6TH APIMEDICA & 5TH APIQUALITY
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Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri

Propolis: intracellular mechanism of action to clarify anti-inflammatory and antioxidant activities

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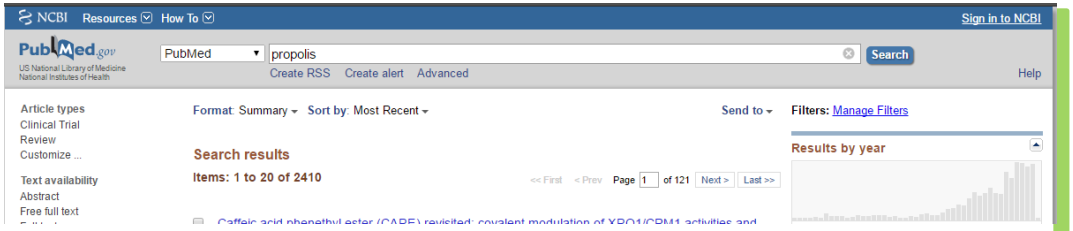
Why have we chosen propolis?

CHANCE

More than
2400 scientific papers
30.000 tons of extract



CHALLENGE

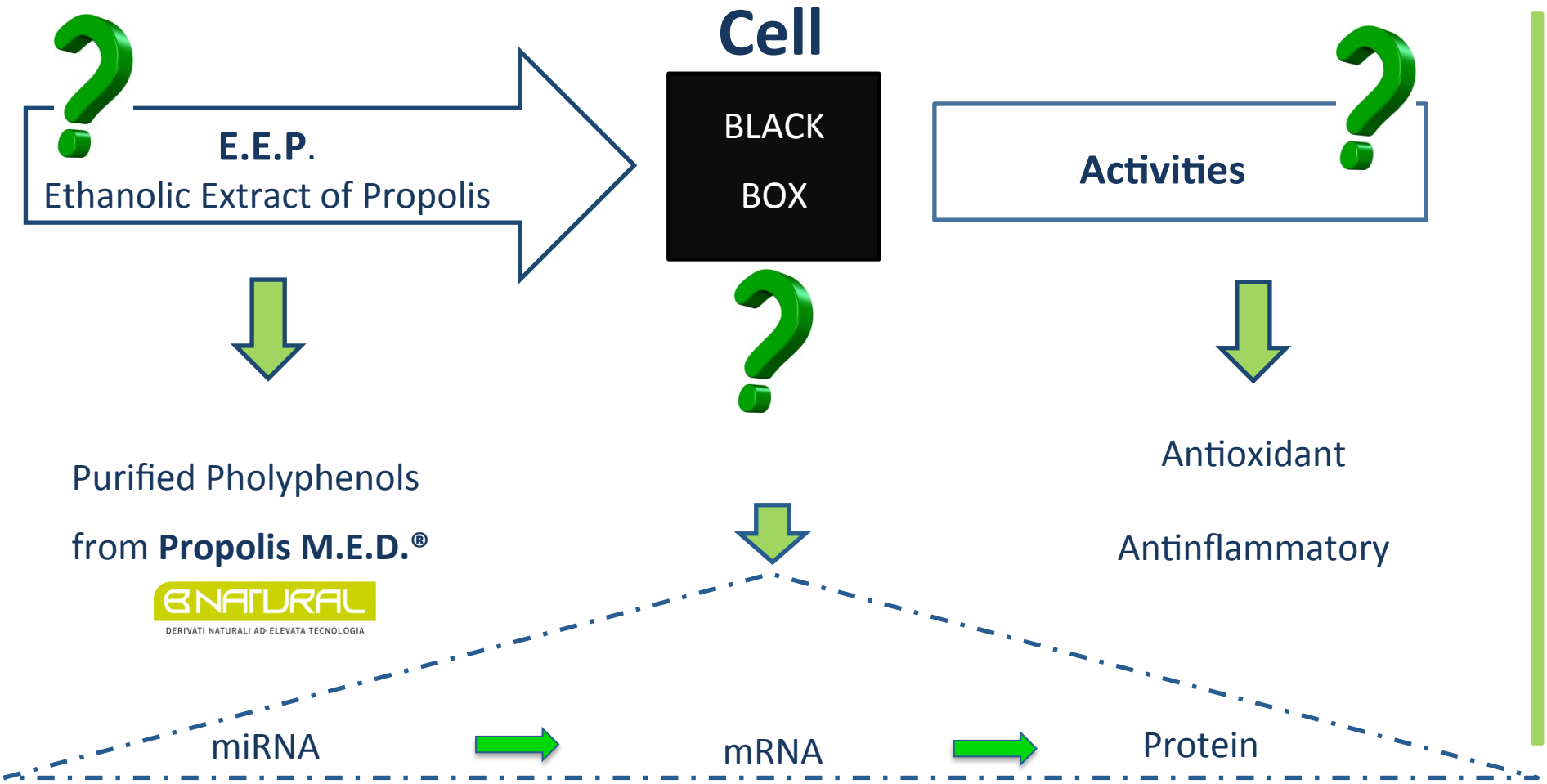
Several activities have been reported without mechanism of action

INNOVATION

From Folk remedy
to definied nutraceutical compound

Bankova VS. 2005 Chemical diversity of propolis and the problem of standardization. Journal of Ethnopharmacology 100, 114–117

Scientific Project – Aim of this study

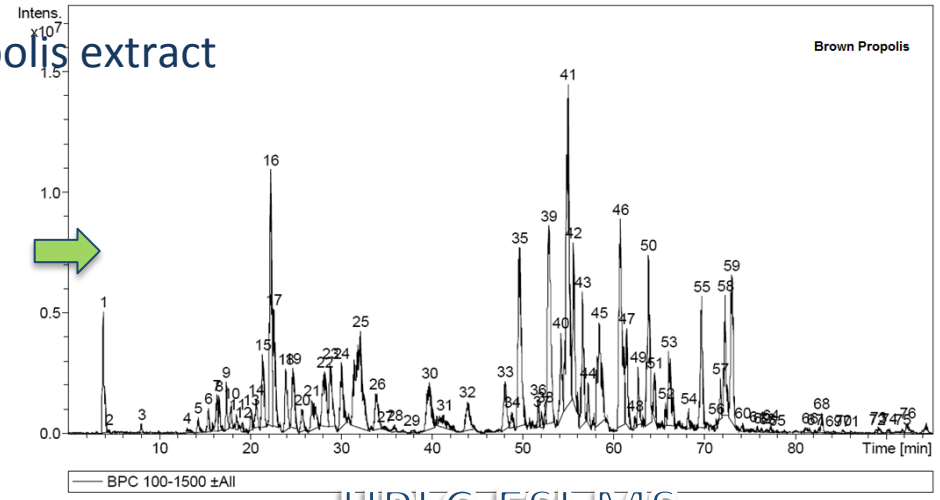


Why propolis M.E.D.® ?

B NATURAL
DERIVATI NATURALI AD ELEVATA TECNOLOGIA

1st Standardized and characterized propolis extract

TOTALS (%)	73,5
Phenolic acids and derivates(%)	8,0
Flavones and Flavonols(%)	29,8
Flavanones and Dihydroflavonols(%)	5,5
Glicolisilated Bioflavonols (%)	30,0

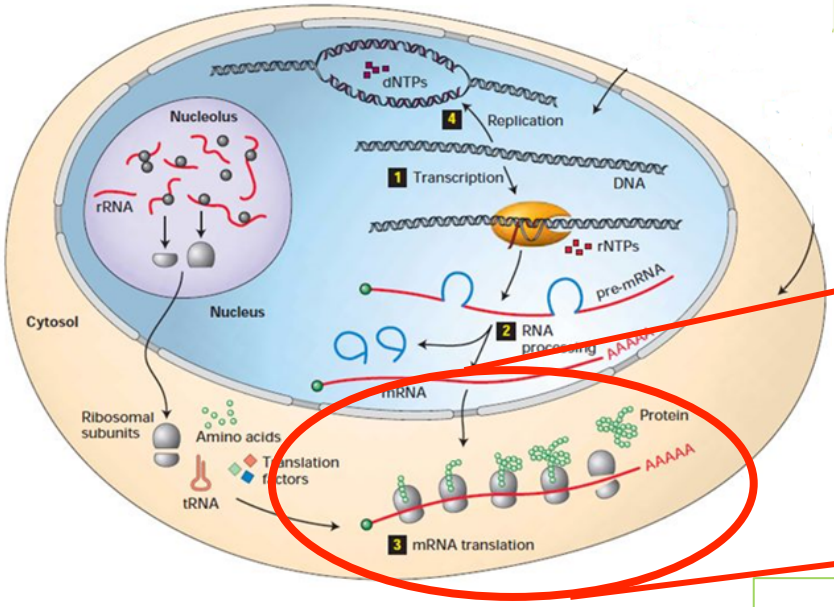


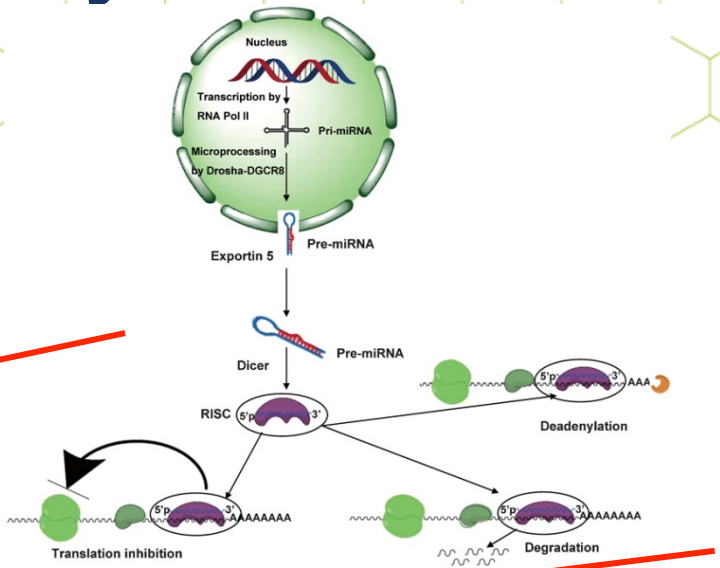
HPLC-ESI-MS

Total Polyphenols (HPLC- ESI –MS) of which 25 % (6 markers)
Chrysin, Galangin, Pinocembrin, Apigenin, Pinobanskin, Quercitin

Volpi N, Bergonzini G. 2006 Analysis of flavonoids from propolis by on-line HPLC-electrospray mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 42, 354-361
Pellati F, Orlandini G, Benvenuti S (2011) HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. J Pharm Biomed Anal 55: 934–948.
S. Falcão, M. Vilas-Boas, L.M. Estevinho, C. Barros, M.R.M. Domingues, S.M. Cardoso, Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds, Anal. Bioanal. Chem. 396 (2010) 887–897.

Role of miRNAS in Protein synthesis





Small non-coding RNA 22nt.
Down regulation of gene expression

They negatively control the translation of RNA in protein and therefore the gene expression

miRNAs are good markers to define antinflammatory and antioxidant action

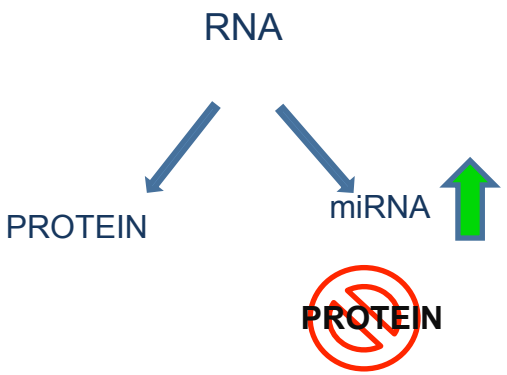
miRNAs appear as interesting mediators in regulating polyphenols biological effects

miRNAs tested by real time PCR

Hsa miRNA	Validated protein target
hsa-miR-203 a-3p	TNF alpha
hsa-miR19a-3p	
hsa-miR-144-3p	Nuclear factor (erythroid-derived 2)-like 2
hsa-miR-153-3p	
hsa-miR-142-5p	
hsa-miR-27a-3p	

Anti inflammatory

Antioxidant



If miRNA increases, the target gene will be not expressed

Xu Y, Fang F, Zhang J, et al. miR-17* suppresses tumorigenicity of prostate cancer by inhibiting mitochondrial antioxidant enzymes. PLoS One. 2010 Dec 22;5(12):e14356.

Studies on activity of propolis:

Evaluation of *in vitro* activity of Purified Polyphenols deriving from brown propolis M.E.D. miRNAs , mRNAs and protein expression in HaCat cells

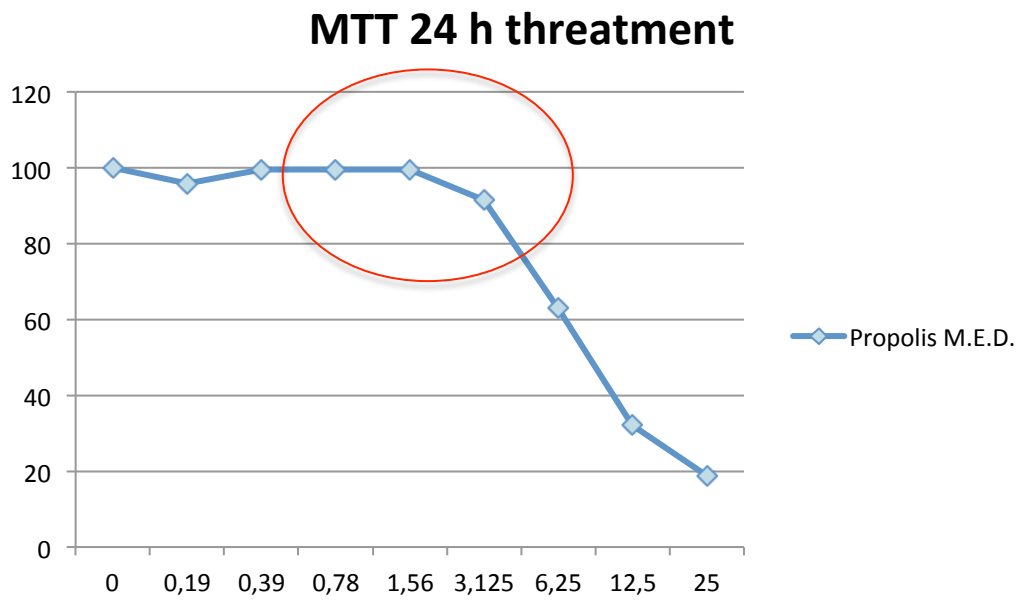
MTT test of Hydroglicerc Extract of Brown Propolis M.E.D

Treatment of HaCat Cells with subtoxic concentrations of propolis extract

Quantification of expression of miRNAs, mRNAs and protein involved in antioxidant and antinflammatory processes

Studies on activity of propolis

MTT test of Hydroglicerc Extract of Brown Propolis M.E.D.

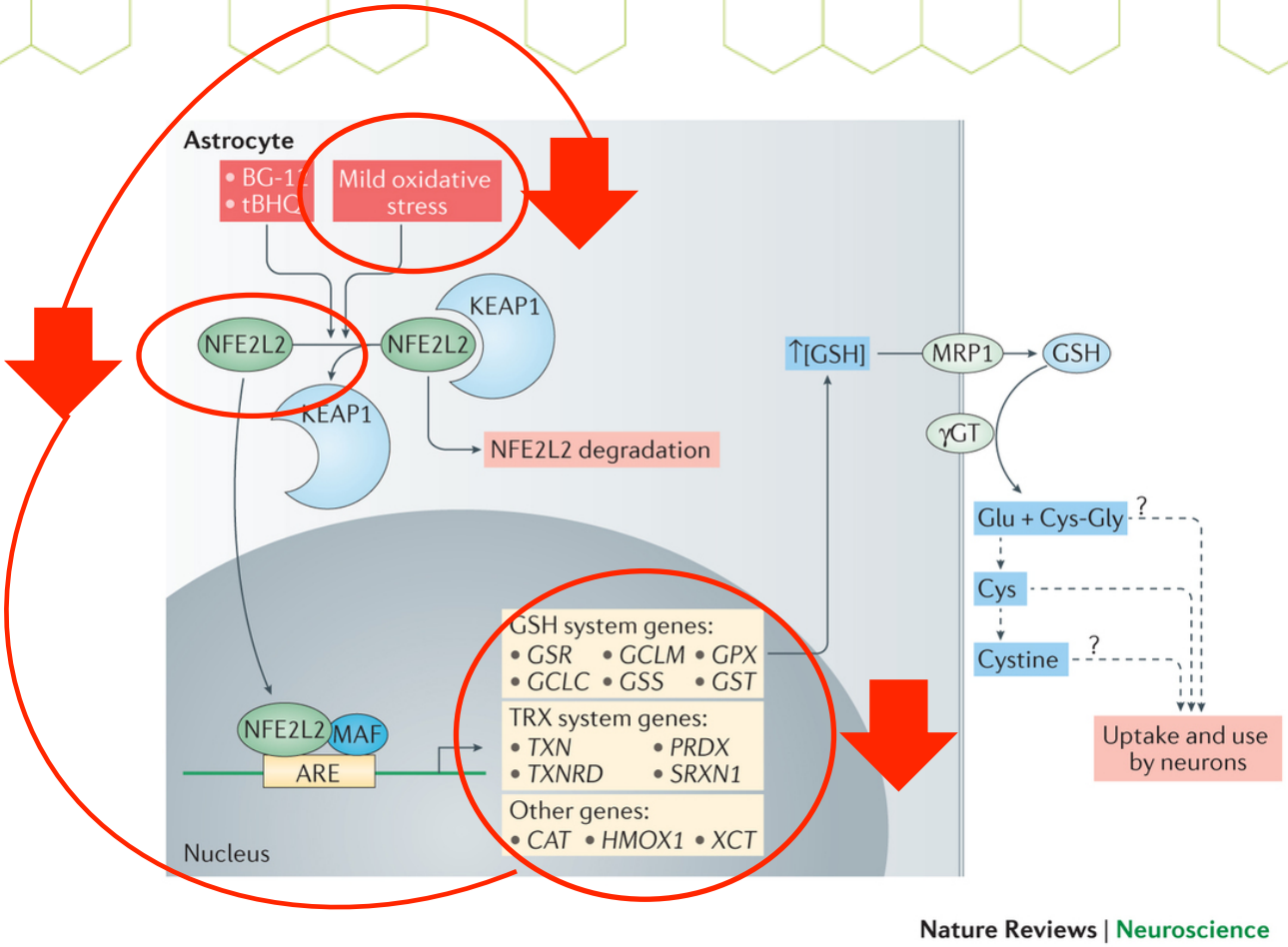


HaCat Cells treated for 24h at three subtoxic concentrations

0,78mg/ml
1,56 mg/ml
3,125 mg/ml

Sample (mg/ml)	0	0,19	0,39	0,78	1,56	3,125	6,25	12,5	25
Propolis M.E.D.	100,00	95,99	99,58	99,6	99,59	91,50	62,88	32,16	18,81

Why Nuclear factor (erythroid-derived 2)-like 2 ?



miRNA ↑

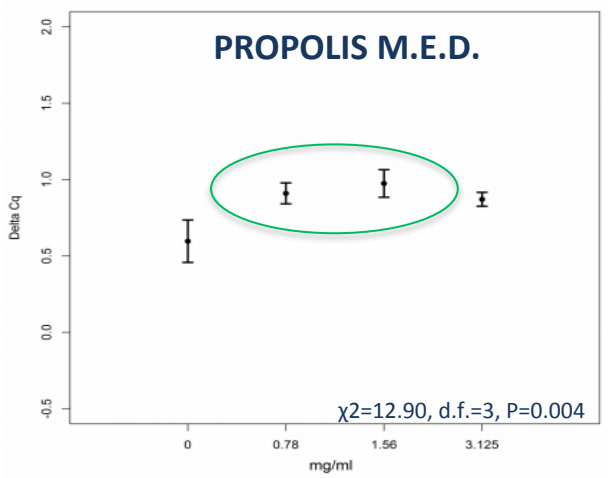
mRNA ↓

Protein ↓

In presence of Oxidative Stress NFE2L2 enhance the production of antioxidant protein

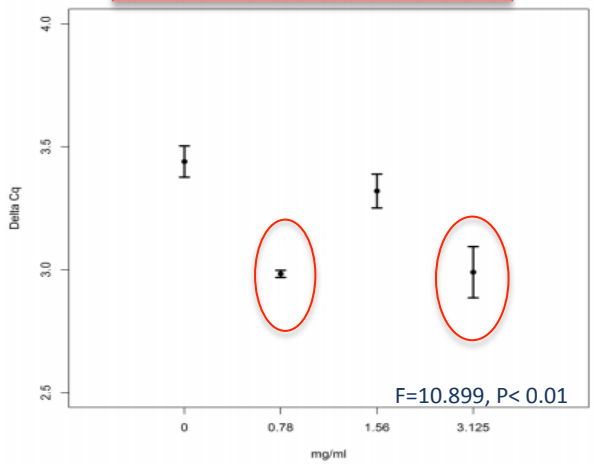
From miRNAs to mRNA

miR-27a-3p



ANTI-OXIDANT

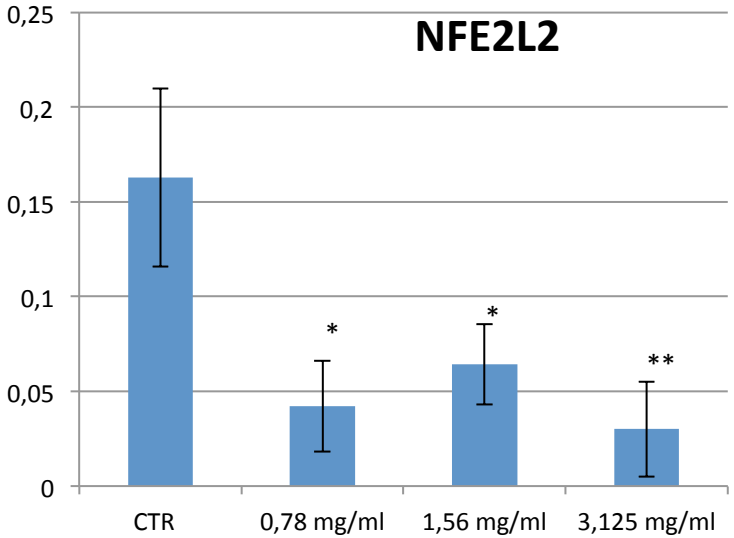
mRNA-NFE2L2



Propolis M.E.D.® is able to modulate the expression of miRNAs and mRNAs involved in antioxidant activities

Target: Nuclear factor (erythroid-derived 2)-like 2

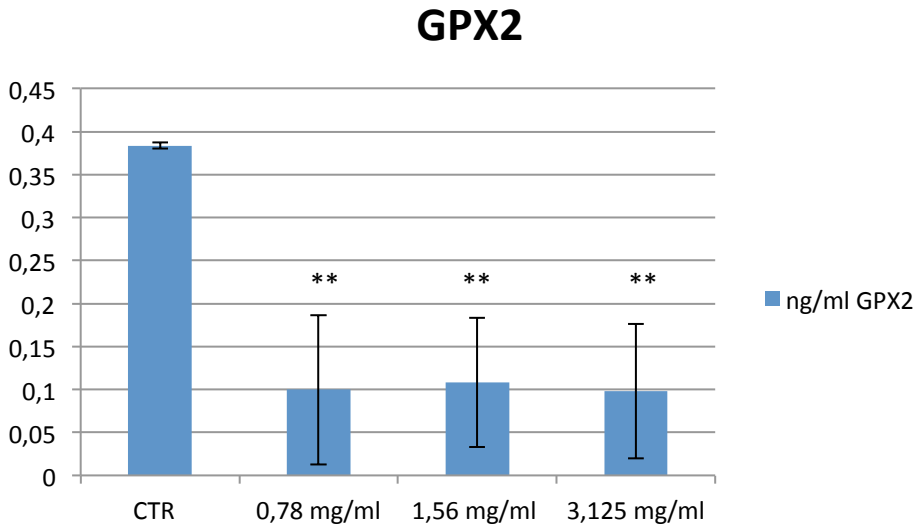
Nuclear factor (erythroid-derived 2)-like 2 pathway



f.=9,49 P<0.01
Bonferroni and Holm: P<0,01; P<0,05

As aspected Propolis M.E.D.® is able to reduce the production of NFE2L2

The expression of Glutathione Peroxidase 2 is reduced after treatment with Propolis M.E.D.® according to the first hypothesis

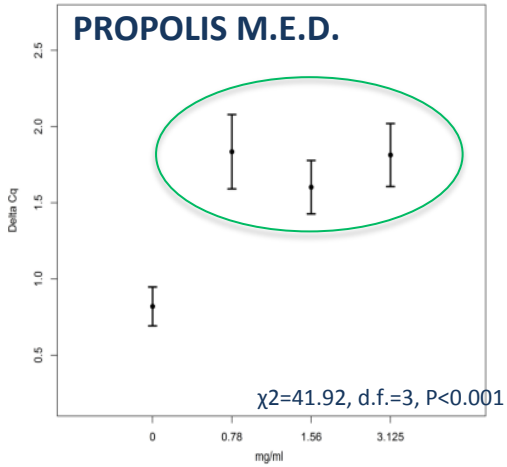


f.= 6,29 P<0.01
Bonferroni and Holm: P<0,05

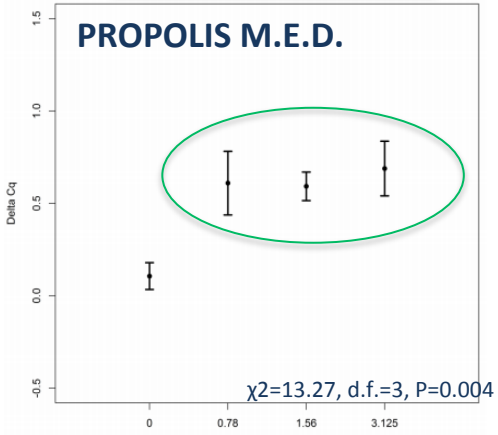
From miRNAs to mRNA

INFLAMMATION

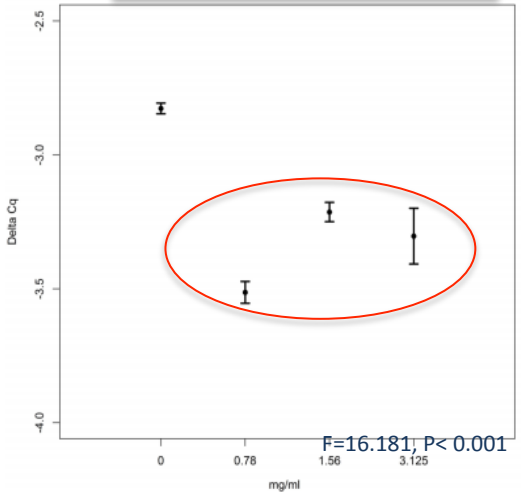
miR-203a-3p



miR-19a-3p

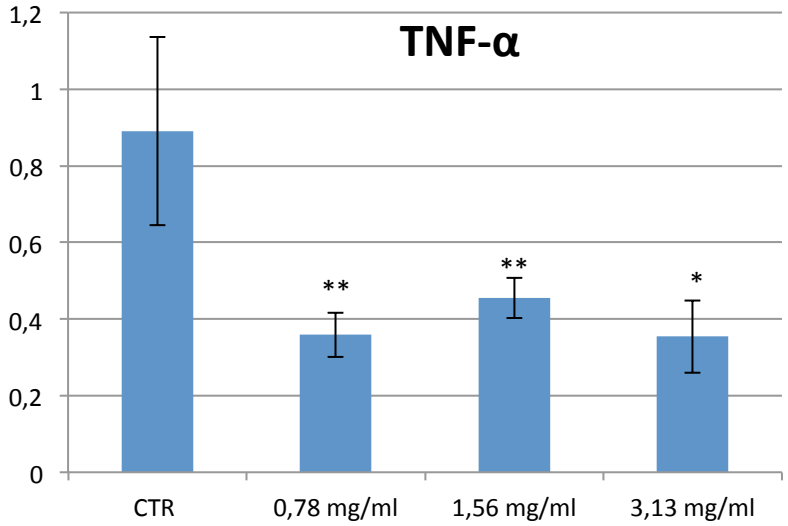


mRNA-TNF-α



Target: TNF alpha

TNF - ALPHA



f.=6,73 P<0.01
Bonferroni and Holm: P<0,01; P<0,05

HaCatCells, after treatment with Propolis M.E.D.®, showed a reduction of the expression of TNF-alpha

Future perspectives: *in vivo* studies

Experiment	Experimental read-out	Groups	Timing of blood sample	N of mice per group	Total mice
1	Metabolites miRnas mRnas and protein expression	Control Treated A Treated B	10 – 20 – 30 days	10	30
2	Metabolites	Control Treated 200 mg/kg Treated 250 mg/kg Treated 300 mg/kg Treated	1h , 2h, 4h, 8h	30	30

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Organismo preposto al Benessere Animale

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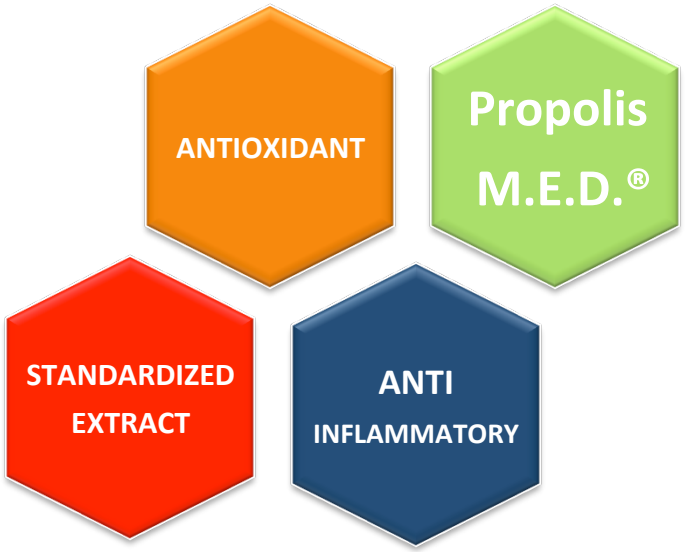
APPROVED

CONCLUSIONS

PURIFIED POLYPHENOLS FROM PROPOLIS M.E.D.® :

Reduce and preserve from oxidative stress acting on NEF2L2 pathway

Reduce the inflammation reducing the production of TNF – alpha



Is possible to use Propolis M.E.D. not only for it's antibacterial activity but also for its proved antioxidant and antibacterial activity

THANK YOU FOR YOUR ATTENTION

Propolis:
intracellular mechanism of action to clarify anti-inflammatory and antioxidant activities

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