In humans M.E.D. Propolis efficacy against the symptoms related to Upper Respiratory Tract Infections and *in vitro* antibacterial activity against antibiotics resistant and susceptible

Zaccaria V.[1],Galeotti F.[2],Garzarella E.U.[3],Esposito C.[3],Gisone L.[1],Pianta S.[1],Volpi N.[2],Daglia M.[3]

1 - B Natural R&D Unit, via Gran Sasso 33, Corbetta (MI)
2 - Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 213/D, 41121 Modena, Italy
3 - Department of Pharmacy, Nutraceutical Lab, University of the Naples, Federico II, Via D. Montesano 49, 80131 Napoli, Italy

ABSTRACT

STUDY OBJECTIVE

Propolis has been traditionally used for centuries for the prevention and treatment of respiratory diseases for its antimicrobial and anti-inflammatory activity. Nevertheless, very few clinical studies show its beneficial effects against upper respiratory tract infections (URTIs). Moreover, the few results of the clinical trials and, more generally, literature data on propolis have the important limitation to be not easily comparable due to the high variability of the different types of propolis studied which were obtained using different extraction methods showing different chemical composition and biological activities. To overcome this issue, standardized propolis extracts were obtained with a new patent Multi Dynamic Extraction (M.E.D.) method. Recent studies have been shown that M.E.D. extracts have *in vitro* and *in vivo* anti-inflammatory and antioxidant activities. Since it is important to clinically prove propolis activities using a propolis extract with a standardized composition, the first aim of this study was to demonstrate that M.E.D. method produces standardized polyphenolic mixtures from poplar-type propolis, with reproducible chemical composition and biological activity (anti-microbial activity), independently from the chemical profile of the starting raw propolis. Then, considering the anti-infammatory activity of propolis, the second aim was to test M.E.D. Propolis extracts to evaluate the actions against URTIs symptoms on 122 volunteers in a randomized, monocentric, double blind clinical trial.

METHODS

Samples analysis and antibacterial activity: nine raw propolis samples, from Europe, America, and Asia, were analyzed for their polyphenol chemical composition by means of HPLC and then combined to obtain three mixtures of propolis, which underwent through M.E.D. process. The chemical composition and the anti-microbial activity against bacteria and fungi involved in upper respiratory tract (see table), gastrointestinal, vaginal and skin (data not shown) infections were determined.

Clinical trial: 122 volunteers showing symptoms related with URTI, were treated with M.E.D. Propolis extract and with placebo for 5 days. After 3 and 5 days, during the outpatient visit, all symptoms were evaluated and oral swab were analysed to determine bacterial infections.

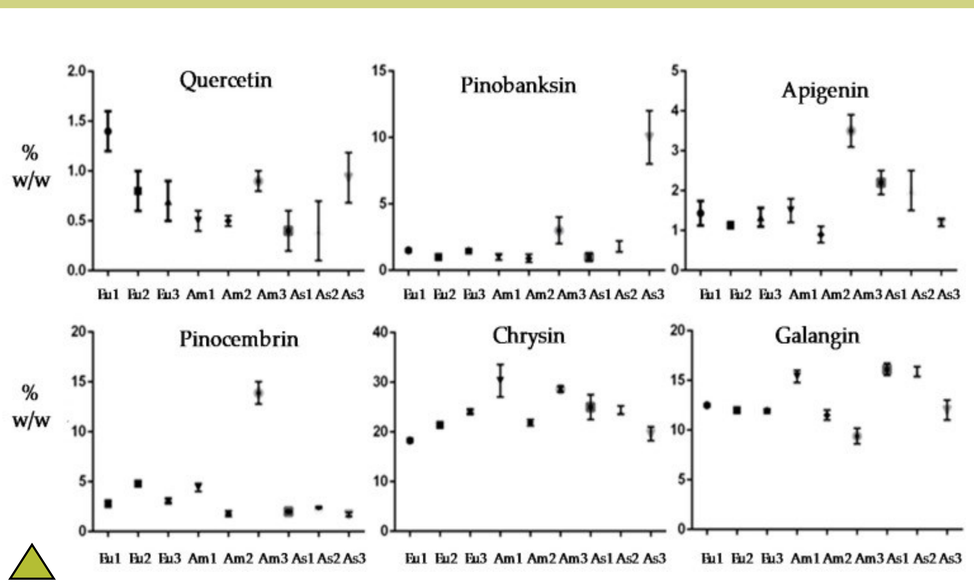
RESULTS

The antimicrobial activity of the three M.E.D. Propolis extracts was tested against microorganism strains representing the major families: Gram-positive, Gram-negative bacteria and fungi. As expected, MIC values showed that the three M.E.D. Propolis extracts exerted antimicrobial activity. Low MIC values (6-24 µg/mL) were found against *Aspergillus niger, Streptococcus pneumonia Penicillin-susceptible, Moraxella catarrhalis, Atopobium vaginae*, and *Neisseria gonorrhoeae*. Moderate activity against Staphylococcus spp and *Gardnerella vaginalis,* (48 µg/mL), low effects on Candida spp and Clostridium spp, (384 µg/mL) were found. No activity was detected against Bacteroides fragilis and Lactobacillus spp. Only MIC values related to URTIs are reported in the table. The results obtained gave comparable MIC values for each extract obtained using the M.E.D. method against the same microorganisms, despite the different geographical origins of the nine raw materials.

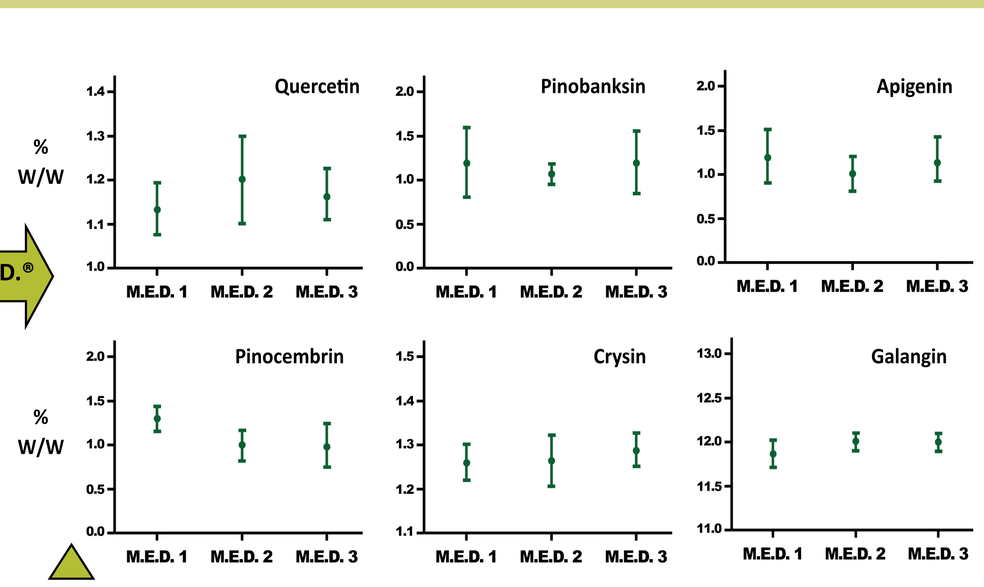
M.E.D. Propolis, resolved all URTI symptoms earlier than control groups even if compared with literature data. Only a small percentage of patients results positive to the oral swab at T0 and treatment with M.E.D. Propolis solved the infections; on other patients the treatment works on inflammatory process.

CONCLUSIONS

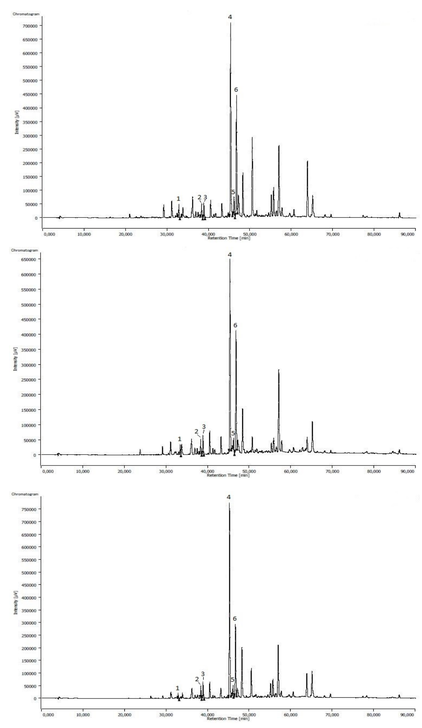
M.E.D. Propolis is active against many different bacterial and fungi spp which cause respiratory, skin, vaginal and gastrointestinal infections. M.E.D. Propolis extracts showed similar chemical compositions and antimicrobial activities, exhibiting no relevant differences against antibiotic-susceptible and antibiotic-resistant strains. Moreover M.E.D. Propolis extracts, had very strong activity against vaginal and gastrointestinal pathogens isolates and no activity against Lactobacilli. These results taken together could suggest a synergic effect of antibacterial activity of propolis M.E.D. extracts against vaginal and gastrointestinal pathogens. Since only few patients shown bacterial infections, this clinical trial showed that the relief from URTI symptoms could be due not only to the well known and established anti-bacterial activity but also to anti-viral and anti-inflammatory action of M.E.D. Propolis, as previously demonstrated *in vitro* by Zaccaria et al. (2017). Further investigations will be needed to verify the *in vivo* antibacterial activity of M.E.D. Propolis to demonstrate the complete action of this kind of propolis in URTIs. The batch-to-batch reproducibility of propolis extracts obtained with the M.E.D. method encourages the design of foods supplements having active dosage to reduce the use of synthetic antibiotics.



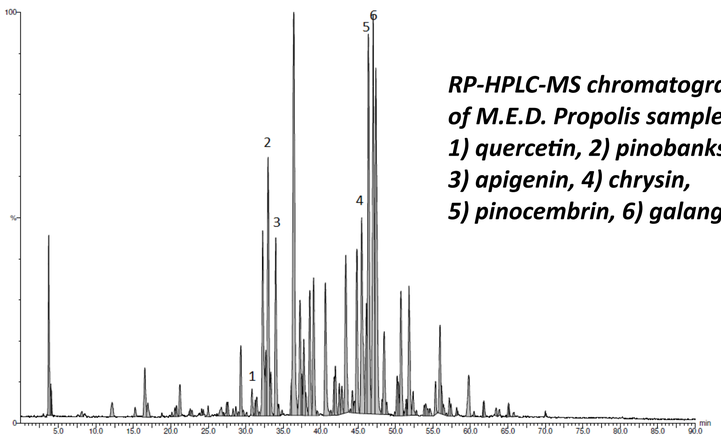
Analysis of Variance: contribution of geographical origin of raw propolis (Eu: Europe; As: Asia; Am: South America) on the relative percentage (% w/w) of each main flavonoid species.



Analysis of Variance: contribution of M.E.D. extracts on the relative percentage (% w/w) of each main flavonoid species and their similar HPLC-UV chromatograms

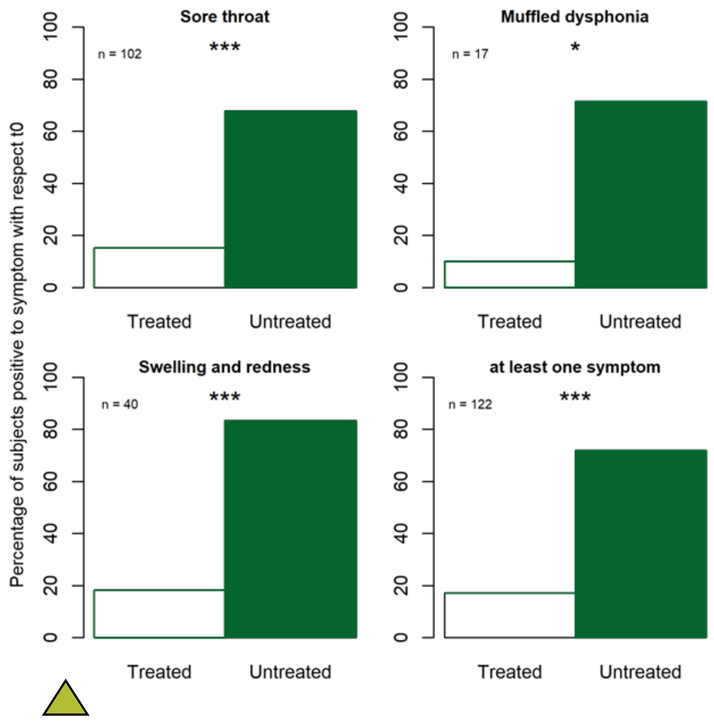


Monocentric, randomized, double-blind, placebo-controlled CLINICAL TRIAL



RP-HPLC-MS chromatogram of M.E.D. Propolis sample 1) quercetin, 2) pinobanksin, 3) apigenin, 4) chrysin, 5) pinocembrin, 6) galangin.

- n= 122 healthy subjects suffering from uncomplicated forms of mild URTI
- 5 days of treatment with 12-24 mg/day of Polyphenols from M.E.D. Propolis
- Primary endpoint was the remission of the symptoms associated to URTIs (Sore throat, Muffled dysphonia, Swelling and redness)



Number of patients with URTI symptoms at t0 and t1; n is for the whole sample including treated and untreated subjects; *: p<0.05, ***: p<0.001.

REMARKS

M.E.D. Propolis can be used to improve both **bacterial** and **viral** mild uncomplicated URTI symptoms within 3 days (half the time of placebo).

Minimum Inihibitory Concentration of Polyphenols from M.E.D. Propolis in MED 1, MED 2 and MED 3 extracts.

STRAIN	CODE	MIC (µg/ml) of Polyphenols		
		MED 1	MED 2	MED 3
Staphylococcus aureus MSSA	L1280	48	48	48
Staphylococcus epidermidis ATCC12228	L147	48	48	48
Moraxella catarrhalis	L3292	6	12	12
Streptococcus pneumoniae Pen-S	L44	3	6	6
Aspergillus niger ATCC10535	L53	12	24	24
Escherichia coli hyperpermeable	G1640	48	96	96
Candida albicans ATCC24443	L4120	192	192	192
Candida albicans ATCC90028	L3023	192	384	384
Lactobacillus gasseri	ND127	750	>750	>750
Lactobacillus acidophilus	ND126	>750	>750	>750
Streptococcus pneumoniae Cli-Eri-R	L1542	6	n.a.	n.a.
Streptococcus pneumoniae Mef (E)+	L1402	6	n.a.	n.a.
Streptococcus pneumoniae Pen-R	L617	3	n.a.	n.a.
Pseudomonas aeruginosa ATCC 21253	L1367	750	n.a.	n.a.
Staphylococcus aureus GISA MSSA	L3797	96	n.a.	n.a.
Staphylococcus aureus GISA MRSA	L3798	48	n.a.	n.a.

BIBLIOGRAPHY:

Zaccaria V, Garzarella EU, Di Giovanni C, Galeotti F, Gisone L, Campoccia D , Volpi N, Arciola CR, Daglia M. 2019 **Multi Dynamic Extraction: An Innovative Method to Obtain a Standardized Chemically and Biologically Reproducible Polyphenol Extract from Poplar-Type Propolis to Be Used for Its Anti-Infective Properties**. Materials, 12, 3746;

Zaccaria, V.; Curti, V.; Di Lorenzo, A.; Baldi, A.; Maccario, C.; Sommatis, S.; Mocchi, R.; Daglia, M. 2017 **Effect of green and brown propolis extracts on the expression levels of microRNAs, mRNAs and proteins, related to oxidative stress and inflammation.** Nutrients, 10, 1090;

Esposito C, Garzarella EU, Caruso D, D'Avino M, Buonomo G, Bocchino B, Sacchi R, Tenore G, Novellino E, Galeotti F, Zaccaria V and Maria Daglia. 2020 **A standardized polyphenol mixture extractef from poplar-type propolis for remission of symptoms of uncompliated Upper Respiratory Tract infections (URTI): a monocentric, randomized, double-blind, placebo-controlled clinical trial**. Submitted