Dipartimento di Scienze del Farmaco
Università degli Studi di Pavia

DETERMINAZIONE DEL CONTENUTO DI GALANGINA NELL'ESTRATTO DI PROPOLI FORNITO DALL' AZIENDA "DOTT. STEFANO FARALLI SCARL" (vedi scheda tecnica allegata)

Reagenti:
- galangina standard (Phytolab GmbH & Co. KG Germany);
- metanolo per HPLC (VWR);
- acido formico (1 M) per HPLC (Sigma-Aldrich).

Campione:
Estratto di propoli fornito dall' Azienda "Dott. Stefano Faralli scarl" con sede in Milano, Piazzale Cadorna 9, 20123 Milano P. IVA 06749480965 e consegnato in data 7 febbraio 2013.
Scheda tecnica del prodotto.

Metodo HPLC-DAD
La determinazione della galangina è stata condotta mediante il metodo proposto da Volpi et al. (*Journal of Pharmaceutical and Biomedical Analysis,* 2006, 42: 354-361. *Analysis of flavonoids from propolis by on-line HPLC-electrospray mass spectrometry.* Volpi N., Bergonzini G.) a cui sono state apportate le seguenti modifiche:

- Sistema HPLC-DAD Agilent 1100 (Agilent, Waldbronn, Germany), costituito da una pompa quaternaria, auto-campionatore con termostato dotato di loop di iniezione da 5 µl, termostato per colonna, rivelatore DAD. Per il controllo del sistema e l'analisi dei dati è stato impiegato il software Chemstation.
- Fase stazionaria: ZORBAX SB C18 150x4,6 mm – 5 µm (Agilent Technologies, Waldbronn, Germany;
- Fase mobile: metanolo e acido formico (0,1%) in gradiente come riportato in tabella1.
- Velocità di flusso: 0,5 ml/min;
- Temperatura della colonna: 25 °C;
- Durata analisi: 115 minuti.
- λ= 395nm

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Tabella 1.

Tempo min	Metanolo % v/v	Acido formico (0,1%) % v/v
0	50	50
20	60	40
50	60	40
100	100	0
105	50	50
115	50	50

Analisi HPLC-DAD

Lo studio è stato condotto nel seguente modo:

1) acquisto dello standard di galangina;
2) messa a punto di un metodo riportato in letteratura che permetta di determinare la galangina nell'estratto di propoli oggetto di studio mediante tecnica HPLC-DAD;
3) analisi del campione.

Dal cromatogramma ottenuto dall'analisi della galangina standard registrato a λ 395 nm si evince che tale sostanza presenta, nelle condizioni operative applicate, tempo di ritenzione (RT) pari a 36,569 min e mostra lo stesso spettro UV-Vis riportato in letteratura per tale sostanza.

I risultati dell'analisi HPLC-DAD condotta sull'estratto di propoli diluito 1:100 con metanolo hanno indicato la presenza di un picco a RT 37,116 min. L'analisi HPLC-DAD dell'estratto di propoli ha permesso pertanto di identificare la galangina sulla base del confronto dei tempi di ritenzione (RT) e degli spettri UV-Vis del composto standard e del corrispondente analita presente nell'estratto (Figura 1 - 4).

Figura 1. Cromatogramma RP-HPLC-DAD ottenuto dall'analisi di una soluzione metanolica di galangina standard

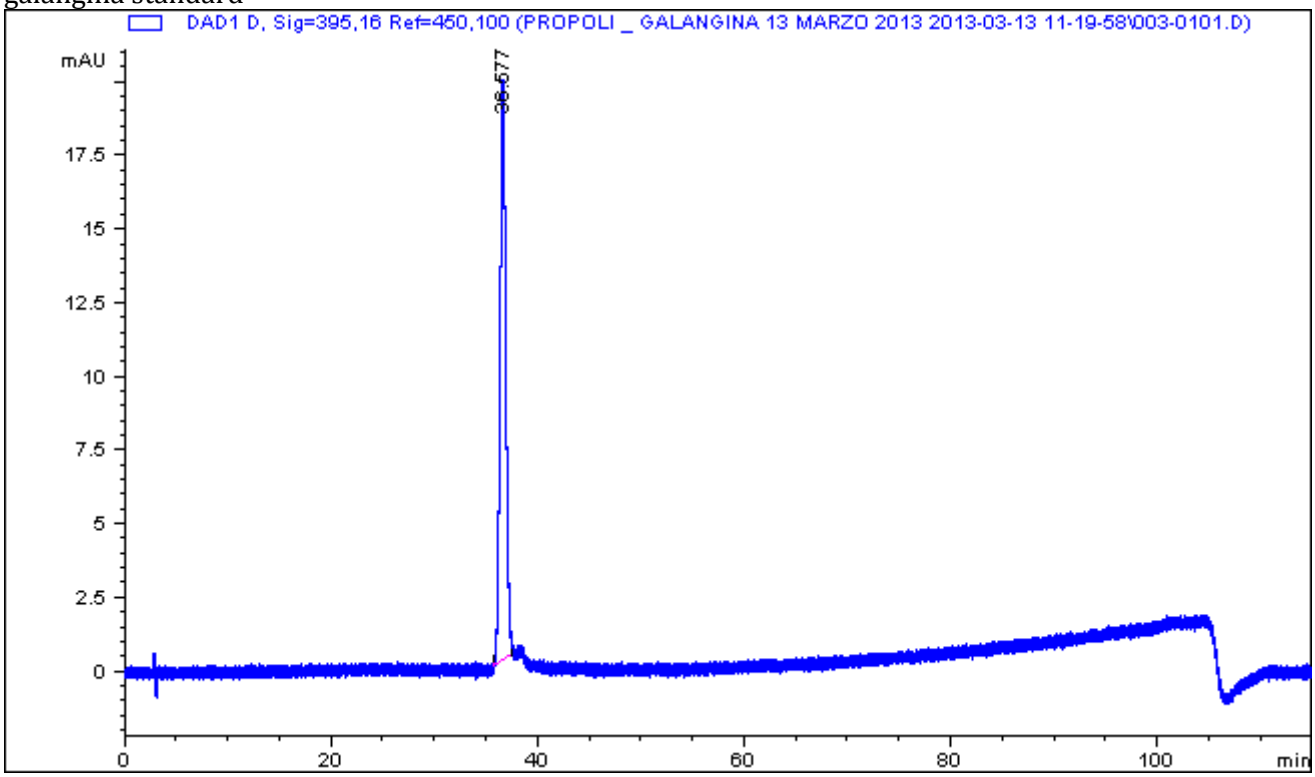

Figura 2. Spettro UV-Vis registrato per il picco con tempo di ritenzione 36,578 min

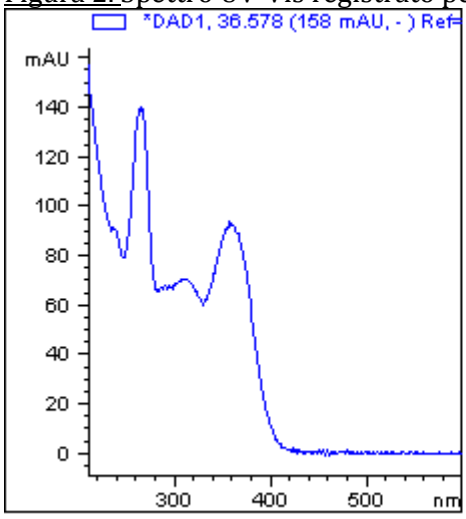

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Figura 3. Cromatogramma RP-HPLC-DAD ottenuto dall'analisi dell'estratto di propoli oggetto di studio diluito 1:100, registrato a λ 395 nm.

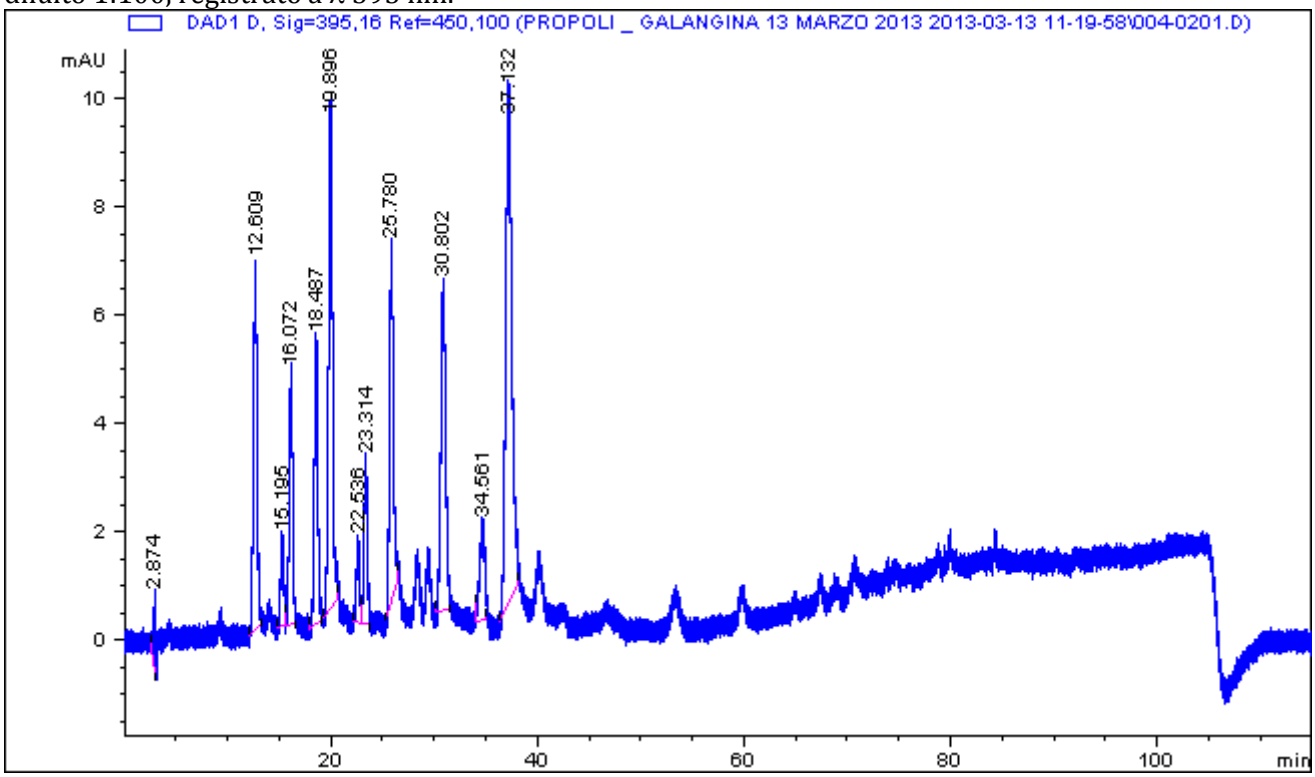
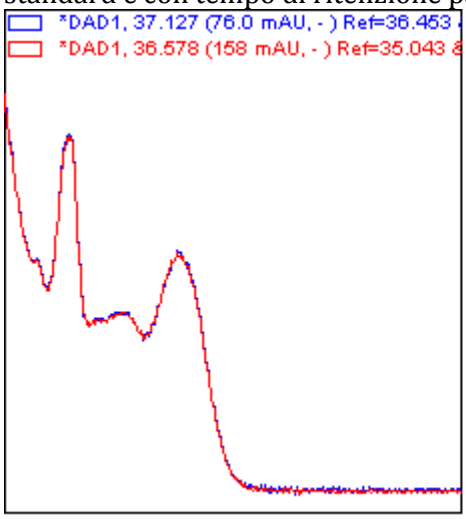


Figura 2. Spettro UV-Vis registrato per il picco con tempo di ritenzione 36,578 min della galangina standard e con tempo di ritenzione pari a 37, 127 min dell'estratto di propoli diluito 1:100.



Si è pertanto proceduto al dosaggio della galangina mediante il metodo delle aggiunte di quantità note di galangina standard (corrispondenti a +50%, +75% e +100% della concentrazione iniziale dell'analita nel campione).
In tabella vengono riportati, l'intervallo di concentrazioni saggiate, l'equazione della retta di regressione e il relativo coefficiente di correlazione.

Intervallo di concentrazioni µg/mL	Equazione della retta di regressione	Coefficiente di correlazione (r)
0-50 µg/mL	y=6,1364x+283,01	r=0,994

Si è proceduto quindi al calcolo della concentrazione della galangina nell'estratto di propoli:
Concentrazione di galangina: 4,612 mg/mL.

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A seguito vengono riportati anche i cromatogrammi registrati a λ 254, 270, 320 nm, indicativi del profilo cromatografico dell'estratto.
Tali cromatogrammi potranno essere utilizzati in seguito per verificare la composizione di estratti di propoli ottenuti nelle stesse condizioni dall'Azienda (Figure 5-7).

Figura 5. Cromatogramma RP-HPLC-DAD ottenuto dall'analisi dell'estratto di propoli oggetto di studio diluito 1:100, registrato a λ 254 nm.

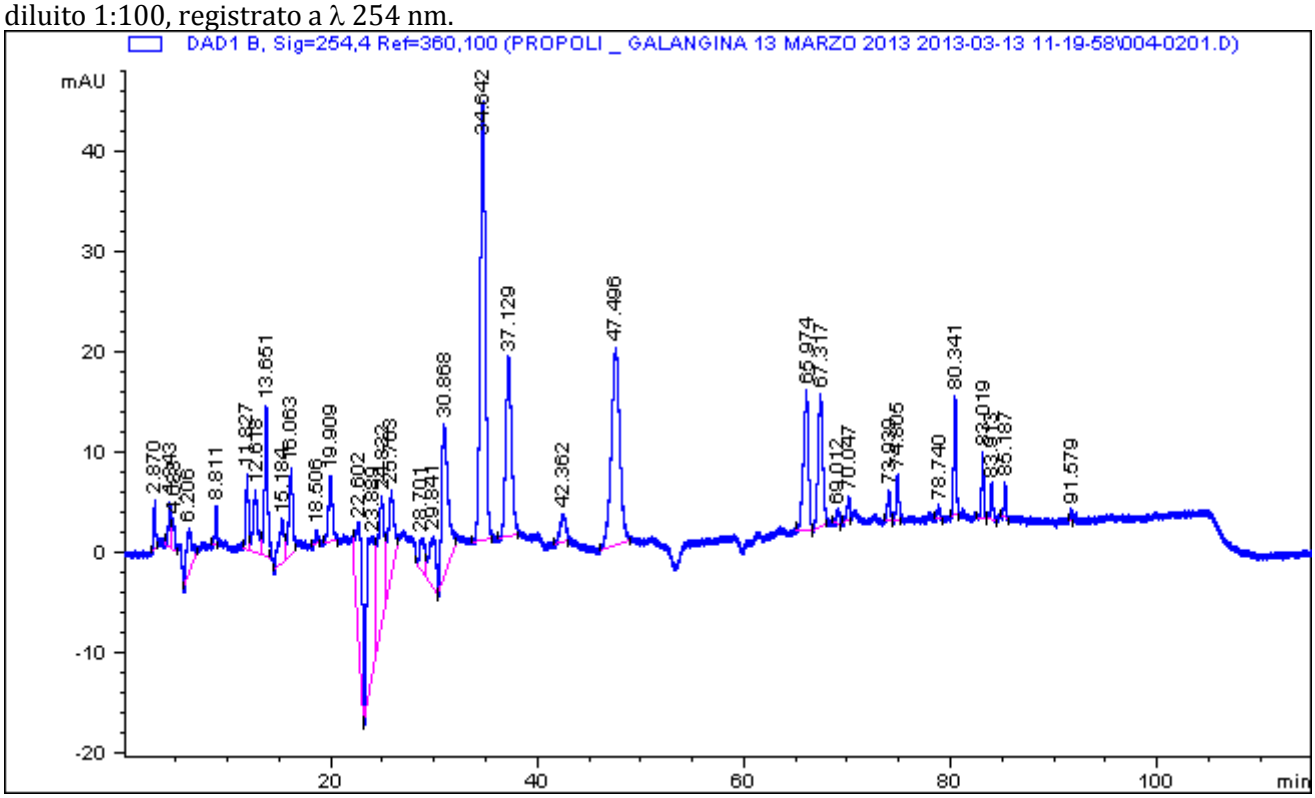


Figura 6. Cromatogramma RP-HPLC-DAD ottenuto dall'analisi dell'estratto di propoli oggetto di studio diluito 1:100, registrato a λ 270 nm.

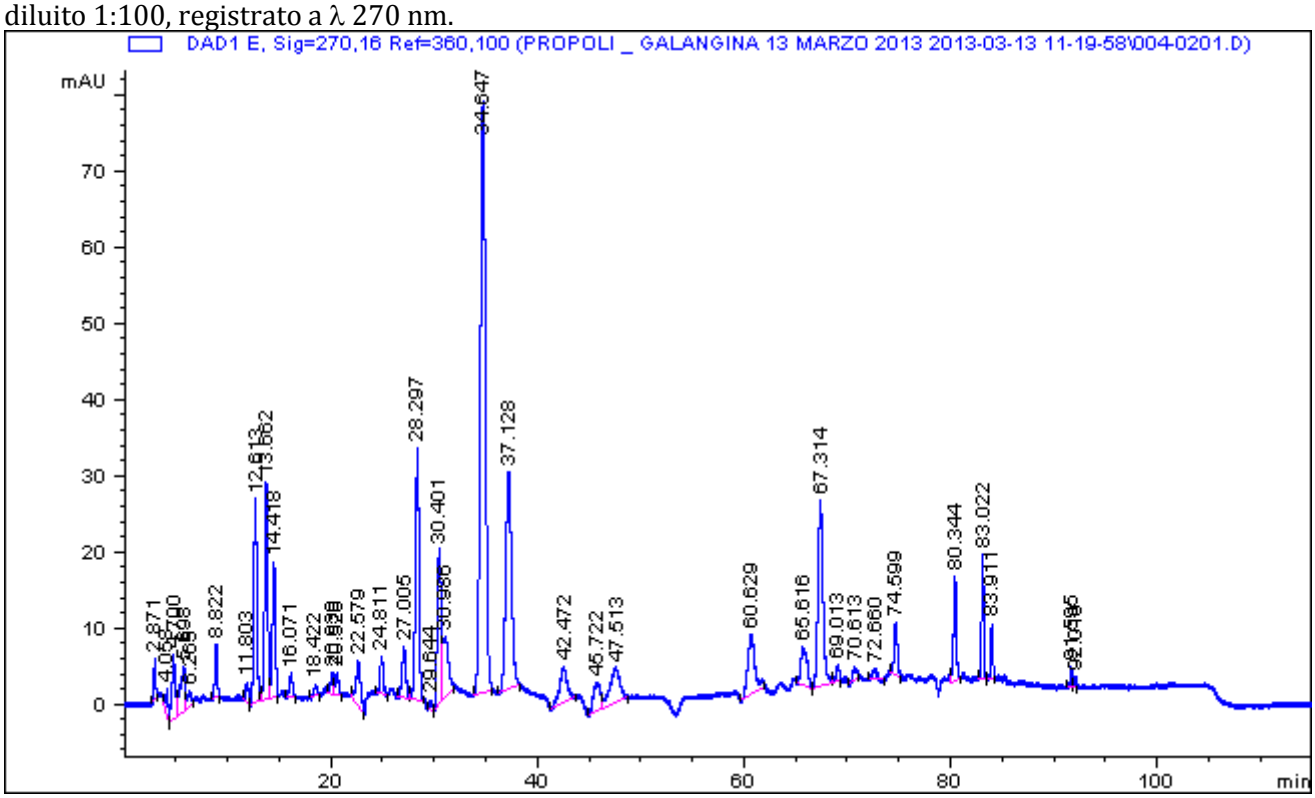
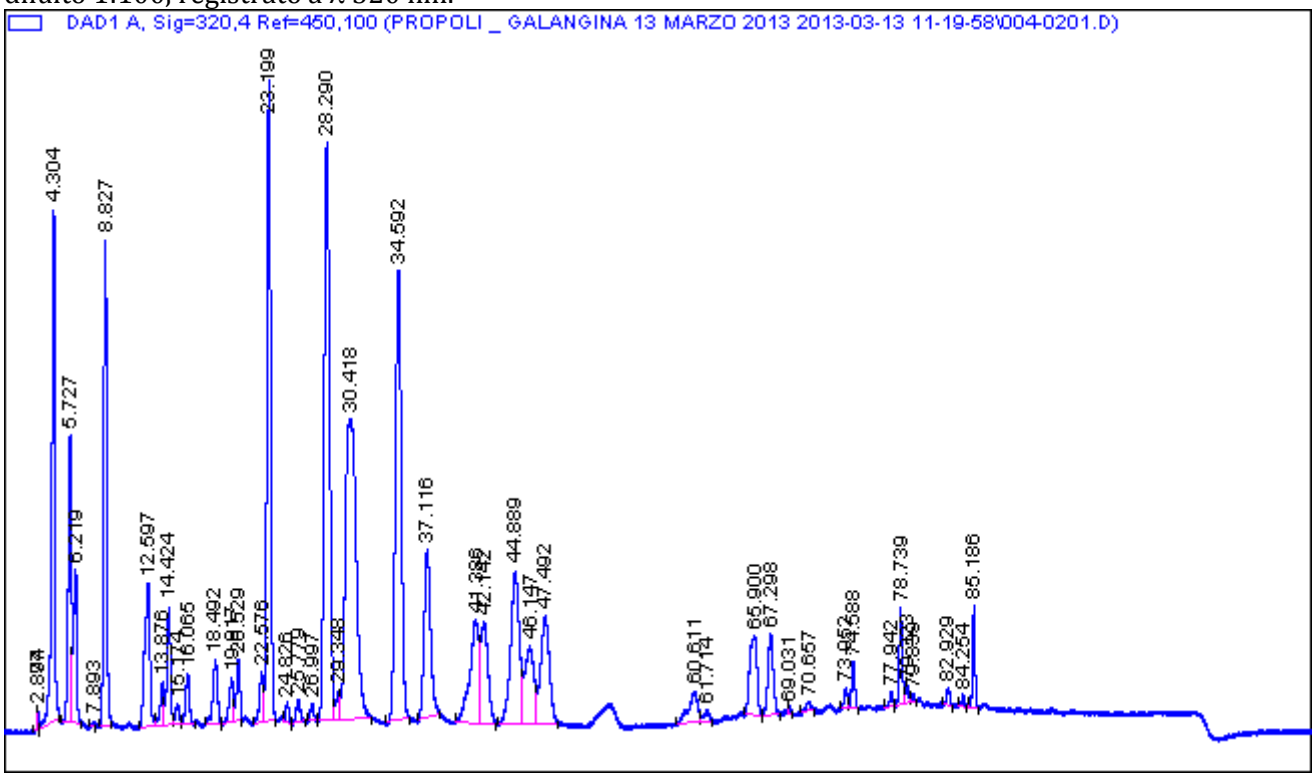


Figura 7. Cromatogramma RP-HPLC-DAD ottenuto dall'analisi dell'estratto di propoli oggetto di studio diluito 1:100, registrato a λ 320 nm.



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PROPOLI

La propoli è una sostanza resinosa che le api raccolgono dalle gemme e dalla corteccia delle piante. Si tratta di una sostanza di origine completamente naturale, di colore variabile dalle tonalità del giallo, fino al rosso, al marrone e al nero e dall'odore caratteristico fortemente aromatico. Ha proprietà antibiotiche (batteriostatiche e battericide), antiinfiammatorie, antimicotiche, antiossidanti ed anti-irrancidenti, antivirali, anestetiche, riepitilizzanti e cicatrizzanti, antisettiche, immunostimolanti, vasoprotettive, antitumorali.
La propoli è costituita essenzialmente da reseine, balsami e cere. Le principali molecole che la caratterizzano sono di natura aromatica e fenolica e tra i suoi componenti si possono riconoscere sostanze molto eterogenee fra loro come acidi grassi, terpeni, aminoacidi, vitamine, sali minerali.
La grande ricchezza di flavonoidi assicura la funzione più preziosa, quella antimicrobica. Questi sono contenuti in grande quantità nella propoli (fino al 20% del peso). L'ape modifica la struttura dei flavonoidi, originariamente presenti nelle piante, idrolizzando il legame glicosidico tra l'aglicone polifenolico e il residuo zuccherino grazie agli enzimi prodotti dalle loro ghiandole salivari. Sono inoltre presenti numerose sostanze ad azione nutritiva e funzionale:
-minerali: Mg, Ca, I, K, Na, Cu, Zn, Mn e Fe;
-vitamine: B_1 (tiamina), B_2 (riboflavina), B_6 (piridossina), C (acido ascorbico), E (tocoferolo), P (flavonoidi);
-enzimi: succinato deidrogenasi, glucosio 6-fosfatasi, fosfatasi acida;
-acidi organici: acido caffeico feniletilestere (CAPE) contenuto nelle resine e nei composti organici, fenolo, adenosintrifosfato (ATP);
-terpeni sono contenuti nelle resine e negli oli essenziali e conferiscono il caratteristico odore alla propoli;
-derivati dell'acido benzoico: acido gentisico, acido salicilico, acido protocatechico, acido-3-ossibenzoico, acido-4-ossibenzoico, acido gallico, acido-4-metossibenzoico;
-derivati dell'acido cinnamico: acido caffeico, acido ferulico, acido isoferulico, acido idrocaffeico, acido p-cumarico, acido o-cumarico, acido m-cumarico;
-cumarine: cumarina, esculetina, scopoletina;
-alcoli: alcol benzilico, alcol cinnamilico, alcol feniletilico, alcol pentenilico, alcol 3,5-dimedossibenzilico
- aldeidi: vanillina, isovanillina, aldeide cinnamica;
- flavoni: 5-idross-7,4'-dimetossiflavone, acacetina, apigenina-dimetiletere7,4', crisina, pectolinarigenina, tettocrisina;
- flavonoli: 3,5-3,5-diidrossi-7,4'-imetossiflavone, betuletolo, ermanina, galangina, isalpinina, isoramnetina, kaempferide, kaempferolo, quercetin-3,3'-dimetiletere, quercetina, ramnazina, ramnetina, ramnocitrina;
- flavanoni: 5-idrossi-7,4'-dimetossiflavone, isosakuranetina, pinocembrina, pinostrobina, sakuranetina;
- diidroflavonoli: pinobaksina, pinobanksina-3-acetato;
- idrocarburi: cariofillene, α-guaiene, β-selinene;
- alcoli sesquiterpenici: β-eudesmolo, guaiolo;
- aminoacidi;
- acidi grassi;
- chetoni;
- steroli, polisaccaridi, lattoni;

L'utilizzo è indicato principalmente per combattere le infezioni dell'apparato respiratorio come raffreddore, mal di gola, influenza. La sua peculiarità è l'ampia versatilità: dalla cura dell'igiene orale, contro alito cattivo, gengiviti, infiammazioni della bocca in genere, alle

cicatrizzazioni lente e difficili da dermatiti, screpolature, foruncoli, eczemi. E' indicata anche per combattere problemi ginecologici.
Oggi la propoli viene estratta con moderne tecniche che ne assicurano la purezza, rendendola disponibile in farmacia in varie forme: soluzione idroalcolica, soluzione glicolica, capsule, collutorio, sciroppi, spray orale e nasale, unguento; utilizzata da sola o associata ad altri componenti che ne completano l'azione.

ARTICOLI DA PUB MED

Dal 2010 ad oggi sono stati pubblicati 455 articoli sulla Propoli.
Poiché i temi trattati da alcune pubblicazioni si sovrappongono, per riassumere i dati presenti in letteratura, i lavori a seguito riportati sono stati suddivisi come segue:

- 33 sulle attività funzionali
- 50 sulle attività terapeutiche
- 50 sugli effetti salutistici positivi
- 33 sull'attività antibatterica
- 13 sull'attività antivirale

PROPOLIS FUNCTIONAL ACTIVITIES

Effect of seasonality on chemical composition and antibacterial and anticandida activities of Argentine propolis. Design of a topical formulation.
Isla MI, Dantur Y, Salas A, Danert C, Zampini C, Arias M, Ordóñez R, Maldonado L, Bedascarrasbure E, Nieva Moreno MI.
Nat Prod Commun. 2012 Oct;7(10):1315-8.

Green Brazilian propolis effects on sperm count and epididymis morphology and oxidative stress.
Capucho C, Sette R, de Souza Predes F, de Castro Monteiro J, Pigoso AA, Barbieri R, Dolder MA, Severi-Aguiar GD.
Food Chem Toxicol. 2012 Nov;50(11):3956-62.

Chemical and functional characterization of Italian propolis obtained by different harvesting methods.
Papotti G, Bertelli D, Bortolotti L, Plessi M.
J Agric Food Chem. 2012 Mar 21;60(11):2852-62.

Catechols in caffeic acid phenethyl ester are essential for inhibition of TNF-mediated IP-10 expression through NF-κB-dependent but HO-1- and p38-independent mechanisms in mouse intestinal epithelial cells.
Mapesa JO, Waldschmitt N, Schmoeller I, Blume C, Hofmann T, Mahungu S, Clavel T, Haller D.
Mol Nutr Food Res. 2011 Dec;55(12):1850-61.

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Food components with anticaries activity.
Gazzani G, Daglia M, Papetti A.
Curr Opin Biotechnol. 2012 Apr;23(2):153-9.

Chemical compositions and antioxidant activities of water extracts of Chinese propolis.
Guo X, Chen B, Luo L, Zhang X, Dai X, Gong S.
J Agric Food Chem. 2011 Dec 14;59(23):12610-6.

Chemical composition of Argentinean propolis collected in extreme regions and its relation with antimicrobial and antioxidant activities.
Vera N, Solorzano E, Ordoñez R, Maldonado L, Bedascarrasbure E, Isla MI.
Nat Prod Commun. 2011 Jun;6(6):823-7.

Caffeic acid phenethyl ester reduces spinal cord injury-evoked locomotor dysfunction.
Kasai M, Fukumitsu H, Soumiya H, Furukawa S.
Biomed Res. 2011 Feb;32(1):1-7.

Ethanol extract of chinese propolis facilitates functional recovery of locomotor activity after spinal cord injury.
Kasai M, Fukumitsu H, Soumiya H, Furukawa S.
Evid Based Complement Alternat Med. 2011;2011.

Caffeic acid phenethyl ester-mediated Nrf2 activation and IkappaB kinase inhibition are involved in NFkappaB inhibitory effect: structural analysis for NFkappaB inhibition.
Lee Y, Shin DH, Kim JH, Hong S, Choi D, Kim YJ, Kwak MK, Jung Y.
Eur J Pharmacol. 2010 Sep 15;643(1):21-8.

PROPOLIS THERAPEUTICAL ACTIVITIES

Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity.
Dias LG, Pereira AP, Estevinho LM.
Food Chem Toxicol. 2012 Dec;50(12):4246-53.

7-epi-nemorosone from Clusia rosea induces apoptosis, androgen receptor down-regulation and dysregulation of PSA levels in LNCaP prostate carcinoma cells.
Díaz-Carballo D, Gustmann S, Acikelli AH, Bardenheuer W, Buehler H, Jastrow H, Ergun S, Strumberg D.
Phytomedicine. 2012 Nov 15;19(14):1298-306.

Geopropolis from Melipona scutellaris decreases the mechanical inflammatory hypernociception by inhibiting the production of IL-1β and TNF-α.
Franchin M, da Cunha MG, Denny C, Napimoga MH, Cunha TM, Koo H, de Alencar SM, Ikegaki M, Rosalen PL.
J Ethnopharmacol. 2012 Sep 28;143(2):709-15.

Bactericidal activity of ethanolic extracts of propolis against Staphylococcus aureus isolated from mastitic cows.
Santana HF, Barbosa AA, Ferreira SO, Mantovani HC.
World J Microbiol Biotechnol. 2012 Feb;28(2):485-91.

The anticancer activity of propolis.
Sawicka D, Car H, Borawska MH, Nikliński J.
Folia Histochem Cytobiol. 2012 Apr 24;50(1):25-37.

Selective inhibition of human type-5 17β-hydroxysteroid dehydrogenase (AKR1C3) by baccharin, a component of Brazilian propolis.
Endo S, Matsunaga T, Kanamori A, Otsuji Y, Nagai H, Sundaram K, El-Kabbani O, Toyooka N, Ohta S, Hara A.
J Nat Prod. 2012 Apr 27;75(4):716-21.

Effective neurofibromatosis therapeutics blocking the oncogenic kinase PAK1.
Maruta H.
Drug Discov Ther. 2011 Dec;5(6):266-78.

In vitro antiproliferative/cytotoxic activity on cancer cell lines of a cardanol and a cardol enriched from Thai Apis mellifera propolis.
Teerasripreecha D, Phuwapraisirisan P, Puthong S, Kimura K, Okuyama M, Mori H, Kimura A, Chanchao C.
BMC Complement Altern Med. 2012 Mar 30;12:27.

Propolis standardized extract (EPP-AF®), an innovative chemically and biologically reproducible pharmaceutical compound for treating wounds.
Berretta AA, Nascimento AP, Bueno PC, Vaz MM, Marchetti JM.
Int J Biol Sci. 2012;8(4):512-21.

Antimicrobial activity, phenolic profile and role in the inflammation of propolis.
Silva JC, Rodrigues S, Feás X, Estevinho LM.
Food Chem Toxicol. 2012 May;50(5):1790-5.

Antimicrobial activity of stingless bee (Trigona sp.) propolis used in the folk medicine of Western Maharashtra, India.
Choudhari MK, Punekar SA, Ranade RV, Paknikar KM.
J Ethnopharmacol. 2012 May 7;141(1):363-7.

Enhanced anti-diabetic activity of a combination of chromium(III) malate complex and propolis and its acute oral toxicity evaluation.
Wu XY, Li F, Zhao T, Mao GH, Li J, Qu HY, Ren YN, Yang LQ.
Biol Trace Elem Res. 2012 Jul;148(1):91-101.

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Protective effect of Brazilian propolis against hepatic oxidative damage in rats with water-immersion restraint stress.
Nakamura T, Ohta Y, Ohashi K, Ikeno K, Watanabe R, Tokunaga K, Harada N.
Phytother Res. 2012 Oct;26(10):1482-9.

Combined treatment of HEDTA and propolis prevents aluminum induced toxicity in rats.
Bhadauria M.
Food Chem Toxicol. 2012 Jul;50(7):2487-95.

Design, synthesis and evaluation of caffeic acid phenethyl ester-based inhibitors targeting a selectivity pocket in the active site of human aldo-keto reductase 1B10.
Soda M, Hu D, Endo S, Takemura M, Li J, Wada R, Ifuku S, Zhao HT, El-Kabbani O, Ohta S, Yamamura K, Toyooka N, Hara A, Matsunaga T.
Eur J Med Chem. 2012 Feb;48:321-9.

Potential utility of hyperbaric oxygen therapy and propolis in enhancing the leishmanicidal activityof glucantime.
Ayres DC, Fedele TA, Marcucci MC, Giorgio S.
Rev Inst Med Trop Sao Paulo. 2011 Nov-Dec;53(6):329-34.

A novel property of propolis (bee glue): anti-pathogenic activity by inhibition of N-acyl-homoserine lactone mediated signaling in bacteria.
Bulman Z, Le P, Hudson AO, Savka MA.
J Ethnopharmacol. 2011 Dec 8;138(3):788-97.

Anti-tumour effects of Egyptian propolis on Ehrlich ascites carcinoma.
Badr MO, Edrees NM, Abdallah AA, El-Deen NA, Neamat-Allah AN, Ismail HT.
Vet Ital. 2011 Jul-Sep;47(3):341-50.

Anti-ulcerogenic effect of aqueous propolis extract and the influence of radiation exposure.
El-Ghazaly MA, Rashed RR, Khayyal MT.
Int J Radiat Biol. 2011 Oct;87(10):1045-51.

Effect of green propolis on oral epithelial dysplasia in rats.
Cavalcante DR, Oliveira PS, Góis SM, Soares AF, Cardoso JC, Padilha FF, Albuquerque Jr RL.
Braz J Otorhinolaryngol. 2011 Jun;77(3):278-84.

Inhibition of melanogenesis by 5,7-dihydroxyflavone (chrysin) via blocking adenylyl cyclase activity.
Kim DC, Rho SH, Shin JC, Park HH, Kim D.
Biochem Biophys Res Commun. 2011 Jul 22;411(1):121-5.

Antimicrobial traits of tea- and cranberry-derived polyphenols against Streptococcus mutans.
Yoo S, Murata RM, Duarte S.
Caries Res. 2011;45(4):327-35. doi: 10.1159/000329181.

Characterisation of protease activity in extracellular products secreted by Giardia duodenalis trophozoites treated with propolis.
David EB, de Carvalho TB, Oliveira CM, Coradi ST, Sforcin JM, Guimarães S.
Nat Prod Res. 2012;26(4):370-4.

Therapeutic effects of propolis essential oil on anxiety of restraint-stressed mice.
Li YJ, Xuan HZ, Shou QY, Zhan ZG, Lu X, Hu FL.
Hum Exp Toxicol. 2012 Feb;31(2):157-65.

The effects of Brazilian and Bulgarian propolis in vitro against Salmonella Typhi and their synergism with antibiotics acting on the ribosome.
Orsi RO, Fernandes A, Bankova V, Sforcin JM.
Nat Prod Res. 2012;26(5):430-7.

Caffeic Acid Phenethyl Ester (CAPE) derived from propolis, a honeybee product, inhibits growth of breast cancer stem cells.
Omene CO, Wu J, Frenkel K.
Invest New Drugs. 2012 Aug;30(4):1279-88.

NBM-HD-3, a novel histone deacetylase inhibitor with anticancer activity through modulation of PTEN and AKT in brain cancer cells.
Huang WJ, Lin CW, Lee CY, Chi LL, Chao YC, Wang HN, Chiou BL, Chen TJ, Huang CY, Chen CN.
J Ethnopharmacol. 2011 Jun 14;136(1):156-67.

Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells.
Barbarić M, Mišković K, Bojić M, Lončar MB, Smolčić-Bubalo A, Debeljak Z, Medić-Šarić M.
J Ethnopharmacol. 2011 Jun 1;135(3):772-8.

Antibacterial effects of Brazilian and Bulgarian propolis and synergistic effects with antibiotics acting on the bacterial DNA and folic acid.
Orsi RO, Fernandes A Jr, Bankova V, Sforcin JM.
Nat Prod Res. 2012;26(4):344-9.

Caffeoylquinic acids are major constituents with potent anti-influenza effects in brazilian greenpropolis water extract.
Urushisaki T, Takemura T, Tazawa S, Fukuoka M, Hosokawa-Muto J, Araki Y, Kuwata K.
Evid Based Complement Alternat Med. 2011;2011:254914.

Caffeic acid phenethyl ester reduces spinal cord injury-evoked locomotor dysfunction.
Kasai M, Fukumitsu H, Soumiya H, Furukawa S.
Biomed Res. 2011 Feb;32(1):1-7.

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Scheda tecnica dell'estratto di propoli fornito dall' Azienda "Dott. Stefano Faralli scarl".

Laboratorio
Dott. Stefano Faralli scarl

PROPOLI
TINTURA MADRE

CERTIFICATO DI ANALISI E SCHEDA TECNICA

CONFORMITA' A FARMACOPEA EUROPEA 7.4

CON METODICA ESTRATTIVA NAVIGLIO

SPECIFICHE	IDENTIFICAZIONE:	RISULTATI
Denominazione	*Propoli*	
Materia prima	TINTURA MADRE	
Parte utilizzata	Resine	
Origine	Italia	
Solvente	Alcool	
SPECIFICHE		
Aspetto	Liquido chiaro	
Colore	Caratteristico	
Odore	Caratteristico	
Grado alcolico	70°% v/v	68° -73°%v/v
Residuo secco a 105°C		
Rapporto di estrazione	1/4	
Metalli pesanti (conforme a Reg. EC 629/2008)		
Piombo	< Max. 3.0 ppm	Max. 3.0 ppm
Cadmio	< Max. 1.0 ppm	Max. 1.0 ppm
Arsenico	< 0,00026 mg/Kg	
Pesticidi	Conforme	Assenti
Ocratossina A	< 0,001mg/Kg	
Aflatossine B1	< Max. 5ppb	Max. 5ppb
Aflatossine B1,B2,G1,G2	< Max. 10ppb	Max. 10ppb
Analisi microbiologica:		
Conta totale batteri	< 1000 ufc/g	Max. 1000 ufc/g
Lieviti e muffe	< 10 ufc/g	Max. 10 ufc/g
Streptococchi fecali	< 2 ufc/g	Max. 10 ufc/g
Stafilococco aureo	< 2 ufc/g	Max. 10 ufc/g

STOCCAGGIO: Conservare in luogo fresco e asciutto, non congelare, tenere al riparo da luce e calore.

ALLERGENI: Non contiene nessuno degli allergeni citati nella Direttiva 2003/89/CE e successivi aggiornamenti.

OGM (In accordo con Reg. 1829/2003 e N° 1830/2003): Non contiene, non deriva e non è costituito da organismi geneticamente modificati.

Codice prodotto: **LOTTO : SF1769DTG028** **Dott. STEFANO FARALLI scarl.**
Codice documento: **MC-028SP**
Prima emissione: **07/11/'12 SCADENZA 11/2017**
RESPONSABILE CONTROLLO QUALITA': DOTT. STEFANO FARALLI

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