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Antimicrobial activity and nutraceutical potential of Tuscan bee-pollens on oxidative and endoplasmic reticulum stress in different cell-based models †

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✓ Bee-pollen is an apicultural product of great interest owing to its high nutritional and therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial, anti-mutagenic, and antitumor effects

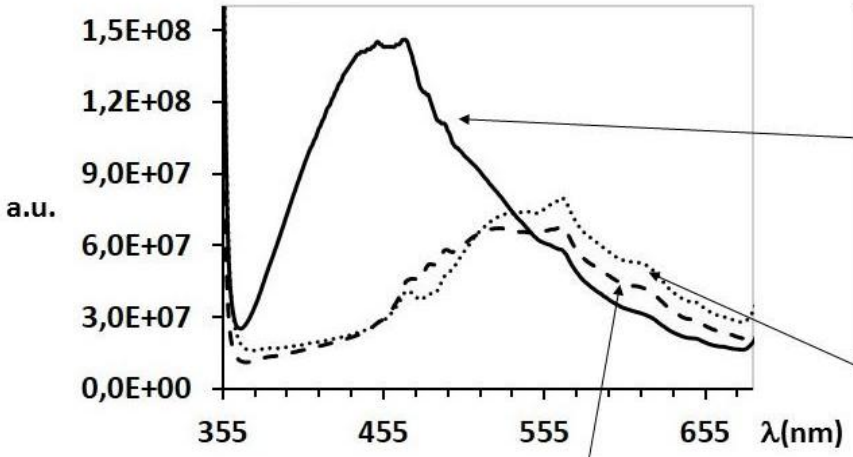
✓ Bee-pollen is an important source of energy, bioactive compounds and proteins for human nutrition

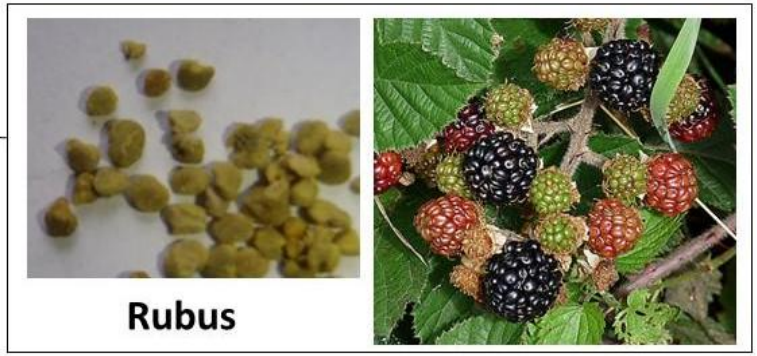
✓ To the best of our knowledge, no data on bee-pollen effects on endoplasmic reticulum stress are available in the literature

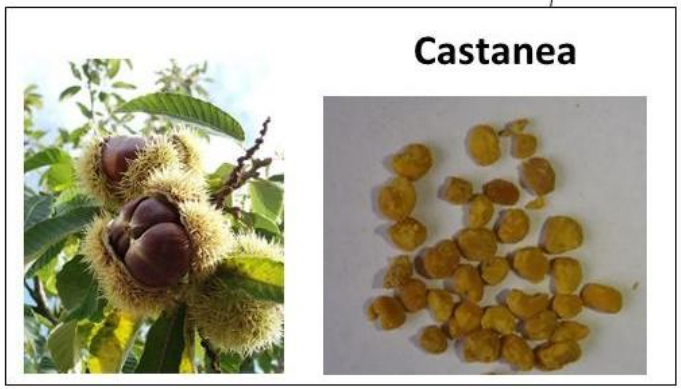
This study aimed to assess the *ex vivo* antioxidant activities and the antimicrobial potential of *Castanea*, *Cistus* and *Rubus* bee-pollens

Besides, we analyzed, for the first time, the effects of *Castanea* bee-pollen on functional properties of human microvascular endothelial cells (HMEC-1) under endoplasmic reticulum (ER) stress condition

In a previous study (*Gabriele et al., 2015*) we investigated the botanical origin, the phytochemical profile and the free-radical scavenging activity of a polyfloral Tuscan bee-pollen separated by color into three botanical families, specifically *Castanea*, *Rubus* and *Cistus*.







***Gabriele M. et al.*, *Ital J Food Sci*. 2015, 27, 248-59**

Castanea bee-pollen showed the better phytochemical content

Castanea spp.

Cistus spp.

Rubus spp.

	Phenolics (mg GAE/g dw)	**Flavonoids** (mg CE/g dw)	**Flavonols** (mg QE/g dw)	**Ascorbic acid** (mg AAE/g dw)	**Anthocyanins** (mg C3GE/L)	**ORAC** (µmol TE/100 g dw)
Castanea	24.8±0.8***	15.9±0.6***	4.8±0.1*	12±0.2***	77.4 ±2.6***	54401±475
Cistus	21.2±0.2	14.2±0.6***	4.9±0.05**	9.1±0.5	52.6±3.5	54001±1720
Rubus	13.5±0.4***	5.9±0.3***	2.5±0.1***	6.8±0.3**	58.5±4.7	51945±1507
Polyfloral	21.3±0.5	11.6±0.4	4.5±0.15	8.5±0.4	51.2±1.2	67770±1292

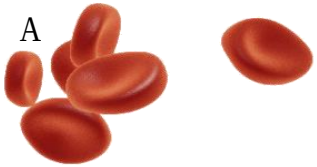
Phytochemical profile and *in vitro* antioxidant activity of polyfloral and *Castanea* spp., *Cistus* spp. and *Rubus* spp. bee pollen extracts.
* different from polyfloral bee pollen, with * $p \leq 0.05$; ** $p \leq 0.01$ and *** $p \leq 0.001$. ***Gabriele M. et al., Ital J Food Sci.* 2015, 27, 248-59

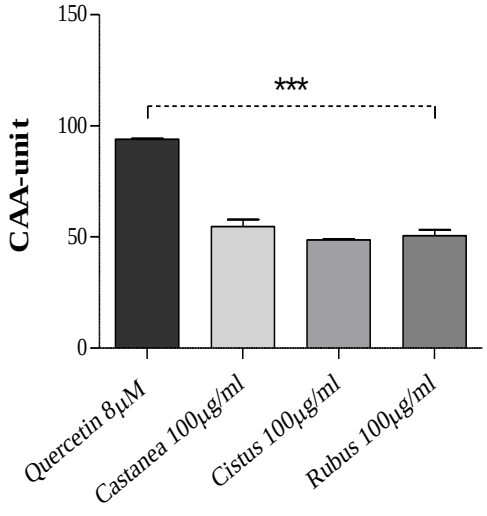
- ✓ ***Ex vivo* antioxidant activity on human erythrocytes**: CAA-RBC and hemolysis test
- ✓ **Antimicrobial activity**: minimum inhibitory concentration (MIC) determination

- ✓ **Cell culture**: Human microvascular endothelial cells (HMEC-1)
- ✓ **Cell functionality**: viability
- ✓ **Intracellular ROS production**: DCFH-DA assay
- ✓ **Gene expression**: real-time PCR

Statistical analysis: One-way ANOVA with Tukey post-hoc test. All data are expressed as mean ± SD of three replicates.

A





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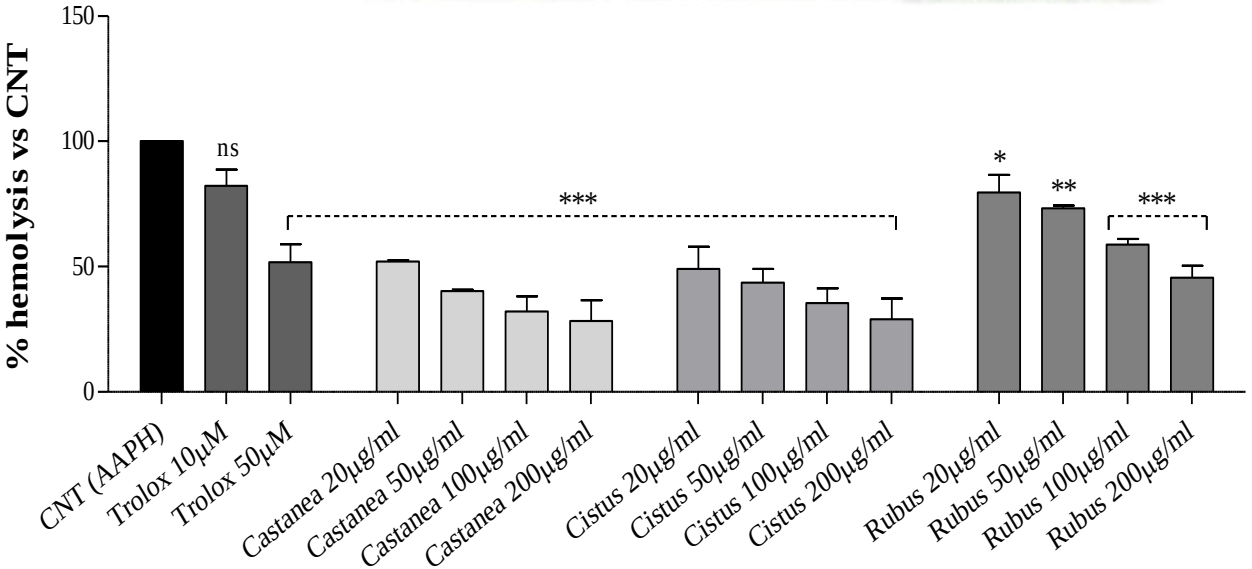


(A) Effects of *Castanea*, *Cistus*, and *Rubus* bee-pollen extracts (100µg/ml) on the cellular antioxidant activity (CAA) of oxidized human erythrocytes. Quercetin (8µM) was used as the reference standard.

(B) Effects of increasing concentrations (20, 50, 100, and 200µg/ml) of *Castanea*, *Cistus* and *Rubus* bee-pollen extracts on erythrocytes AAPH-induced oxidative hemolysis. Trolox (10 and 50µM) was used as astandard.

Results were expressed as mean ± SD. One-way ANOVA with Tukey's multiple comparison test: *significantly different from CNT (AAPH-treated cells), *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Strains	Minimum inhibitory concentration (MIC) values		
	Castanea	*Cistus*	*Rubus*
Escherichia coli	10 mg/ml	10 mg/ml	-
Salmonella Typhimurium	10 mg/ml	10 mg/ml	-
Enterobacter erogene	-	10 mg/ml	-
Enterococcus faecalis	-	5 mg/ml	10 mg/ml
Staphylococcus aureus	10 mg/ml	5 mg/ml	10 mg/ml

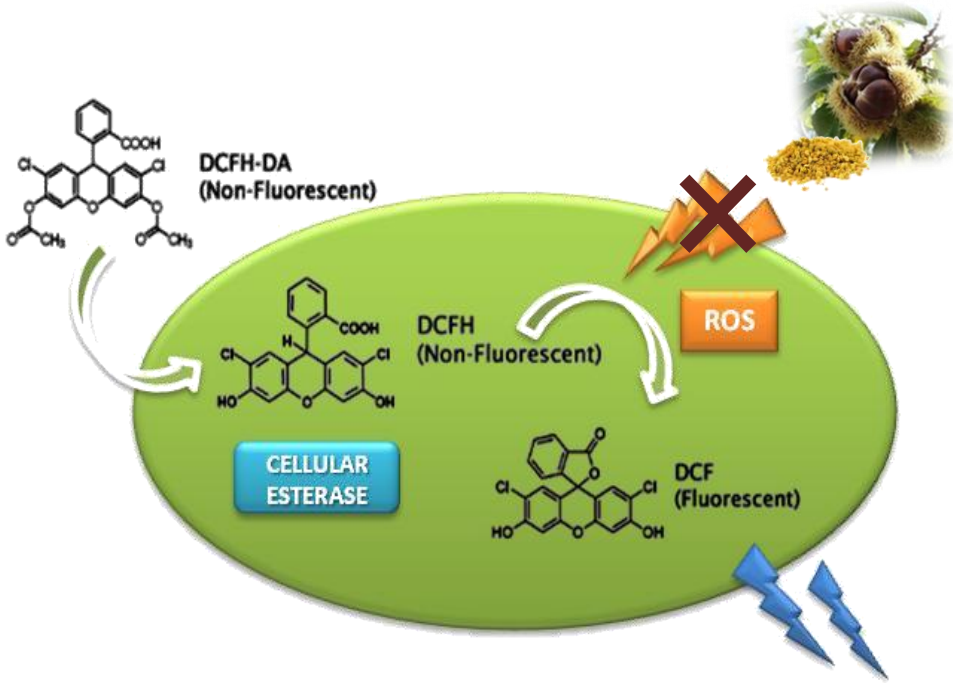
Minimum inhibitory concentration (MIC) values of *Castanea, Cistus*, and *Rubus* bee-pollen extracts on selected pathogen strains growth (O.D. 660 nm).

Thapsigargin
HMEC-1 treated cells

- CHOP
- IL-6
- COX-2
- ICAM-1
- ROS

Castanea bee-pollen
pre-treatment

- CHOP
- IL-6
- COX-2
- ICAM-1
- ROS



Effects of 1 hour pre-treatment with 10µg/ml *Castanea* bee-pollen extract on HMEC-1 exposed 2 hours to 0.3µM thapsigargin (thaps)

✓ Bee-pollen samples contained high levels of phytochemicals, good *in vitro* and *ex vivo* antioxidant activities, as well as antibacterial action.

✓ Our findings suggest a preventive action in protecting HMEC-1 from ER-stress induced by thapsigargin exposure.

✓ Bee-pollens, especially *Castanea* species, represent a good natural antibacterial and a potential nutraceutical product useful in the prevention of free radical and ER-stress-associated diseases.

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