Monograph

M.E.D.® Propolis

Characterized Polyphenols Complex

Respiratory Health

PROPOLIS AND BEEKEEPING DERIVATIVES

Propolis

Propolis is a natural source of polyphenols: raw propolis contains more than 50% and more than 300 different polyphenols. It is a resinous matrix that bees (*Apis mellifera*) collect from gems, exudates and plants, resulting in a heterogeneous mixture of many substances harvested, processed and used by bees to close hive holes and to protect it: Propolis functions as the immune system of the beehive. Since propolis is derived from tree resins, it is sometimes classified according to the plant source and/or geographical origin. These two factors could influence the chemical composition and the biological activities of propolis (Kosalec et al., 2004; Burdock et al., 1998; Teixeira et al., 2005). In Europe and, in general, about 45th parallel, bees collect resin mainly from Poplar plants, producing the so called brown propolis. Among the most representative metabolites of propolis there are flavonoids, terpenoids, phenolic acids, phenolic esters and sugars in different proportions. In literature there are hundreds of studies supporting the healthy properties of propolis, such as gastroprotective, hepatoprotective, immunomodulatory, wound healing and antidiabetic activities. These propolis properties are ascribed to three main activities namely antioxidant, anti-inflammatory and antimicrobial.

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M.E.D.® Propolis

In literature, several solvents to extract propolis' compounds are reported: water, absolute ethanol, ethanol-water mixtures (80, 90, and 96%), glycerol, methanol, hexane, acetone, DMSO, supercritical CO_2 and chloroform (Gómez-Caravaca et al., 2006; Miguel et al., 2010; Netı´kova' et al., 2013; Sforcin & Bankova, 2011). However, none of them is able alone to obtain an extract with all the most important active compounds and moreover not all of them are considered food grade. B Natural has taken up the challenge developing and positing a new extraction process method of propolis able to totally extract the active compounds present in brown propolis: the Multi Dynamic Extraction patented method (N.001425516). This method stems from the need to totally extract the active molecules present in raw propolis, it involves varying alcoholic grade of ethanol to extract polyphenolic compounds with different solubility.
The extract obtained with Multi Dynamic Extraction method contains the integral complex of polyphenols naturally present in propolis, it is pure from inactive resins and it is rich in polyphenols; in particular, obtained propolis extract called M.E.D.® Propolis, is characterized and standardized by the presence of a biological-ly active polyphenol complex, identified in six major polyphenols: galangin, chrysin, pinocem-brin, apigenin, pinobanksin, quercetin, having a relative concentration in the extract always greater than 25% (w/w) determined in HPLC-ESI-MS. Due to the natural complexity of propolis raw materials, in order to assure and guarantee the constant quantity and quality of active compounds, in all M.E.D.® Propolis extracts the total polyphenols and the characterized polyphenols complex are determined and quanti-fied by HPLC-ESI-MS (Volpi & Bergonzini, 2006). To ensure a continuous supply of produce extracts with rich and similar chemical profile M.E.D.® Propolis derives by a mixture of raw materials, previously selected, coming from different geo-graphical origins (Europe, South America and North Asia) but deriving from bees that collect resins mainly from buds of Salicaceae (in particular *Populus spp.*) tree. Multi Dynamic Extraction method is flexible and it can be adapted to raw materials with different chemical composition. Moreover, M.E.D.® Propolis extracts are free from organic contaminants and soluble in aqueous solvents and/or organic mixtures (Galeotti et al., 2016). Using Multi Dynamic Extraction method it is possible to obtain a M.E.D.® Propolis mother extract suitable to be dissolved in low alcoholic grade, glycerin and water-glycerin soluble products with high content of a characterized polyphenol com-plex. Galeotti et al. (2018) from University of Modena and Reggio Emilia demonstrated that, starting from the mother extract obtained with Multi Dynamic extract it can be possible to produ-ce extracts dissolved in different solvents with a similar chemical composition even if with different titration of active compounds as shown in fig.1.

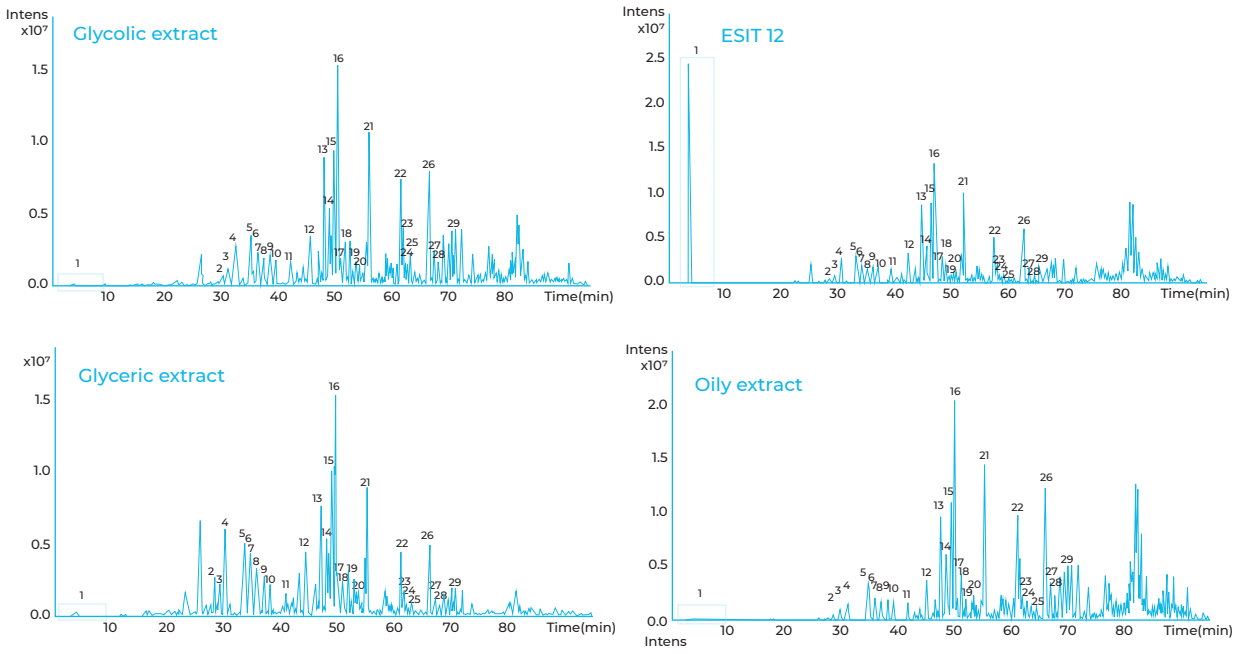

Fig.1 HPLC-MS chromatogram of M.E.D.® Propolis extracts

Moreover, also the antioxidant activity was measured by DPPH test. A direct consequence of the same composition is a quite similar antioxidant activity when calculated for mg of polyphenols so we can produce finished products having a defined composition-activity relationship (Tab.1).

Propolis Finished Products	Polyphenols Content	Microg Trolox/mg Polyphenols
ESIT 12 (w/w)	**16.5 ± 0.8%**	**74.0 ± 4.2%**
Oily extract (w/v)	**24.0 ± 1.4%**	**79.4 ± 4.2%**
Glycolic extract (w/v)	**81.2 ± 3.7%**	**71.7 ± 3.5%**
Glyceric extract (w/v)	**26.2 ± 1.6%**	**74.4 ± 3.8%**
Hydroalcolic extract (w/v)	**69.7 ± 2.0%**	**76.0 ± 4.1%**

Tab.1 Polyphenols content and antioxidant activity of the various propolis preparations measured by the radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reported as µg trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent/mg polyphenols. Data are illustrated as mean ± standard errors.

Pill

M.E.D.® Propolis extracts are standardized products and contain the same characterized polyphenol complex in all pharmaceutical forms (HPLC-ESI-MS), this mean that it is possible to use the best extract for your application sure of having the same active compounds in all of them and in all batches.

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Activities

Propolis has been used since ancient times as folk remedy. Nowadays in literature there are hundreds of studies supporting the healthy properties such as antibacterial, antiviral, antifungal, antiulcer, antioxidant, antiradiation, hepatoprotective, antitumor, antimutagenic, anti-angiogenic, cyto- and chemopreventive, antinflammatory, wound healing, immunomodulating (immunostimulating and immunosuppressive in autoimmune diseases), cardioprotective (antimyocardial injury, antithrombogenic, antihypertensive, antiarrhythmic), local anesthetic, regenerative (cartilaginous and bone tissue, dental pulp) and food preservative activities (Fidalgo et al., 2011; Mathivanan et al., 2013; Lofty, 2006; Marcucci, 1995; Burdock, 1998; Castaldo et al., 2002 Sforcin et al. 2001; 2007; Banskota et al. 2001; Bankova, 2005c; 2014). It was recently demonstrated that the combination of polyphenolic species is essential for the biological activity of propolis (Boisard et al., 2015).

Antioxidant and Antinflammatory

One of the most studied properties of propolis is its antioxidant capacity (Kurek-Gorecka et al., 2013). The main compounds responsible for this activity are polyphenols, which show higher radical scavenging activity than most common antioxidants, such as vitamin C and vitamin E (Banskota et al., 2000). Antioxidant activity is a very important topic since many syndromes are linked to an imbalance between the antioxidant defense system and the production of free radicals (Favier 1997). Oxidative stress and inflammation are closely related phenomena. In fact, the oxidative stress causes inflammation, which in turn induces oxidative stress and causes the emergence of a chronic inflammatory state (Liaudet et al., 2009). Recently, it has been shown that chronic inflammation is a predisposing factor for the onset of some diseases such as atherosclerosis, neurodegenerative diseases and cancer (Ishibashi, 2013). The antioxidant activity of flavonoids could be the basis of the anti-inflammatory activity (Robak & Gryglewski, 1996) due to their structure, their ability to penetrate the cell lipid membrane (Saija et al., 1995) and their own ability to modulate the expression of closely related anti-inflammatory genes (Sperandio, 2006). As anti-inflammatory agent, propolis has been shown to inhibit the synthesis of prostaglandins, activate the thymus, help the immune system by promoting phagocytic activity, stimulate cellular immunity and improve curative effects in epithelial tissues (Casaroto et al., 2010). Inflammation represents a fundamental biological process that stands at the foreground of a large number of acute and chronic pathological conditions. Complex inflammatory networks, involving countless cellular and humoral components, orchestrate critical aspects of diseases. Inflammation represents an adaptive response to any condition perceived as potentially dangerous to the host, and which aims at the removal of the danger, the induction of tissue repair, and the restoration of tissue homeostasis (Okin and Medzhitov, 2012).

Using standardized M.E.D.® Propolis extracts, thanks to the studies conducted at University of Pavia and published by Zaccaria, Curti et al. (2017, 2019) it was possible to clarify in vitro the intracellular anti-inflammatory and antioxidant mechanisms, in vivo the antioxidant activity and the bioavai-lability, and in vitro the antibacterial activity. To better understand the intracellular mechanism of action, microRNAs (miRNAs) were chosen to investigate the epigenetic action of M.E.D.® Propolis since miRNAs play a very important role in the regulation of gene expression. They are a class of endogenous non-coding RNA, consisting of about 22 nucleotides, which are able to regulate gene expression at the post-transcriptional level. They exert their functions by binding

complementary sequences on messenger RNA (mRNA) targets, interfering with the translation process and preventing or altering gene expression. If miRNAs increase, the target gene will not be expressed (Bartel et al 2004). M.E.D.® extract of propolis containing a characte-rized polyphenols complex, was active on all miRNAs tested suggesting that brown propolis has a great epigenetic activity. In turn, characte-rized Polyphenol complex showed a great modulatory capacity in modifying the expression levels of mRNAs as miRNAs target, and the corresponding proteins as TNF-α, NFE2L2 and GPX2 in physiological conditions. Based on the results of this research, the antioxidant and anti-inflammatory effects attributed to characterized polyphenol complex of M.E.D.® Propolis could be due to modulation of the levels of certain miRNAs invol-ved in these pathways (Tab.2).

miRNA (RT-PCR)		mRNA (RT-PCR)		Protein (ELISA)	
miR 27a-3p	↑	NFE2L2	↓	(pro oxidant marker)	↓
miR 17-3p	↓	GPX2	↑	(antioxidant marker)	–
miR 2o3a-3p	↑	TNF α	↓	(pro inflammatory cytokine)	↓
miR 19a-3p	↑	TNF α	↓	(pro inflammatory cytokine)	↓

Tab.2 miR-19a-3p and miR-203a-3p, which target mRNA coding for TNF-α, were significantly upregulated by propolis at all tested concentration ($\chi 2 = 13.27$, df = 3, p = 0.004), ($\chi 2 = 41.92$, df = 3, $p < 0.001$) when compared to the control. As far as miR-27a-3p is concerned, it regulates NFE2L2 expression. A significant increase was registered ($\chi 2 = 12.90$, df = 3, p = 0.004), compared to the control sample. TNF-α protein concentrations confirmed the expression levels of mRNAs. Significant decrease in expression levels were measured at all tested concentration s for brown propolis ($F = 6.7292$, $p < 0.05$), compared to the control sample.

Moreover, characterized polyphenol complex of M.E.D.® Propolis reduces the production of proinflammatory cytokine (TNF-α) and increase the production of antioxidant defenses in vitro through an epigenetic mechanism of action (Fig.2) (Zaccaria et al., 2017).
These results were also confirmed in other *in vitro* study for the evaluation of the antinflammatory activity of M.E.D.® Propolis extracts using human fibroblasts (ATCC-CRL-2703) after induction of the inflammatory events by Lipopolysaccharide (LPS). The amount of TNF-α after administration of M.E.D.® Propolis extract was evaluated and compared to untreated control. According to the use condition, cell cultures were treated with 0.05 / 0.1 mg/ml to test the systemic antinflammatory activity and 1.25/2.5 mg/cm^2 to evaluate the local antinflammatory activity.
The cell treatment with M.E.D.® Propolis extract has highlighted a significant reduction of the TNF-α release in the cells subjected to inflammatory stress, in all the considered experimental conditions; the products show an effective anti-inflammatory activity;
According to obtained and analyzed data, M.E.D.® Propolis extract at the concentration 0.05 mg/ml reduces the pro-inflammatory cytokine release until 73% while at the concentration 0.1 mg/ml reduces TNF-α until 101.3% with statistically significant differences ($p<0.01$) com-pared to positive control with inflammation induced by LPS. (Fig.3)

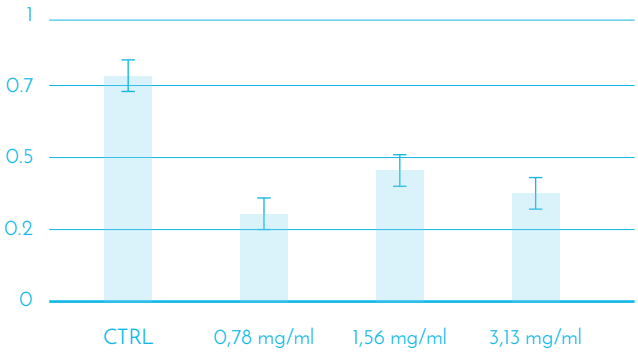

Fig.2 TNF-α levels in HaCat cells treated with increasing concentrations of brown propolis extract (0.78-3.125 mg/mL); with statistically significant differences ($p < 0.01$) between treated and untreated cell cultures as reported in the text.

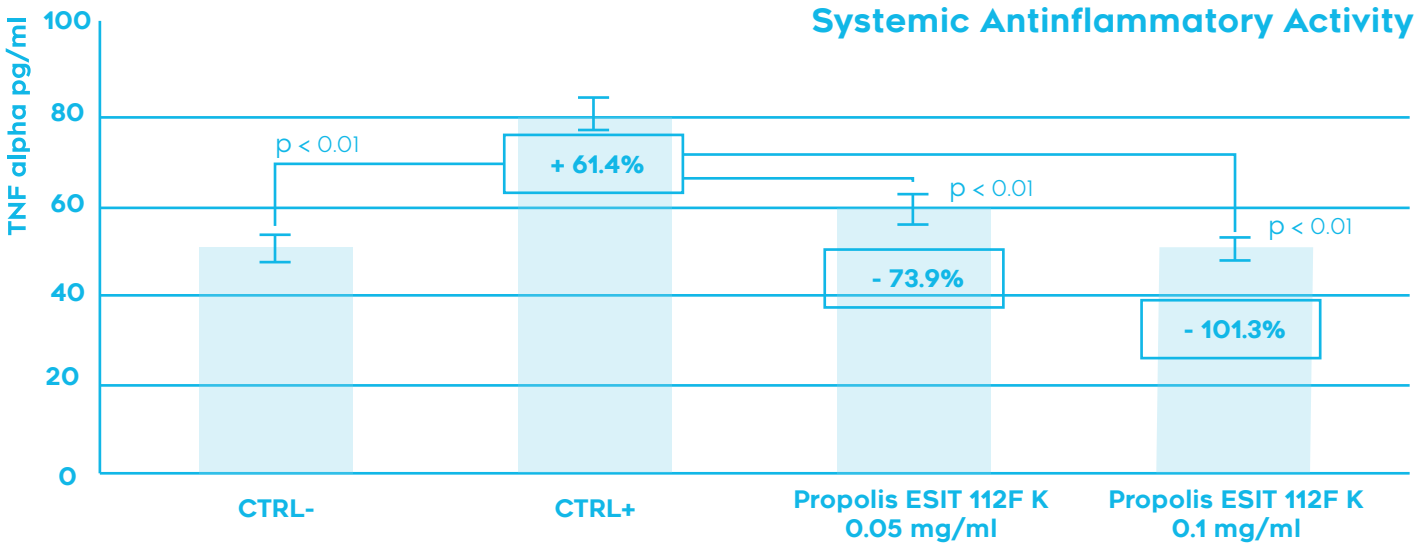

Fig.3 Dosage on TNF-α in cell culture CTR-, CTR+ and treated with tested product. The results are expressed as mean value ± s.e. (expressed in pg/ml) and as % variation (mean value ± s.e.) compared to CTR- ; anti-inflammatory activity is expressed as reduction od cytokine compared to CTR+ .

As regards the local anti-inflammatory activity, the cell treated with M.E.D.® Propolis extract has highlighted a significant reduction of the TNF-α release in the cells subjected to inflammatory stress, in all the considered experimental condi-tions; the products show an effective anti-inflam-matory activity;

In particular, M.E.D.® Propolis extract at the concentration 1.25 mg/cm² reduces the pro-inflam-matory cytokinee release until 91.6% while at the concentration 2.5 mg/cm² reduces TNF-α until 96.7% with statistically significant differences (p<0.01) compared to positive control with inflam-mation induced by LPS. (Fig.4)

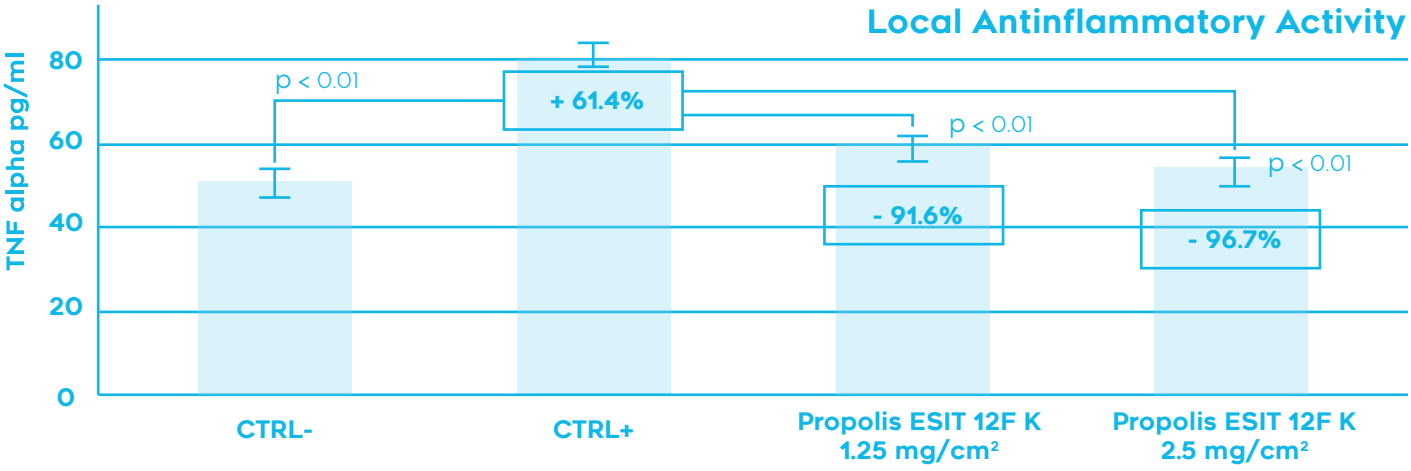

Fig.4 Dosage on TNF-α in cell culture CTR-, CTR+ and treated with tested product. The results are expressed as mean value ± s.e. (expressed in pg/ml) and as % variation (mean value ± s.e.) compared to CTR- ; anti-inflammatory activity is expressed as reduction od cytokine compared to CTR+ .

In 2019 Curti, Zaccaria et al. investigated about the antioxidant activity of characterized polyphe-nol complex of M.E.D.® Propolis in *in vivo* studies using animal model (mice); three doses of propolis extracts were prepared and incorpora-ted in the experimental animal pellet at three concentrations (500 mg/kg/day, 250 mg/kg/day, 100 mg/kg/day) of characterized polyphenol complex. Each group was treated with one of the obtained pellet ad libitum for 30 days. The extrapolation of animal dose to human dose was performed through normalization to body surface area (BSA) using the following formula: animal dose = HED x Human Km /Animal Km (where human Km factor is 37 for a human and animal Km factor is 3 for a mouse)

(Regan-Shaw et al., 2008).
At the end of the treatment the differences in the expression of proteins involved in antioxidant pathway (ELISA) and the presence of propolis metabolites was evaluated in the liver. Chronic administration of M.E.D.® Propolis was able to increase the endogenous antioxidant defenses in mice (Fig.5).

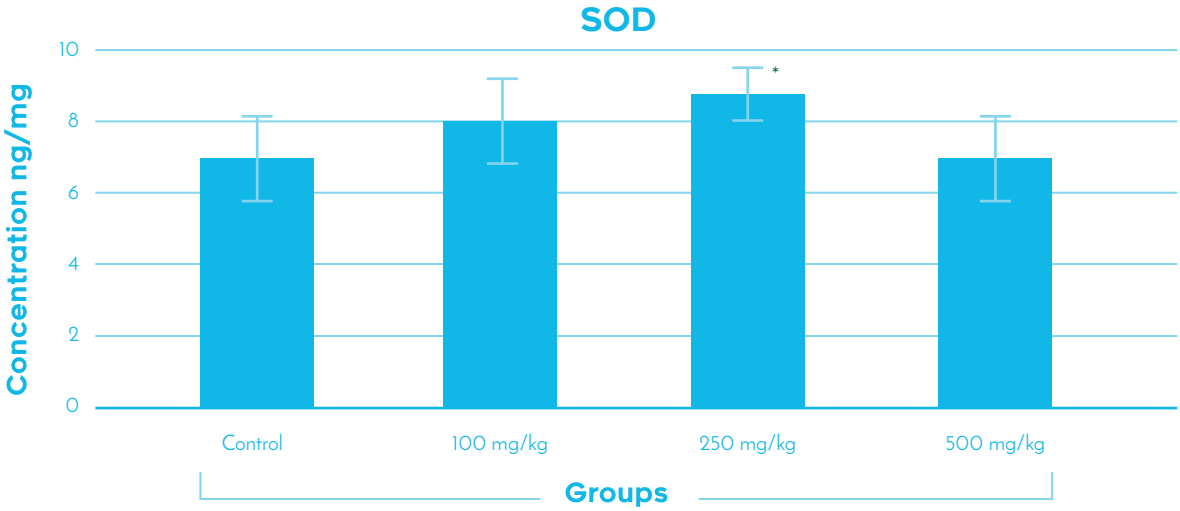

Fig.5 The average SOD-1 concentration in the control group and in mice treated with different dosages of propolis expressed in pg/mg of soluble liver protein. A significant difference could be detected after the prolonged treatment, with 250 mg/kg of propolis extract compared to the control (* means a p = 0.0106).

After acute administration with a single bolus of a M.E.D.® Propolis extract, the bioavailability was evaluated using galangin as maker.
These studies confirmed that galangin is bioavailable and it is immediately absorbed and metabolized in galangin glucuronide, as literature data neither galangin nor its metabolites are accumulated in the liver (Fig 6a/b).

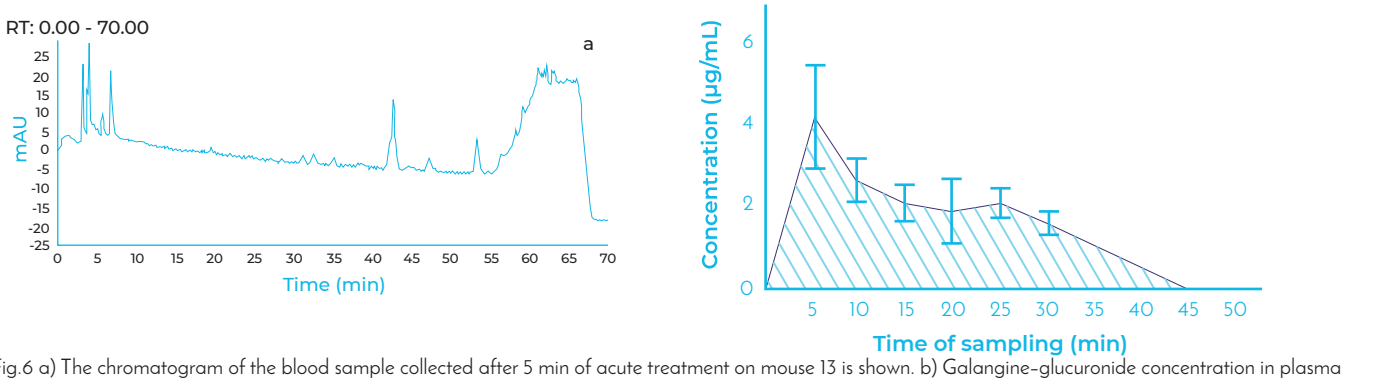

Fig.6 a) The chromatogram of the blood sample collected after 5 min of acute treatment on mouse 13 is shown. b) Galangine-glucuronide concentration in plasma samples collected at different times is shown: after 5 min this metabolite reaches its highest concentration in plasma; then, the concentration maintains a plateau; finally, after 45 min from the treatment, it is no longer detectable. Asterisk indicates significantly different from the other time points ($p < 0.05$).

M.E.D.® Propolis reduces the production of proinflammatory cytokine (TNF-α) and increase the production of antioxidant defenses in vitro through an epigenetic mechanism of action. Chronic administration of M.E.D.® Propolis was able to increase the endogenous antioxidant defenses in mice; galangin is bioavailable and it is immediately absorbed and metabolized.

Antimicrobial activity

Since ancient times, propolis has been used for its antimicrobial properties.
Nowadays, due to the increase of antibiotic resistance and thanks to the increased attention of scientific community and consumers on natural products, propolis is considered for new applications to reduce the use of antibiotic drugs.
The most known and studied activity of propolis is the antibacterial one. Using a panel of many microorganisms from clinical isolates and ATCC library, different species and strains, both sensitive and resistant to antibiotics, researchers from University of Pavia and Modena (Italy) evaluated the antibacterial activity of M.E.D.® Propolis extract.
The results suggested that propolis was active against many different bacterial and fungi spp which causes skin, respiratory, vaginal and gastrointestinal infections.
The main infective agents responsible for skin disease are those of the genus Staphylococci (Hanses, 2017) for which M.E.D.® Propolis extracts were particularly active (MIC value: 24-94 µg/ml) in both antibiotic susceptible and resistant strains. No activity was found, for the concentra-tions tested, against *P. acnes* (MIC value: >750 µ g/ml) a common skin organism, usually associa-ted to acne vulgaris, but also to postoperative and device-related infections (Perry & Lambert, 2011). Traditionally propolis is used in cold flu diseases involving upper respiratory tract infections (Wagh, 2013) in fact M.E.D.® Propolis extracts highlighted a strong activity against Streptococci spp in resistant and susceptible strains to Penicillin, and resistant to Clindamycin and Erithromycin, Macrolide and Erythromycin (MIC value: 3-6 µg/ml). *M. catarrhalis* is involved in the upper respiratory tract infections as well as in otitis (Ren et al., 2016). Against these bacteria, M.E.D.® Propolis extracts were particularly active (MIC value: 6-12 µg/ml). *A. niger* is a mold reported as one of the causes of pneumonia (Person et al., 2010) against which tested extracts were very active (MIC value: 12-24 µg/ml).
Lactobacilli spp are commonly used as probiotics to maintain vaginal and gastrointestinal health and microflora (Sungur et al., 2017). Propolis extracts did not show any antibacterial activity against these microorganisms (MIC value: > 750 µg/ml). On the contrary, M.E.D.® Propolis extracts, had very strong activity against vaginal and gastrointestinal pathogens such as *N. gonorrhoeae*, *G. vaginalis*, *A. vaginae* isolates (MIC value: 12-48 µg/ml) and good activity against Candida spp (MIC value: 190-380 µ g/ml). M.E.D.® Propolis extracts were active against Clostridium spp strains (*Clostridium difficile* MIC value: 380 µg/ml) and moreover, it was demonstrated that Lactobacilli are able to prevent *Clostridium difficile*-associated diarrhea (Goldenberg et al., 2013). These results taken together could suggest a synergic effect of antibacterial activity of M.E.D.® Propolis extracts against vaginal and gastrointestinal pathogens and the probiotic activity of lactobacilli whose growth is not inhibited.
Different mechanisms of action have been proposed: propolis inhibits bacterial mobility (Mirzoeva et al., 1997); pinocembrin acts as quorum sensing inhibitor (Savka et al., 2015); galangin blocks the adhesion of *S. aureus* (Cushnie et al., 2007); propolis, both in vivo and in vitro, inhibits peptidoglycan synthesis, more precisely the glucosyltransferase production and activity in *S. sorbinus* and *S. mutans* (Parolia et al., 2010; Duarte et al., 2006; Koo et al., 2000; Lotfy et al., 2006; Ikeno et al., 1991); propolis reduces the symptoms of bacterial peptidoglycan-induced colitis by inhibiting mainly the production of pro-inflammatory cytokines in macrophages (Fitzpatrick & Wang, 2001, Banskota, 2001).

Many researchers have demonstrated propolis antifungal activity against *Candida albicans* (Santos et al., 2008; Dias et al., 2007; Borrelli et al., 2002) as well as against some other yeasts such as *C. tropicalis* and *C. krusei* that are equally sensitive (Vardar-Unlu et al., 2008). Combinations of certain antimycotic drugs with propolis increa-sed their activity on *C. albicans* (Martins et al., 2002). Through *in vitro* and *in vivo* experimen-ts, the antifungal activity was also shown against some plant fungi (Martins et al., 2002).

Biofilm production by bacterial pathogens represents a virulence factor often difficult to control. About 65% of nosocomial infections are associated with biofilm formation, and they are 10 to 1000 times more difficult to eliminate with an otherwise successful treatment.
In some instances, the mechanism for enhanced antimicrobial resistance is believed to involve alteration or change in gene expression leading to a phenotypic difference between the planktonic and sessile condition of bacterial pathogens. The sessile forms are more resistant as they produce exopolysaccharide, have different growth characte-ristics and take up nutrients and drugs differently from their planktonic counterparts.
Many Staphylococcus species are known to produ-ce biofilms during different infectious processes, which involve the adhesion of organism onto devices, the bacterial multiplication on the device surface and the biofilm formation, which represen-ts the starting point of infection. Biofilm formation is therefore a validated target for the prevention and treatment of staphylococcal infections.
In a recent research, characterized polyphenol complex of M.E.D.® Propolis was able to inhibit 24 hours aged biofilm of *Staphylococcus aureus*.

Strain	Experiments	Polyphenols (mg/ml)		
		MIC	MBIC	MBEC
Staphilococcus aureus 3988	N.1	4.69	9.38	18.76
	N.2	4.69	18.76	28.14

Tab.3 Evaluation of antimicrobial activity of Propolis M.E.D. characterized polyphenol complex on Staphylococcus aureus Biofilm.

Propolis M.E.D.® exerts very good antimicrobial activity against Gram positive, Fungi and some Gram negative microorganisms showing very low MIC values. Thanks to the standardi-zation of propolis extraction method, it is possible to obtain reproducible extract profiles that can be used to establish the correlation between quantity of characterized polyphenol complex and the antibacterial activity. Then the use of these propolis extracts could be useful to reduce the application of antibiotics in humans and animals.

UPPER RESPIRATORY TRACT

The upper respiratory system includes the nose, nasal cavity, pharynx, and larynx with subglottic area of trachea. Upper respiratory tract infection (URTI) is a nonspecific term used to describe acute infections involving the nose, paranasal sinuses, pharynx and larynx which serve as gateways to the trachea, bronchi, and pulmonary alveolar spaces; of them rhinitis and sinusitis usually coexist and are concurrent in most individuals; thus, the correct terminology is now rhinosinusitis (Meltzer et al. 2004).
Respiratory tract diseases affect a large number of people worldwide: in the European Union , for instance, 7% of hospital admissions are linked to respiratory illnesses, which are responsi-ble for approximately 12% of all-cause deaths. Upper and lower respiratory tract infections (RTIs) are common conditions for which medical advice is regularly sought, and their manage-ment relies on the use of prescription and over-the-counter (OTC) medicines (Hamoen et al. 2014). The World Health Organization alerts that about 80% of antibiotics are used in the community, of which about 20 e 50% are used inappropriately (WHO, 2017)
The clinical syndrome of URTI comprises a variety of symptoms. Cough is usually the main symp-tom; other symptoms are nasal blockage/ob-struction/congestion, discolored nasal discharge, fever and headache. Symptoms typically peak after 2-3 days, and then gradually clear. However, the cough may persist after the infection has gone. This is because inflammation in the airways, caused by the infection, can take a while to settle. It may take 2-3 weeks, after other symp-toms have gone, for a cough to clear completely (Cherry et al 2004).

URTI are mainly infectious diseases resulting from interplay between microbial load (viral and bacterial) and immune response. Host defense mechanisms consist of both cellular immune responses and release of soluble chemical factors, which operate in the body through a complex interaction with cytokines and other mediators (Fokkens et al 2012). Literature data showed that during acute upper respiratory infections, interleukin 1, IL-8, IL-6, and TNF-α were overexpressed compared to baseline, and all except TNF-α decreased signifi-cantly by 2-4 weeks later. Thus, cytokines are likely to participate in regulation of respiratory virus induced inflammation (Gelardi et al 2007).
The most common bacteria in URTI are those belonging to the “infernal trio” (*Streptococcus pneumoniae, Haemophilus influenzae and Mora-xella catarrhalis*) and Staphylococcus aureus (Heikkinen et al 2003; Payne et al 2007).
Infant and young children are prone to developing upper respiratory tract infections, especially those who attend day care centers, which often result in bacterial complications expecially acute otitis media. Literature data reported that 29% to 50% of all URTI develop into otitis media (OM) (Revai et al 2007). OM is a common disease in children, with an incidence range from 6% to 64%. The disease is primarily caused by: *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella Catarrhalis* (Bakaletz 1995).OM describes an inflammatory process within the middle ear space that is gene-rally associated with accumulation of fluid and that may lead to hearing loss, learning difficulties, and delays in language development.

Sinusitis is common in persons with viral upper respiratory tract infection. The major pathogens of acute bacterial sinusitis are *Streptococcus pneu-moniae* and *Hemophilus influenzae*, followed by a-hemolytic and b-hemolytic streptococci, *Staphylococcus aureus*, and anaerobes. In the past decade, *S. anginosus* and methicillin-resistant *S. aureus* (MRSA) has been increasingly recognized as a cause of bacterial sinusitis in children and adole-scents (Botting et al., 2008).

Acute bacterial pharyngitis and tonsillopharyngitis usually occur during the colder months. The most common cause is group A b-hemolytic Streptococcus (*S. pyogenes*), which is responsible for 15–30% of all cases of pharyngitis in children and for 10% in adults.
Laryngitis is an acute or chronic inflammation of laryngeal structures. The most common etiological agents of acute laryngitis are: Hemophilus influenzae type B, b-hemolytic streptococci, *Moraxella catarrhalis, Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus.*

Tracheitis is an inflammatory process of the larynx, trachea, and bronchi. Most conditions that affect the trachea are bacterial or viral infections; however, irritants and dense smoke can injure the epithelium of the trachea and increase the likelihood of infections.
The most frequent causes of tracheitis are *S. aureus*' group A b-hemolytic streptococci, *M. catarrhalis*, H. influenzae type B, Klebsiella species, Pseudomonas species, anaerobes, *Mycoplasma pneumoniae*, and influenza A virus (H1N1).

Why M.E.D.® Propolis?

Bee products have been used empirically for centuries especially for the treatment of respiratory diseases. Propolis can significantly reduce the number and severity of nighttime asthma attacks, improve pulmonary function and reduce inflammation (Khayyal et al., 2003). Subjects with pharyngitis treated with propolis showed a significant positive trend in symptom relief with a reduction in sore throat, fever, adenomegaly, pharyngeal erythema and exudate with the only exception in the nasal secretion that showed no clear signs of improvement (Di Pierro et al., 2016). Propolis can be effective in relieving symptoms of allergic rhinitis by inhibiting the release of histamine (Shinmei et al., 2009). In addition, propolis is able to reduce allergic pulmonary inflammation in murine model through the involvement of lung inflammatory cells and the decrease of polymorphonuclear inflammatory cells (de Farias et al., 2014).
In particular, the antibacterial activity of M.E.D.® Propolis and it's proved antinflammatory and antioxidant activity (as previously discussed: Zaccaria et al., 2017, Curti et al., 2019, Zaccaria et al., 2019) suggest using the characterized polyphenol complex to treat the URTI.

Next, the minimum inhibitory concentration of M.E.D.® Propolis characterized polyphenol complex against pathogens involved in URTI:

STRAIN	CODE	MIC (µg/ml) Polyphenols
Staphylococcus aureus GISA MSSA	L3797	96
Staphylococcus aureusATCC	6538P	24
Staphylococcus aureus GISA MRSA	L3798	48
Staphylococcus aureus MRSA macrolide-resistant	ND060411	96
Staphylococcus aureus MRSA	L4064	96
Staphylococcus aureus MSSA ATCC 25923	L1280	48
Streptococcus pyogenes	L49	12
Streptococcus pneumoniae clindamycin/erithromycin resistant	L1542	6
Streptococcus pneumoniae macrolide/erithromycin resistant	L1402	6
Streptococcus pneumoniae penicillin-resistant	L3917	3
Pseudomonas aeruginosa ATCC 27853	L1367	> 750
Moraxella catarrhalis	L3292	6
Haemophilus influenzae ATCC	51907	750

Table 4. Minimum Inihibitory Concentration of M.E.D.® Propolis characterized polyphenol complex

In the beginning of 2019, a clinical trial began, randomized double blind placebo control study, in colla-boration between University of Naples and UCCP di San Giorgio del Sannio (BM) Italy to investigate activity of M.E.D.® Propolis on the incidence of URTI.

How, where and how much can you use M.E.D.® Propolis extracts?

In the treatment of URTI, the most common galenic forms used are: oral and nasal spray, effervescent tablets, capsules, syrups and orosoluble tablets. For these applications we suggest:
Hydrogliceric extract **GREIT®** for analcoholic spray, syrup etc.
Dry extract **ESIT®** for orosoluble tablets, effervescent tablets, capsules, candies,etc.
Hydroalcoholic extract **EPE®** spray, drops, etc.

All these extracts are standardized and available with different concentration of polyphenols while maintaining the characterized polyphenol complex of M.E.D.® Propolis In all these extracts of M.E.D.® Propolis the identification and quanti-fication of total polyphenols are performed by means HPLC-ESI-MS.

In alternative, or in addition B Natural produces synergy with M.E.D.® Propolis melts with other active ingredients:
Flavoxale® dry extract (M.E.D.® Propolis and Manuka® honey)
Propolsave® available in powder and hydroglice-ric version (M.E.D.® Propolis and polysaccharides from Opunxia®) to enhance mucohadesivity and time in situ of active compounds.
Propolfenol® available in powder and hydroglice-ric version (Propolis M.E.D.®, cathechins and OPCs)

M.E.D.® Propolis is already used in medical devices thanks to its mucoadhesive properties able to promote barrier effect.
Moreover, B Natural has got several formulation for oral spray in bulk, also without alcohol, ready to be packaged.
Thanks to our R&D team expertise in propolis and in extraction methods, B Natural is open to develop new products, formulation and synergies with and for the customers.

Dosages

The suggested minimum daily intake of M.E.D.® Propolis characterized polyphenols complex was calculated starting from ten times the highest concentration able to inhibit the growth of the tested microorganisms and crossing these values with the traditional use of M.E.D.® Propolis in food supplements having these applications.

Daily intake: polyphenols of M.E.D. ® Propolis			
	Acute	Chronic	Topic*
Adults	15 mg	8 mg	7.5 mg
Children	7.5 mg	5 mg	7.5 mg

Maximum oral daily intake of polyphenols suggested: 1000 mg.
*Oral Spray, orosoluble tablets

Notes

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