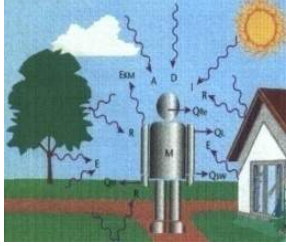
5TH APIQUALITY & 6TH APIMEDICA
INTERNATIONAL SYMPOSIUM
ROME, 22-25 NOVEMBER 2016



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

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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**
- **Antiartherosclerotic activity - fat-soluble pollen extract**
- **Activity on osteoporosis – *Cistus ladaniferus* – PM ≤ 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 Pratice 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

Producers

Competion with cheap products without quality control

Public Health

(Contamination with pesticides, heavy metals, etc)

Customer/ Patient

Pollen composition and standartisation 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)

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,
Romany,
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

Centro de Estudos Farmacêuticos

Portugal

Identification of Floral Origin

UNIVERSIDADE DE COIMBRA

Acacia dealbata

Bignonia spp.

B r a s s i c a

Camellia sinensis L.

Cocus nucifera L.

Coleostephus myconis

Digitalis purpurea L.

Galactites tomentosus L.

Ligustrum sp.

Lotus sp.

Olea europaea L.

Tilia sp..

Viburnum tinus L.

Zea mays L.

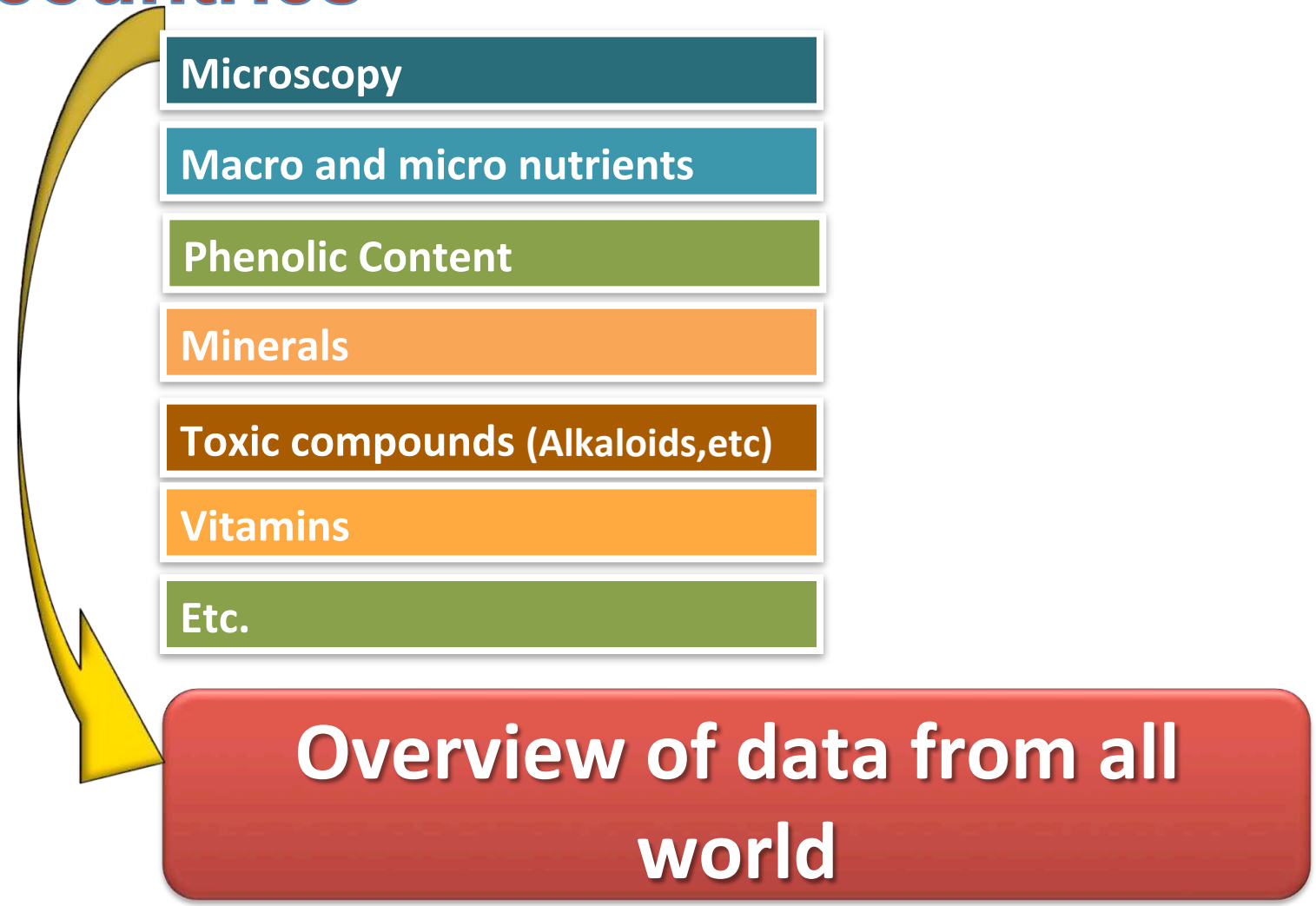
Etc.

Etc.

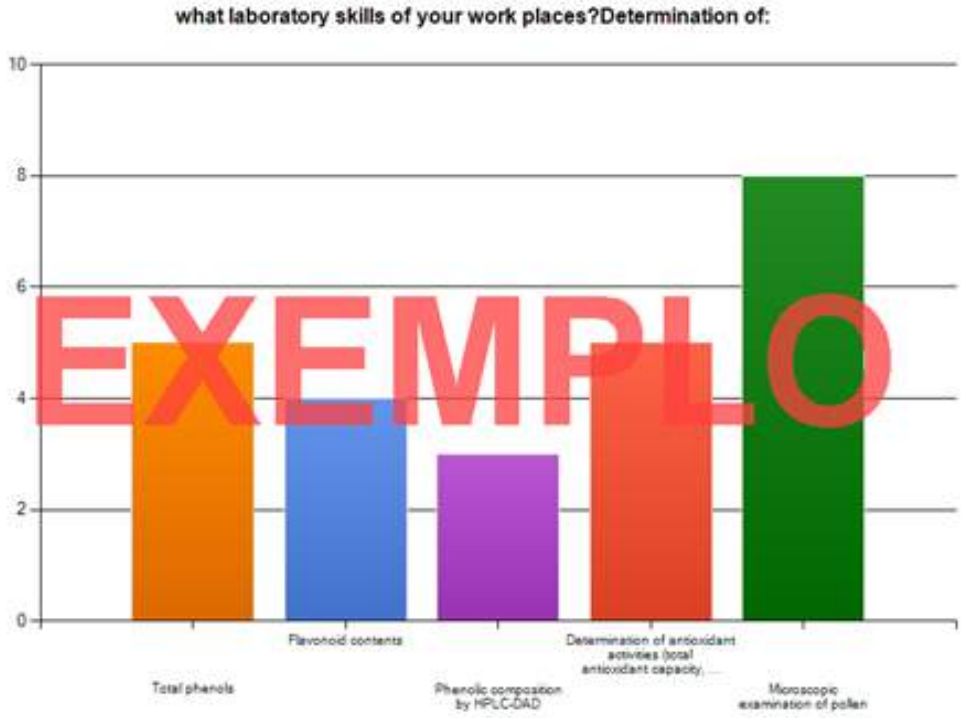
Espécies com exploração apícola (várias zonas do globo).*AMÂNCIO, D. C. - Compostos bioativos do pólen. Tese de mestrado. Faculdade de Farmácia, Universidade de Coimbra (2014) 30-32.*

Campos M.G, Anjos O:. & Amâncio D.

Results from various countries



- **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, antioxydant 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

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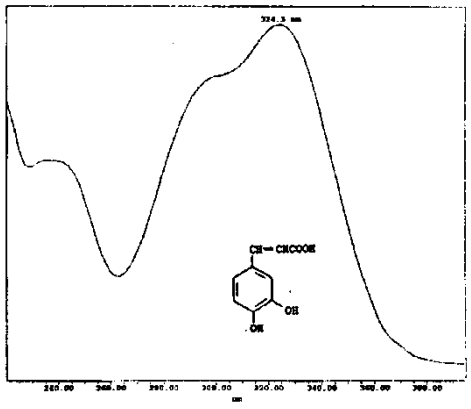
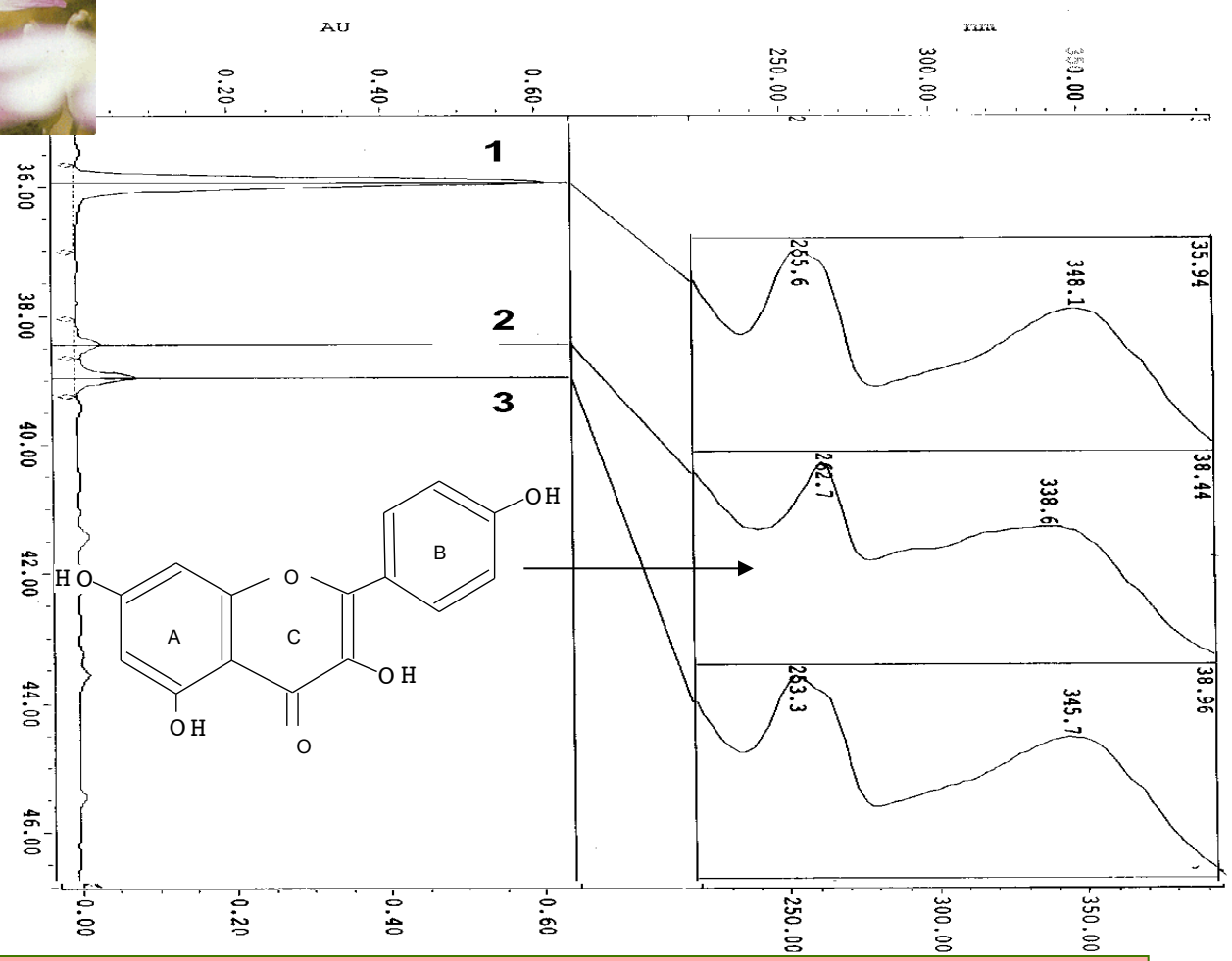
Bee POLLEN





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 officinalis

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

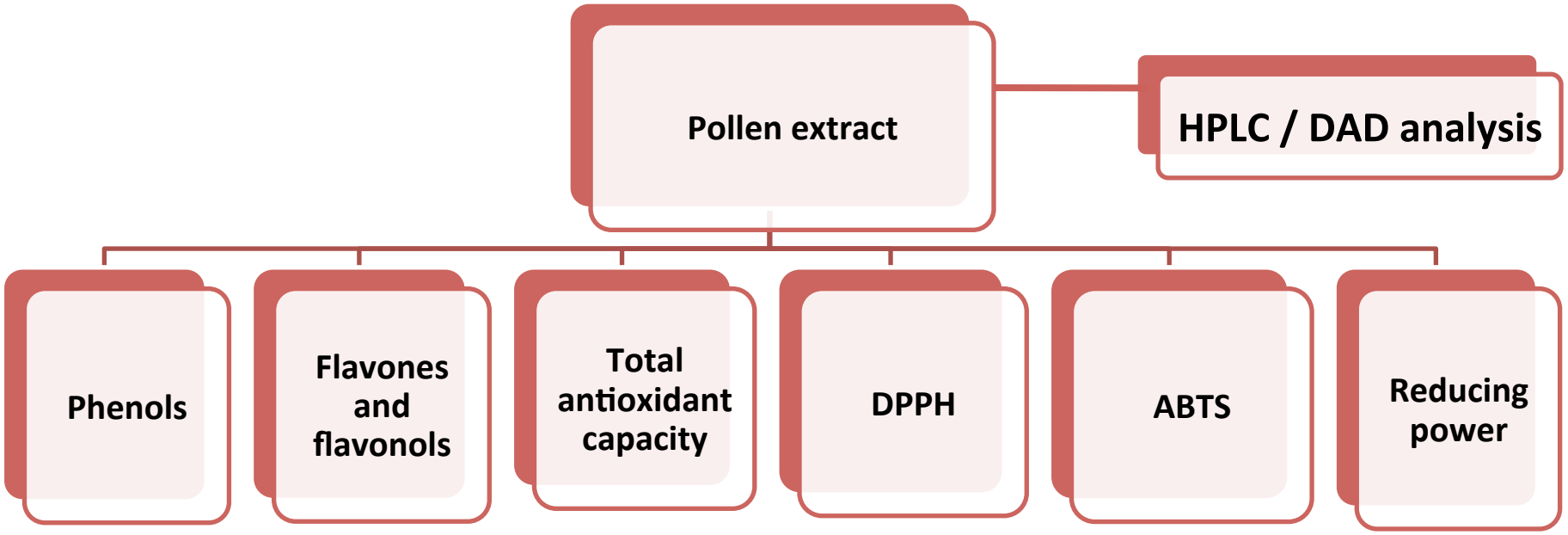


Table 1: Phenolics,flavones and flavonol content correlated to Phosphomolybdate assay (total antioxidant capacity) (mg/g of pollen)

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

•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

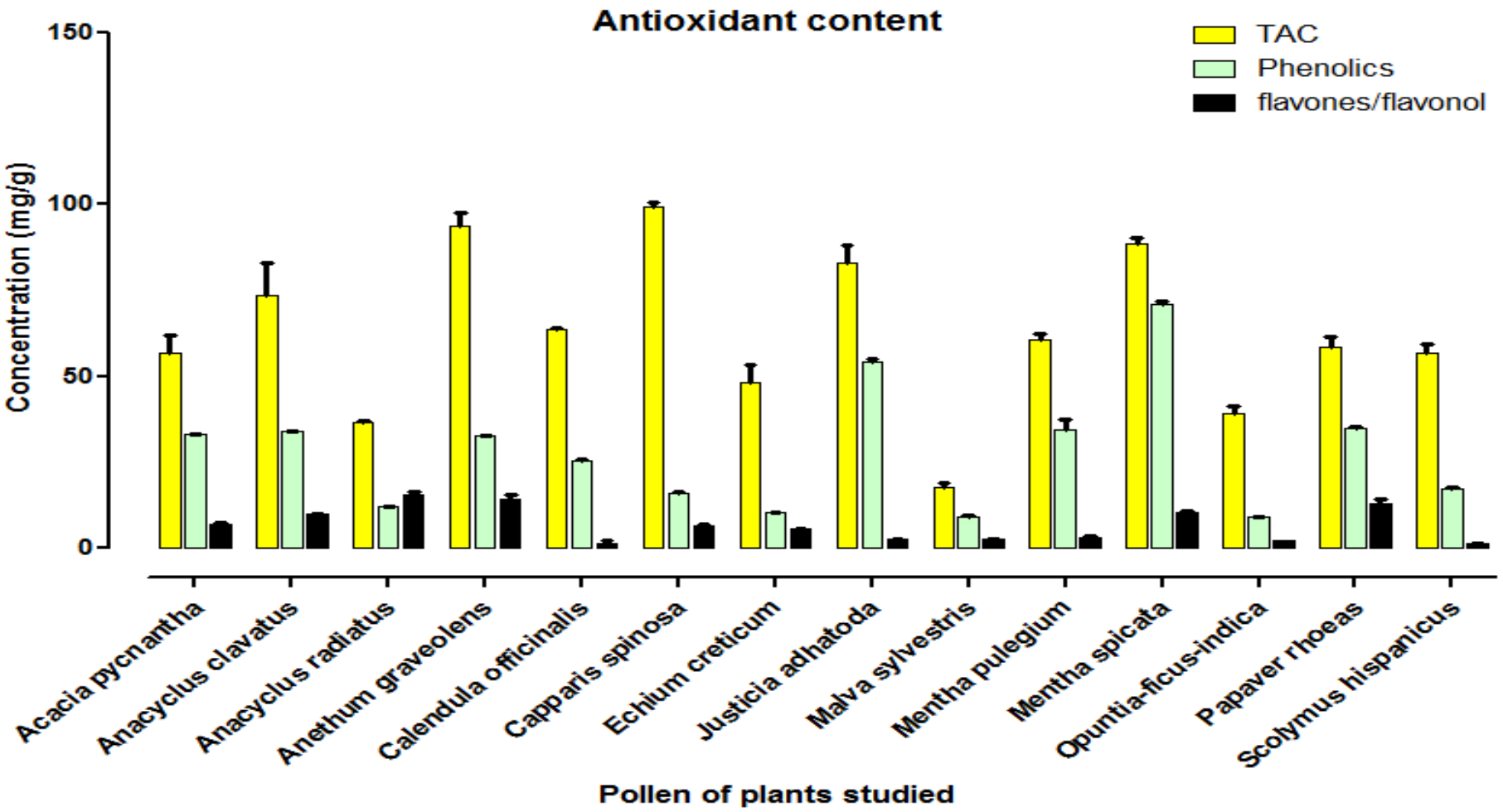


Figure 1: Phenol, flavones and flavonol Content, and Phosphomolybdate assay (total antioxidant capacity) (mg/g of pollen)

Table 2: Correlation between the specie and the Antioxidant activities (DPPH, ABTS and RP) expressed as IC_{50} (mg/ml) for ABTS, DPPH and EC_{50} for RP.

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

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

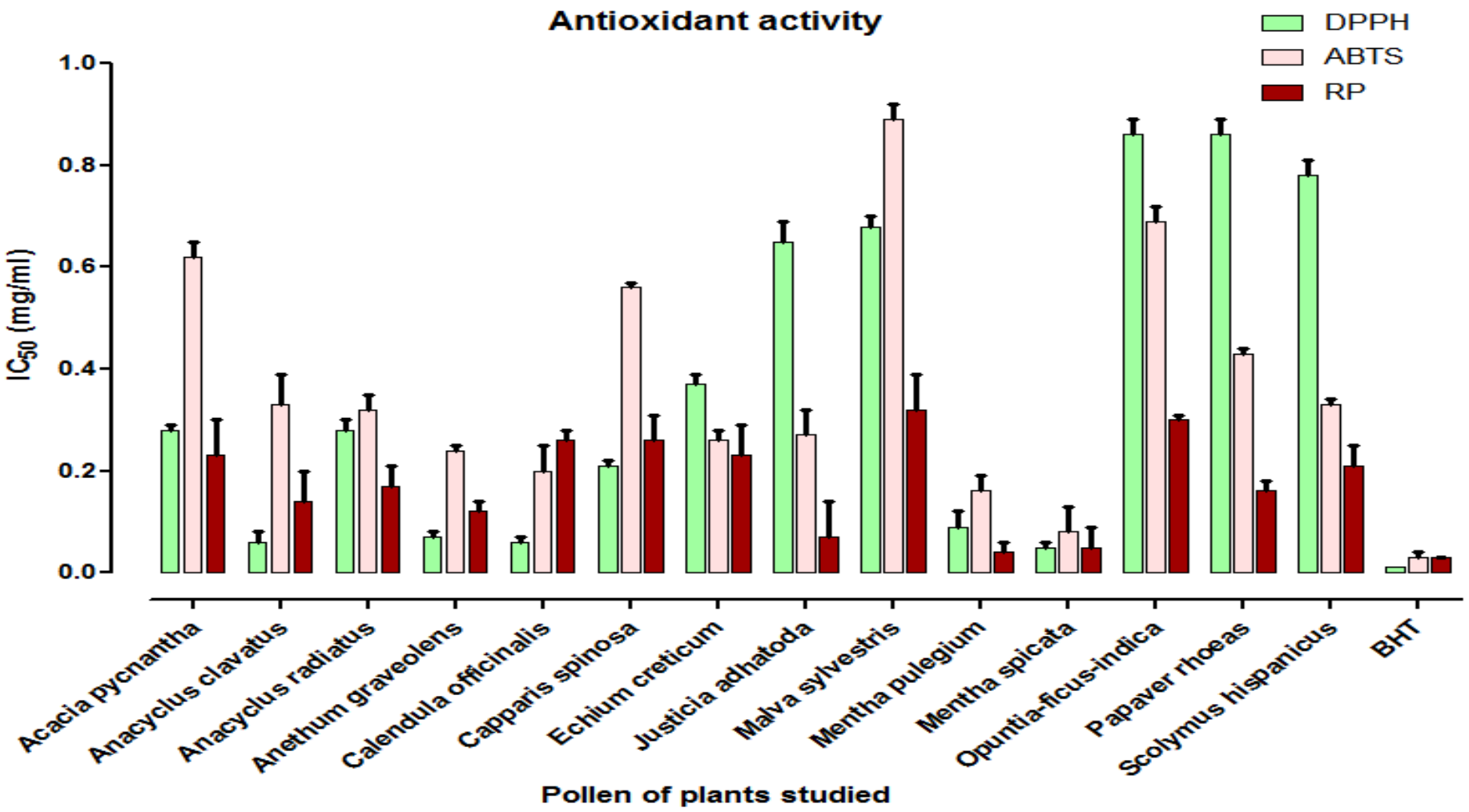
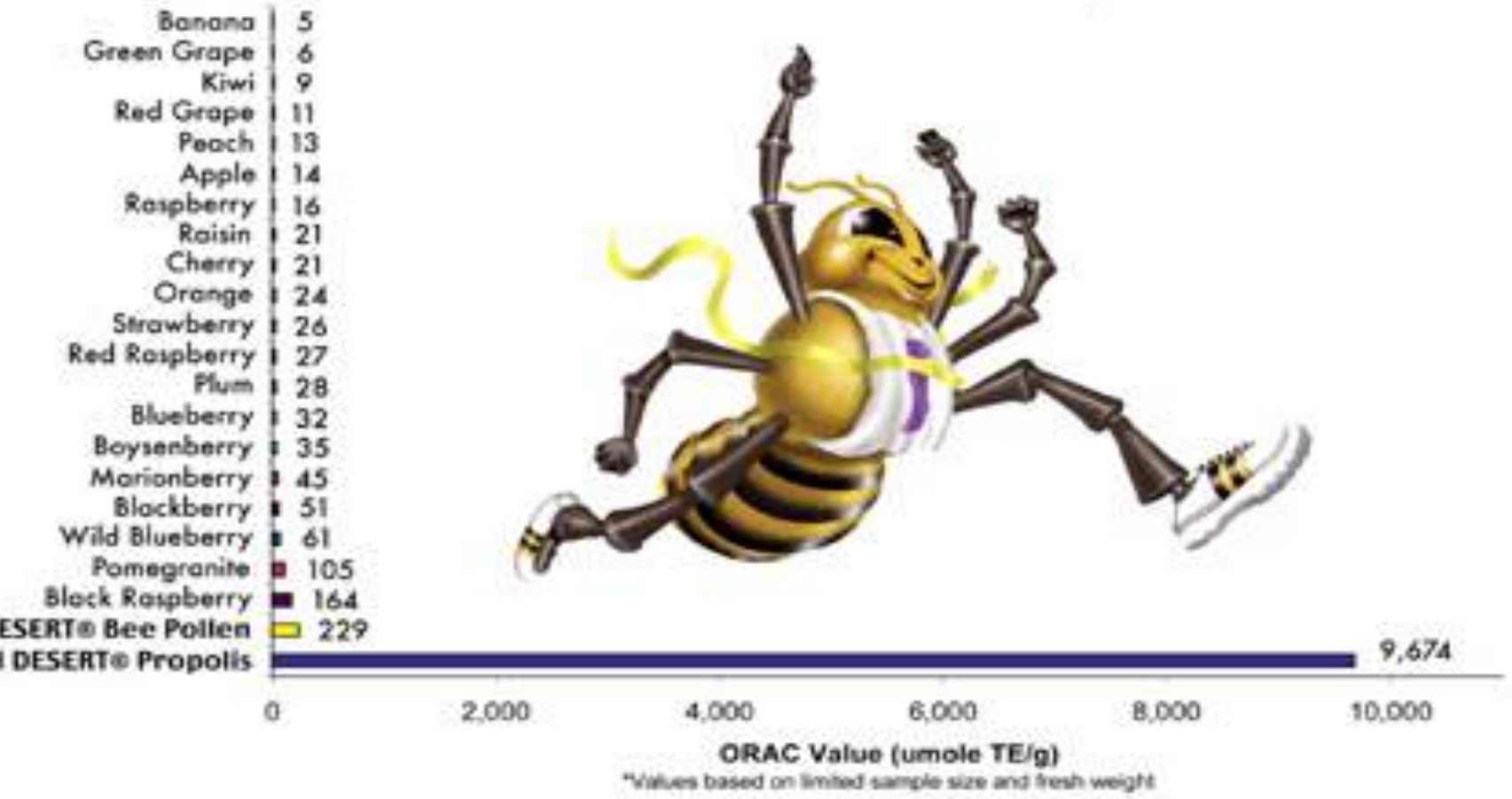


Figure 2 : Antioxidant activities (DPPH, ABTS and Reducing power) expressed as IC_{50} (mg/ml).

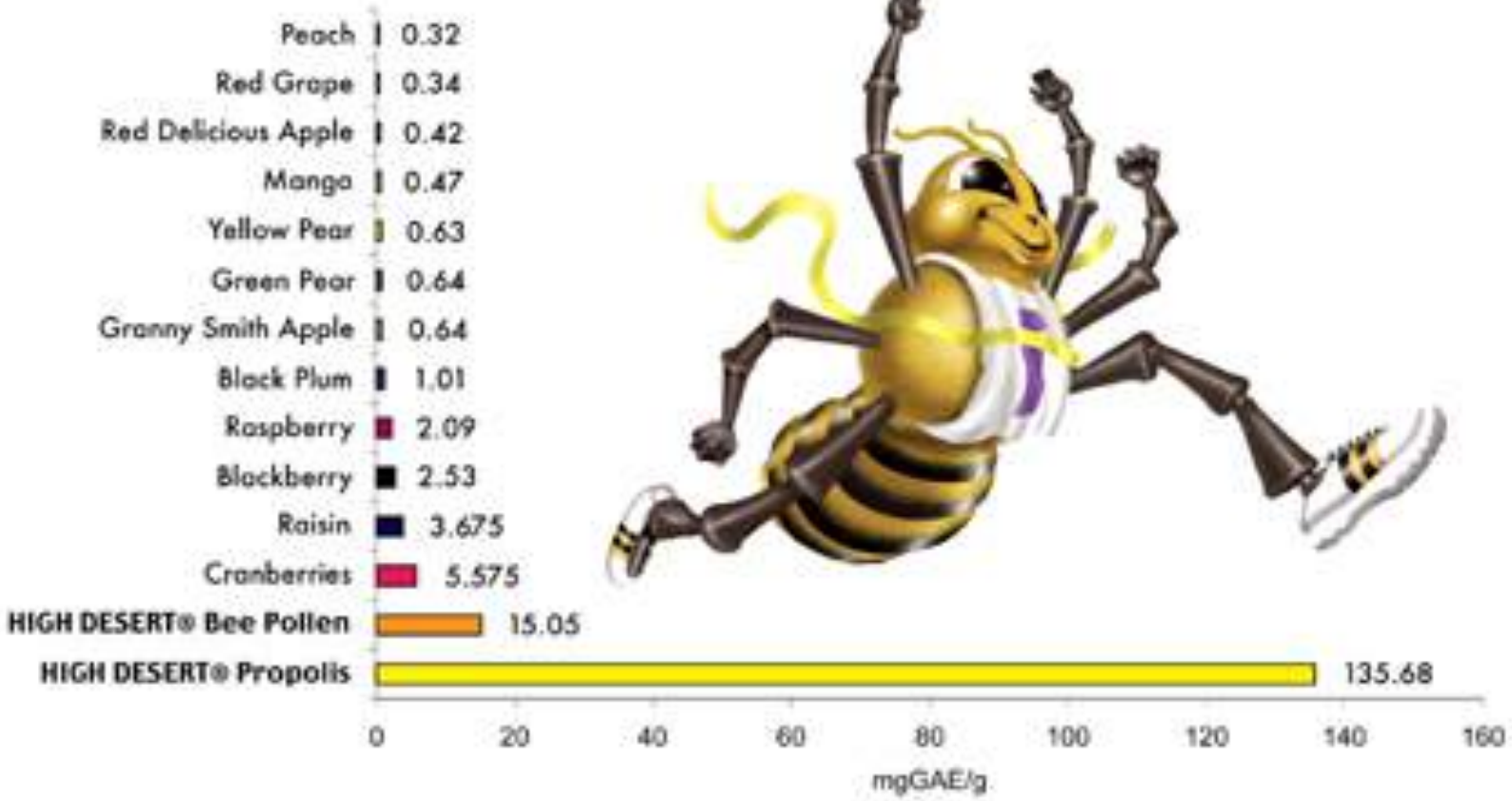
ORAC (Oxygen Radical Absorbance Capacity) Chart

Whole Food Antioxidant Activity

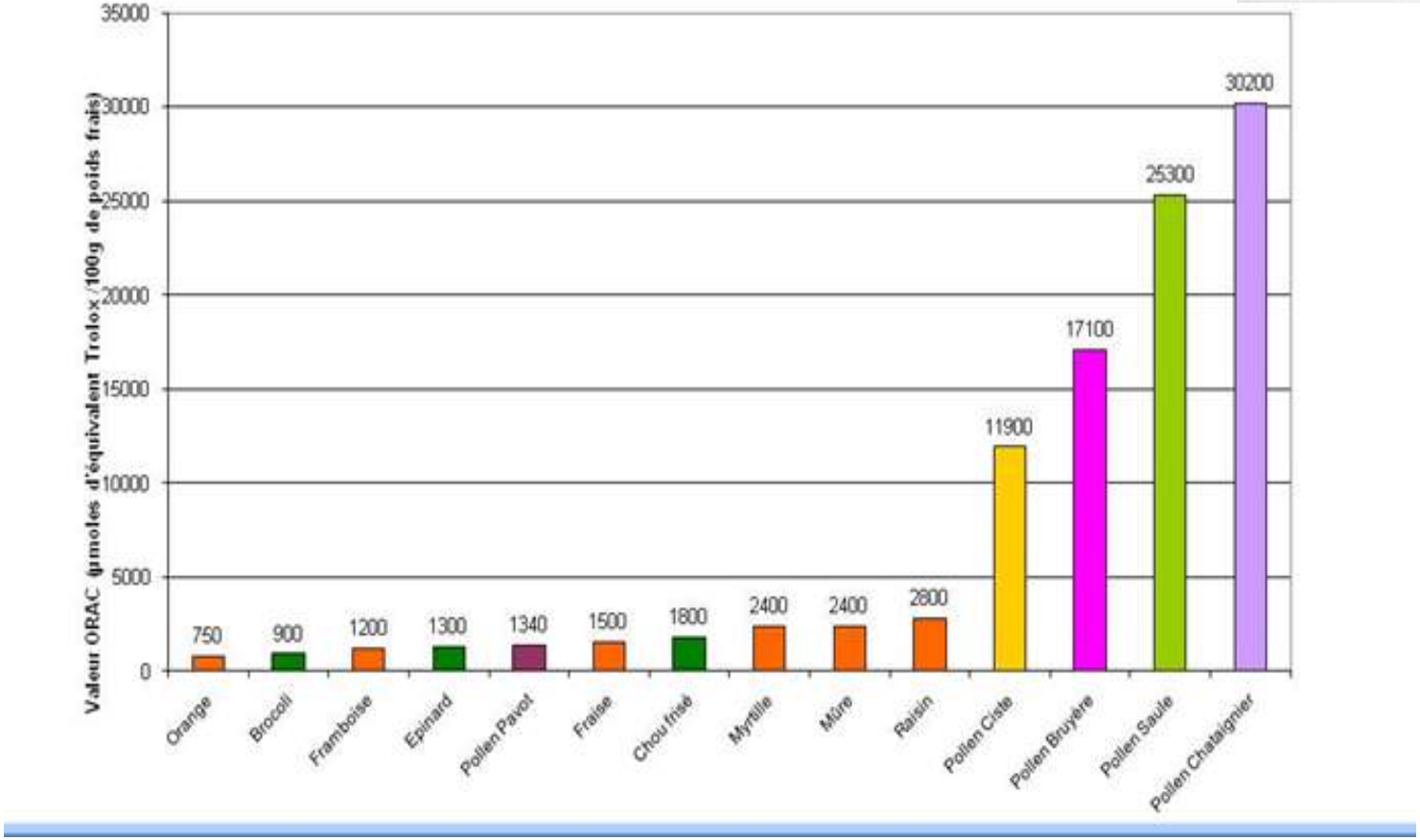


*Values based on limited sample size and fresh weight

Total Polyphenol Content

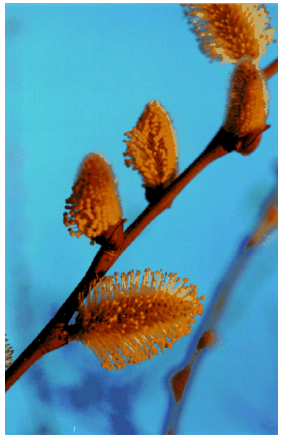


Antioxidant capacity of pollen



Analytical methods

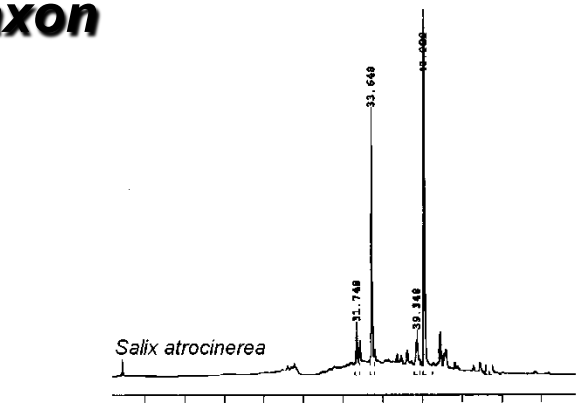
Polyphenolic profiles (phenolic acids & flavonoids)
Identification of the *taxon*
Herbarium samples

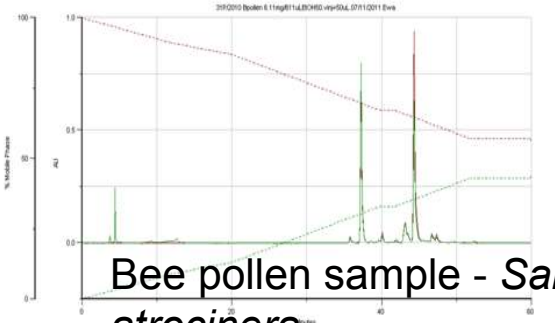


sediment

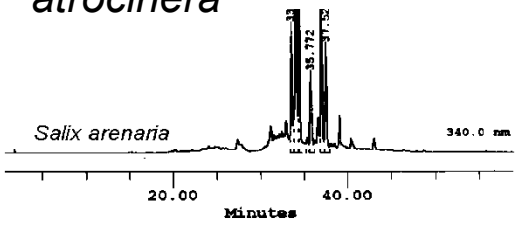
SAMPLE PREPARATION

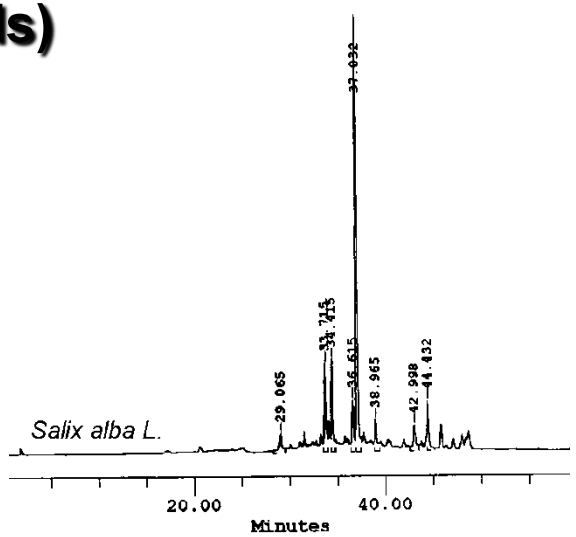
- ✓**ethanol 50% extraction**
- ✓ **vortex + ultrasounds**
- ✓ **centrifugation**

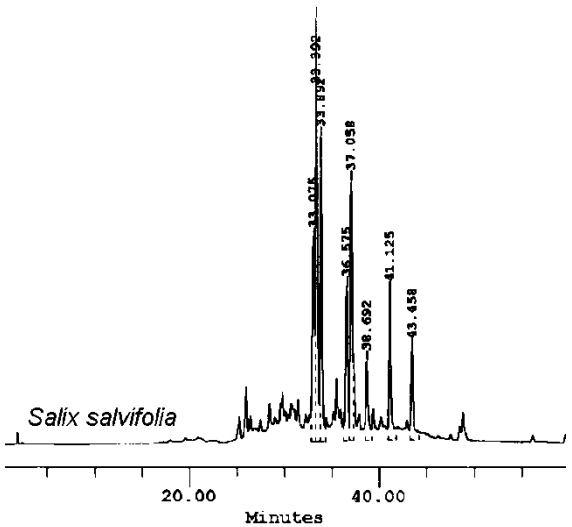




Bee pollen sample - *Salix atrocinerea*

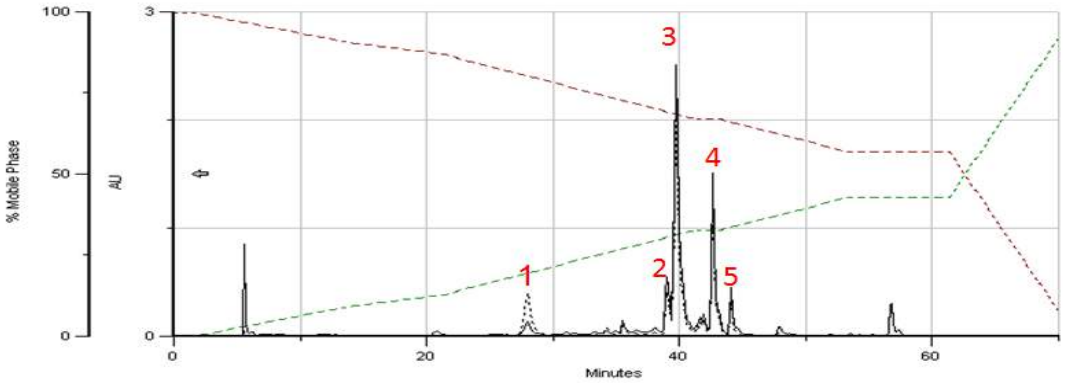


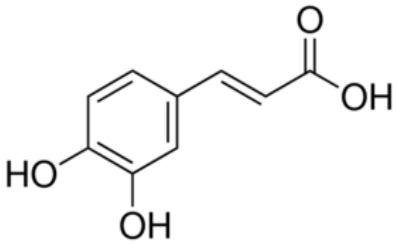




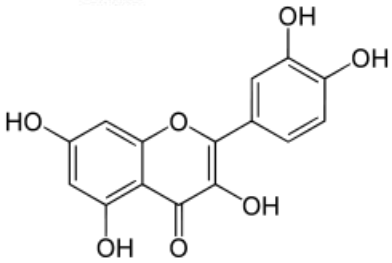
HPLC/DAD λ 220-400nm

Anethum graveolens

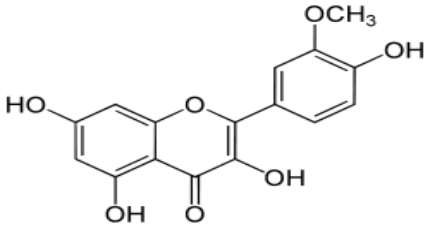




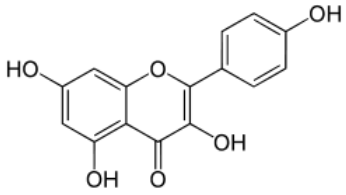
1: Caffeic acid



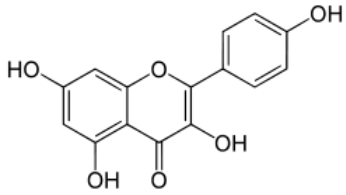
2: Quercetine



3: Isorhamnetin



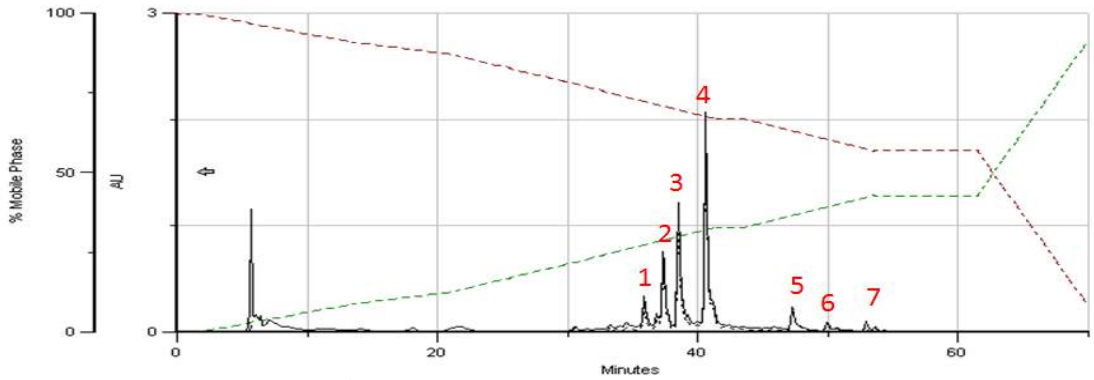
4: Kaempferol

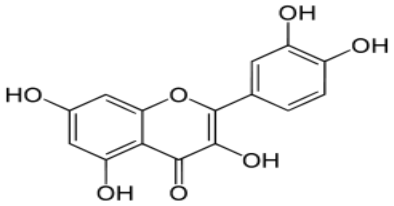


5: Kaempferol

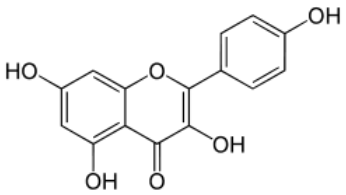
- Haematological and hypoglycemic effets
- Microbiological effects

Capparis spinosa

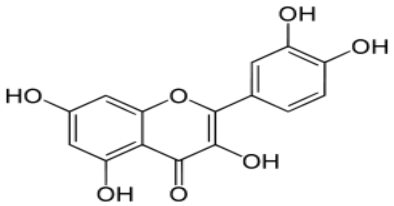




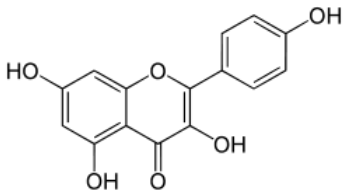
1: Quercetine



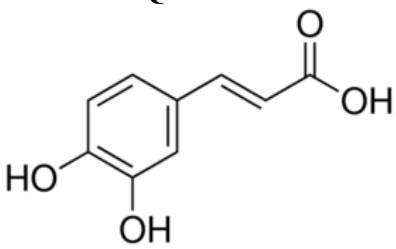
2: Kaempferol



3: Quercetine



4: Kaempferol



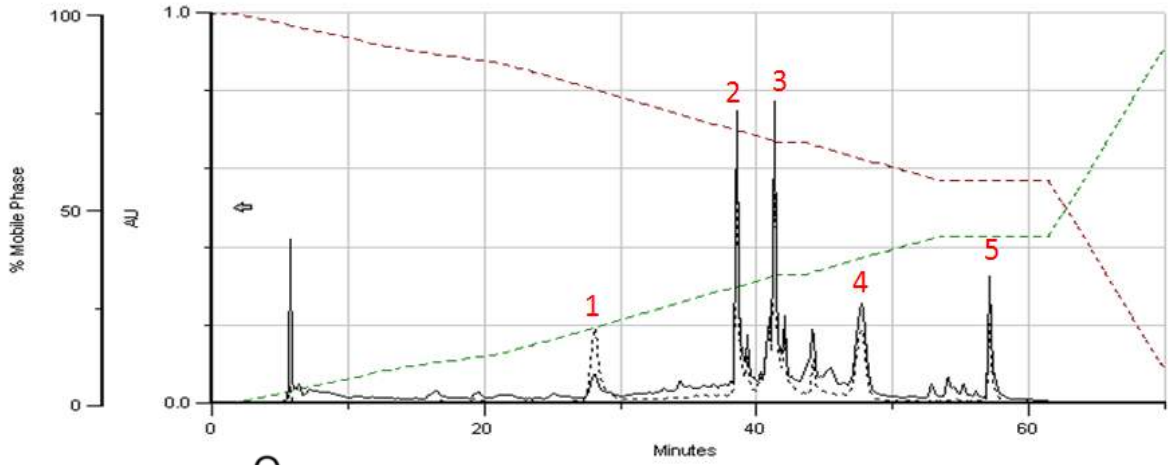
5: Caffeic acid

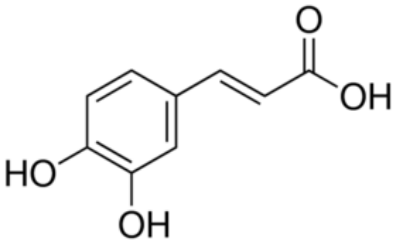
6: Lignans or tanins

7: Lignans or tanins

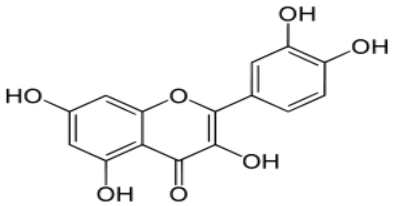
- Antiarthritic
- Antibacterial activity

Calendula officinalis

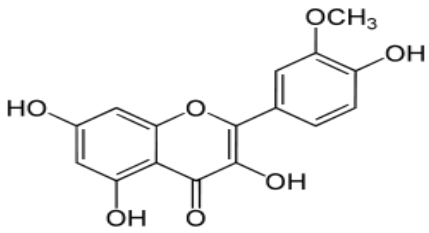




1: Caffeic acid



2: Quercetine



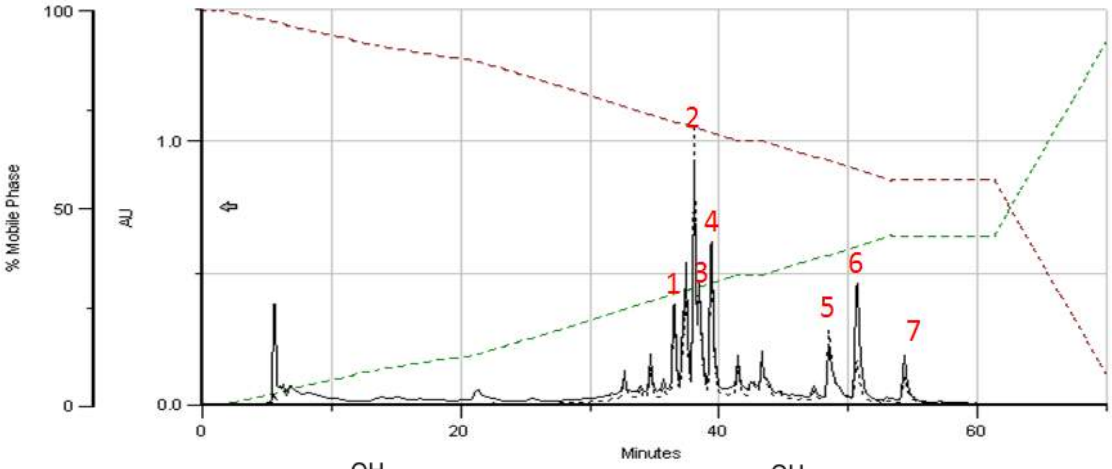
3: Isorhamnetin

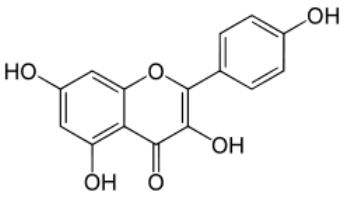
4 :Lignans or tannins

5: Lignans or tanins

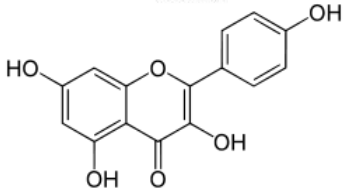
- cytotoxic and anti-tumor activity
- anti inflammatory activity

Papaver rhoeas

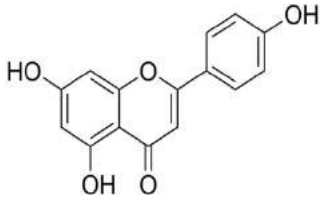




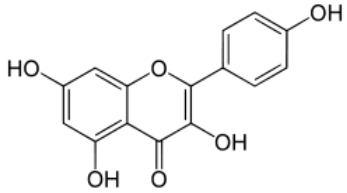
1: Kaempferol



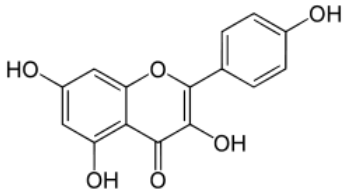
2: Kaempferol



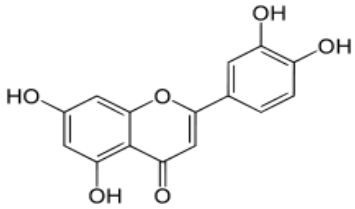
3: Apigenin



3: Kaempferol



4: Kaempferol



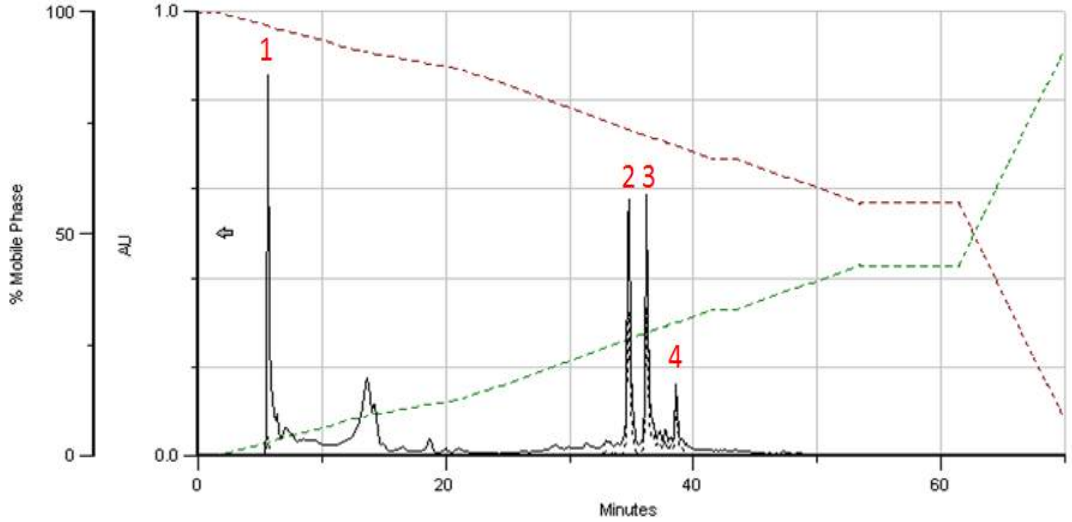
5: Luteolin

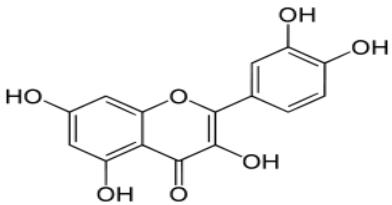
6: Lignans or tannins

7: Lignans or tanins

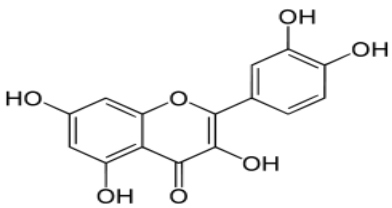
- Antioxidant activity
- Antimicrobial activity

Malva sylvestris



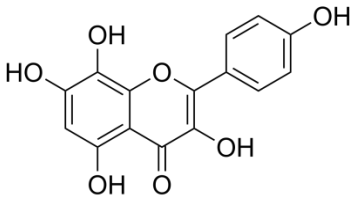


1: alkaloid



2: Quercetin

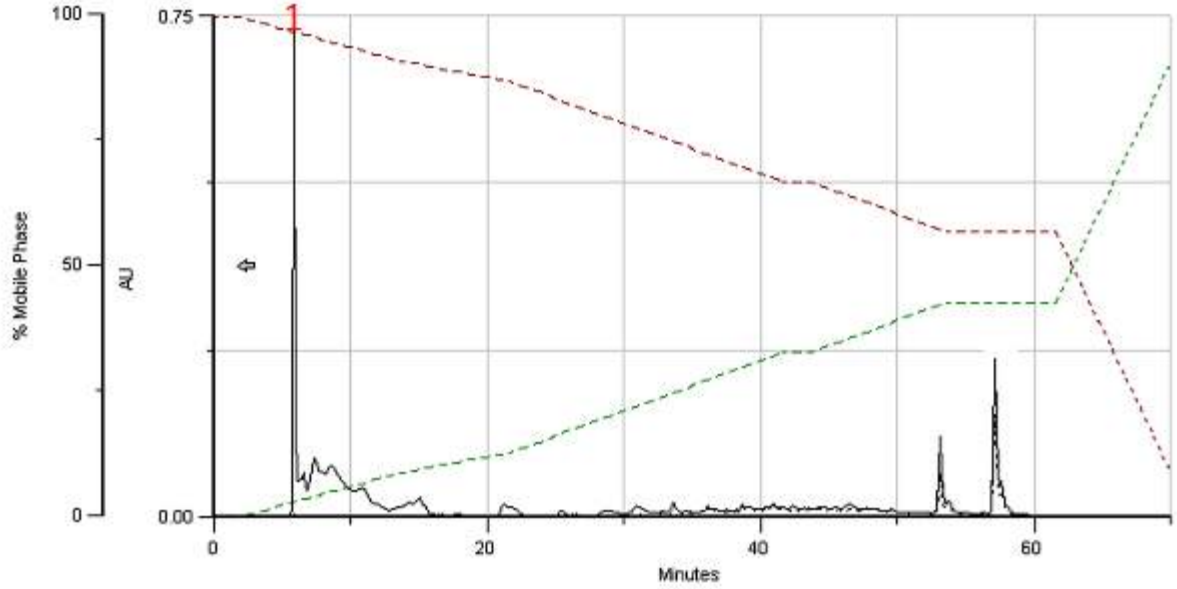
3: Quercetin



4: Herbacetin

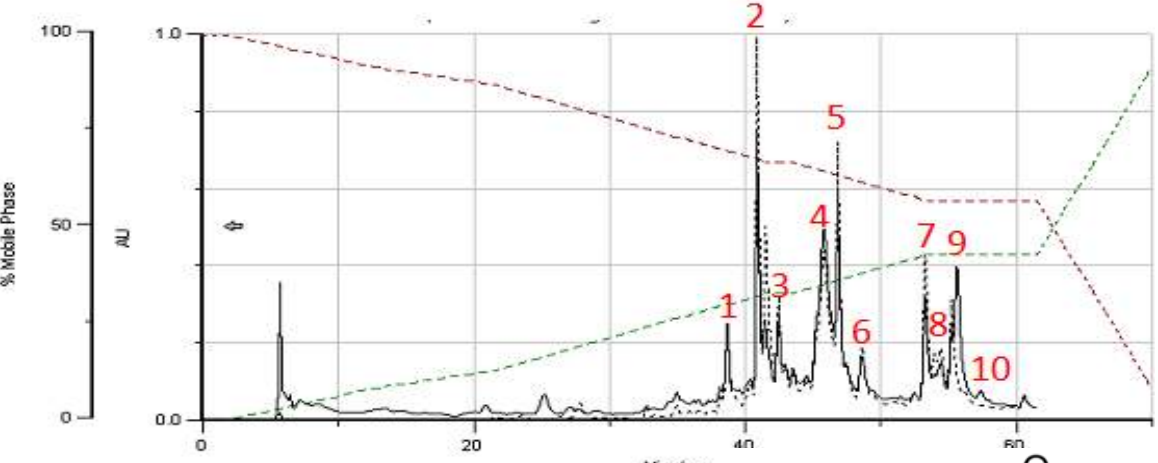
- Cutaneous wound healing
- Hepatoprotective effects

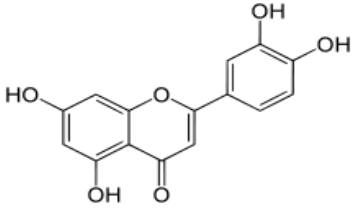
Scolymus hispanicus



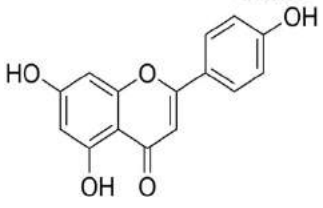
1: alkaloid

Mentha Spicata

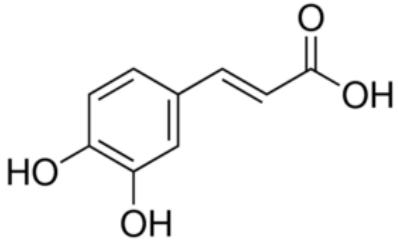




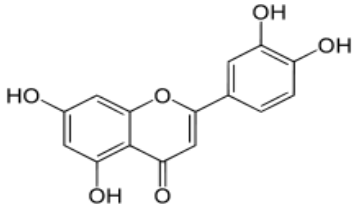
1: Luteolin



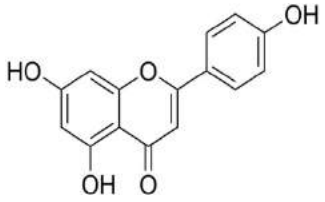
2: Apigenin



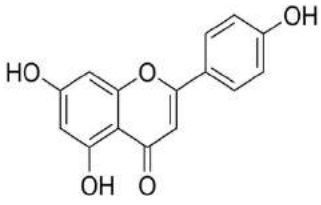
3: Caffeic acid



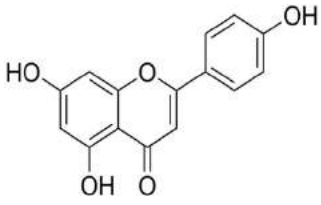
4: Luteolin



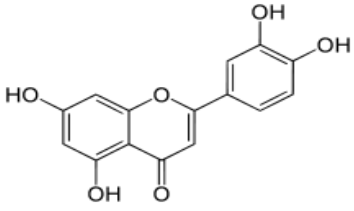
7: Apigenin



8: Apigenin

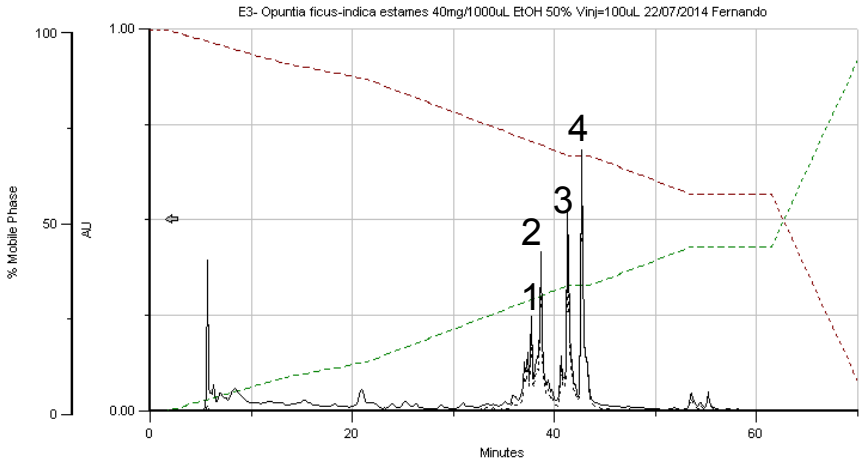


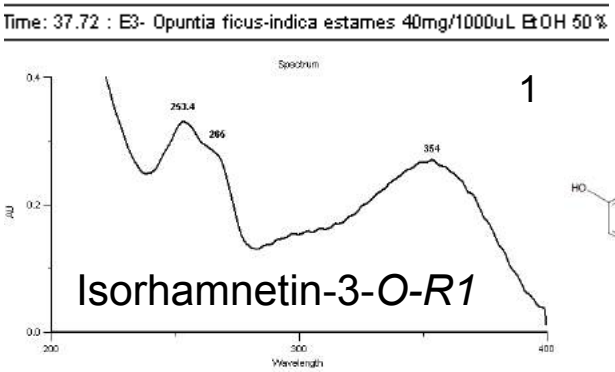
9: Apigenin

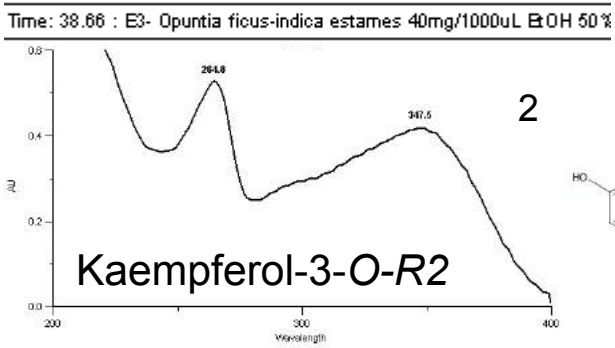


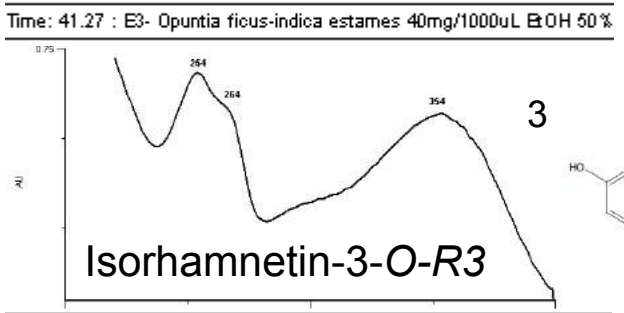
10: Luteolin

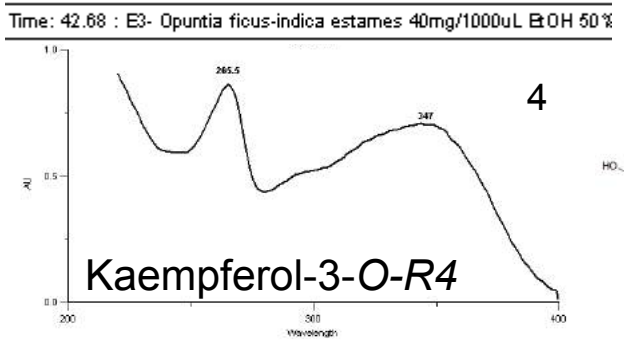
Opuntia-ficus-indica pollen

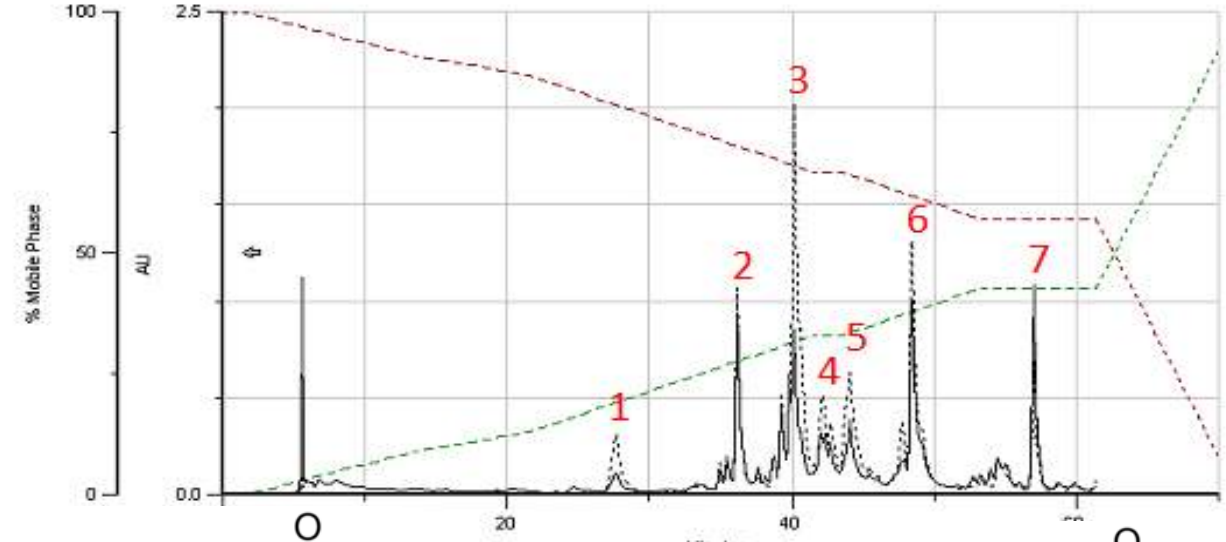




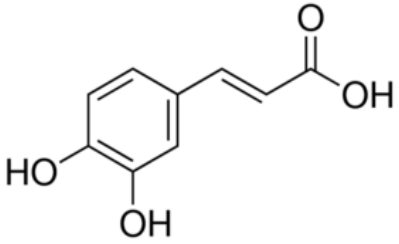




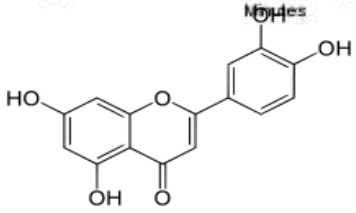




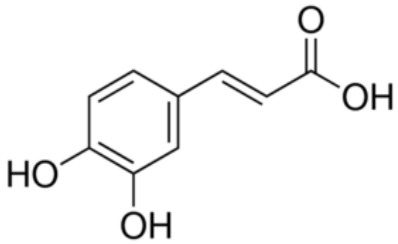
Anacyclus clavatus



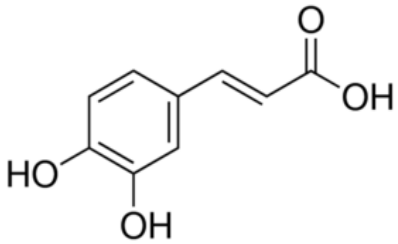
1: Caffeic acid



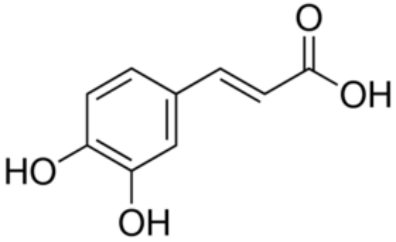
2: Luteolin



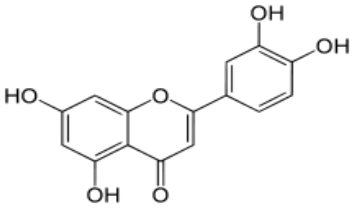
3: Caffeic acid



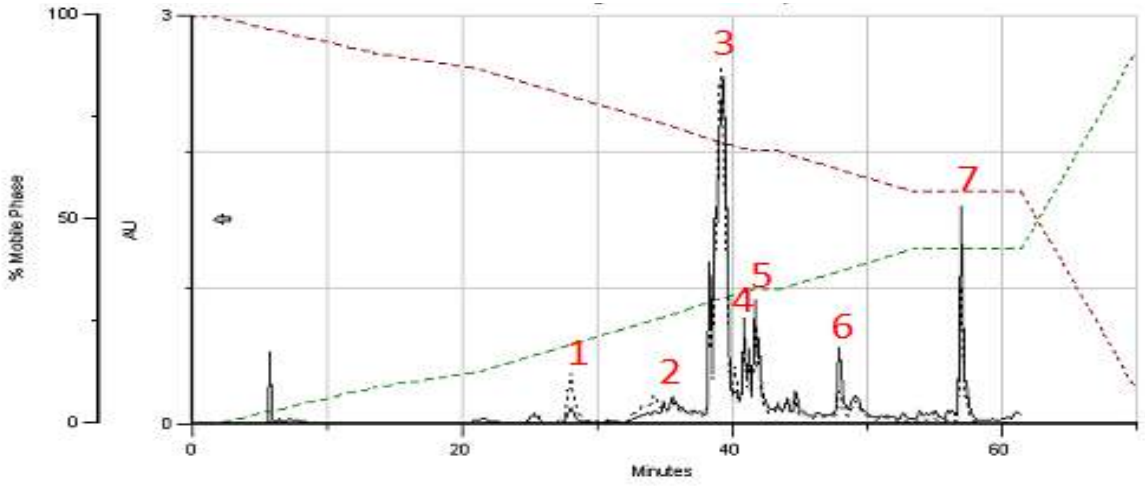
4: Caffeic acid



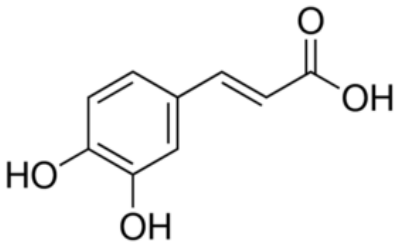
5: Caffeic acid



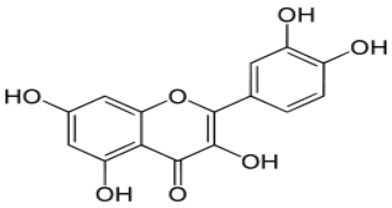
6: Luteolin



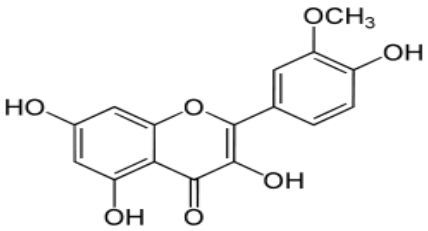
Justicia adhatoda



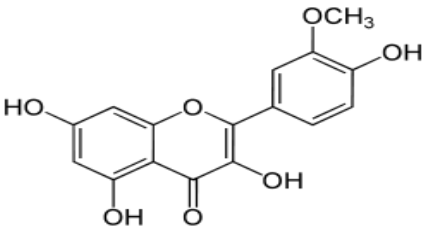
1: Caffeic acid



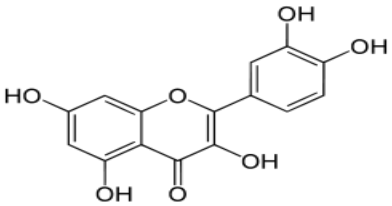
2: Quercetin



3: Isorhamnetin

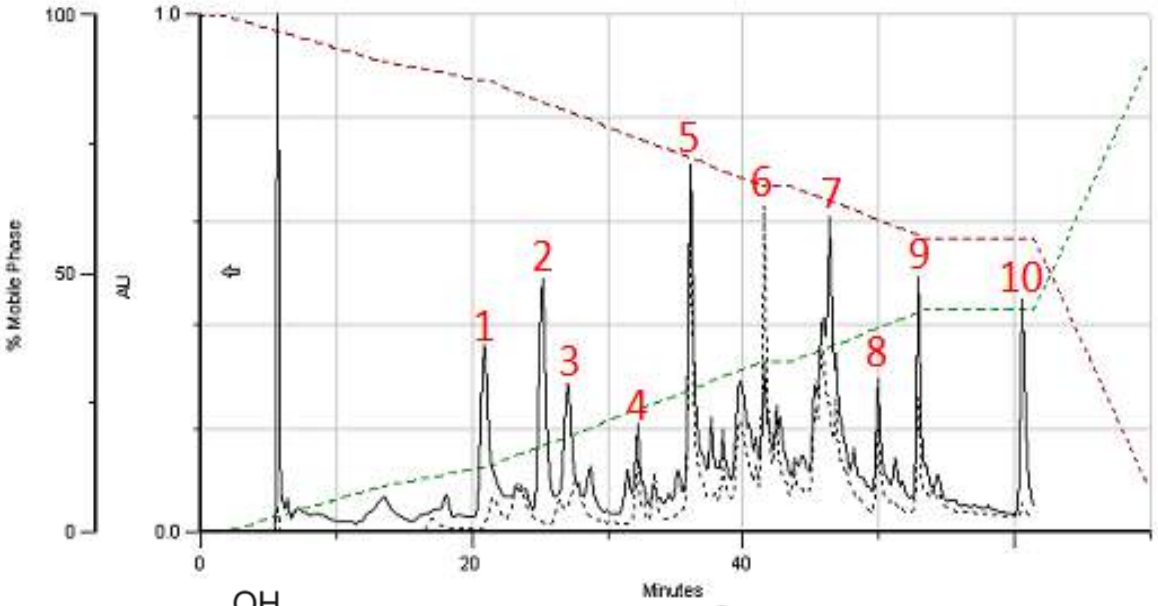


4: Isorhamnetin

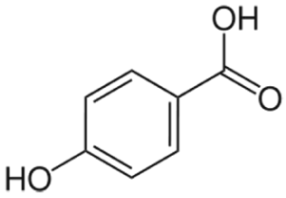


6: Quercetin

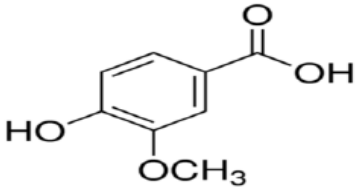
7:Lignan or tanin



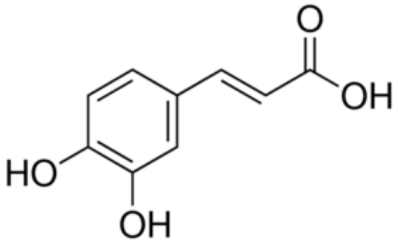
Mentha pulegium



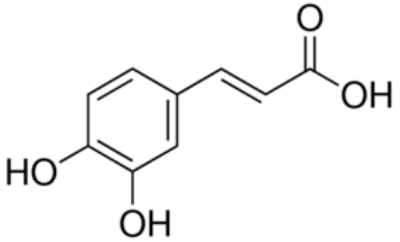
1: p-hydroxybenzoic



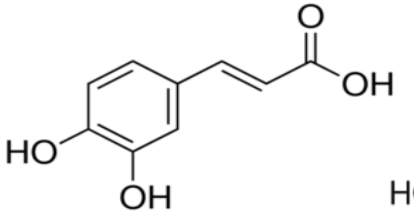
2: vanillic acid



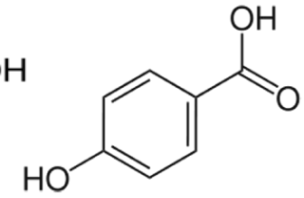
3: Caffeic acid



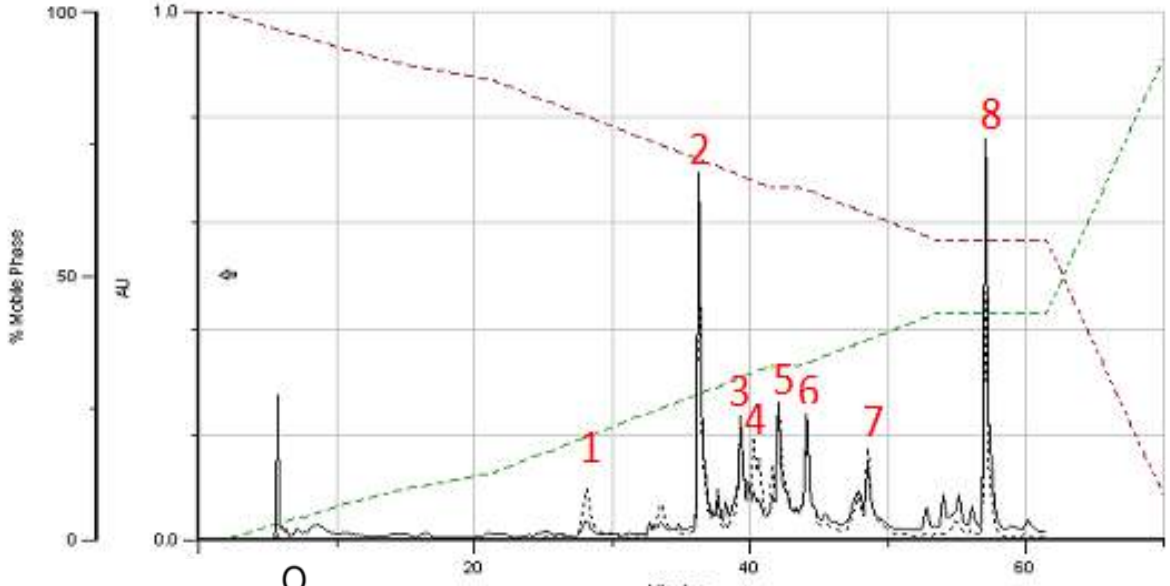
4: Caffeic acid



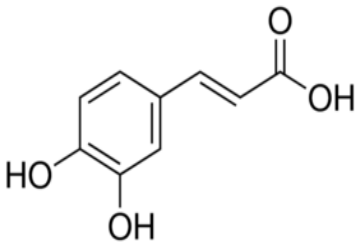
5: Caffeic acid



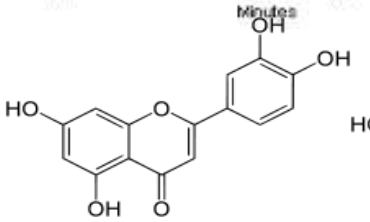
6: p-hydroxybenzoic



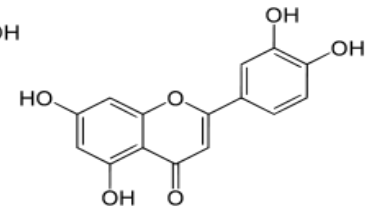
Anacyclus radiatus



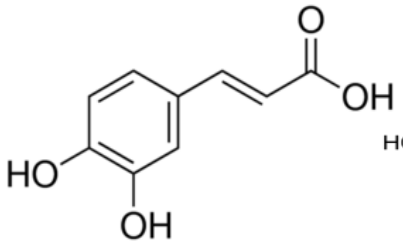
1: Caffeic acid



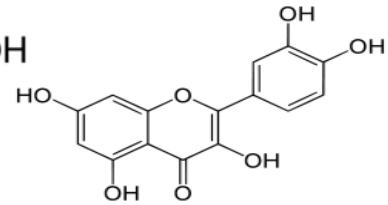
2: Luteolin



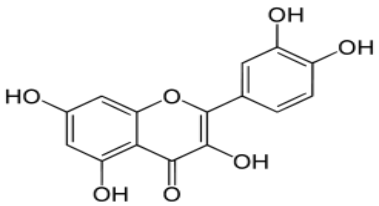
3: Luteolin



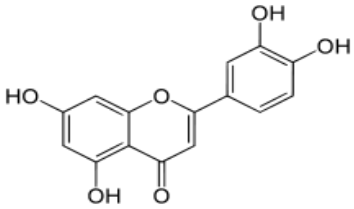
4: Caffeic acid



5: Quercetine

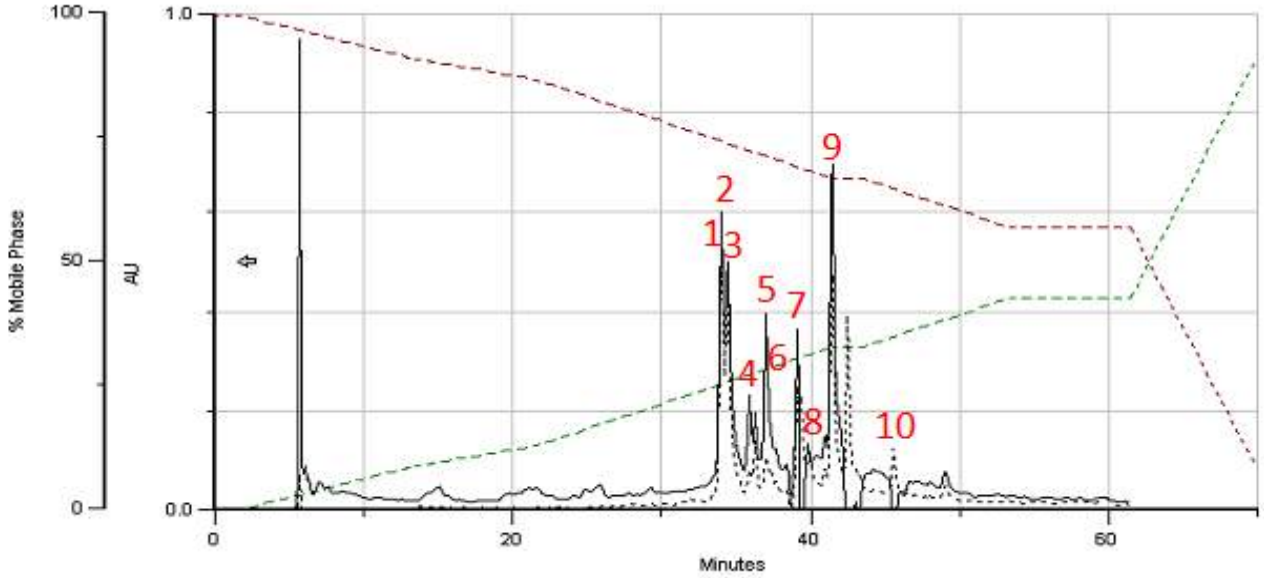


6: Quercetine

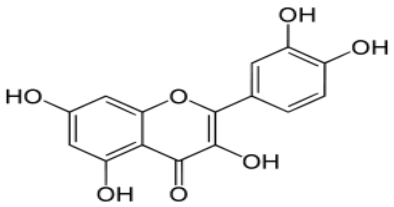


7: Luteolin

8:Lignan or tanin

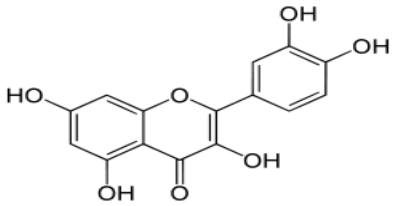


Acacia pycnantha



3: Quercetin

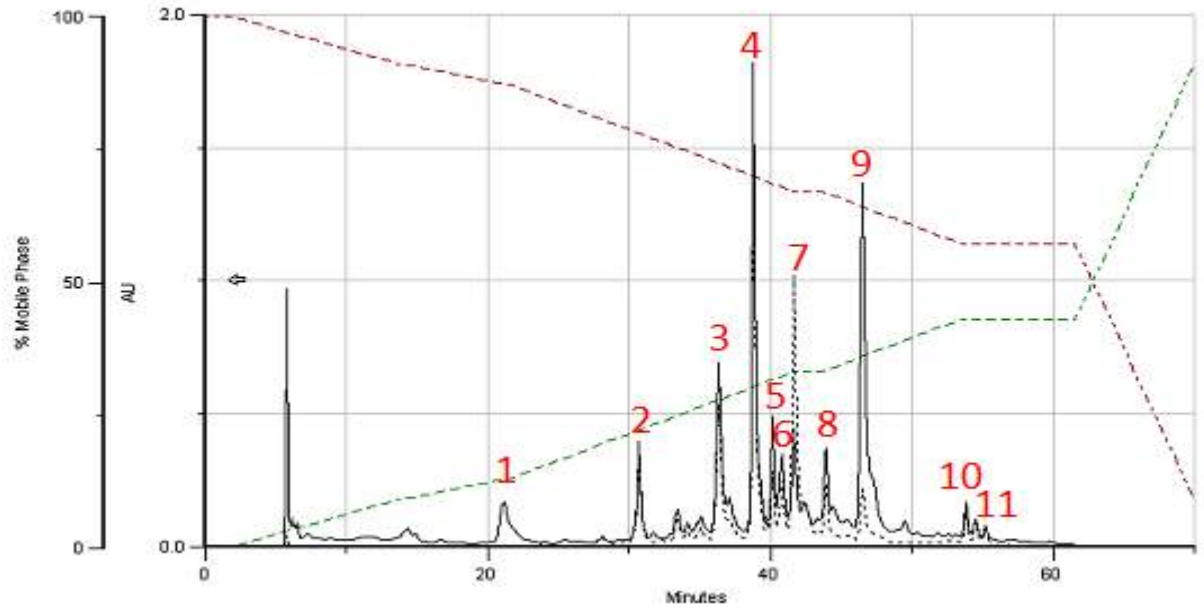
4: Chalcone



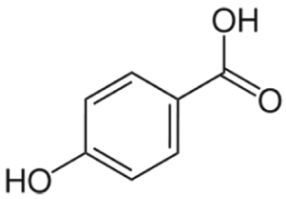
5: Quercetin

8: Chalcone

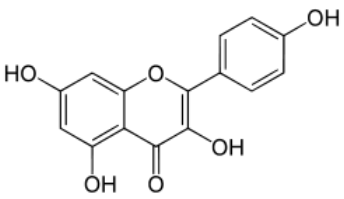
9: Chalcone



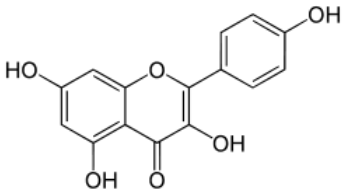
Echium creticum



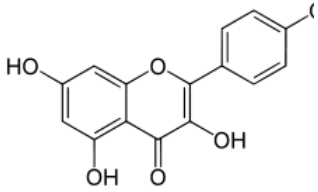
1: p-hydroxybenzoic



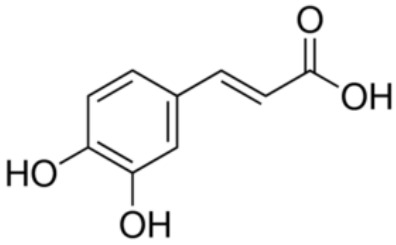
2: Kaempferol



4: Kaempferol

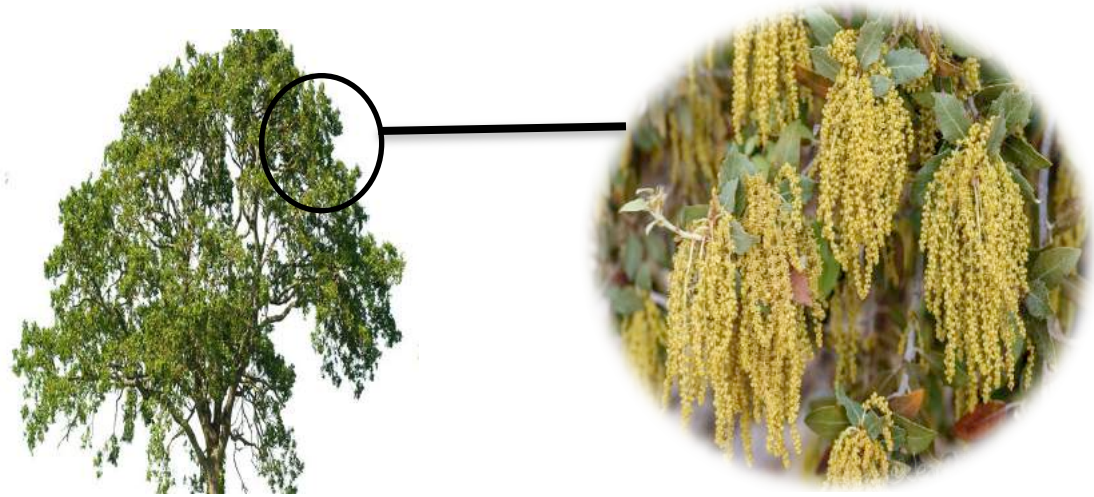


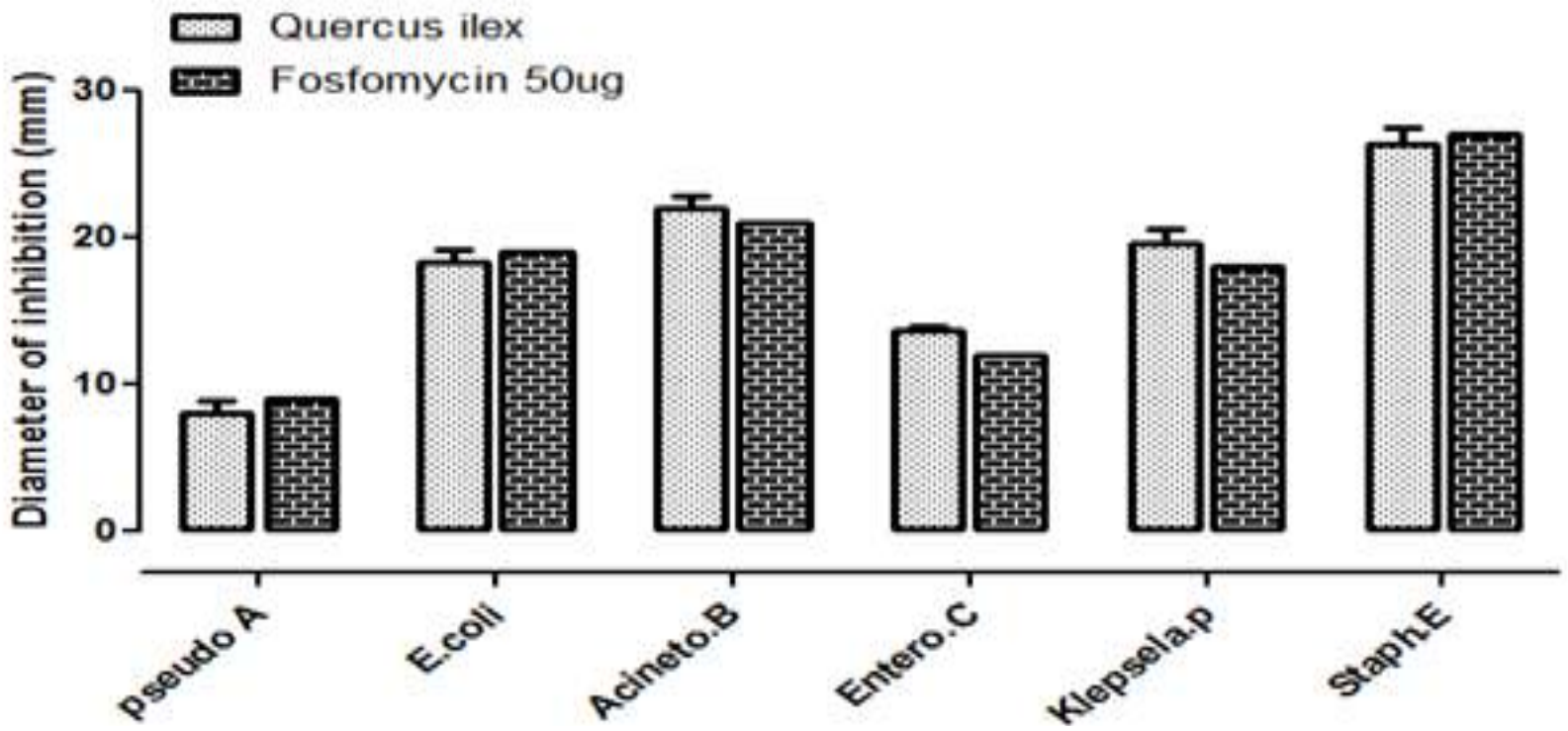
5: Kaempferol



9: Caffeic acid

Escherichia coli
Pseudomonas aeruginosa
Enterobacter cloacae
Acinetobacter baumannii
Klepsela 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

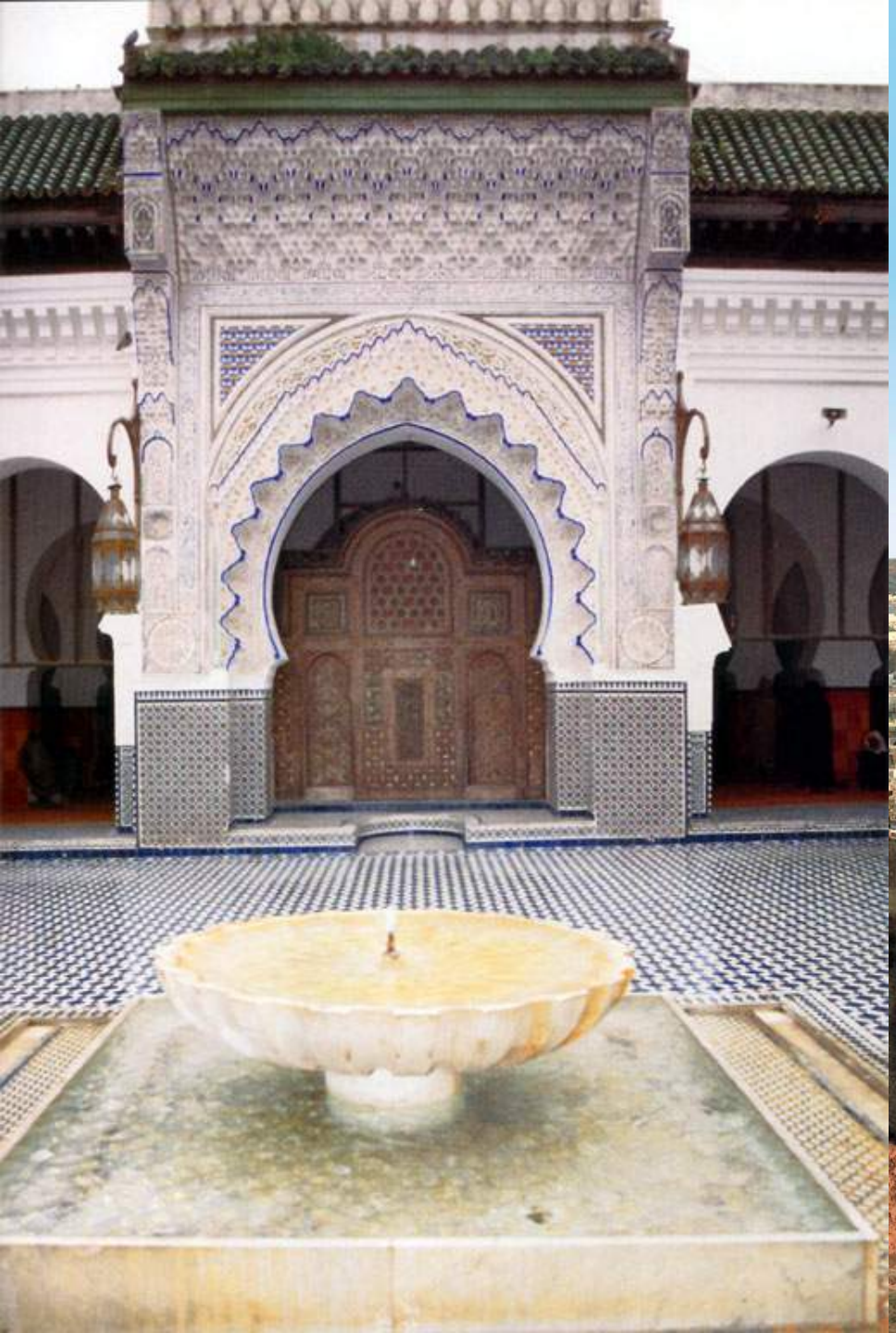
  Alkaloids

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