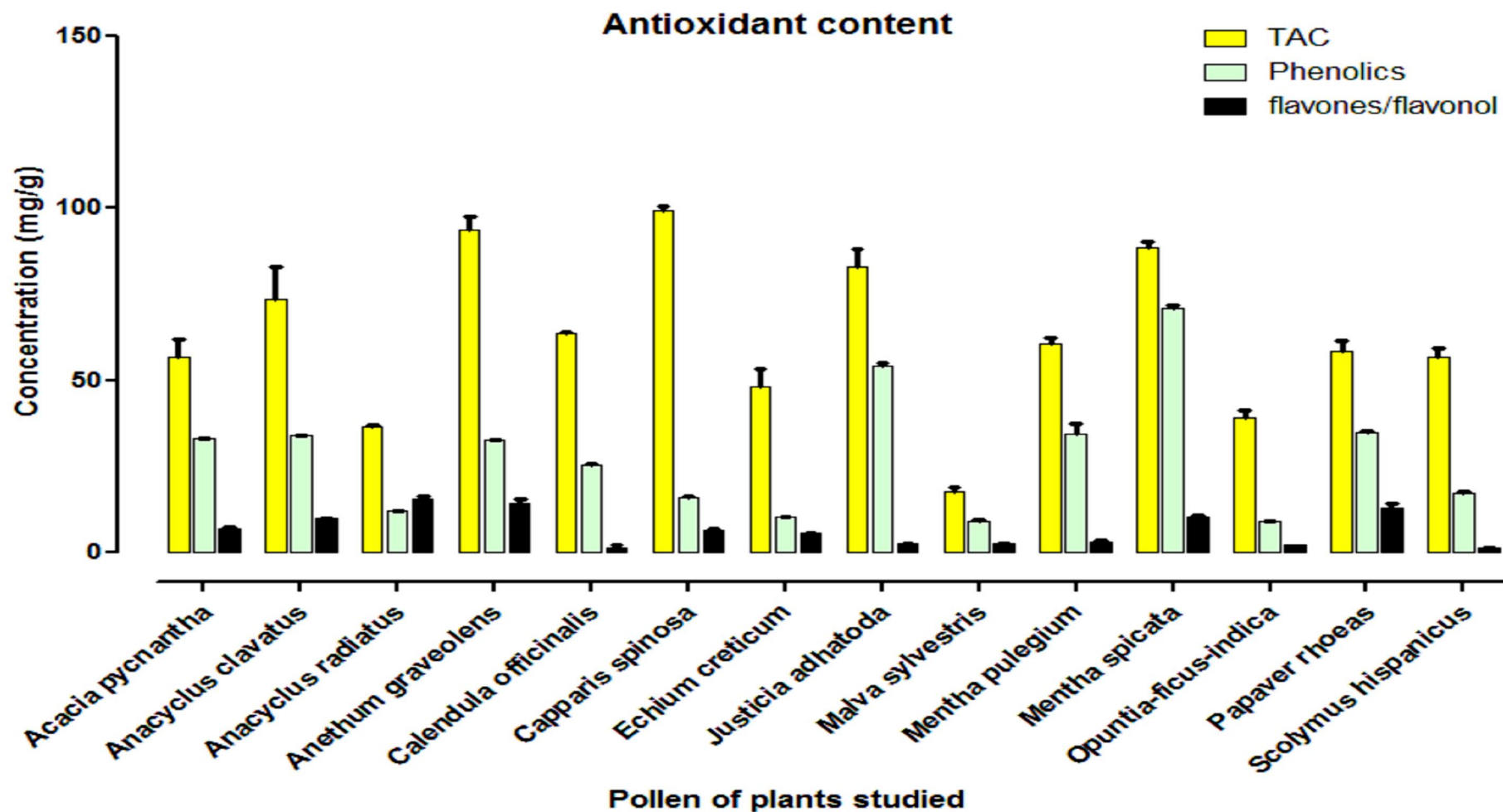




**Table 1:** Phenolics, flavones and flavonol content correlated to Phosphomolybdate assay (total antioxidant capacity) (mg/g of pollen)

sample	Phenolics*	flavones and flavonol**	TAC***
<i>Acacia pycnantha</i>	33.25±0.09 <sup>c</sup>	6.95±0.26 <sup>c</sup>	56.64±5.42 <sup>c</sup>
<i>Anacyclus clavatus</i>	34.02±0.19 <sup>c</sup>	9.77±0.37 <sup>b</sup>	73.40±9.62 <sup>ab</sup>
<i>Anacyclus radiatus</i>	12.04±0.2 <sup>f</sup>	15.44±1.14 <sup>a</sup>	36.61±0.36 <sup>cd</sup>
<i>Anethum graveolens</i>	32.57±0.19 <sup>c</sup>	14.38±1.21 <sup>a</sup>	93.84±3.70 <sup>a</sup>
<i>Calendula officinalis</i>	25.51±0.31 <sup>d</sup>	1.41±0.66 <sup>d</sup>	63.68±0.38 <sup>c</sup>
<i>Capparis spinosa</i>	16.18±0.34 <sup>e</sup>	6.37±0.68 <sup>c</sup>	99.54±0.90 <sup>a</sup>
<i>Echium creticum</i>	10.38±0.07 <sup>fg</sup>	5.55±0.17 <sup>c</sup>	48.26±4.92 <sup>cd</sup>
<i>Justicia adhatoda</i>	54.05±0.96 <sup>b</sup>	2.69±0.04 <sup>d</sup>	83.03±5.15 <sup>ab</sup>
<i>Malva sylvestris</i>	9.20±0.12 <sup>f</sup>	2.50±0.03 <sup>d</sup>	17.84±1.03 <sup>e</sup>
<i>Mentha pulegium</i>	34.63±2.62 <sup>c</sup>	2.97±0.50 <sup>d</sup>	60.74±1.63 <sup>c</sup>
<i>Mentha spicata</i>	71.20±0.72 <sup>a</sup>	10.43±0.2 <sup>b</sup>	88.48±1.64 <sup>a</sup>
<i>Opuntia-ficus-indica</i>	9.22±0.05 <sup>f</sup>	2.31±0.01 <sup>d</sup>	39.29±1.88 <sup>cd</sup>
<i>Papaver rhoeas</i>	34.84±0.69 <sup>c</sup>	12.95±1.29 <sup>a</sup>	58.48±2.85 <sup>c</sup>
<i>Scolymus hispanicus</i>	17.44±0.15 <sup>e</sup>	1.27±0.07 <sup>d</sup>	56.64±2.85 <sup>c</sup>

\*Concentration in mg GAE (gallic acid equivalents)/g of pollen; \*\* concentration in mg QE (quercetin equivalents)/g of pollen;  
 \*\*\*concentration in mg AAE (ascorbic acid equivalents)/g of pollen

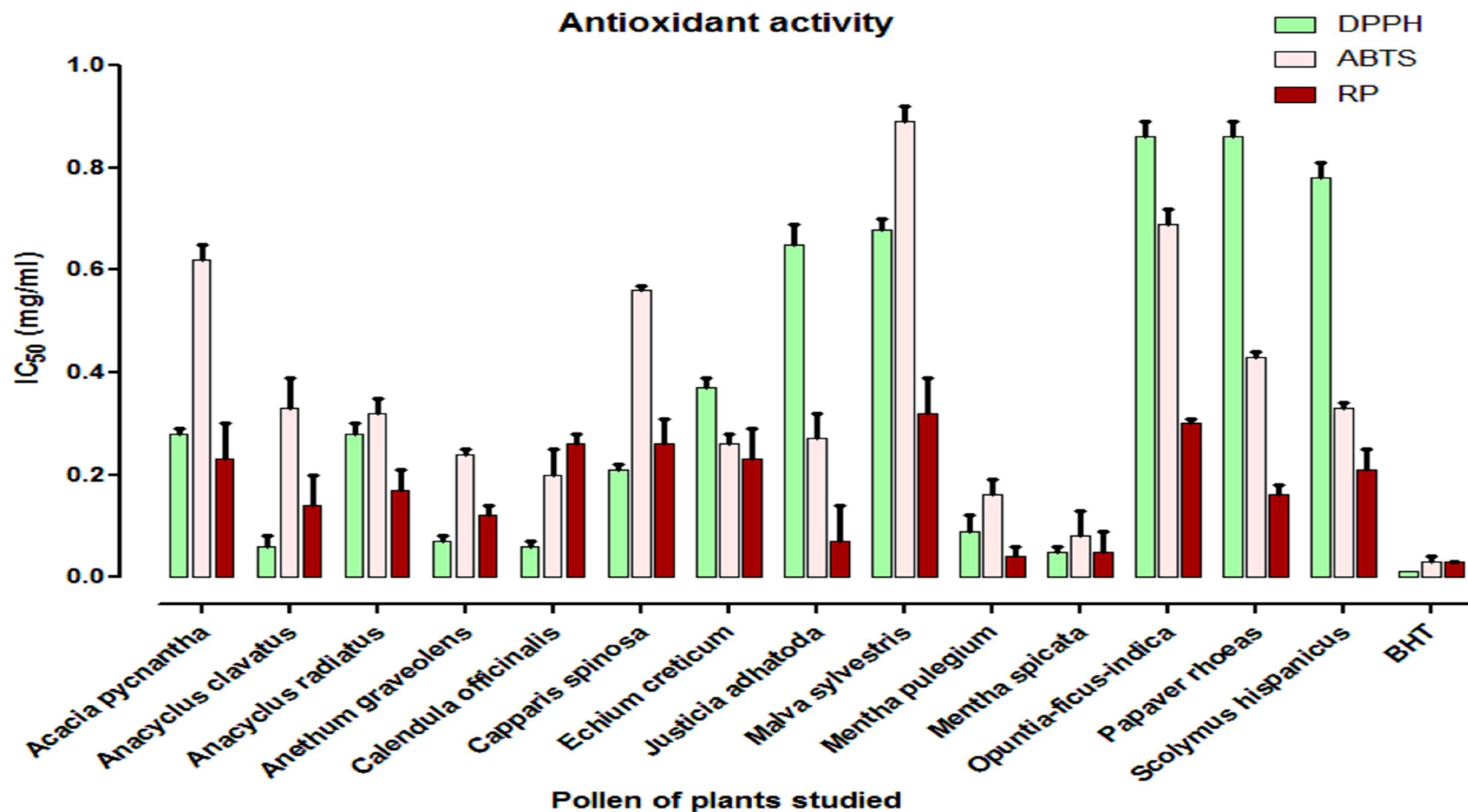


**Figure 1:** Phenol, flavones and flavonol Content, and Phosphomolybdate assay (total antioxidant capacity) (mg/g of pollen)

**Table 2:** Correlation between the specie and the Antioxidant activities (DPPH, ABTS and RP) expressed as IC<sub>50</sub> (mg/ml) for ABTS, DPPH and EC<sub>50</sub> for RP.

Sample	DPPH	ABTS	RP
<i>Acacia pycnantha</i>	0.28±0.01 <sup>e</sup>	0.62±0.03 <sup>b</sup>	0.23±0.07 <sup>c</sup>
<i>Anacyclus clavatus</i>	0.06±0.02 <sup>g</sup>	0.33±0.06 <sup>c</sup>	0.14±0.06 <sup>ef</sup>
<i>Anacyclus radiatus</i>	0.28±0.02 <sup>e</sup>	0.32±0.03 <sup>cd</sup>	0.17±0.04 <sup>e</sup>
<i>Anethum graveolens</i>	0.07±0.01 <sup>g</sup>	0.24±0.01 <sup>cd</sup>	0.12±0.02 <sup>g</sup>
<i>Calendula officinalis</i>	0.06±0.01 <sup>g</sup>	0.20±0.05 <sup>cde</sup>	0.26±0.02 <sup>b</sup>
<i>Capparis spinosa</i>	0.21±0.01 <sup>f</sup>	0.56±0.01 <sup>bc</sup>	0.26±0.05 <sup>b</sup>
<i>Echium creticum</i>	0.37±0.02 <sup>d</sup>	0.26±0.02 <sup>cd</sup>	0.23±0.06 <sup>c</sup>
<i>Justicia adhatoda</i>	0.65±0.04 <sup>c</sup>	0.27±0.05 <sup>cd</sup>	0.07±0.07 <sup>h</sup>
<i>Malva sylvestris</i>	0.68±0.02 <sup>c</sup>	0.89±0.03 <sup>a</sup>	0.32±0.07 <sup>a</sup>
<i>Mentha pulegium</i>	0.09±0.03 <sup>g</sup>	0.16±0.07 <sup>cde</sup>	0.04±0.02 <sup>i</sup>
<i>Mentha spicata</i>	0.05±0.01 <sup>g</sup>	0.08±0.05 <sup>f</sup>	0.05±0.04 <sup>i</sup>
<i>Opuntia ficus-indica</i>	0.86±0.03 <sup>a</sup>	0.69±0.03 <sup>b</sup>	0.30±0.01 <sup>a</sup>
<i>Papaver rhoeas</i>	0.86±0.03 <sup>a</sup>	0.43±0.01 <sup>c</sup>	0.16±0.02 <sup>e</sup>
<i>Scolymus hispanicus</i>	0.78±0.03 <sup>b</sup>	0.33±0.01 <sup>c</sup>	0.21±0.04 <sup>cd</sup>
<i>BHT</i>	0.009±0.0001 <sup>gh</sup>	0.003±0.01 <sup>f</sup>	ND
<i>Ascorbic acid</i>	ND	ND	0.003±0.0001 <sup>j</sup>

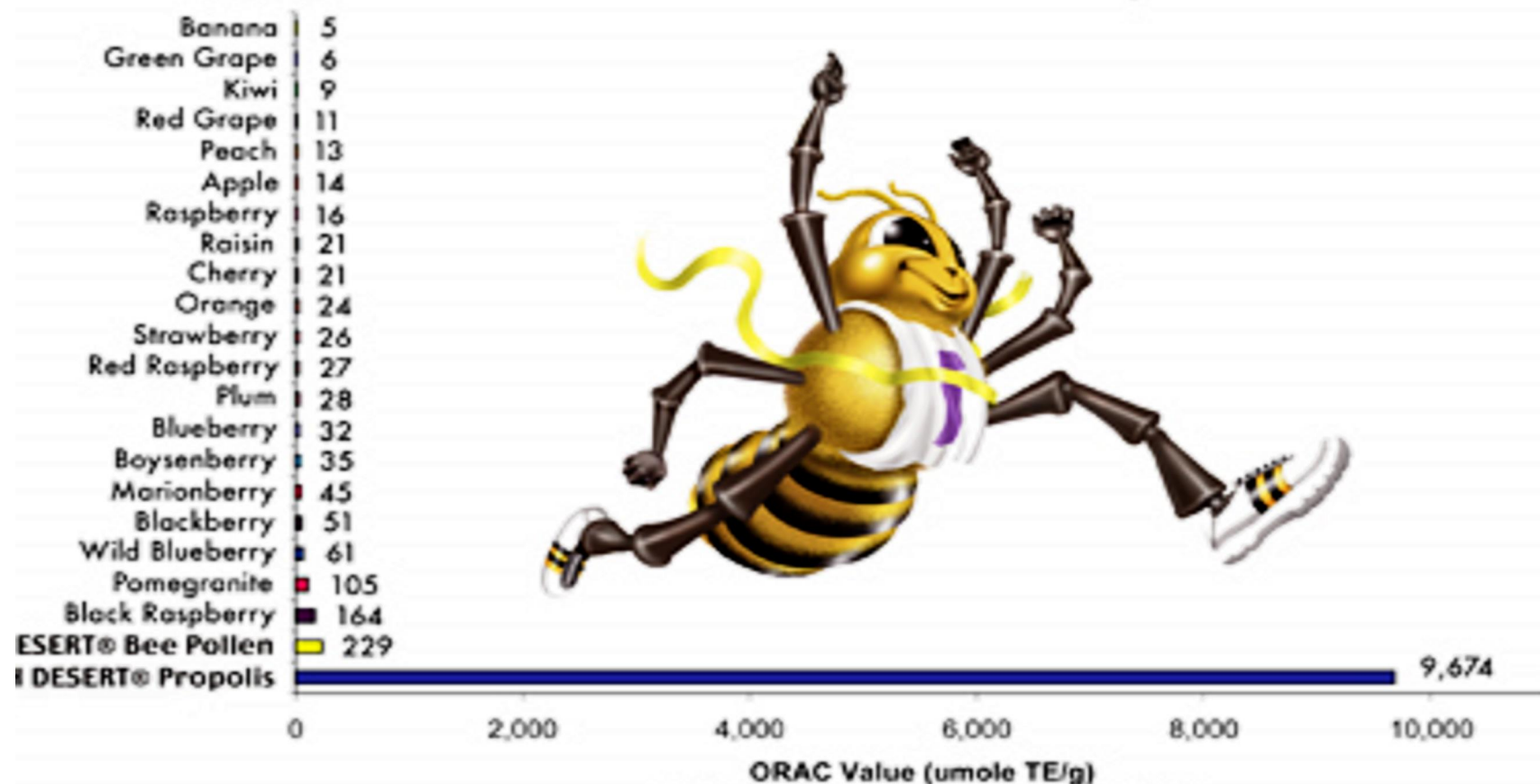
Values in the same column followed by the same letter are not significantly different by the Tukey's multiple range test (p<0.05); data are the means of three replicates. ND =not detected



**Figure 2 :** Antioxidant activities (DPPH, ABTS and Reducing power) expressed as  $IC_{50}$  (mg/ml).

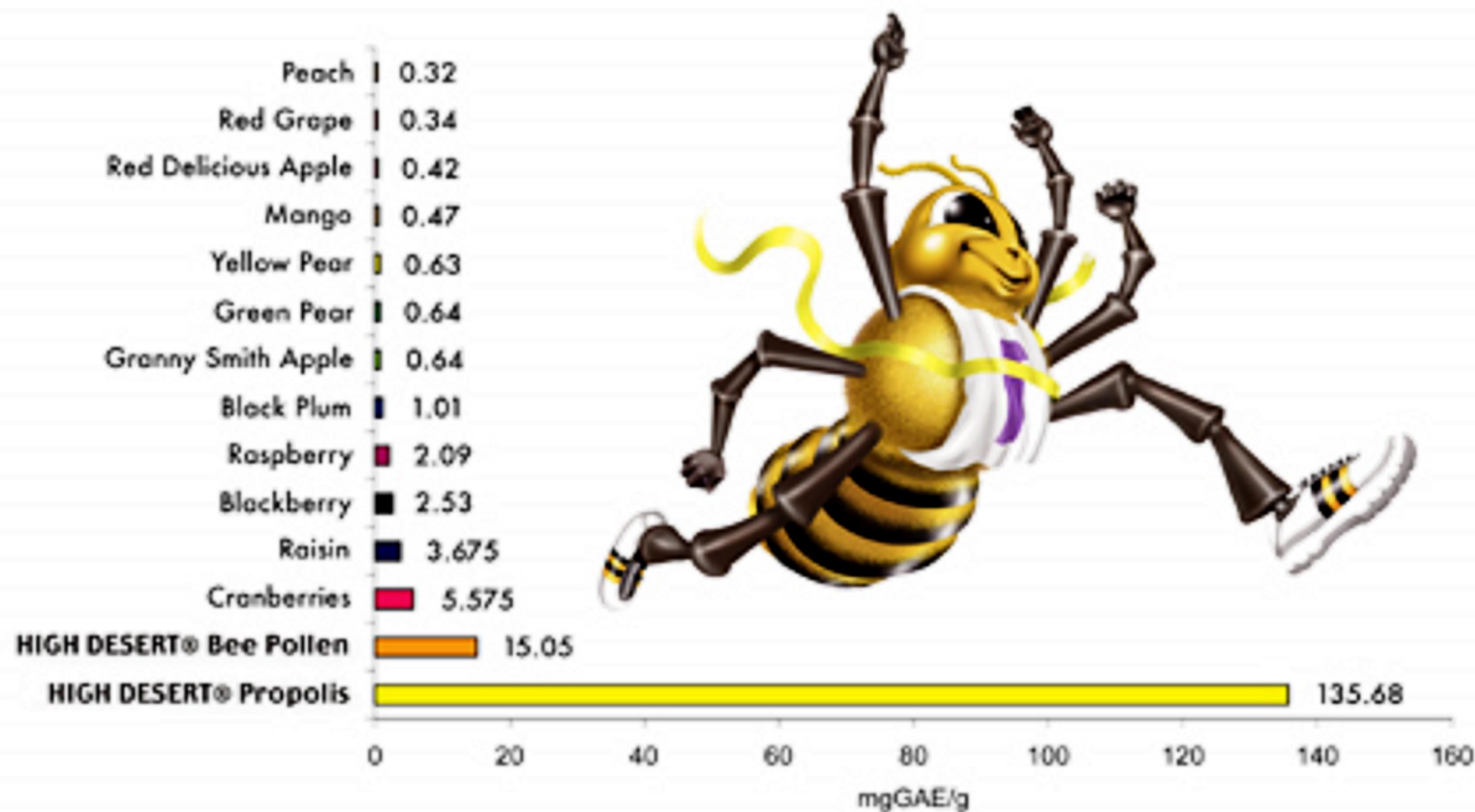
# ORAC (Oxygen Radical Absorbance Capacity) Chart

## Whole Food Antioxidant Activity



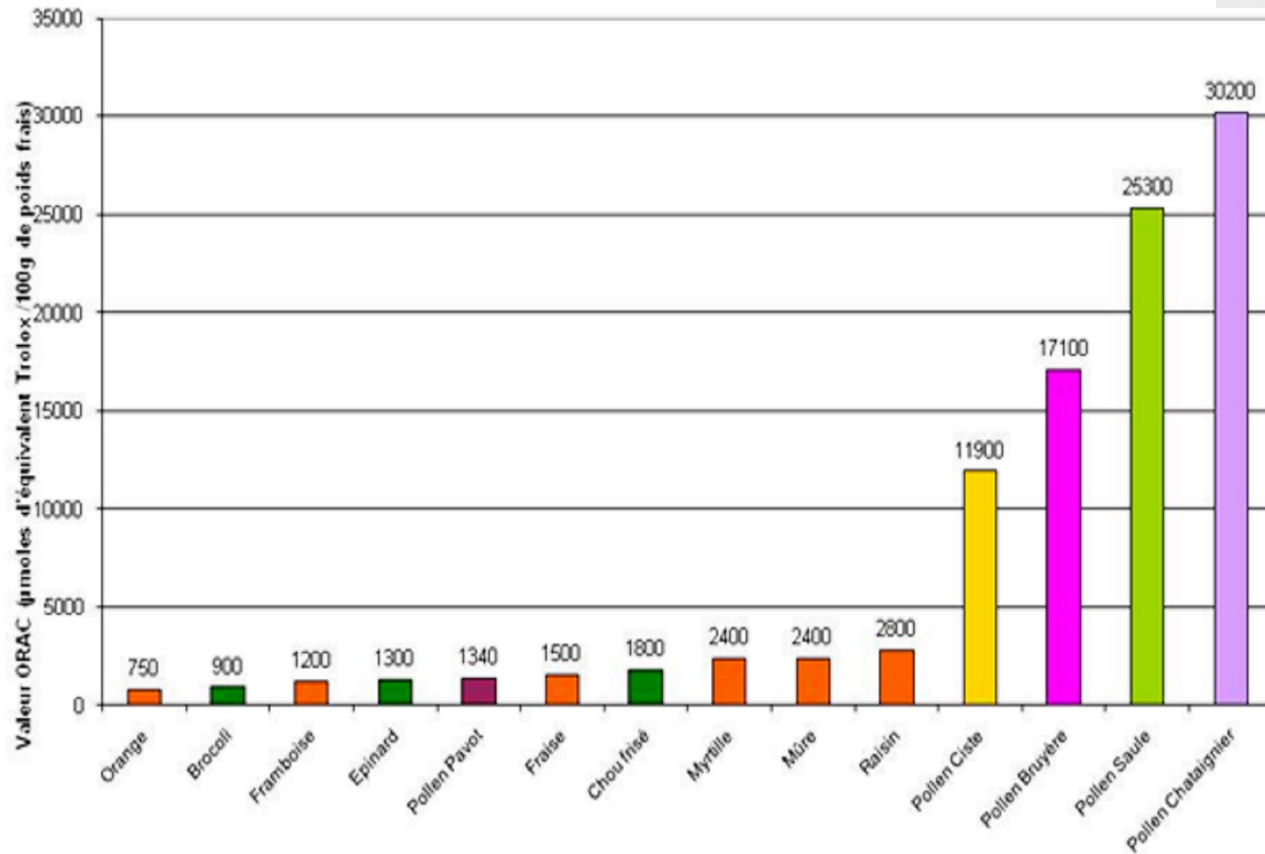
\*Values based on limited sample size and fresh weight

## Total Polyphenol Content





## Antioxidant capacity of pollen





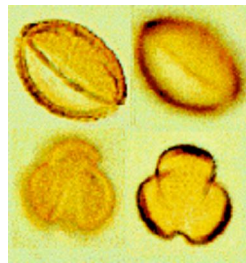
# Analytical methods



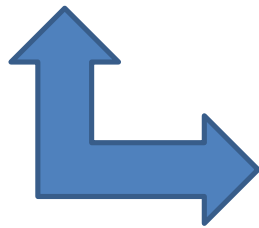
Polyphenolic profiles (phenolic acids & flavonoids)

Identification of the *taxon*

*Herbarium samples*



sediment



SAMPLE

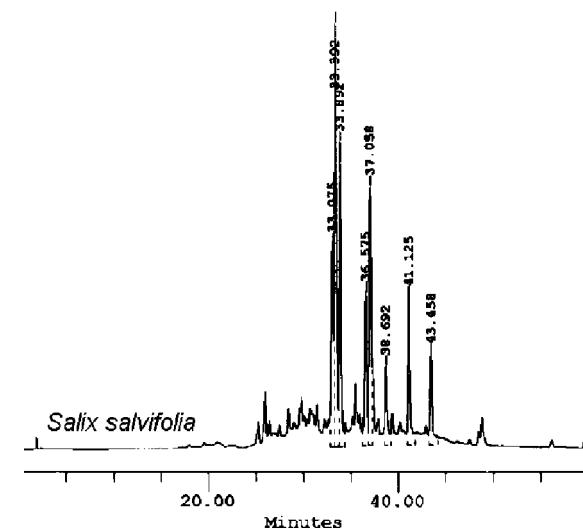
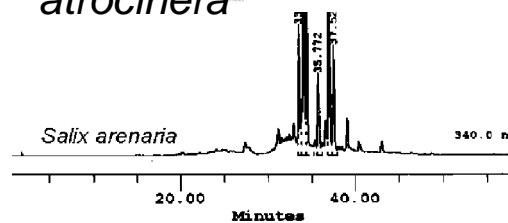
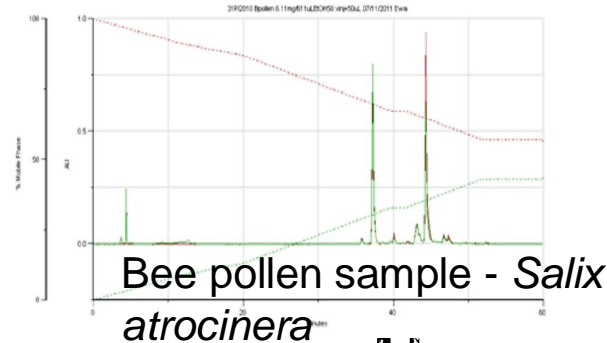
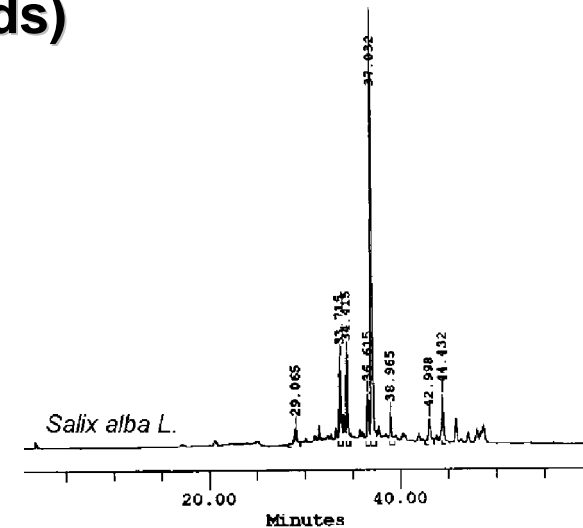
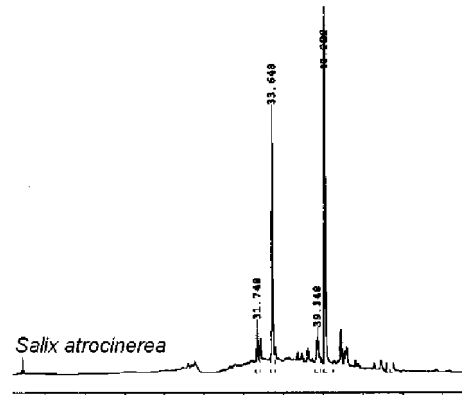
PREPARATION  
✓ ethanol 50%

extraction

✓ vortex +

ultrasounds

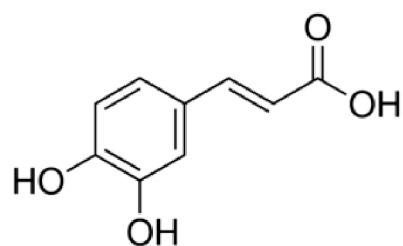
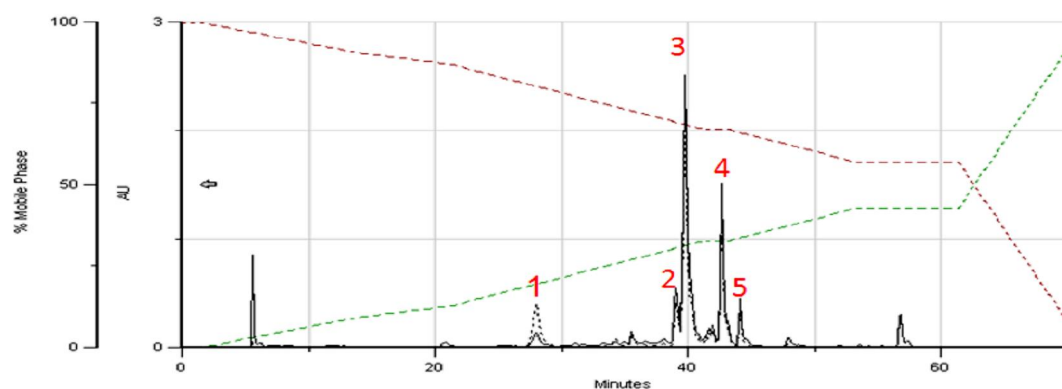
✓ centrifugation



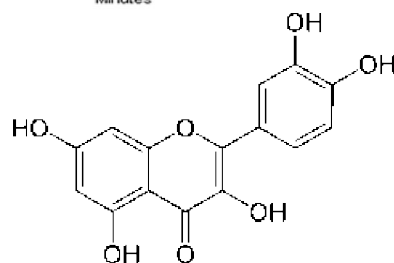
HPLC/DAD

$\lambda$  220-400nm

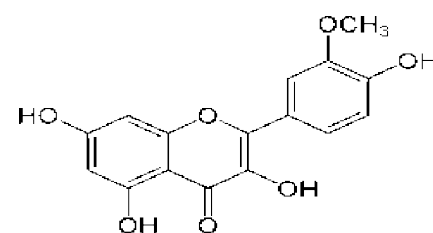
## *Anethum graveolens*



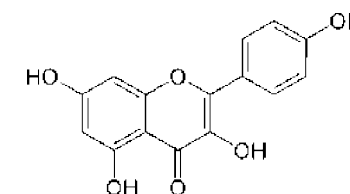
**1: Caffeic acid**



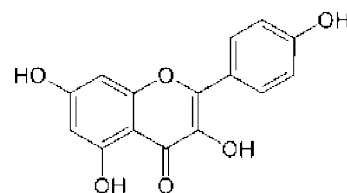
**2: Quercetine**



**3: Isorhamnetin**



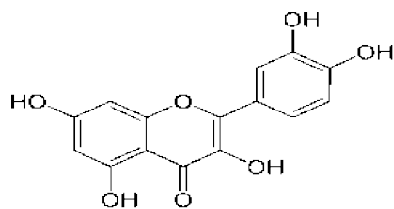
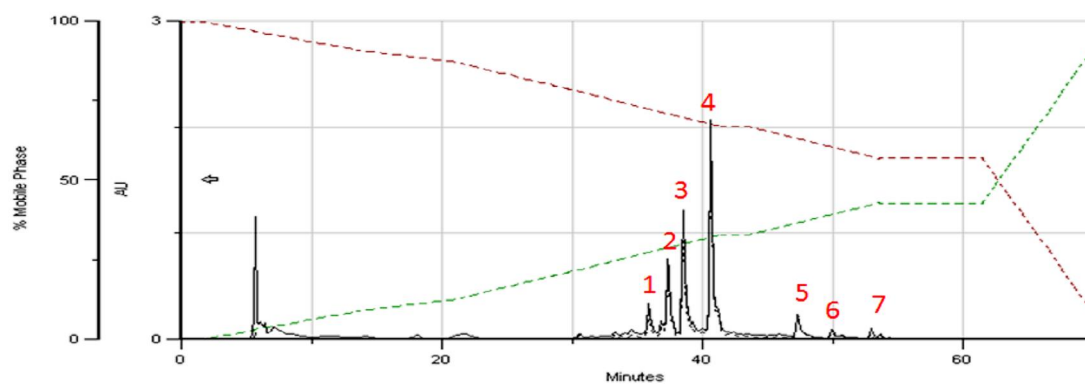
**4: Kaempferol**



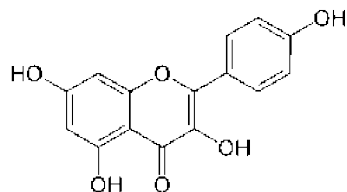
**5: Kaempferol**

- Haematological and hypoglycemic effects
- Microbiological effects

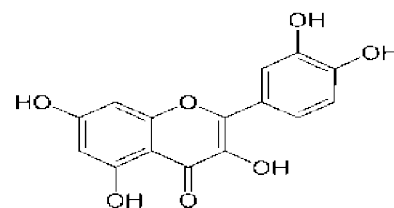
## *Capparis spinosa*



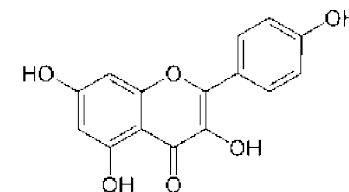
**1: Quercetine**



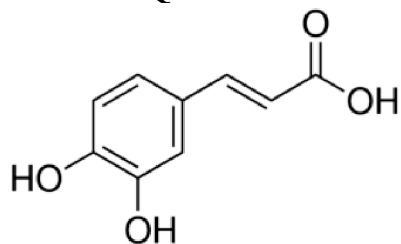
**2: Kaempferol**



**3: Quercetine**



**4: Kaempferol**



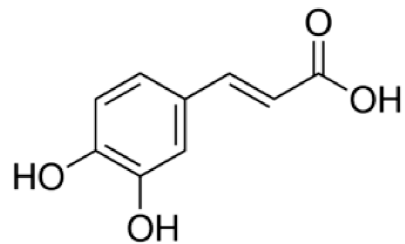
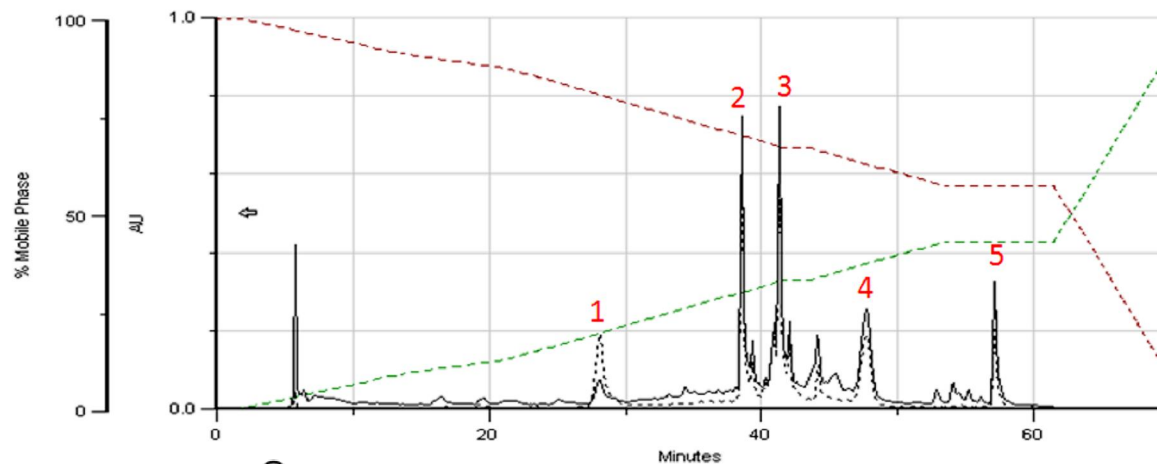
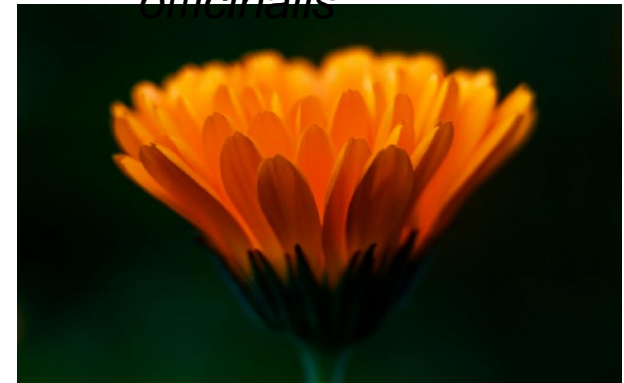
**5: Caffeic acid**

**6: Lignans or tanins**

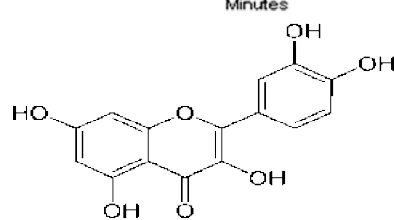
**7: Lignans or tanins**

- Antiarthritic
- Antibacterial activity

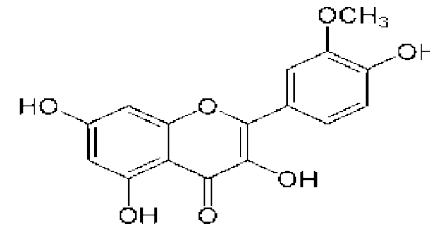
*Calendula  
officinalis*



**1:** Caffeic acid



**2:** Quercetine



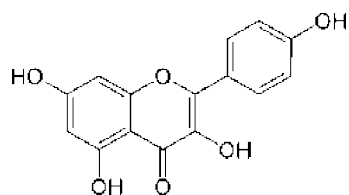
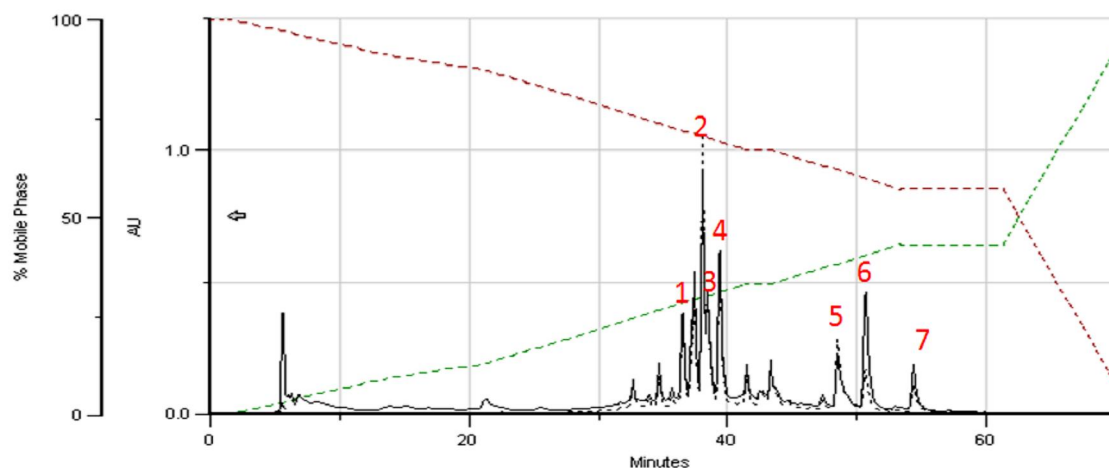
**3:** Isorhamnetin

**4 :**Lignans or tannins

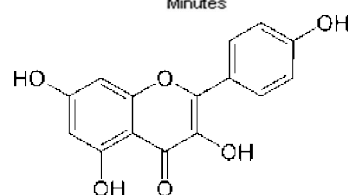
**5:** Lignans or tanins

- cytotoxic and anti-tumor activity
- anti inflammatory activity

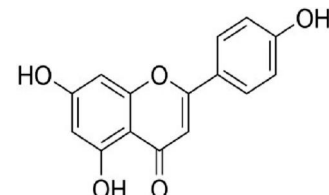
## *Papaver rhoeas*



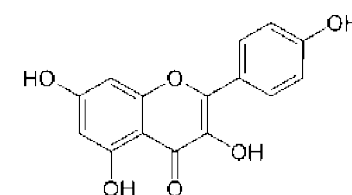
**1: Kaempferol**



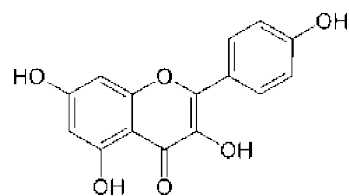
**2: Kaempferol**



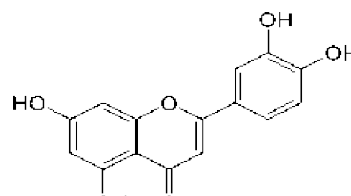
**3: Apigenin**



**3: Kaempferol**



**4: Kaempferol**



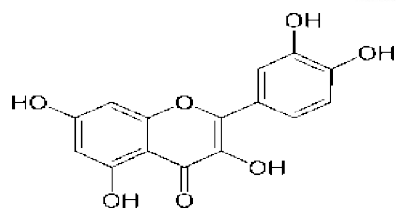
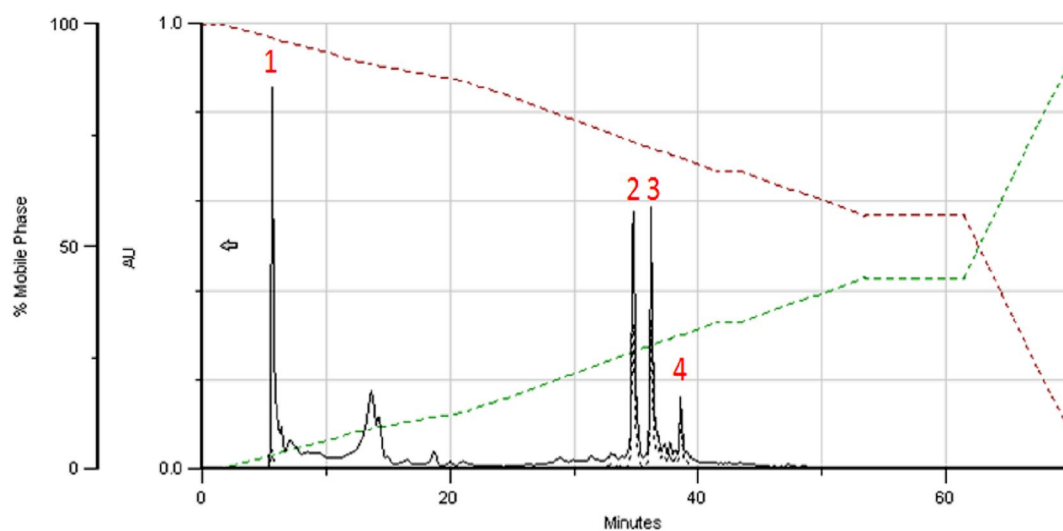
**5: Luteolin**

**6: Lignans or tannins**

**7: Lignans or tanins**

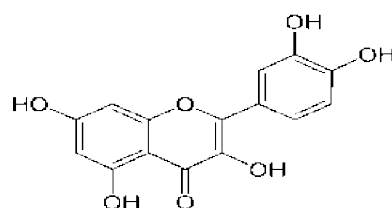
- Antioxidant activity
- Antimicrobial activity

## *Malva sylvestris*

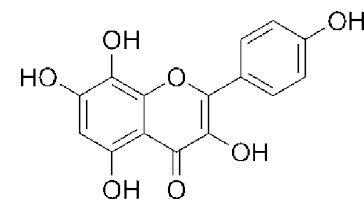


**1:** alkaloid

**2:** Quercetin



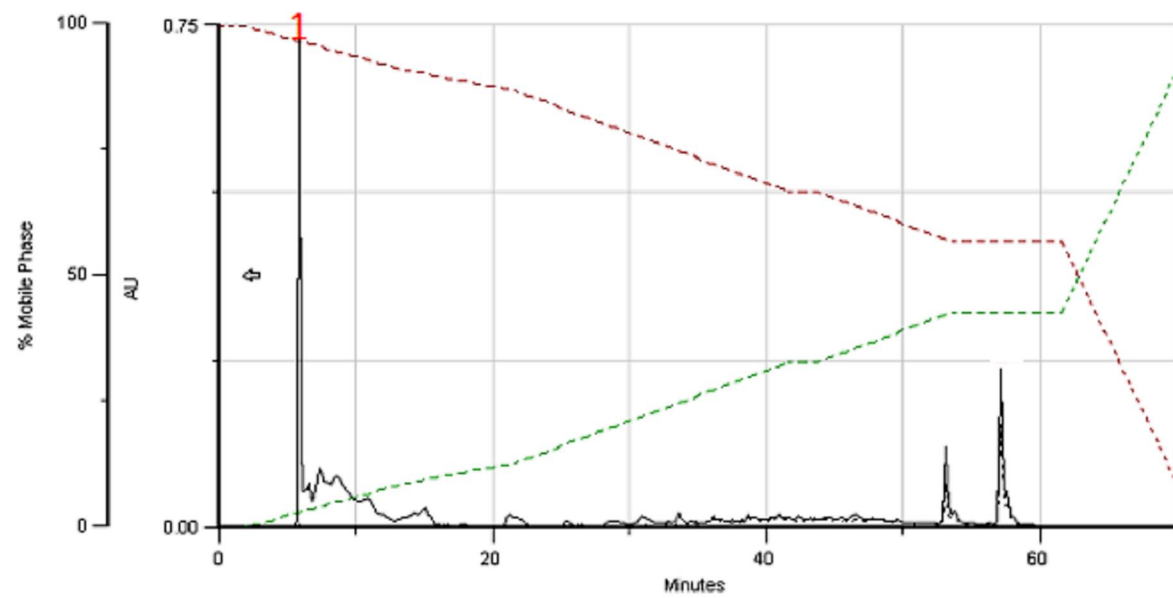
**3:** Quercetin



**4:** Herbacetin

- Cutaneous wound healing
- Hepatoprotective effects



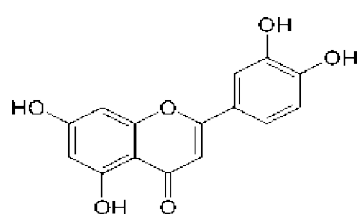
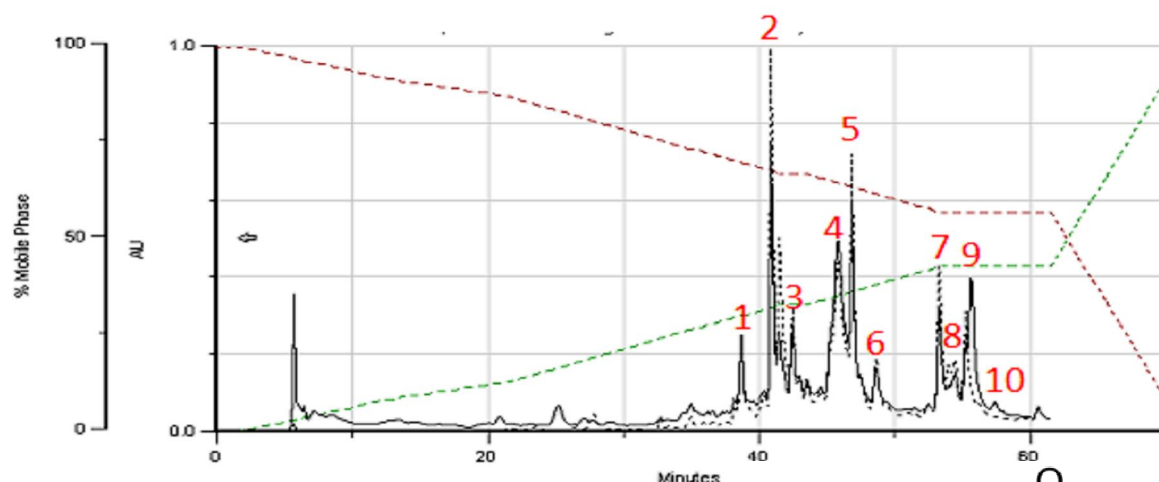


**1: alkaloid**

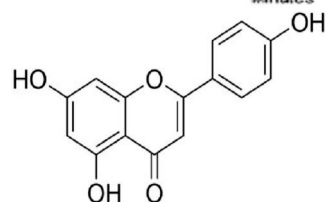
*Scolymus hispanicus*



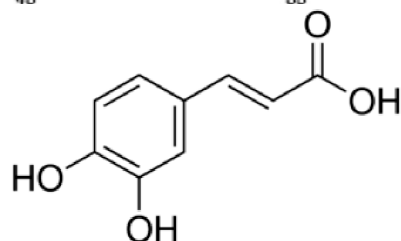
## *Mentha Spicata*



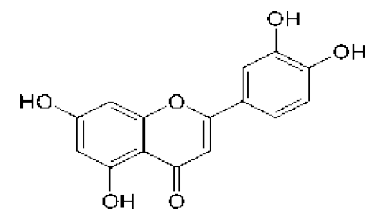
**1: Luteolin**



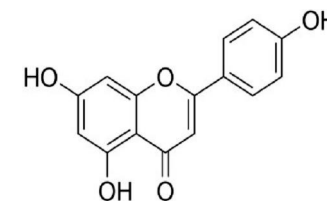
**2: Apigenin**



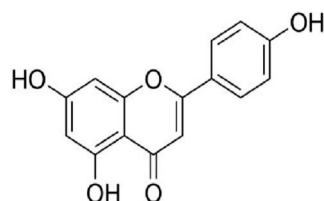
**3: Caffeic acid**



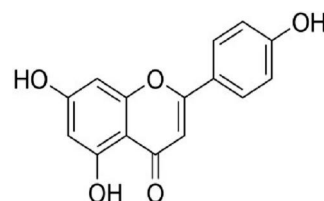
**4: Luteolin**



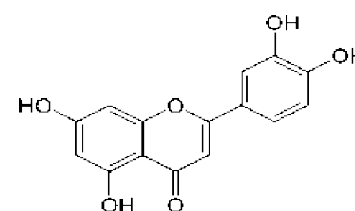
**7: Apigenin**



**8: Apigenin**

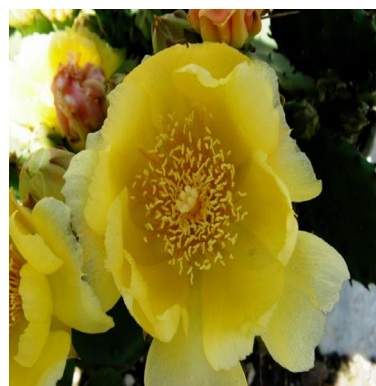
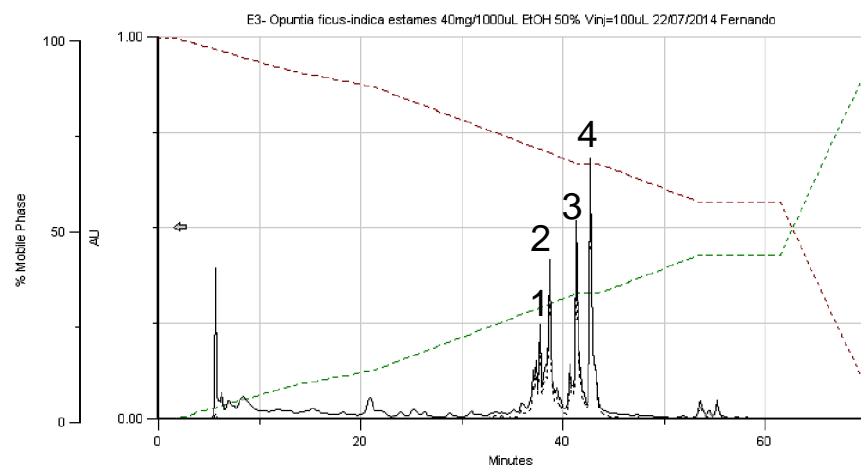


**9: Apigenin**

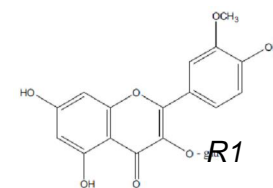
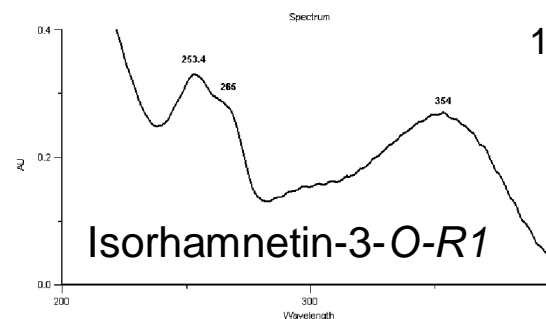


**10: Luteolin**

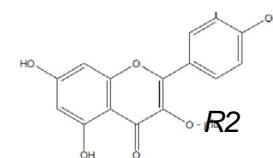
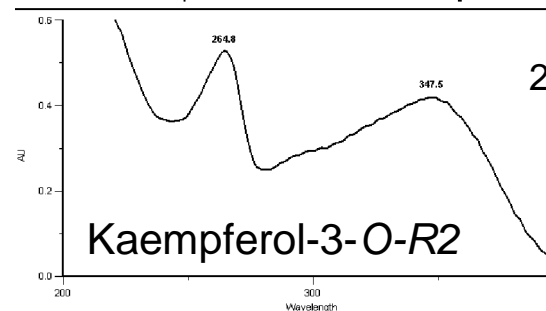
# Opuntia-ficus-indica pollen



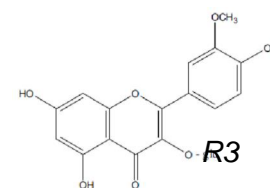
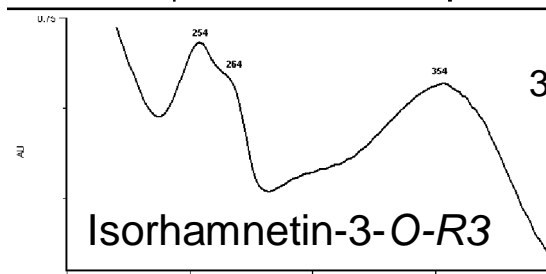
Time: 37.72 : E3- Opuntia ficus-indica estames 40mg/1000uL EtOH 50%



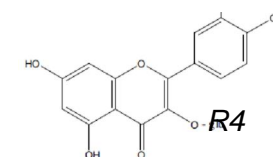
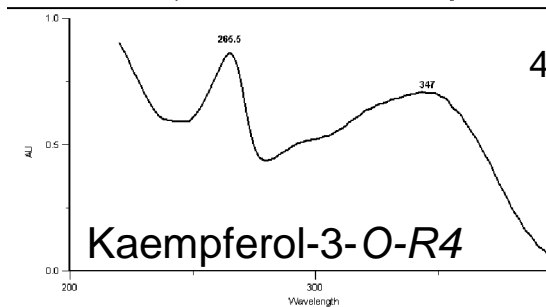
Time: 38.66 : E3- Opuntia ficus-indica estames 40mg/1000uL EtOH 50%

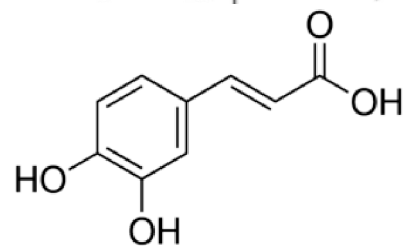
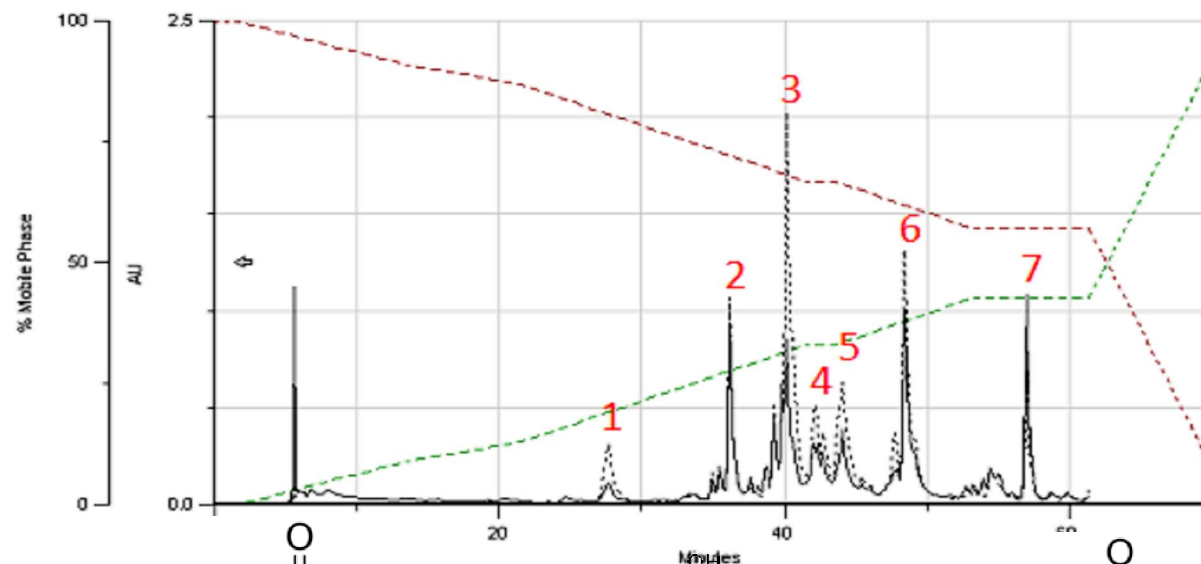


Time: 41.27 : E3- Opuntia ficus-indica estames 40mg/1000uL EtOH 50%

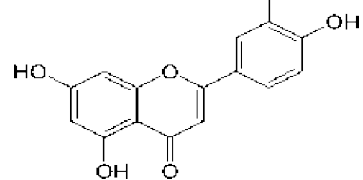


Time: 42.68 : E3- Opuntia ficus-indica estames 40mg/1000uL EtOH 50%

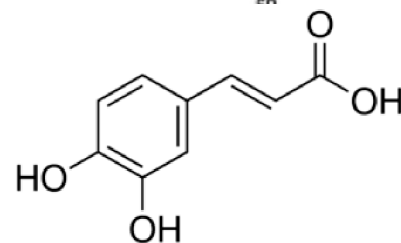




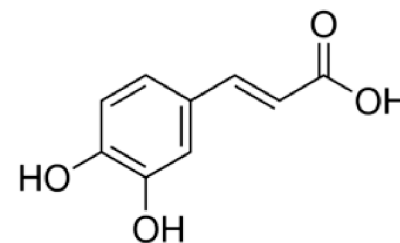
**1: Caffeic acid**



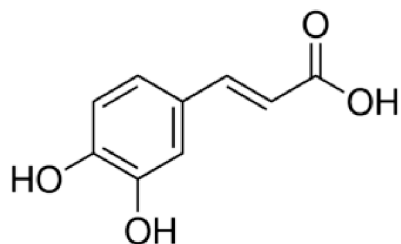
**2: Luteolin**



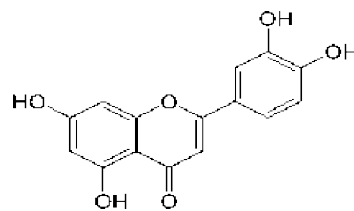
**3: Caffeic acid**



**4: Caffeic acid**



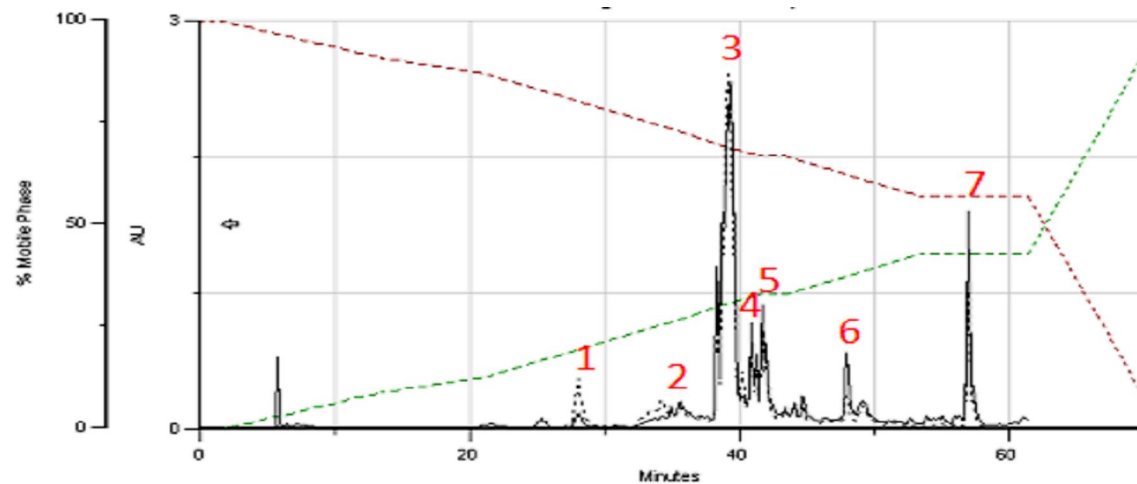
**5: Caffeic acid**



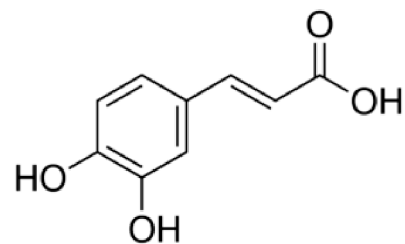
**6: Luteolin**

*Anacyclus clavatus*

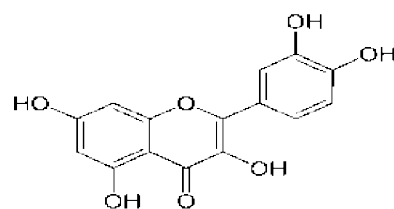




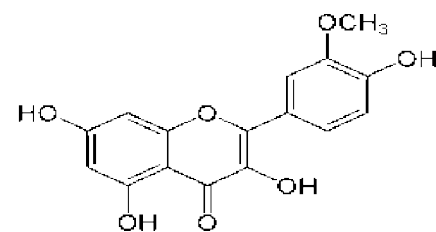
*Justicia adhatoda*



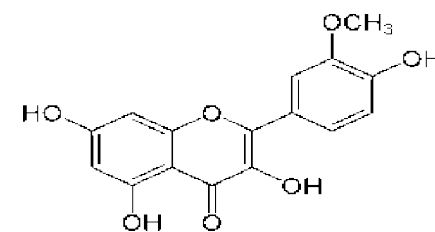
**1:** Caffeic acid



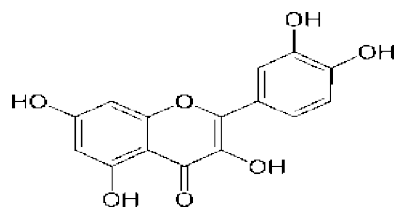
**2:** Quercetin



**3:** Isorhamnetin

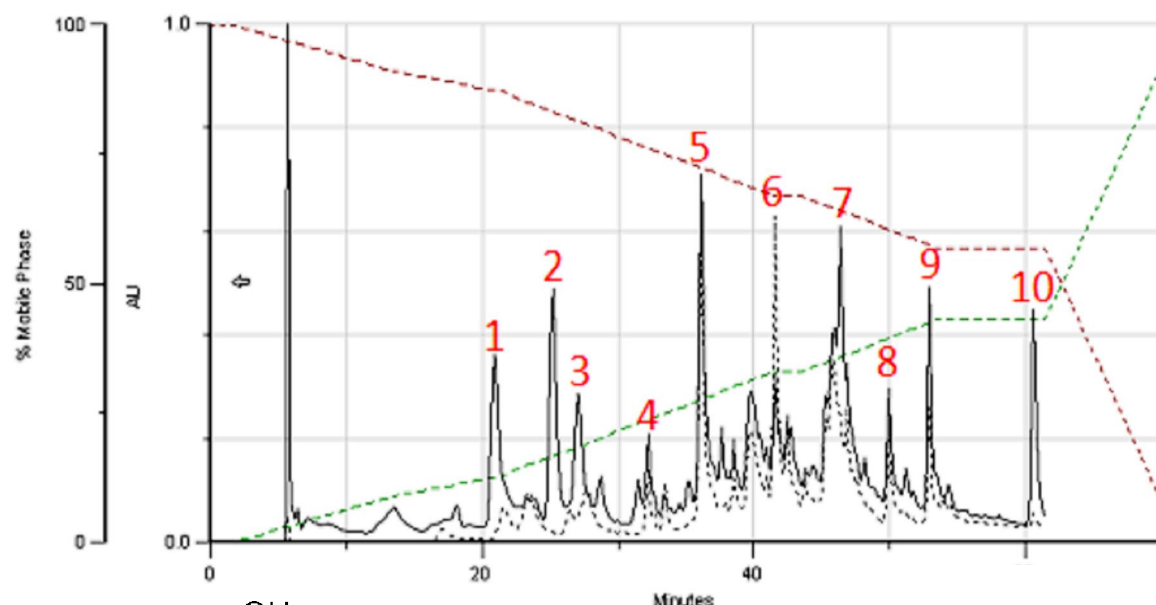


**4:** Isorhamnetin

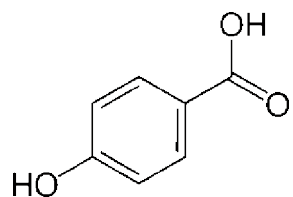


**6:** Quercetin

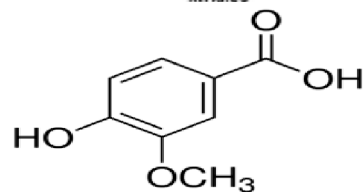
**7:** Lignan or tannin



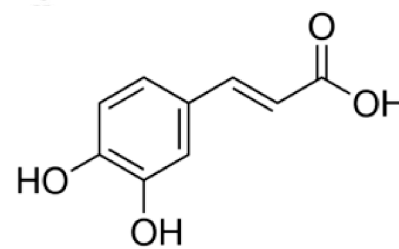
*Mentha pulegium*



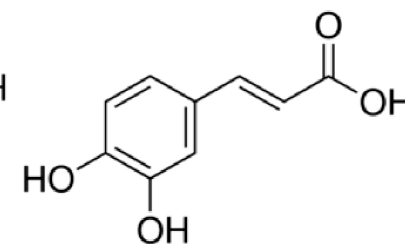
**1:** p-hydroxybenzoic



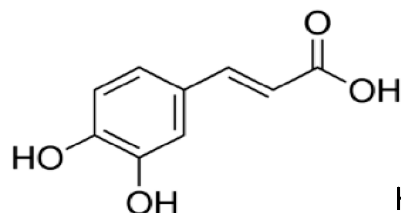
**2:** vanillic acid



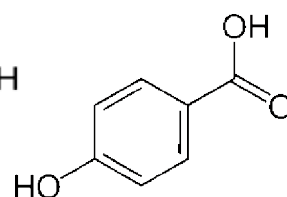
**3:** Caffeic acid



**4:** Caffeic acid

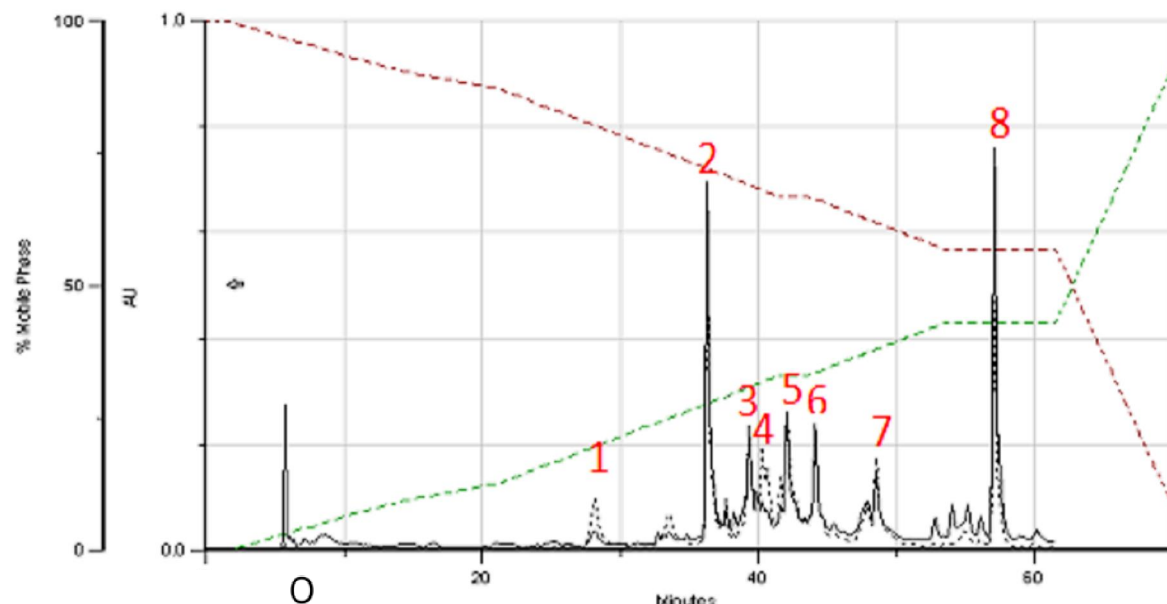


**5:** Caffeic acid

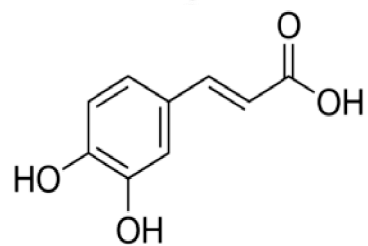


**6:** p-hydroxybenzoic

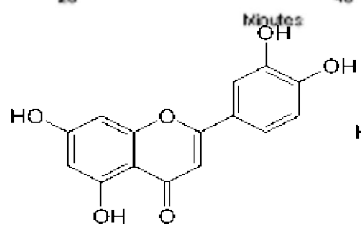




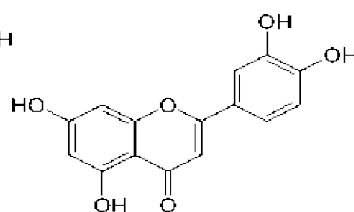
*Anacyclus  
radiatus*



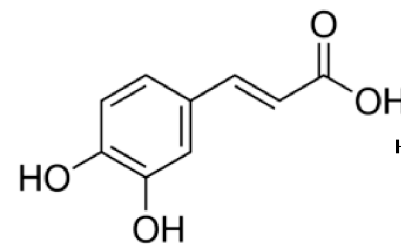
**1:** Caffeic acid



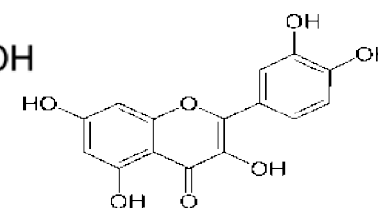
**2:** Luteolin



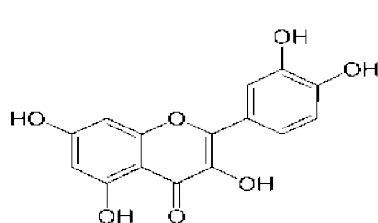
**3:** Luteolin



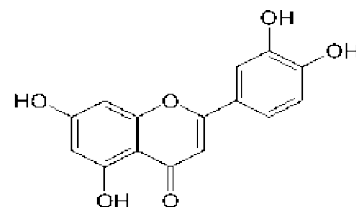
**4:** Caffeic acid



**5:** Quercetine

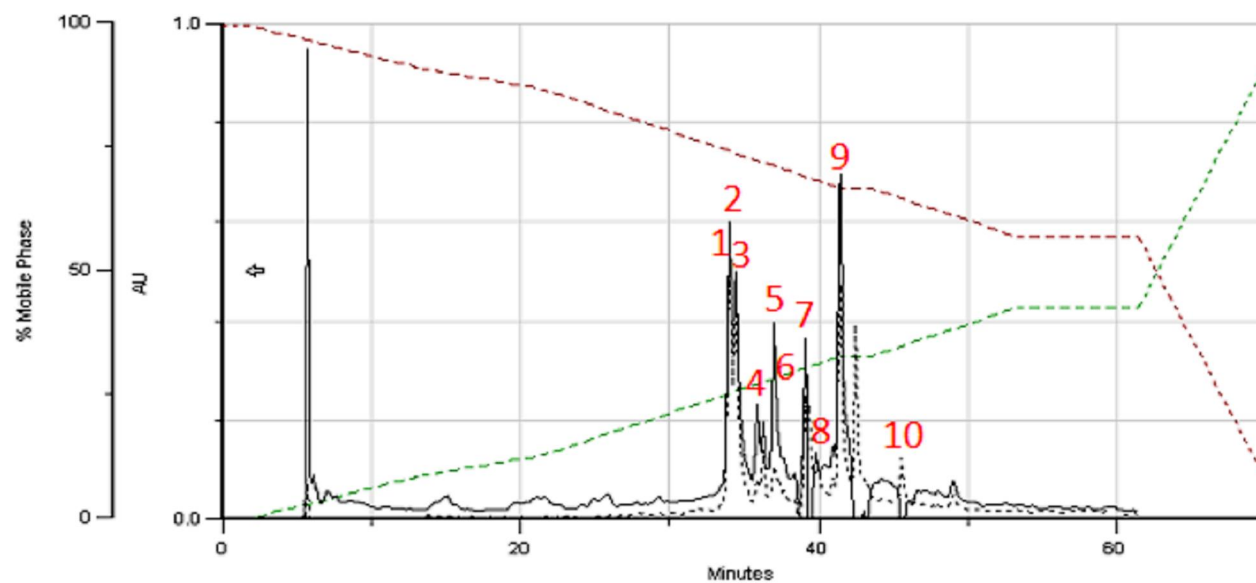


**6:** Quercetine

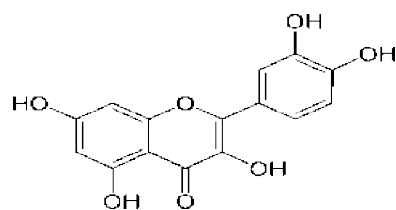


**7:** Luteolin

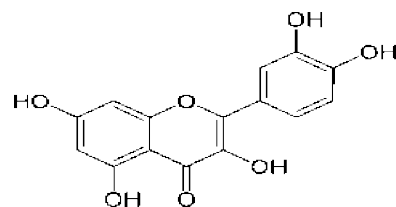
**8:** Lignan or tannin



*Acacia  
pycnantha*



**3: Quercetin**

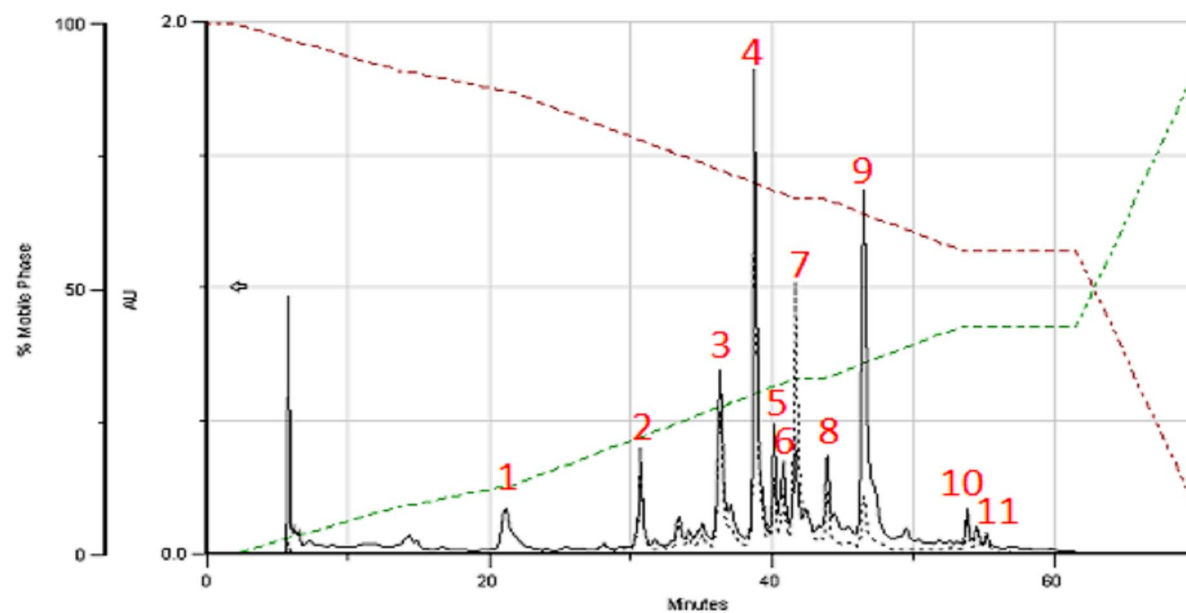


**4: Chalcone**

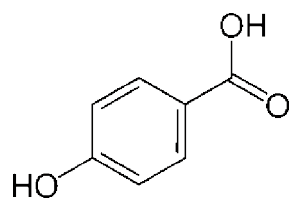
**5: Quercetin**

**8: Chalcone**

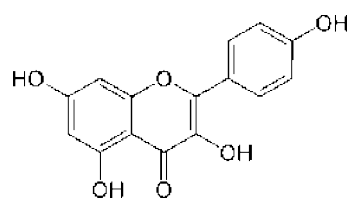
**9: Chalcone**



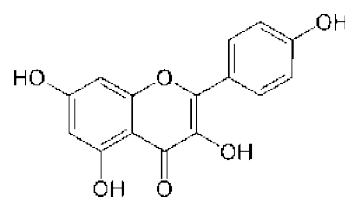
*Echium creticum*



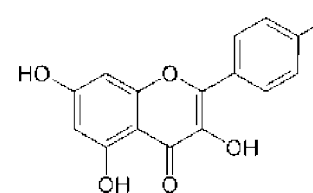
**1:** p-hydroxybenzoic



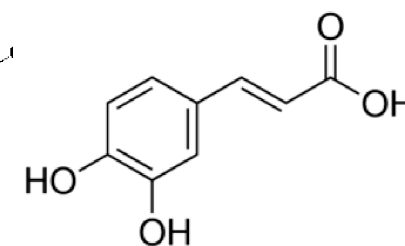
**2:** Kaempferol



**4:** Kaempferol

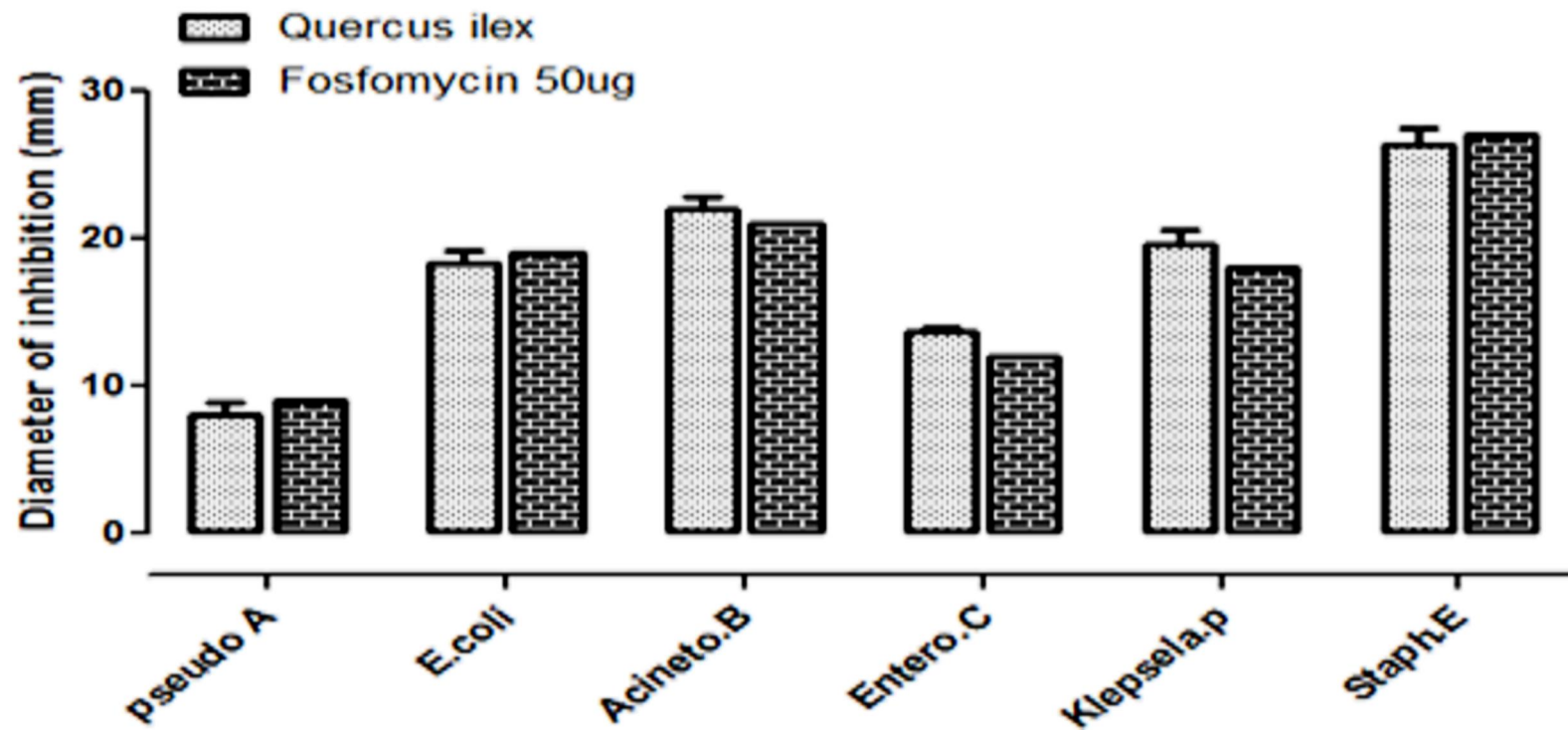


**5:** Kaempferol



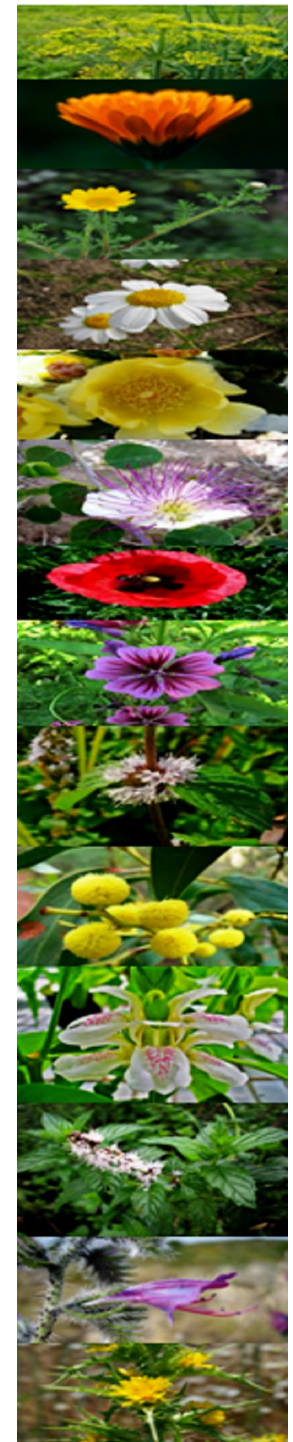
**9:** Caffeic acid

*Escherichia coli*  
*Pseudomonas aeruginosa*  
*Enterobacter cloacae*  
*Acinetobacter baumannii*  
*Klebsella pneumonia*  
*Staphylococcus aureus*





The present findings are the first to be published with fresh hand collect pollen from these selected plants and indicate that this matrix is a good source of phenolic compounds especially polyphenolics as flavones and flavonols like the derivatives of **apigenin**, **luteolin**, **quercetin**, **isorhamnetin** and phenolic acids such as **Caffeic**, *p*-hydroxybenzoic acid, and **Vanillic acid**. All these compounds in the studied plants have certainly a contribution for the bioactivity study. This can be used as a start point to study other potential application for pollen as anti-inflammatory or even as antimicrobial drugs.





## Taxon passport

- “ Variability in the colour of the pellet
- “ Microscopic analysis
- “ HPLC/DAD phenolics profile
- “ Physico-chemical analysis
  - Proteins & Free aminoacids
  - Lipids
  - Sugars
  - Minerals
  - Vitamins
  - Alkaloids



Bioactivity (DPPH; ORAC; TBARS;  
Anti-inflammatory, antimutagenic,  
Antimicrobial, antiprotozoan,  
etc

Caloric value: 381Kcal/100g

# RESEARCH GAPS

- + Validate more floral sources
- + Determination of toxic contaminants
- + Contamination by genetic modified plants
- + ...





Many thanks  
for your attention!  
Merci beaucoup

شكرا



Fez, Morocco

