



6TH APIMEDICA

&

5TH APIQUALITY



Istituto Zooprofilattico Sperimentale
del Lazio e della Toscana M. Aleandri

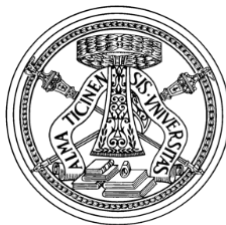


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SYMPOSIUM

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22-25 NOVEMBER 2016
NOVOTEL ROMA EUR

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

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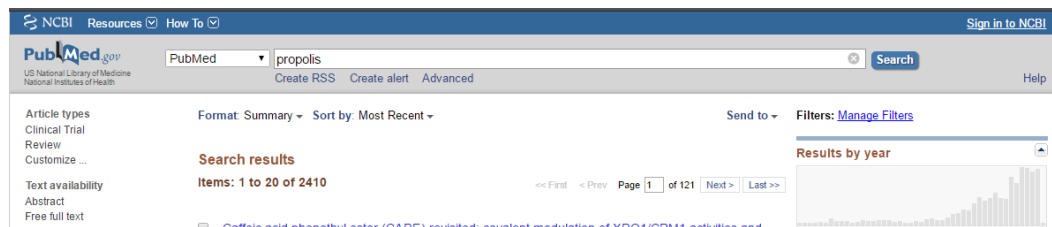
Why have we chosen propolis?



CHANCE



More than
2400 scientific papers
30.000 tons of extract



CHALLENGE



Several activities
have been reported
without mechanism
of action

INNOVATION

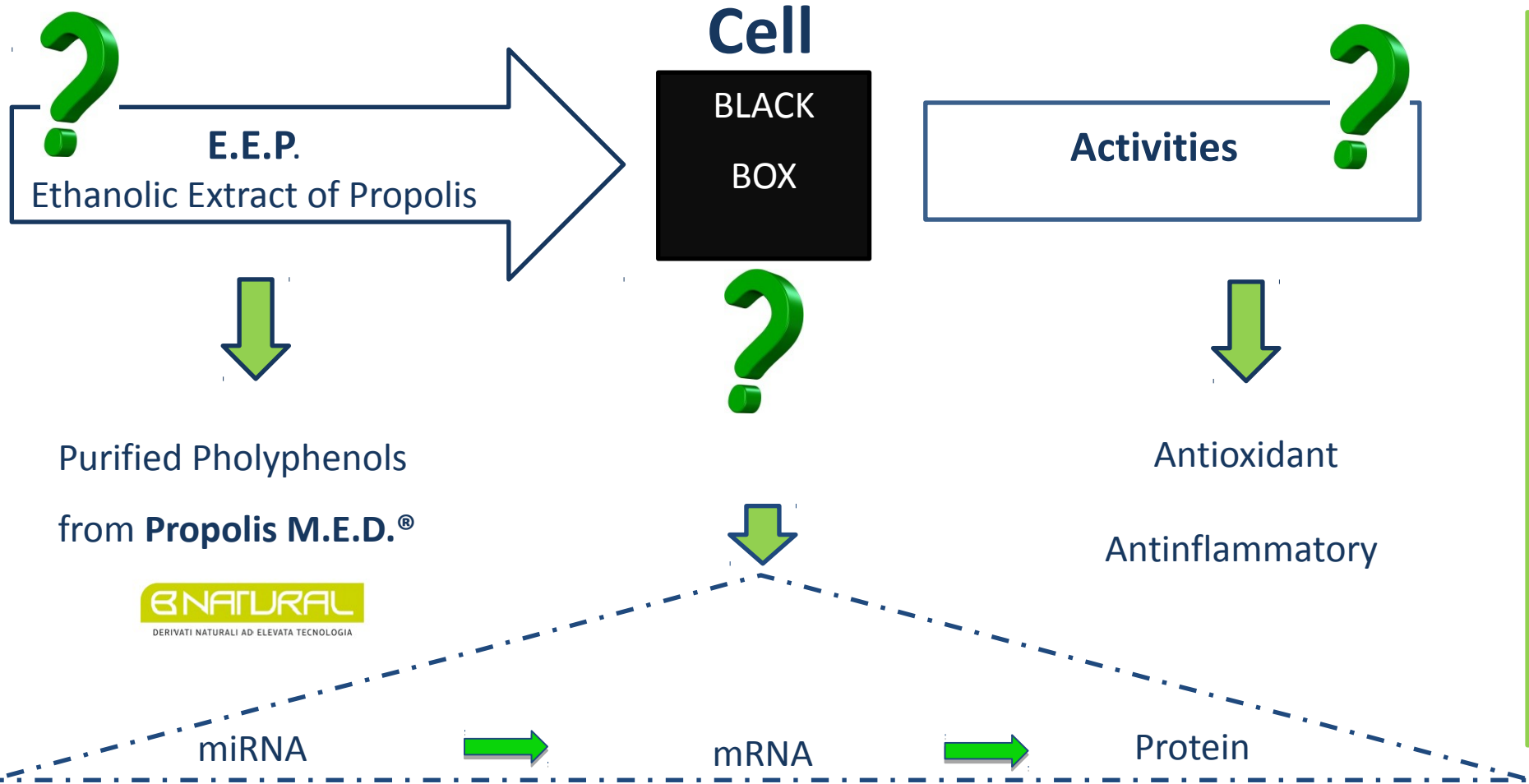


From Folk remedy
to defined nutraceutical compound





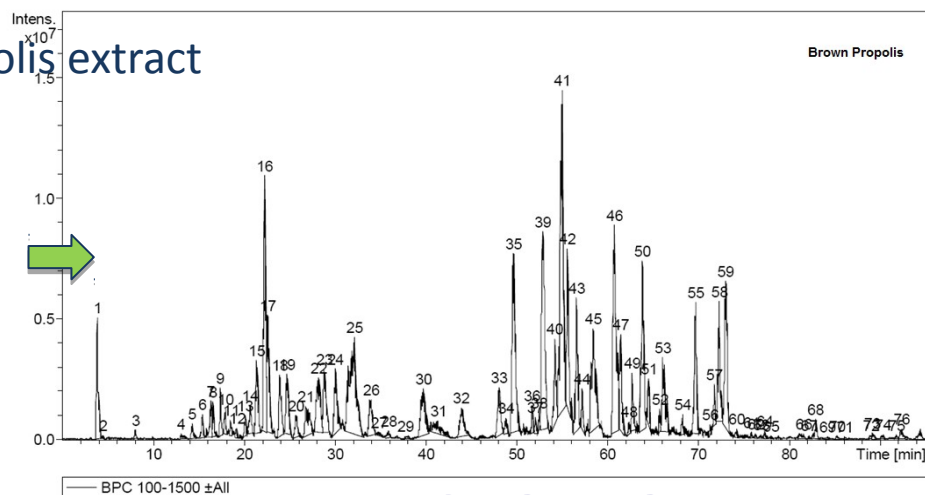
Scientific Project – Aim of this study



Why propolis M.E.D.[®] ?

Standardized and characterized propolis extract

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

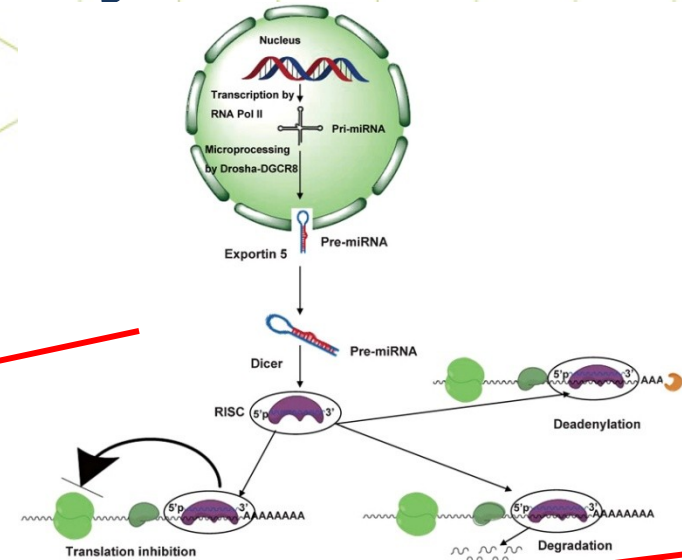
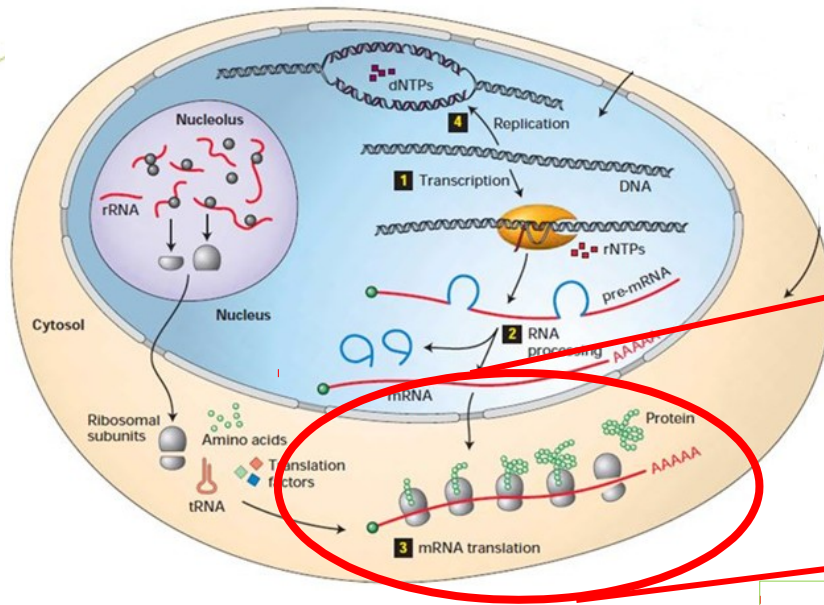


HPLC-ESI-MS

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



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
antiinflammatory and antioxidant action

miRNAs appear as interesting mediators in
regulating polyphenols biological effects

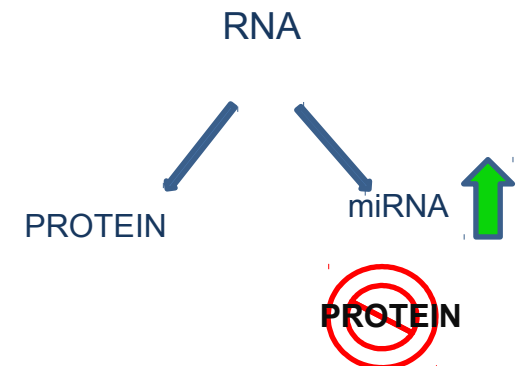


miRNAs tested by real time PCR

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

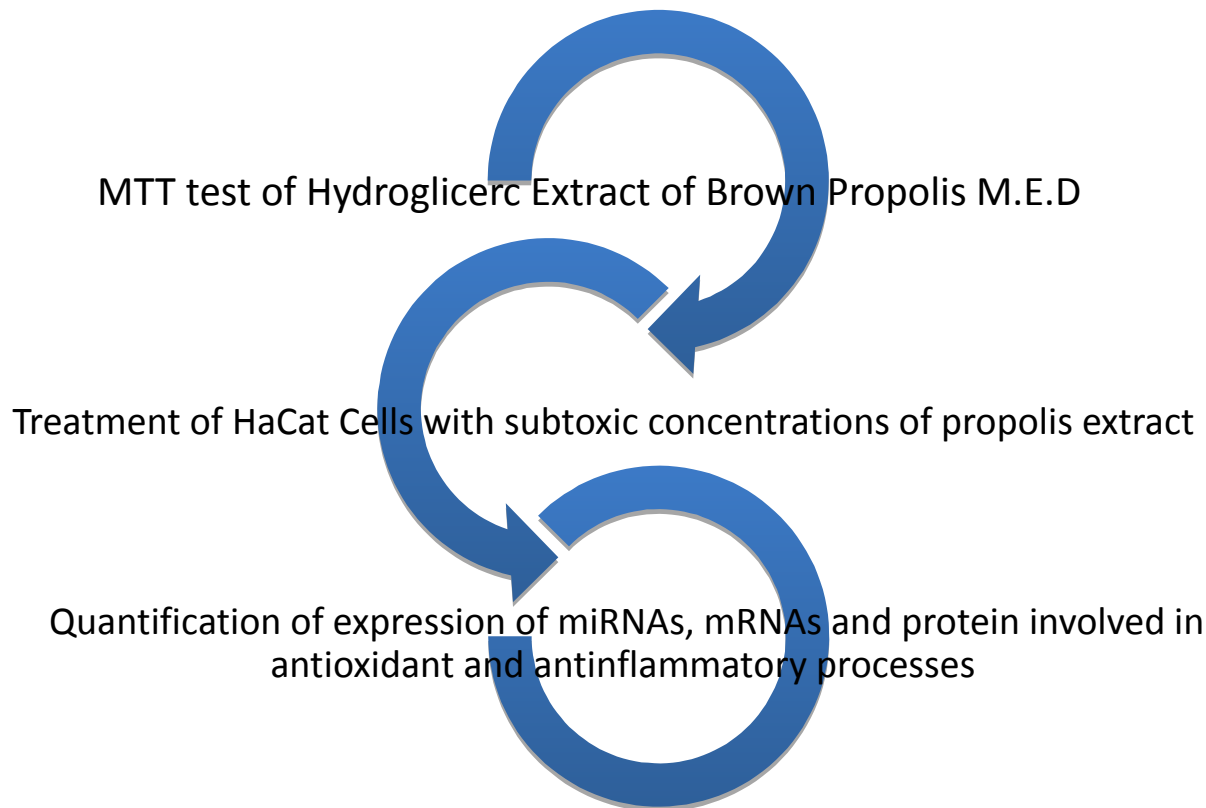


If miRNA increases, the target gene will be not expressed

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

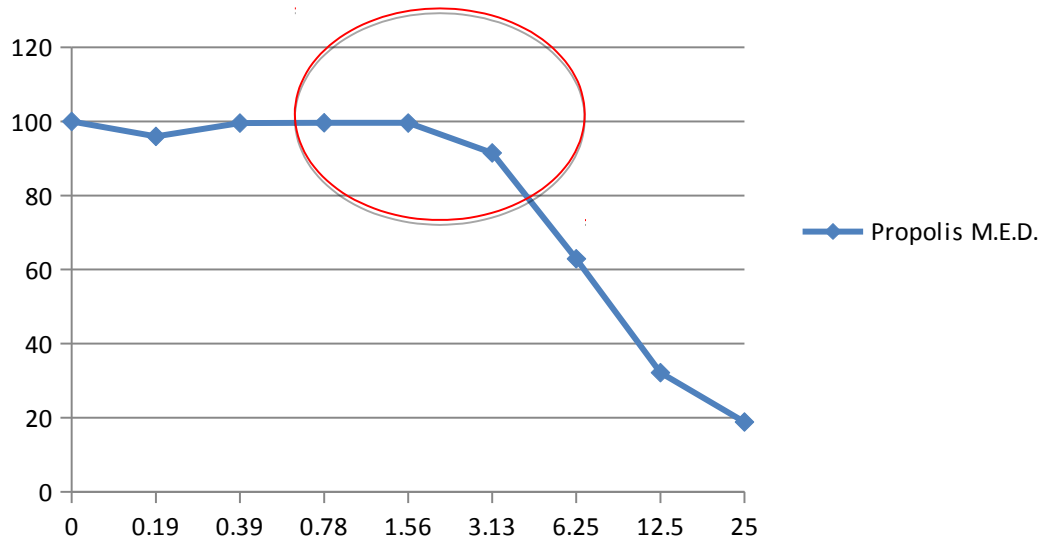




Studies on activity of propolis

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

MTT 24 h threatment

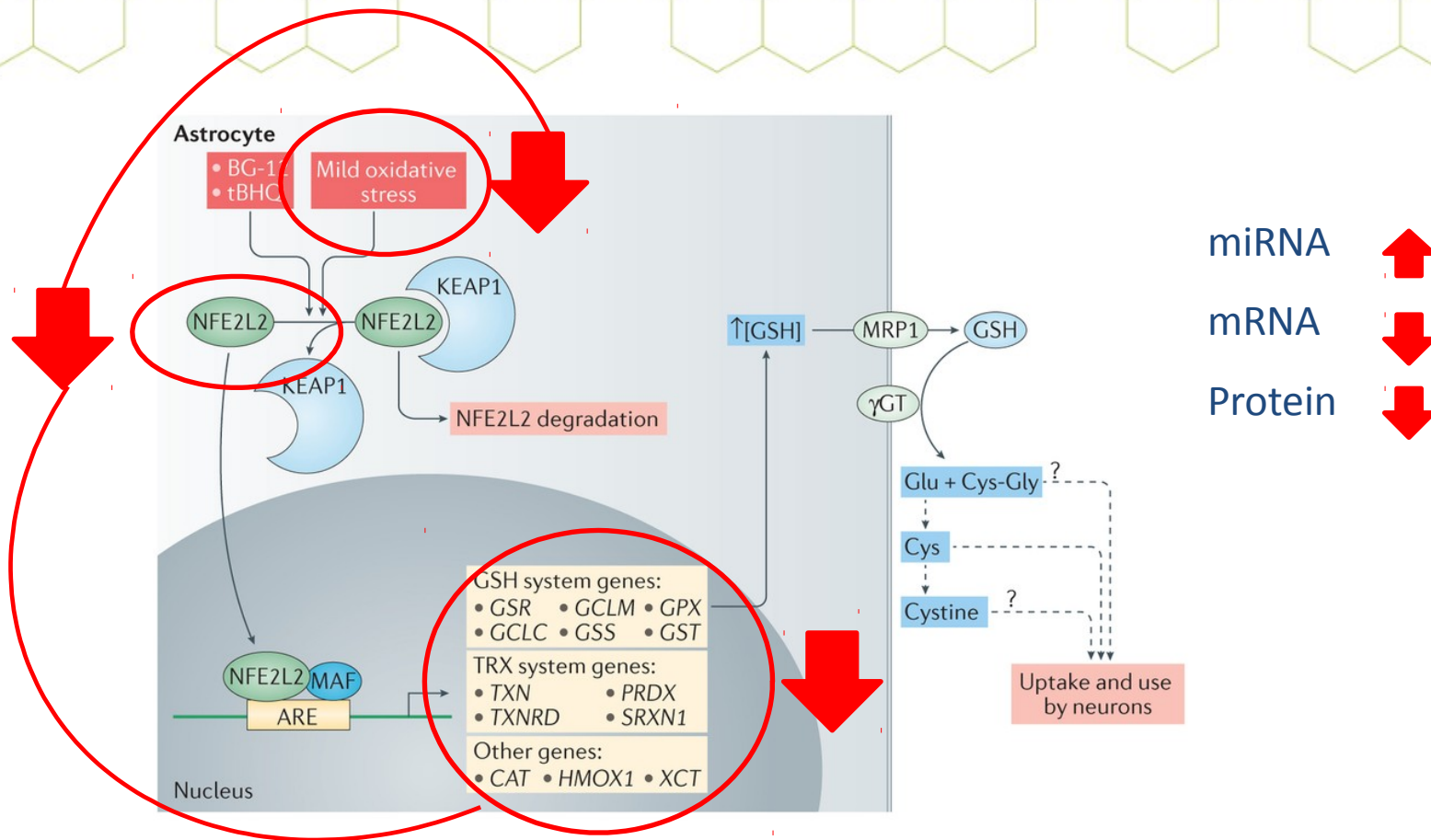


HaCat Cells treated for 24h at three subtoxic concentrations

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 ?



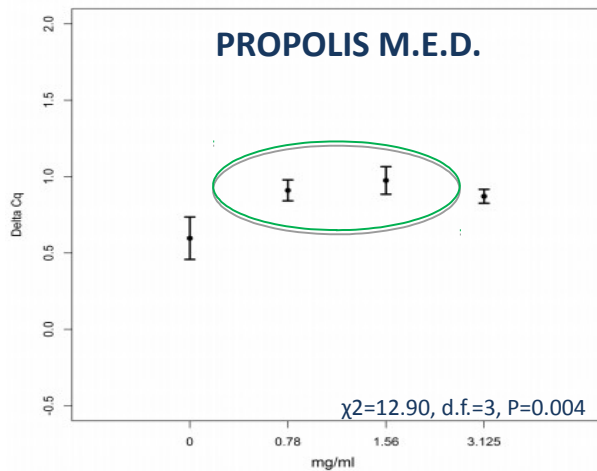
Nature Reviews | Neuroscience

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

From miRNAs to mRNA



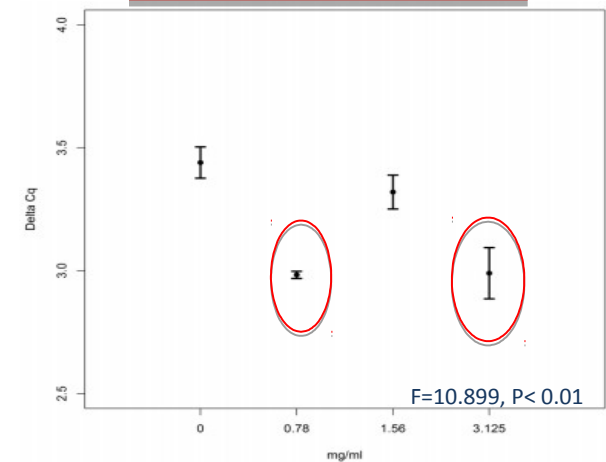
miR-27a-3p



ANTI-OXIDANT



mRNA-NFE2L2



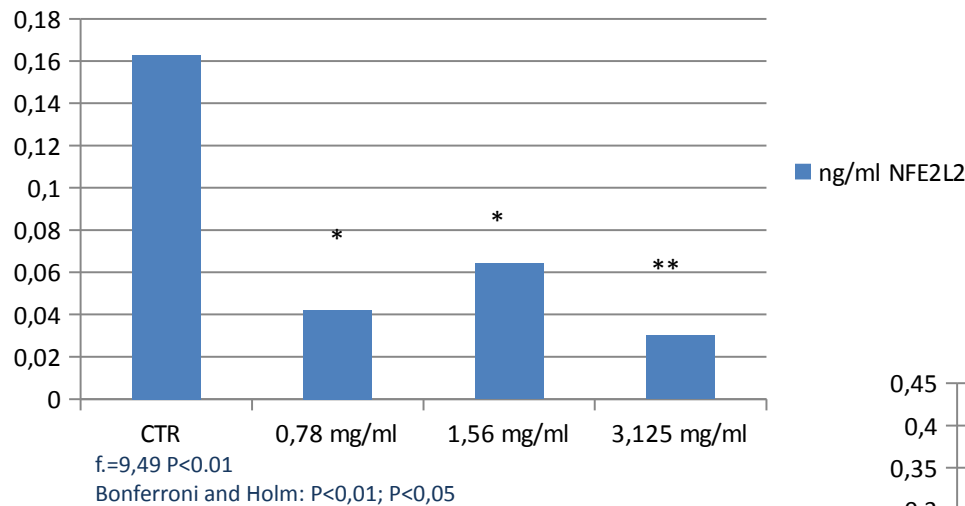
Propolis M.E.D.[®] is able to modulate the expression of miRNAs and mRNAs involved in antioxidant activities

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

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



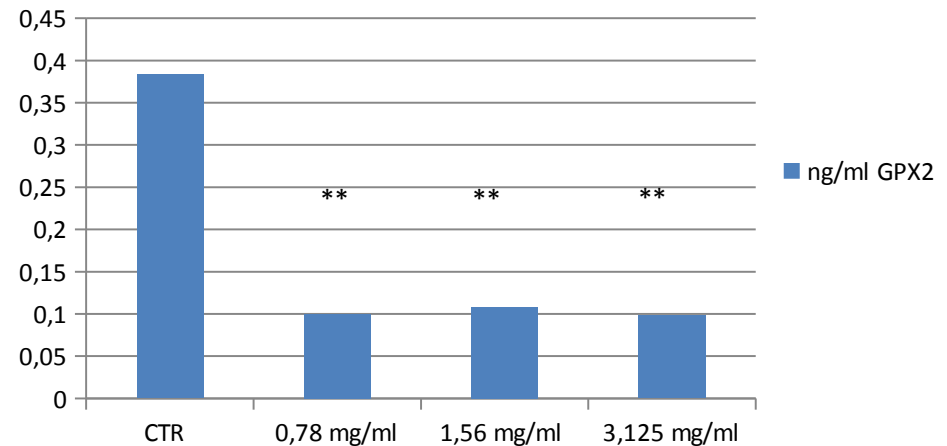
NFE2L2



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

As expected Propolis M.E.D.[®] is able to reduce the production of NFE2L2

GPX2



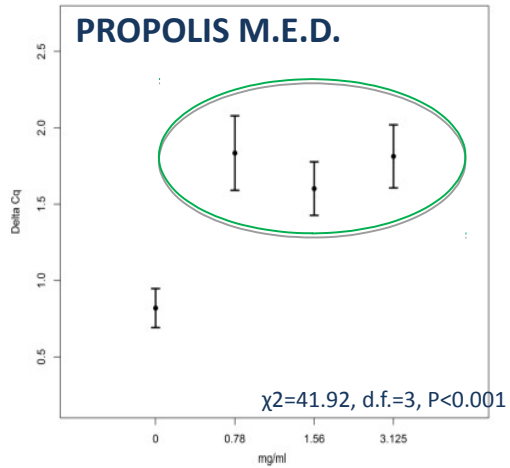
f.= 6,29 P<0.01
Bonferroni and Holm: P<0,05

From miRNAs to mRNA

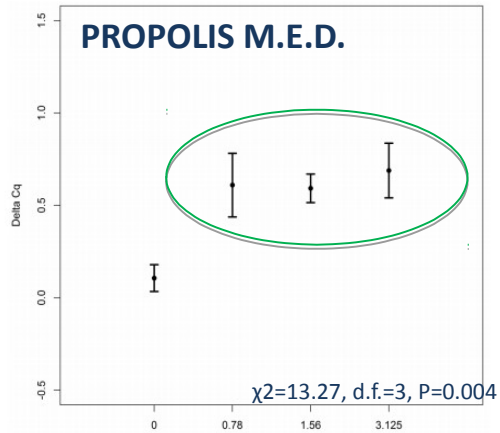


miR-203a-3p

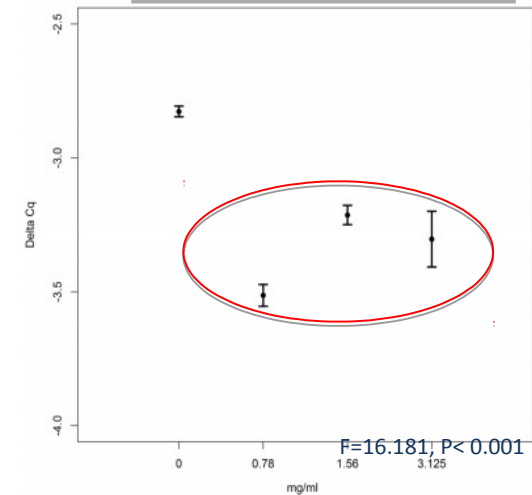
INFLAMMATION



miR-19a-3p

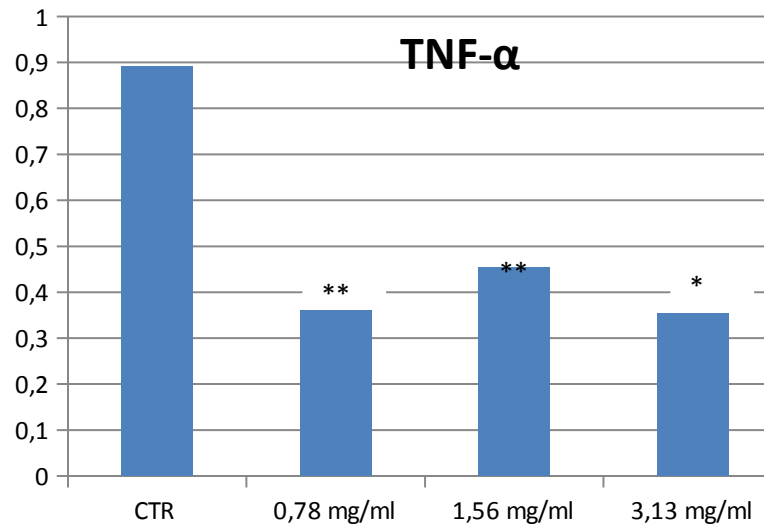


mRNA-TNF- α



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	10 – 20 – 30 days	10	30
		Treated A			
		Treated B			

2	Metabolites	Control	1h , 2h, 4h, 8h	30	30
		Treated 200 mg/kg			
		Treated 250 mg/kg			
		Treated 300 mg/kg			
		Treated			

UNIVERSITA' DEGLI STUDI DI PAVIA
Organismo preposto al Benessere Animale

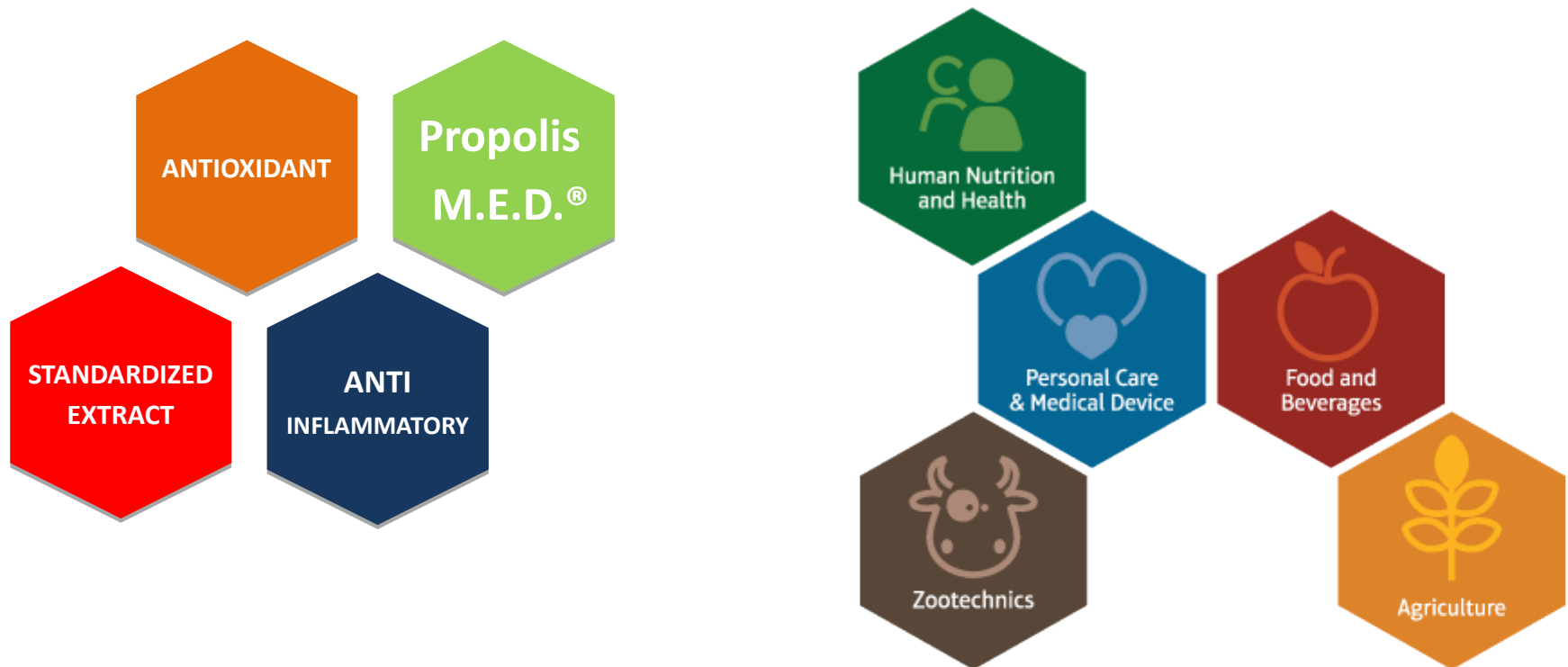


CONCLUSIONS

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



B NATURAL
PROPOLIS AND BEEKEEPING DERIVATIVES

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