UNIVERSIDAD DE LA REPÚBLICA
URUGUAY

Facultad de Ciencias
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ANTIOXIDANT ACTIVITY OF URUGUAYAN PROPOLIS: IN VITRO AND CELLULAR ASSAYS

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PROPOLIS

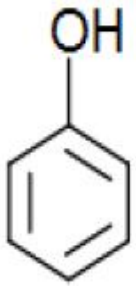
Resin	**50 – 55 %**
Wax	30 – 40 %
Polen	5 %
Oils	1 – 5 %
Others	5 – 10 %

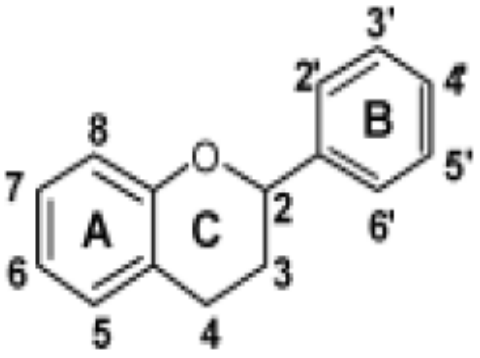
Active Components

POLYPHENOLS (flavonoids, aromatic carboxylic acids and esters)

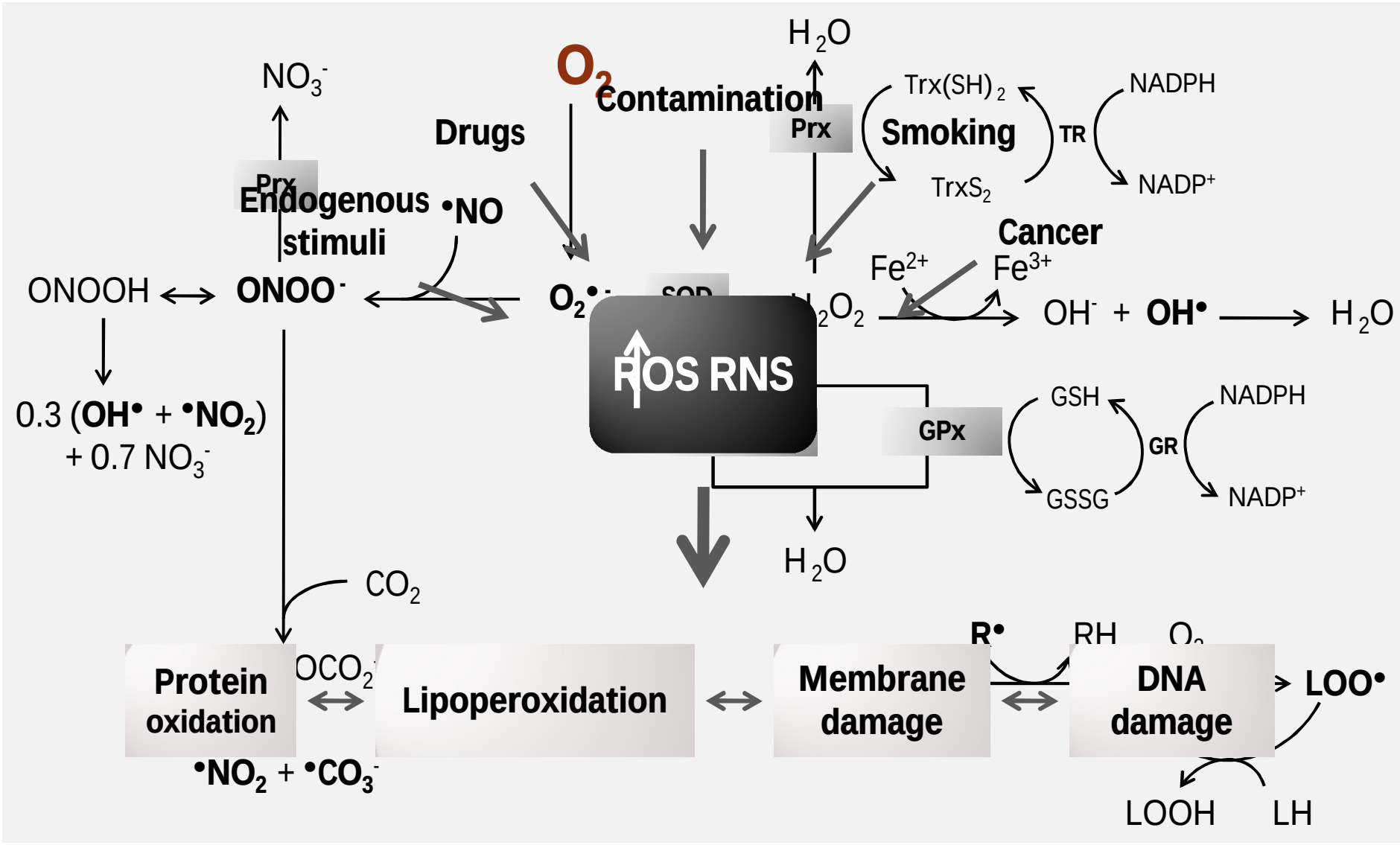
Propolis bioactivity:

- **Antibacterial**
- **Antiviral**
- **Anti-inflamatory**
- **Antioxidant**





PROPOLIS AS NATURAL ANTIOXIDANTS

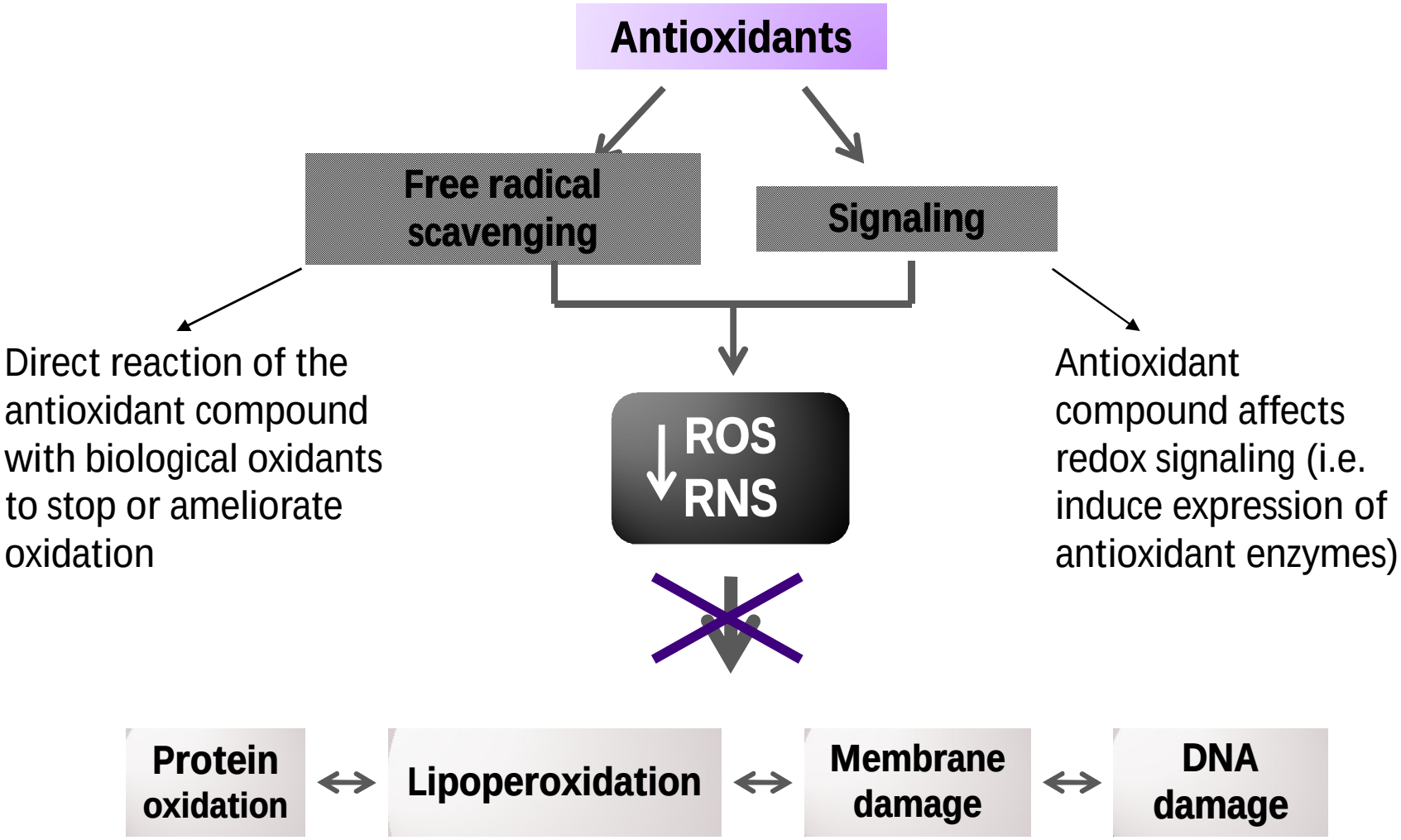


PROPOLIS AS NATURAL ANTIOXIDANTS

OXIDATIVE STRESS

- Aging
- Cardiovascular and neurodegenerative diseases
- Cancer

PROPOLIS AS ANTIOXIDANTS



PROPOLIS AS ANTIOXIDANTS

There is no single method to comprehensively determine antioxidant capacity

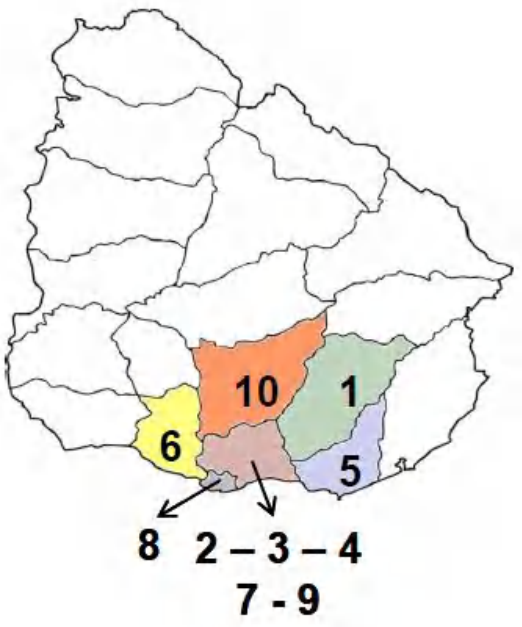
- ***In vitro*** **radical scavenging**
- **Cellular assays**
- ***In vivo*** **antioxidant response**

Antioxidant: a compound able to reduce the oxidative cellular state

Jones, D.P. Redefining oxidative stress. *Antioxid. Redox Signal*. 2006, 8, 1865-

URUGUAYAN PROPOLIS

- Raw propolis
- Southern region of Uruguay
- Late Spring/ early Summer
- Ethanolic extracts, EEP 40 g/L



EEP	Absorbance at 294 nm
1	418
2	156
3	292
4	1060
5	137
6	154
7	1414
8	364
9	850
10	1080

Total Polyphenols and Flavonoids

EEP	[Polyphenols] (mg/g propolis)	[Flavonoids] (mg/g propolis)
1	85 ± 6	22 ± 2
2	44 ± 3	11 ± 2
3	44 ± 2	13 ± 1
4	141 ± 24	45 ± 4
5	35 ± 1	4 ± 1
6	33 ± 3	7 ± 1
7	176 ± 26	54 ± 3
8	75 ± 3	22 ± 3
9	128 ± 14	35 ± 3
10	146 ± 18	41 ± 2

✓**Similar to argentinean and south Brazil propolis**

(Isla , J. Ethnopharmacol. 2001)

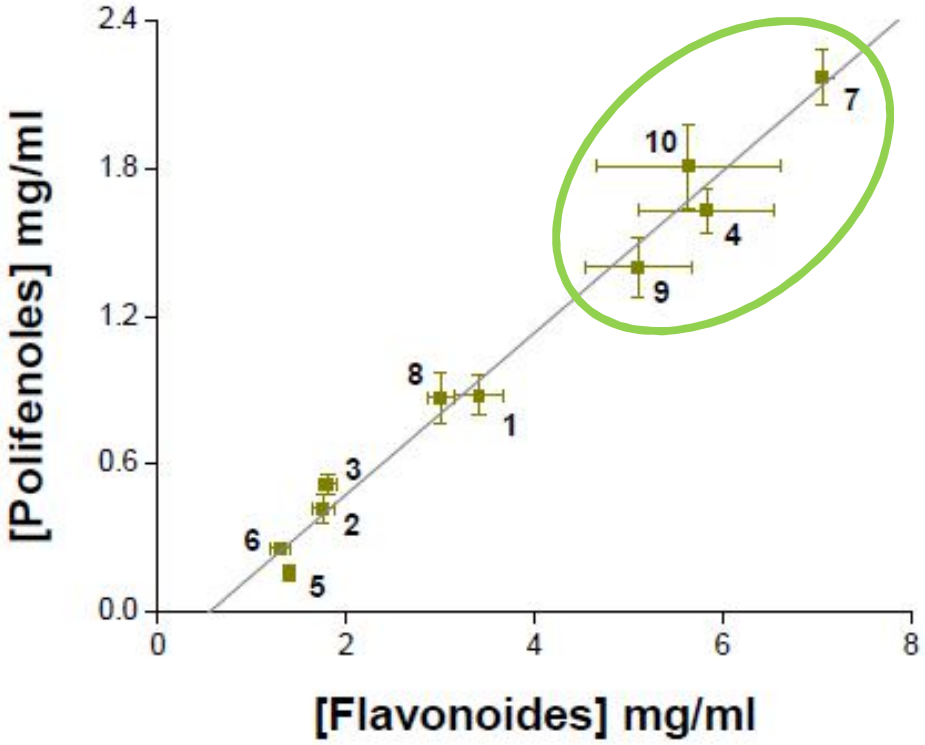
✓**Higher than tropical Brazil propolis** *(Mendes da Silva, Food Chem. 2006)*

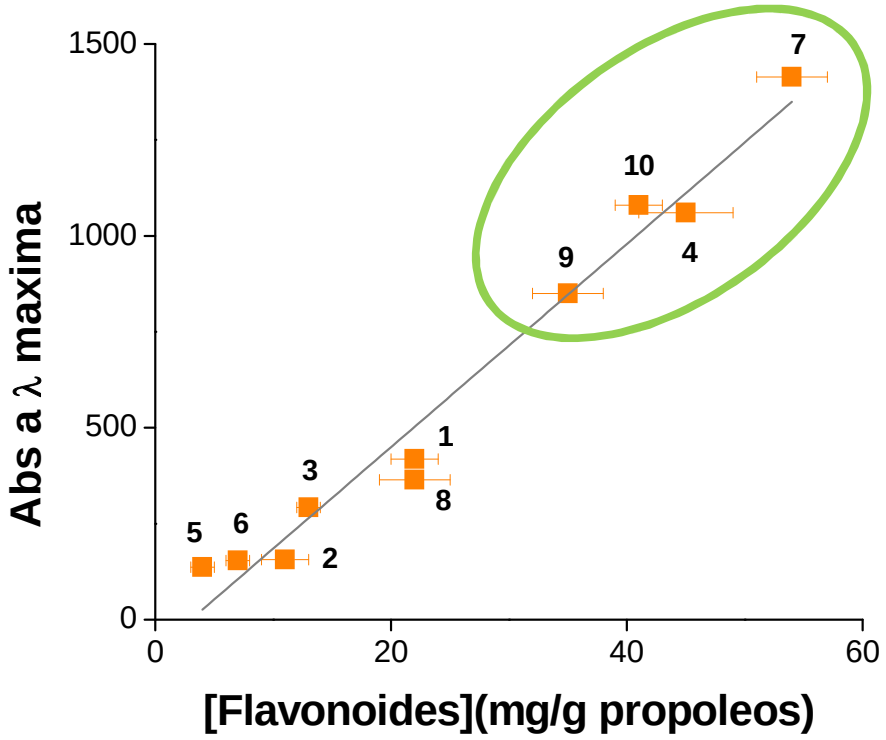
✓**3300-fold higher than honey**

(Gheldof , J. Agric. Food Chem. 2002)

✓**3-fold higher than red wine**

(Davalos, J. Agric. Food Chem. 2004)





✓ **The UV absorbance is a simple and rapid way to estimate the total content of polyphenols in the propolis extract**

Polyphenol composition of propolis extracts (RP-HPLC)

Table 2. Flavonoid and Aromatic Acid Constituents of Propolis Extracts Determined by HPLC

		mg/g propolis for EEP									
retention time (min)	compound	1	2	3	4	5	6	7	8	9	10
4.97	caffeic acid	ND[a]	ND	ND	ND	4.03	ND	ND	3.69	ND	2.01
5.19	ferulic acid	1.62	ND	ND	ND	ND	ND	ND	ND	ND	ND
35.93	naringin	ND	ND	ND	ND	ND	ND	5.94	1.55	3.72	ND
36.69	hesperetin	2.53	0.90	2.56	5.10	ND	ND	ND	ND	ND	ND
39.76	fisetin	2.86	1.11	2.20	6.21	0.73	ND	5.95	2.43	4.48	ND
44.97	luteolin	ND	ND	ND	0.86	ND	ND	1.63	0.19	0.74	0.86
46.43	quercetin	ND	0.53	ND	0.79	ND	ND	0.94	0.4	0.57	0.71
47.94	galangin	0.40	ND	ND	ND	ND	ND	1.37	ND	ND	1.30
49.31	pinobanskin	0.21	ND	0.62	1.02	ND	ND	0.35	1.16	0.44	0.67
50.92	kaempferol	0.60	ND	ND	0.87	ND	ND	1.19	ND	1.15	0.99
51.39	pinocembrin	5.01	1.60	ND	1.26	2.52	ND	1.66	0.82	1.24	6.87
51.63	CAPE[b]	ND	ND	ND	6.87	ND	ND	11.21	ND	8.05	7.65
56.35	galangin 3- methyl ether	16.15	7.87	6.19	8.76	5.24	0.94	7.94	ND	4.52	ND
58.32	apigenin	1.86	3.00	1.62	4.35	0.45	0.04	5.91	2.37	3.66	3.99
65.30	chrysin	4.39	2.03	0.35	8.73	0.92	ND	8.16	3.60	5.44	8.94
66.80	unknown	0.32	0.24	ND	1.91	ND	1.86	3.07	ND	1.81	3.11
78.07	techtochrysin	0.08	0.08	0.07	0.04	0.20	0.18	ND	0.06	ND	ND

[a] ND, not detected. [b] CAPE, caffeic acid phenethyl ester.

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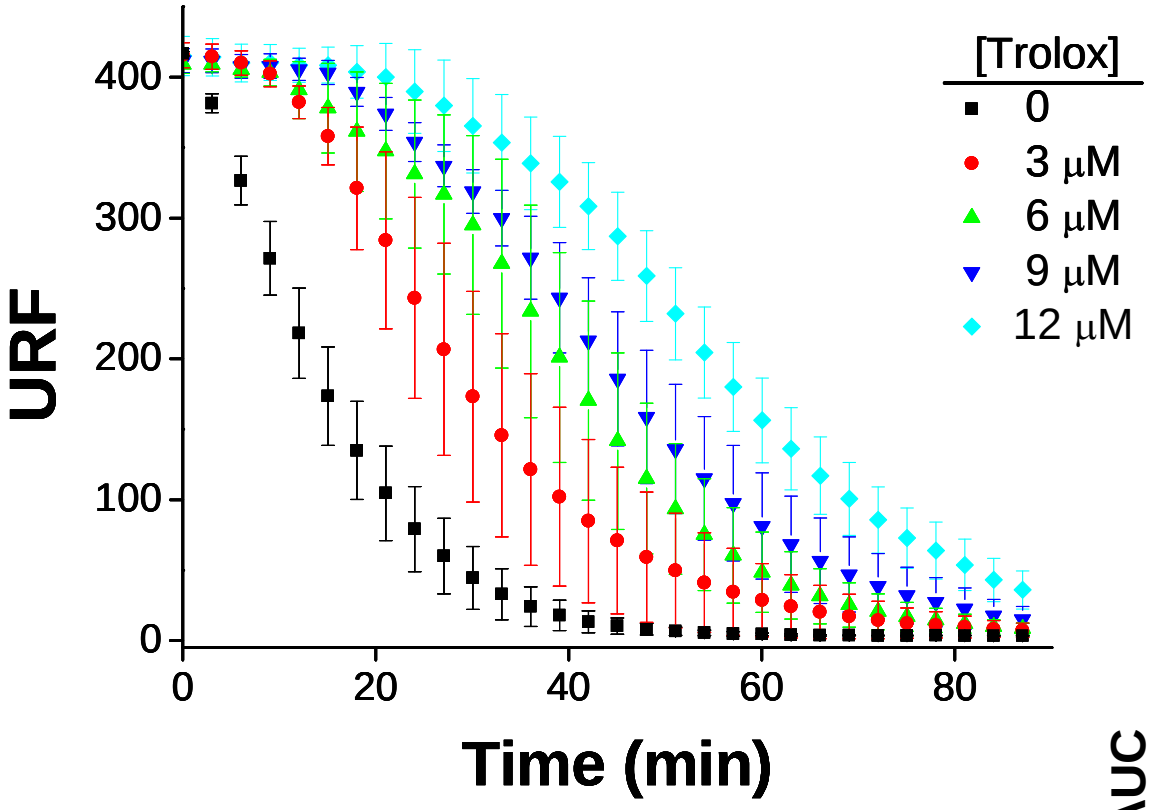
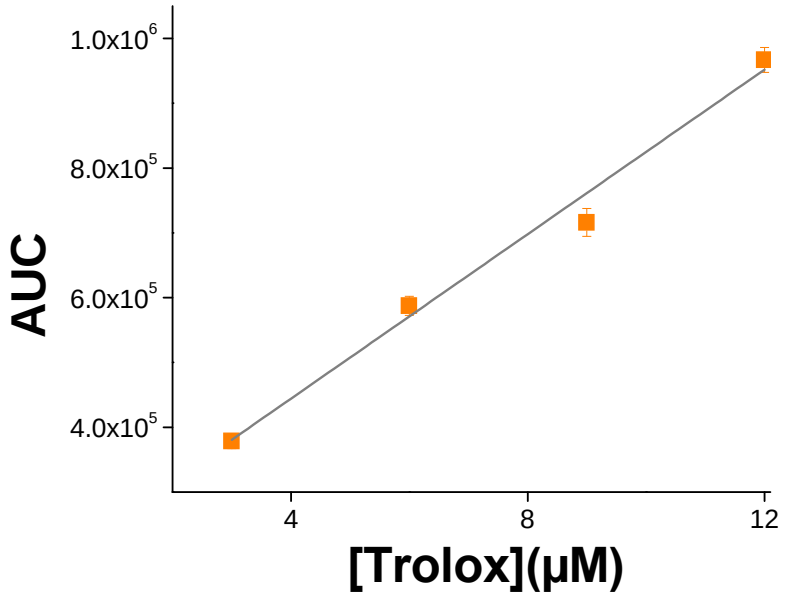
IN VITRO ASSAYS: ORAC





- competition method
- assess peroxyl radical scavenging
- express as Trolox equivalents

ORAC

$$\text{valor ORAC} = \frac{\text{concentracion Trolox equivalente}}{[\text{EEP}](\text{mg/l})}$$

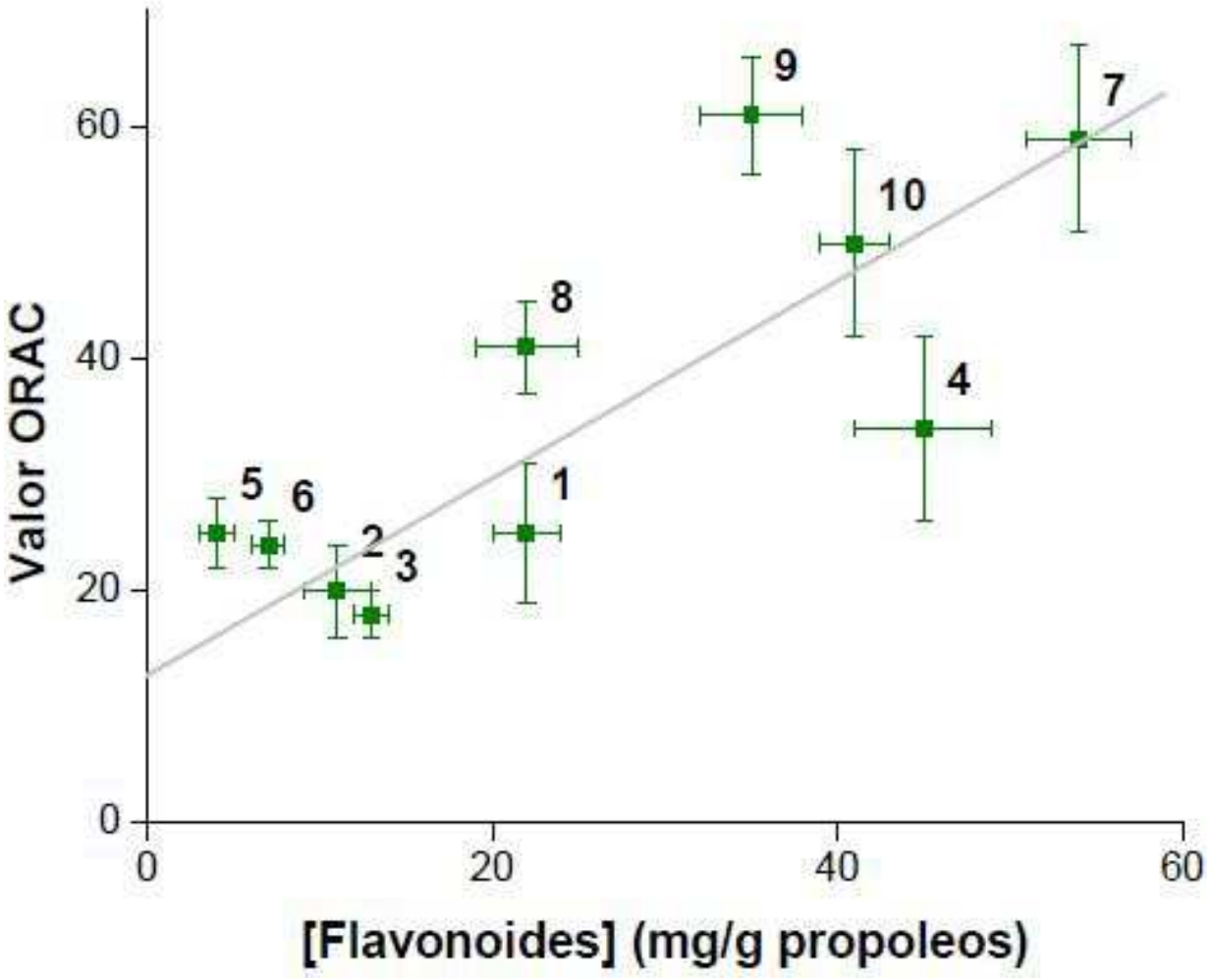
EEP	1	2	3	4	5	6	7	8	9	10
ORAC Value (µmoles Trolox eq/mg propolis)	2.5 ± 0.6	2.0 ± 0.4	1.8 ± 0.2	7.5 ± 0.8	2.5 ± 0.3	2.4 ± 0.2	9.0 ± 0.8	2.6 ± 0.4	7.1 ± 0.5	8.0 ± 0.8

EE7 ≥ EE10 ≥ EE4 ≥ EE9

EE8 = EE1 = EE5 = EE6 ≥ EE2 ≥ EE3

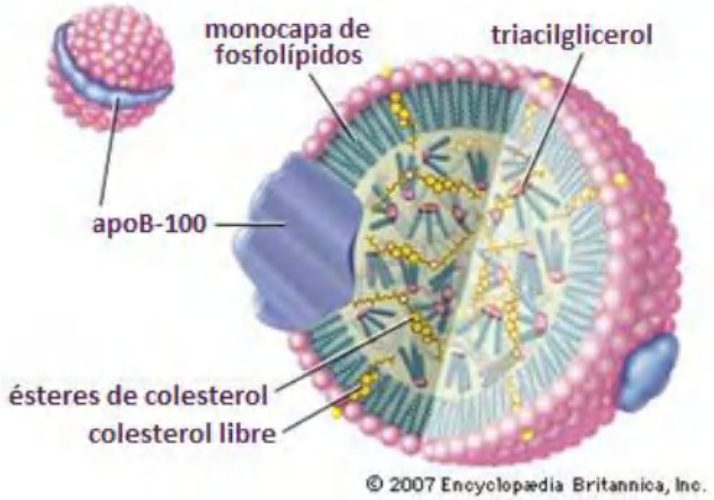
- ✓ **500-fold higher than honey** *(Gheldof , J. Agric. Food Chem. 2002)*
- ✓ **150-fold higher than red wine** *(Davalos, J. Agric. Food Chem. 2004)*

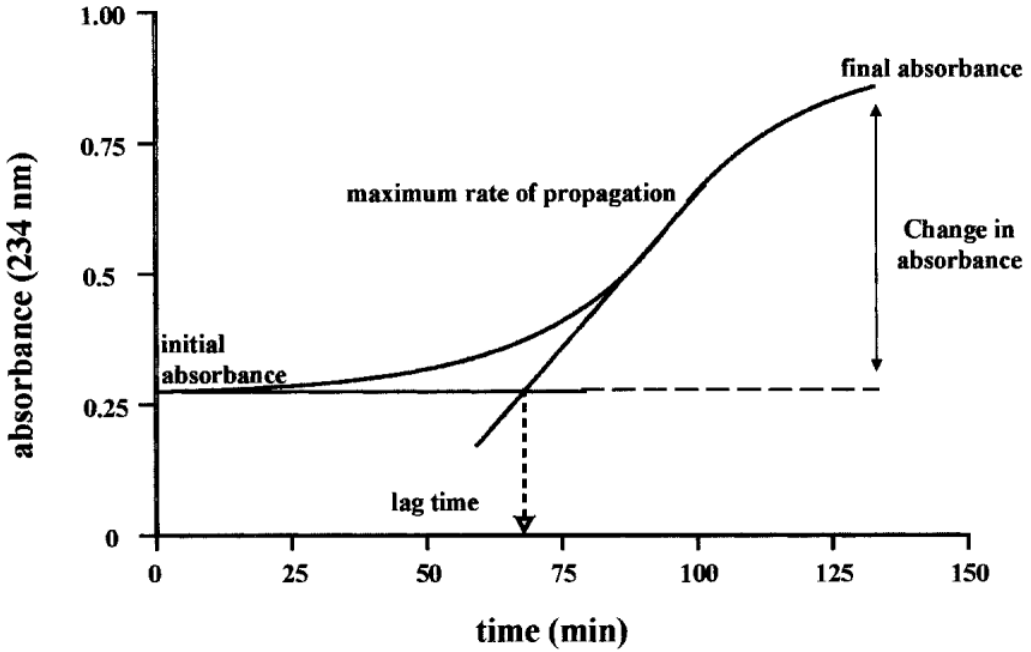
ORAC value correlates with flavonoid content



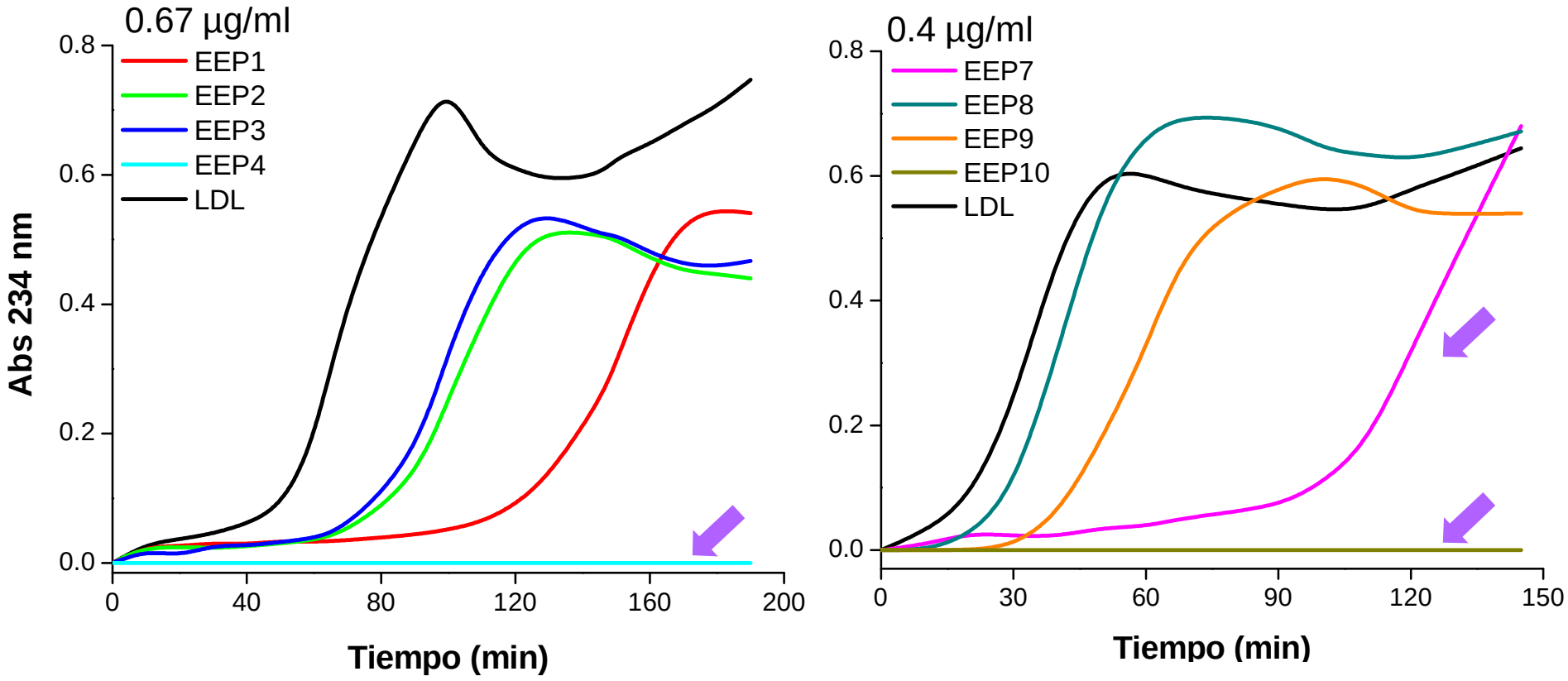
IN VITRO ASSAYS: inhibition of LDL oxidation

- LDL = Low Density Lipoprotein
- Apolipoprotein: ApoB-100
- Transport of cholesterol
- Oxidized LDL associated with atherosclerosis





Inhibition of LDL oxidation by propolis



✓ **EEP high in polyphenols retarded LDL oxidation (lag or exponential phase)**

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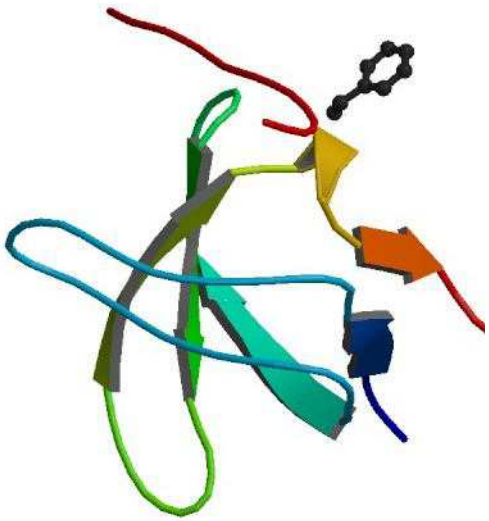
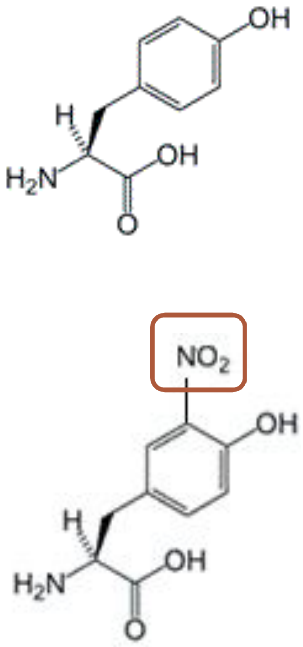
IN VITRO ASSAYS: inhibition of tyrosine oxidation

- Tyrosine: target residue for post-translational modifications:





Phosphorylation: cellular signaling

Oxidation:

Nitrotyrosine

$$Y \xrightarrow[-e^-]{X^\bullet} Y^\bullet \xrightarrow{{}^\bullet NO_2} Y\text{-}NO_2$$

Increased under pathological conditions

Antioxidant: A

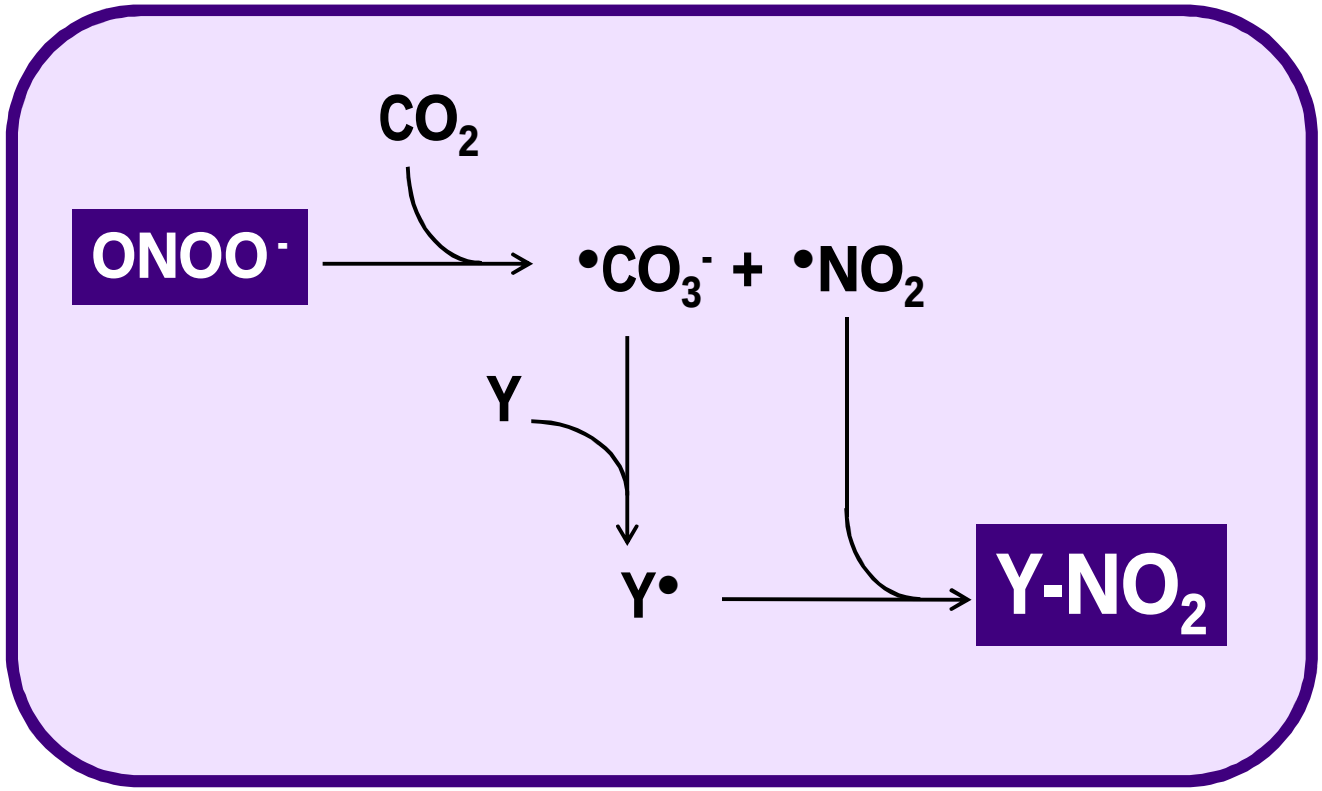
$$A + Y^\bullet \longrightarrow A^\bullet + Y$$

$$A + X^\bullet \longrightarrow A^\bullet + XH$$

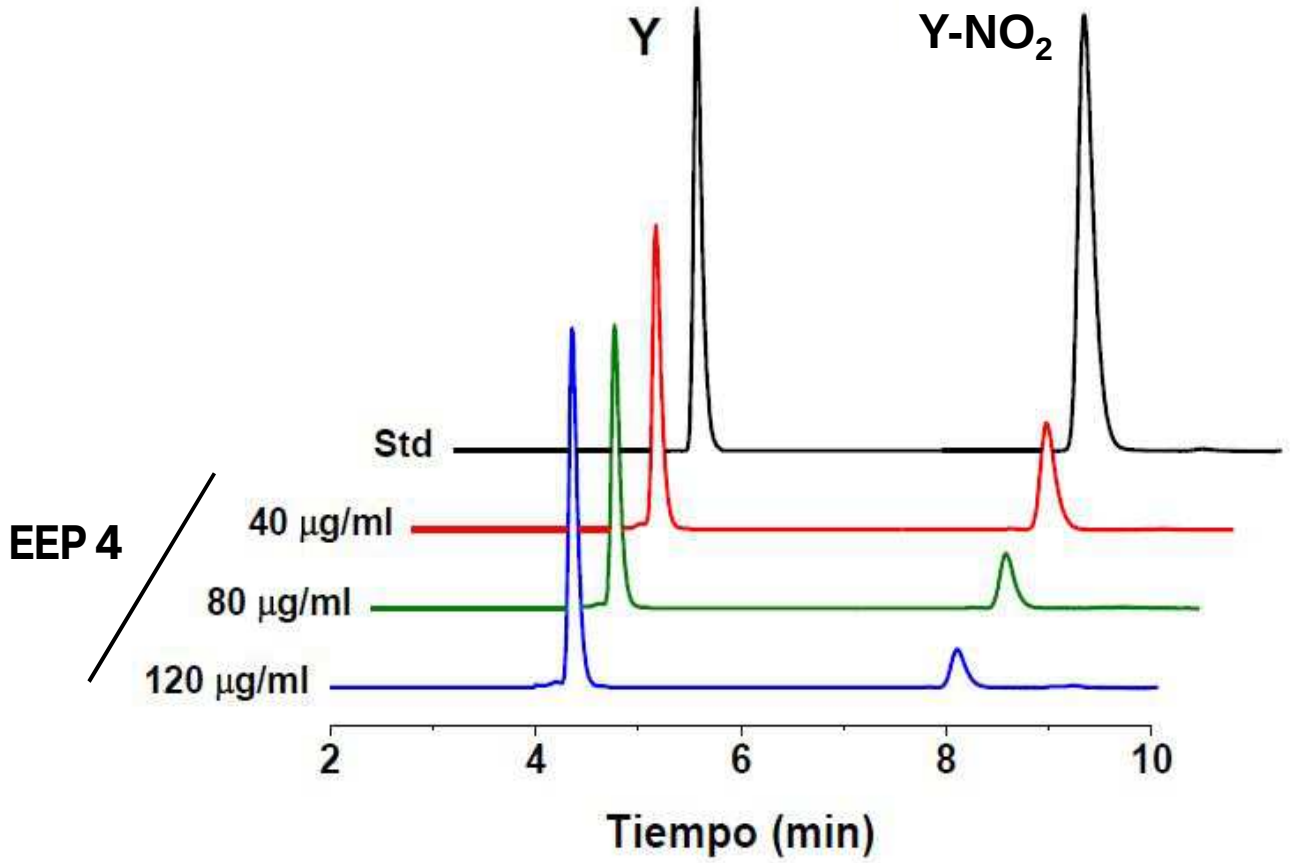
$$A + {}^\bullet NO_2 \longrightarrow A^\bullet + NO_2^-$$

Nitro-tyrosine formation by ONOO-

- NO_2-Y biological formation is a radical mechanism
- Peroxynitrite (ONOO-) can form NO_2-Y:



TYROSINE OXIDATION INHIBITION BY PROPOLIS



✓ **EEP4 (high polyphenols) prevented Y-NO_2 formation in a dose-dependent manner**

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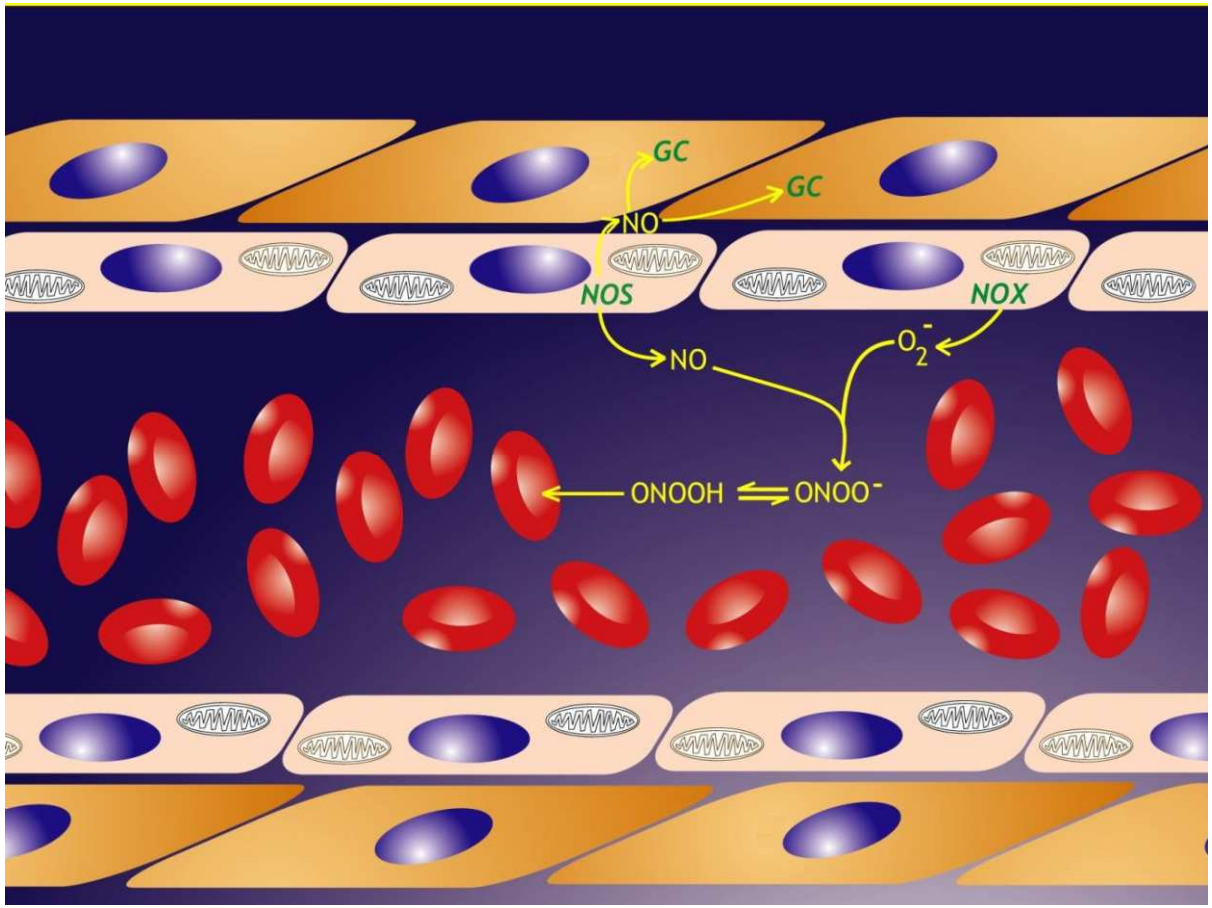
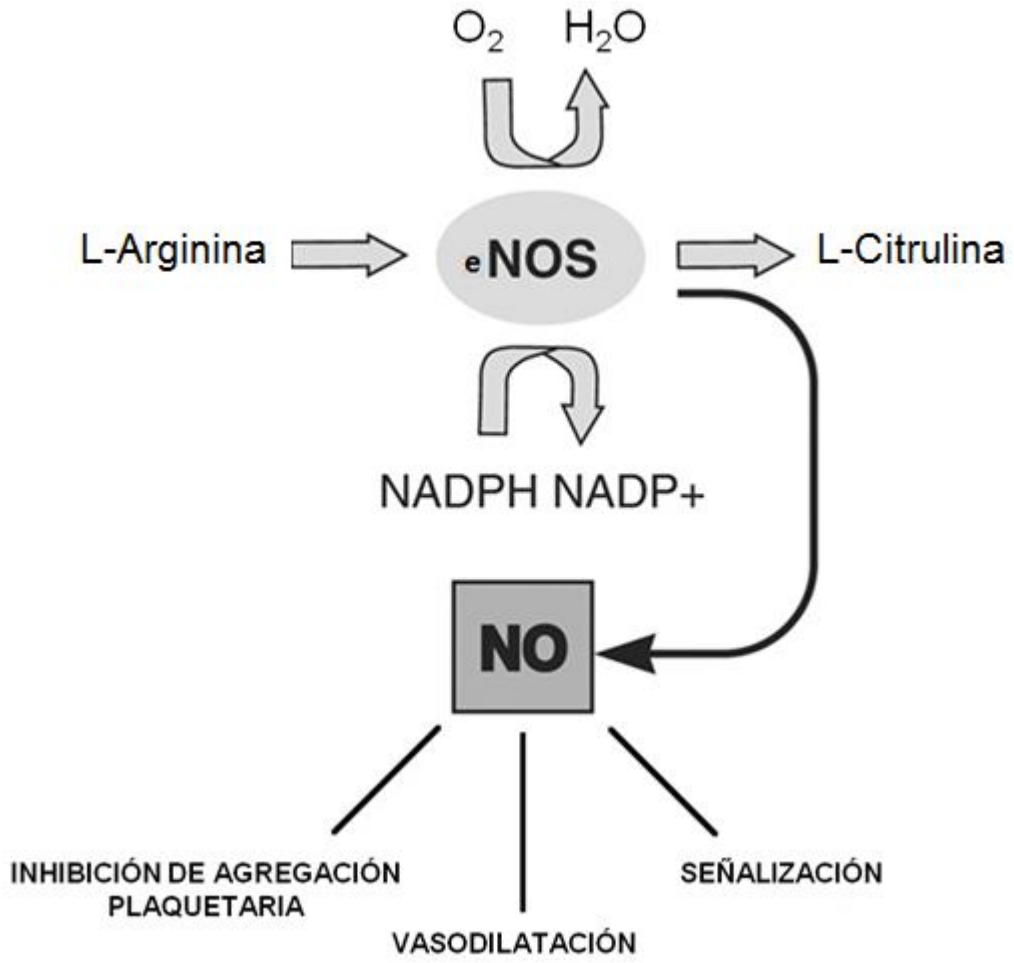
CELLULAR ASSAYS:
EFFECT OF PROPOLIS ON ENDOTHELIAL CELLS

- INDUCE EXPRESSION OF eNOS
- INHIBIT NOX4

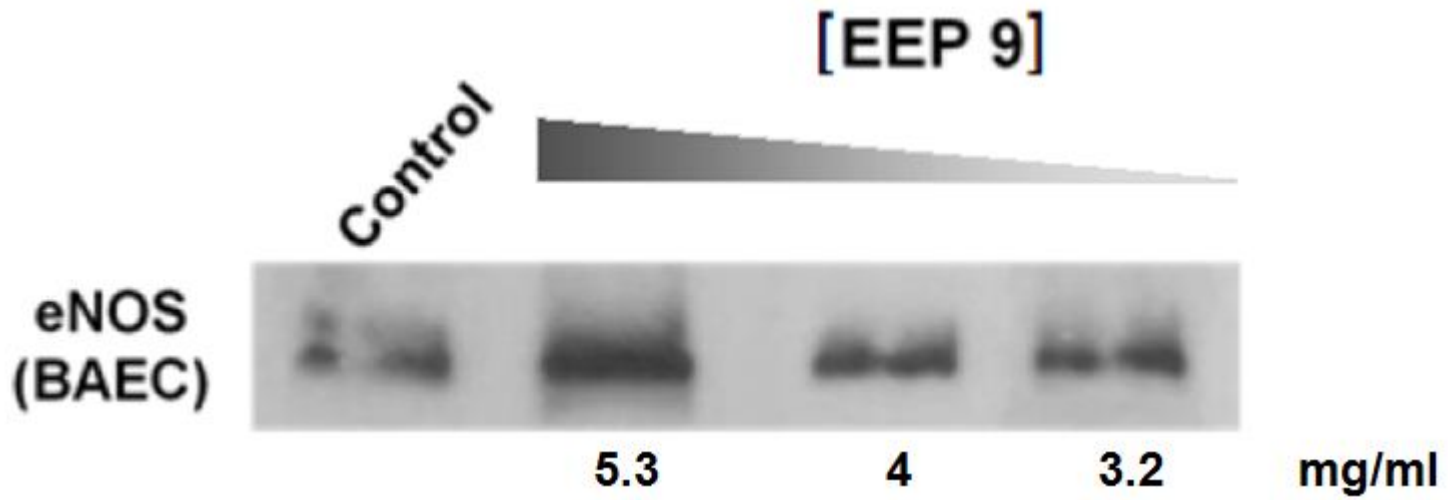


Nitric oxide synthase (NOS)



- NOS-3 , eNOS
- Constitutive expression in the endothelium
- •NO (from eNOS) keeps vascular homeostasis

Induction of eNOS expression by propolis



✓ **EEP 9 (high in polyphenols) significangtly induced eNOS expression**

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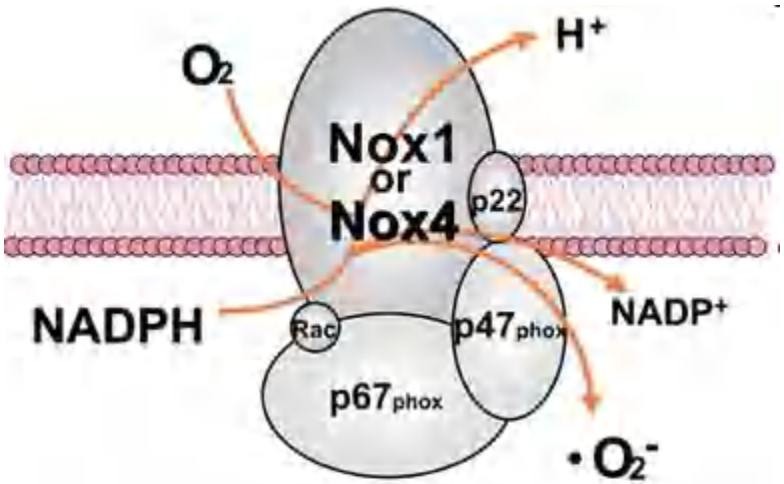
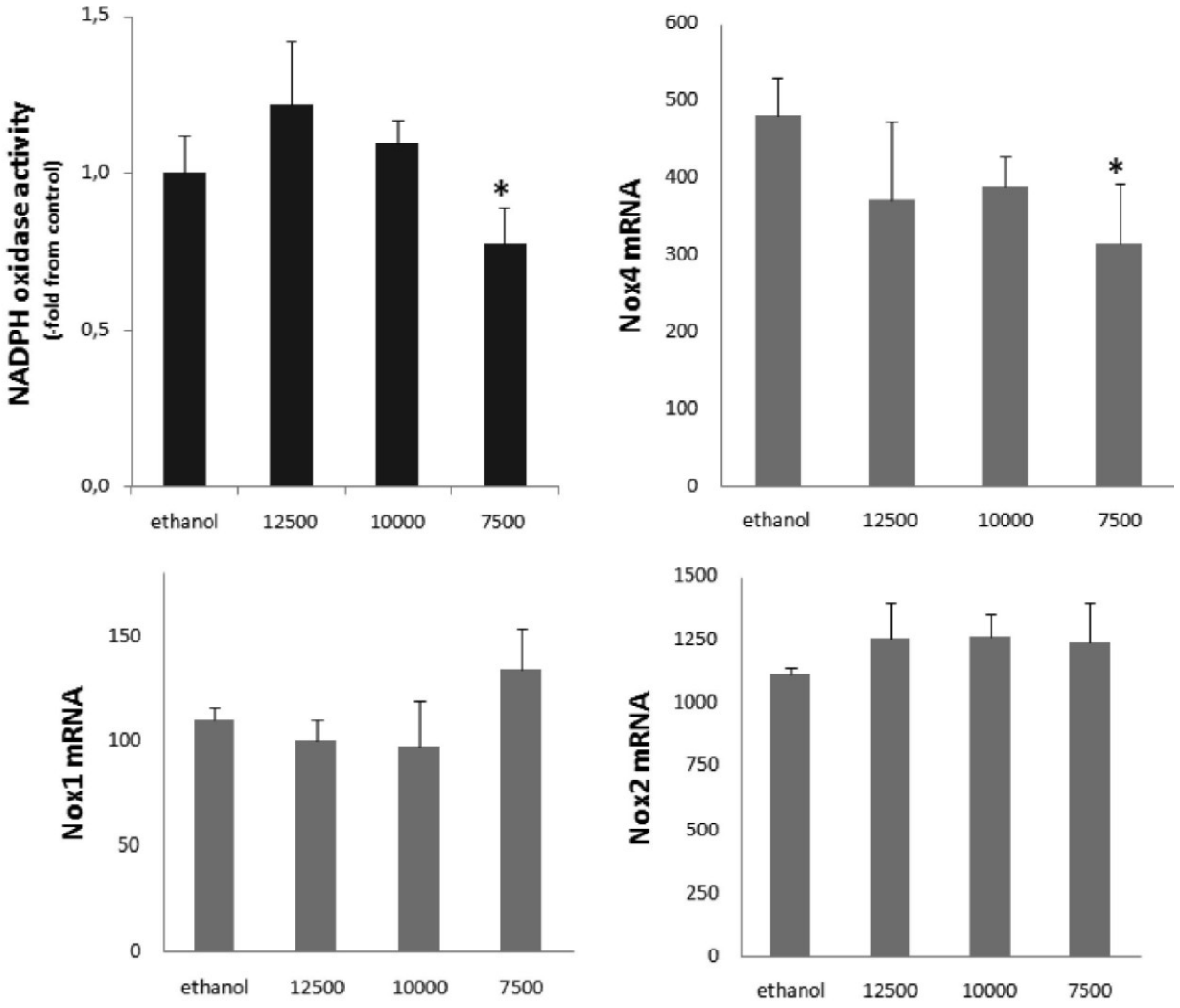
NADPH oxidase (NOX)



- Membrane enzyme
- Activated by assembling of subunits
- NOX1 and NOX4, vascular isoforms
- Uses NADPH and O2 to form superoxide radical anion ($O2^{\cdot -}$)

INHIBITION OF NOX4 BY PROPOLIS



- ✓ **EEP9 decreased NOX activity and mRNA NOX4 expression**

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Conclusions

✓ **The antioxidant capacity of uruguayan propolis was assayed in vitro and cellular assays**

✓ **ORAC 8000 μmol Trolox eq/g propolis (higher than other natural products)**

✓ **Efficiently inhibits LDL oxidation and tyrosine nitration**

✓ **Flavonoid composition (RP-HPLC) indicates poplar-tree origin**

✓ **Induces eNOS expression and NOX4 inhibition (high NO bioavailability in endothelium)**

Thanks to

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