# research / publications

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## In-house Researcher / Science Hotline

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# 1. Immune-system boosting

## 1.1. Xanthine oxidase inhibitory activity and hypouricemia effect of Propolis in rats


| | |
|---|---|
| Authors | Yoshizumi K, Nishioka N, Tsuji T |
| Link | Yakugaku Zasshi. 2005 Mar;125(3):315-21. |
| Research Institute | Fancl Corporation Central Research Laboratory, Yokohama 244-0806, Japan. kayoshizu@fancl.co.jp |



### Abstract

The xanthine oxidase (XOD) inhibitory activity of Propolis from China and Brazil was measured.

The Propolis from both place were seen to have XOD inhibitory activity. However, a stronger tendency was shown in the Propolis from China. The compounds in each the Propolis were measured quantitatively.

A great deal of chrysin, galangin, and caffeic acid phenetyl ester were found in the Propolis from China, an abundance of p-coumaric acid and Artepillin C in the Propolis from Brazil.

Therefore it was revealed that the Propolis compounds are very different depending on their place of origin. The XOD inhibitory activity of these five compounds was measured. Caffeic acid phenetyl ester had the strongest activity, with chrysin and galangin next; p-coumaric acid and Artepillin C showed weak XOD inhibitory activity. We evaluated the hypouricemic effect of Propolis from China on hyperuricemia induced by the uricase inhibitor, oxonic acid (500 mg/kg p.o., 1 h before the test drugs), and measured plasma uric acid values in rats. Oral Propolis had a hypouricemic effect 2 h after its administration to oxonate-pretreated rats.

These results suggested that a continuous intake of Propolis may be effective for the prevention and the treatment of gout and hyperuricemia.

## 1.2. Immunomodulation produced by a Green Propolis extract on humoral and cellular responses of mice immunized with SuHV-1.

| | |
|---|---|
| Authors | Fischer G, Conceicao FR, Leite FP, Dummer LA, Vargas GD, Hubner Sde O, Dellagostin OA, Paulino N, Paulino AS, Vidor T. |
| Link | Vaccine. 2007 Jan 26;25(7):1250-6. |
| Research Institute | Centro de Biotecnologia, Laboratorio de Virologia e Imunologia, Faculdade de Veterinaria, Universidade Federal de Pelotas (UFPel), CP 354, 96010-900 Pelotas, RS, Brazil. |


### Abstract

Despite recent technological advances in vaccine production, most the vaccines depend on the association with adjuvant substances.

This work evaluated the adjuvant capacity of an ethanol extract of green Propolis associated to inactivated Suid herpesvirus type 1 (SuHV-1) vaccine preparations.

Mice inoculated with SuHV-1 vaccine plus aluminum hydroxide and 5mg/dose of Propolis extract presented higher levels of antibodies when compared to animals that received the same vaccine without Propolis. The use of SuHV-1 vaccine with Propolis extract alone did not induce significant levels of antibodies, however it was able to increase the cellular immune response, evidenced by the increase in the expression of mRNA to IFN-gamma. Besides, Propolis increased the percentage of protected animals against challenge with a lethal dose of SuHV-1.

The effect of green Propolis extract on the humoral and cellular immune responses may be exploited for the development of effective vaccines.

## 1.3. Effect of *Baccharis dracunculifolia* D.C. (Asteracea) extracts and its isolated compounds on macrophage activation.

| | |
|---|---|
| Authors | Missima F, da Silva Filho AA, Nunes GA, Bueno PC, de Sousa JP, Bastos JK, Sforcin JM. |
| Link | J Pharm Pharmacol. 2007 Mar;59(3):463-8. |
| Research Institute | Departamento de Microbiologia e Imunologia, Instituto de Biociencias-UNESP, 18618-000 Botucatu, SP, Brazil. |


### Abstract

*Baccharis dracunculifolia* D.C. (Asteraceae), a shrub which grows wild in Brazil, is the main botanical source of Brazlian Green Propolis. Since Brazilian Propolis shows an immunomodulatory activity, the goal of this work was to evaluate the action of *B. dracunculifolia* extracts and some of its isolated compounds on reactive oxygen intermediate (H(2)O(2)) production by macrophages obtained from male BALB/c mice. The results showed that the leaf (Bd-L) (25, 50, and 100 microg mL(-1)), leaf rinse (Bd-LR) (25 microg mL(-1)), and the root (Bd-R) (25 microg mL(-1)) extracts enhanced H2O2 release by macrophages. A phytochemical study of the root and leaves of *B. dracunculifolia* was carried out. The chromatographic fractionation of Bd-R, using several techniques, afforded the isolation of baccharis oxide (1), friedelanol (2), viscidone (11), 11-hydroxy-10,11-dihydro-euparin (12), and 6hydroxy-tremetona (13), while Bd-LR gave the following isolated compounds: baccharis oxide (1), friedelanol (2), isosakuranetin (3), aromadendrin-4'-methyl ether (4), dihydrocumaric acid (5), baccharin (6), hautriwaic acid lactone (7), hautriwaic acid acetate (8), drupanin (9), and cumaric acid (10). Among the isolated compounds, baccharis oxide (1) and friedelanol (2) increased H2O2 production at a concentration of 100 microM. This is the first time that the presence of compounds 7, 8, 12, and 13 in *B. dracunculifolia* has been reported. Based on these results it is suggested that the crude extracts and some isolated compounds from *B. dracunculifolia* display an immunomodulatory action.

## 1.4. Adjuvant effect of Green Propolis on humoral immune response of bovines immunized with bovine herpesvirus type 5.

| | |
|---|---|
| Authors | Fischer G, Cleff MB, Dummer LA, Paulino N, Paulino AS, de Oliveira Vilela C, Campos FS, Storch T, D'Avila Vargas G, de Oliveira Hubner S, Vidor T. |
| Link | Vet Immunol Immunopathol. 2007 Mar 15;116(1-2):79-84. |
| Research Institute | Centro de Biotecnologia, Universidade Federal de Pelotas (UFPel), Campus Universitario, Caixa Postal 354, 96010-900, Pelotas, RS, Brazil. |


### Abstract

Despite recent technological advances in vaccine production, most vaccines depend on the association with adjuvant substances. In this study, Propolis, which has been attracting the attention of researchers due to its bioactive properties, was evaluated as an immunological adjuvant. The association of 40mg/dose of an ethanolic extract of green Propolis with an inactivated oil vaccine against bovine herpesvirus type 5 (BoHV-5), resulted in a significant increase (P=0.01) in the neutralizing antibody levels, comparing to the bovines that received the same vaccine without Propolis. Besides, Propolis increased the percentage of animals with high antibody titers (above 32). Phenolic compounds such as Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) and the derivatives of cinnamic acid besides other flavonoid substances were abundant in the Propolis extract used, and they could be the main substances with adjuvant action. The effect of the green Propolis extract on the humoral immune response can be exploited in the development of new vaccines.

## 1.5. Brazilian Propolis Action on Macrophages and Lymphoid Organs of Chronically Stressed Mice.


| | |
|---|---|
| Authors | Fabiane Missima and José Maurício Sforcin. |
| Link | Evid. Based Complement. Altern. Med. 2008; 5:71-75 [Abstract] [Full Text] [PDF] |
| Research Institute | Department of Microbiology and Immunology, Biosciences Institute, UNESP, 18618-000 Botucatu, S.P., Brazil. |



### Abstract

Stress is a generic term that summarizes how psychosocial and environmental factors influence physical and mental well-being. The interaction between stress and immunity has been widely investigated, involving the neuroendocrine system and several organs. Assays using natural products in stress models deserve further investigation. Propolis immunomodulatory action has been mentioned and it has been the subject of scientific investigation in our laboratory. The aim of this study was to evaluate if and how Propolis activated macrophages in BALB/c mice submitted to immobilization stress, as well as the histopathological analysis of the thymus, bone marrow, spleen and adrenal glands. Stressed mice showed a higher hydrogen peroxide (H2O2) generation by peritoneal macrophages, and Propolis treatment potentiated H2O2 generation and inhibited nitric oxide (NO) production by these cells. Histopathological analysis showed no alterations in the thymus, bone marrow and adrenal glands, but increased germinal centers in the spleen. Propolis treatment counteracted the alterations found in the spleen of stressed mice. New research is being carried out in order to elucidate Propolis immunomodulatory action during stress.

# 2. Detoxifying

## 2.1. Chemical constituents in *Baccharis dracunculifolia* as the main botanical rigin of southeastern Brazilian Propolis.

| | |
|---|---|
| Authors | Park YK, Paredes-Guzman JF, Aguiar CL, Alencar SM, Fujiwara FY. |
| Link | J Agric Food Chem. 2004 Mar 10;52(5):1100-3. |
| Research Institute | Department of Food Science, College of Food Engineering, State University of Campinas, P.O. Box 6177, 13083-970, Campinas, Sao Paulo, Brazil. |


### Abstract

Previously, it was reported that one group of Propolis (Group 12) was identified in southeastern Brazil, and the botanical origin of the Propolis was *Baccharis dracunculifolia* resinous exudates. It was also observed that honeybee (Africanized Apis mellifera) mainly visited the leaf buds or unexpanded leaves of *B. dracunculifolia* but rarely expanded leaves. *B. dracunculifolia* is dioecious with male and female inflorescences, and RPHPLC of the ethanolic extracts of the respective male and female bud resinous exudates showed the same profiles. RPHPLC profiles of Propolis G12 leaf buds and unexpanded and expanded leaves of *B. dracunculifolia* showed similarity, but unexpanded leaves quantitatively decreased in chemical constituents as compared with leaf buds. In the case of expanded leaves, all chemical constituents were severely decreased or disappeared. Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) was also identified in both Propolis and resinous exudates, and both ethanolic extracts contained the highest concentrations of this compound as compared with the rest of the chemical constituents.

## 2.2. Suppression of dioxin mediated aryl hydrocarbon receptor transformationmain botanical origin on dioxin toxicity.

| | |
|---|---|
| Authors | Park YK, Fukuda I, Ashida H, Nishiumi S, Guzman JP, Sato HH, Pastore GM. |
| Link | Biosci Biotechnol Biochem. 2004 Apr;68(4):935-8. |
| Research Institute | State University of Campinas, College of Food Engineering (UNICAMP), Department of Food Science, Laboratory of Food Biochemistry, SP, Brazil. |


### Abstract

Present study demonstrated that the ethanolic extracts of Propolis containing higher concentrations of flavonoids suppressed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced aryl hydrocarbon receptor transformation in a dose-dependent manner. The IC(50) values of Propolis group 3 and group 12 were 1.2 and 3.6 microg/ml, respectively, indicating that Propolis showed stronger antagonistic effects as compared with vegetable extracts.

## 2.3. Origin and Chemical Variation of Brazilian Propolis.

| | |
|---|---|
| Authors | Salatino A, Teixeira EW, Negri G, Message D. |
| Link | Evid Based Complement Alternat Med. 2005 Mar;2(1):33-38. |
| Research Institute | University of São Paulo, Institute of Biosciences, Department of Botany, C. Postal. 11461, 05422-970, São Paulo, SP, Brazil. |


### Abstract

Propolis is a hive product containing chiefly beeswax and plant-derived substances such as resin and volatile compounds. Propolis has been used as an antiseptic and wound healer since ancient times and interest for the product has increased recently. Probably few plant species contribute as major resin sources. Green Propolis derives mainly from vegetative apices of *Baccharis dracunculifolia* (alecrim plants). However, wide variation detected in the chemical composition suggests contributions from alternative resin plant sources. Predominant components of the resin of green Propolis are cinnamic acids, chiefly compounds bearing prenyl groups. Terpenoid compounds, such as sesqui, di and pentacyclic triterpenoids, have been detected in many, but not all, samples investigated. Propolis research has uncovered potentialities of substances previously isolated from plants and has detected constituents of plant origin that would hardly be known otherwise.

## 2.4. Neuroprotection by Brazilian Green Propolis against In vitro and In vivo Ischemic Neuronal Damage.

| | |
|---|---|
| Authors | Shimazawa M, Chikamatsu S, Morimoto N, Mishima S, Nagai H, Hara H. |
| Link | Evid Based Complement Alternat Med. 2005 Jun;2(2):201-207. |
| Research Institute | Department of Biofunctional Molecules, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu, 502-8585 Japan. |


### Abstract

We examined whether Brazlian Green Propolis, a widely used folk medicine, has a neuroprotective function in vitro and/or in vivo. In vitro, Propolis significantly inhibited neurotoxicity induced in neuronally differentiated PC12 cell cultures by either 24 h hydrogen peroxide (H(2)O(2)) exposure or 48 h serum deprivation. Regarding the possible underlying mechanism, Propolis protected against oxidative stress (lipid peroxidation) in mouse forebrain homogenates and scavenged free radicals [induced by diphenyl-p-picrylhydrazyl (DPPH). In mice in vivo, Propolis [30 or 100 mg/kg; intraperitoneally administered four times (at 2 days, 1 day and 60 min before, and at 4 h after induction of focal cerebral ischemia by permanent middle cerebral artery occlusion)] reduced brain infarction at 24 h after the occlusion. Thus, a Propolis-induced inhibition of oxidative stress may be partly responsible for its neuroprotective function against in vitro cell death and in vivo focal cerebral ischemia.

## 2.5. Antimicrobial activity of flavonoids.

| | |
|---|---|
| Authors | Cushnie TP, Lamb AJ. |
| Link | Int J Antimicrob Agents. 2005 Nov;26(5):343-56. |
| Research Institute | School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen, AB10 1FR, UK. |


### Abstract

Flavonoids are ubiquitous in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, Propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity. Moreover, several groups have demonstrated synergy between active flavonoids as well as between flavonoids and existing chemotherapeutics. Reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to inter- and intra-assay variation in susceptibility testing. However, several high-quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement. In addition, numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase. It has also been proposed that sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function, and that licochalcones A and C inhibit energy metabolism. Other flavonoids whose mechanisms of action have been investigated include robinetin, myricetin, apigenin, rutin, galangin, 2,4,2'-trihydroxy-5'-methylchalcone and lonchocarpol A. These compounds represent novel leads, and future studies may allow the development of a pharmacologically acceptable antimicrobial agent or class of agents.

## 2.6. Suppressive effects of ethanolic extracts from Propolis and its main botanical origin on dioxin toxicity.


| | |
|---|---|
| Authors | Park YK, Fukuda I, Ashida H, Nishiumi S, Yoshida K, Daugsch A, Sato HH, Pastore GM. |
| Link | J Agric Food Chem. 2005 Dec 28;53(26):10306-9. |
| Research Institute | Department of Food Science, College of Food Engineering, State University of Campinas, P.O. Box 6177, Campinas, SP, Brazil. |



### Abstract

Suppressive effects of ethanolic extracts prepared from Propolis group 12 and its main botanical origin (leaf bud of *Baccharis dracunculifolia*) on transformation of the aryl hydrocarbon receptor (AhR), the initial action of dioxin toxicity, were investigated. It was found that suppressive effects of Propolis on AhR transformation were relatively higher than those of resins of its botanical origin in cell-free system and in Hepa-1c1c7 cells. When the composition of chemical ingredients was measured, Propolis contained slightly higher amounts of flavonoid aglycones as compared with its botanical origin with the same characteristics. Moreover, antiradical activity, one of the typical biological activities of flavonoids, in Propolis was also slightly higher than that in its botanical origin. These results indicate that not only Propolis but also its botanical origin contains high amounts of flavonoid aglycones and that both of them are useful dietary sources for flavonoids with a potency to prevent dioxin toxicity.

## 2.7. Duration-dependent hepatoprotective effects of Propolis extract against carbon tetrachloride-induced acute liver damage in rats.


| | |
|---|---|
| Authors | M Bhadauria, SK Nirala, and S Shukla. |
| Link | *Adv Ther.* 2007; 24: 1136. [MEDLINE Citation] |
| Research Institute | Reproductive Biology, and Toxicology Laboratory, School of Studies in Zoology, Jiwaji University, Gwalior, India. monikabhadauria@rediffmail.com. |



### Abstract

Propolis is a natural product produced by bees that was discovered through the study of traditional cures and knowledge of indigenous people throughout the world. It is rich in vitamins A, B, C, and E, and in amino acids, copper, iron, manganese, and zinc.

The investigators studied the duration-dependent hepatoprotective effects of Propolis extract (200 mg/kg, orally) against carbon tetrachloride (CCl 4; 1.5 mL/kg, intraperitoneally)-induced liver damage in rats. Administration of CCl 4 caused a sharp elevation in the activity of serum transaminases and serum alkaline phosphatase.

A significant depletion in hepatically reduced glutathione was observed with significantly enhanced hepatic lipid peroxidation. After CCl 4 administration, glycogen contents and activities of alkaline phosphatase, adenosine triphosphatase, and succinic dehydrogenase were significantly decreased, whereas total protein contents and activity of acid phosphatase were increased in the liver and kidney.

Propolis extract reversed alterations in all parameters when administered within 6, 12, and 24 h of toxicant exposure. Propolis therapy produced duration-dependent protection, with maximal protection achieved at 24 h after CCl 4 exposure.

It is believed that Propolis in its natural form has general pharmacologic value and marked hepatoprotective potential because of its composition of minerals, flavonoids, and phenolic compounds.

## 2.8. Protective effects of Propolis on inorganic mercury induced oxidative stress in mice.

| | |
|---|---|
| Authors | Zhao JQ, Wen YF, Bhadauria M, Nirala SK, Sharma A, Shrivastava S, Shukla S, Agrawal OP, Mathur R |
| Link | Indian J Exp Biol. 2009 Apr;47(4):264-9. |
| Research Institute | College of Animal Science and Technology, Yunnan Agricultural University, Kunming 650 201, PR China |


### Abstract

Protective potential of Propolis was evaluated against mercury induced oxidative stress and antioxidant enzymatic alterations in mice liver.

Exposure to mercuric chloride (HgCl2; 5 mg/kg; ip) induced oxidative stress by increasing lipid peroxidation and oxidized glutathione level along with concomitant decrease in glutathione and various antioxidant enzymes. Mercury intoxication deviated the activity of liver marker enzymes in serum.

Conjoint treatment of Propolis (200 mg/kg; po) inhibited lipid peroxidation and oxidized glutathione level, whereas increased glutathione level. Activities of antioxidants enzymes, i.e., superoxide dismutase, catalase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase were also restored concomitantly towards control after propolis administration. Release of serum transaminases, alkaline phosphatase, lactate dehydrogenase and y-glutamyl transpeptidase were significantly restored towards control after propolis treatment.

Results suggest that propolis augments the antioxidants defense against mercury induced toxicity and provides evidence that it has therapeutic potential as hepatoprotective agent

## 2.9. Propolis Prevents Aluminum-Induced Genetic and Hepatic Damages in Rat Liver.


| | |
|---|---|
| Authors | Hasan Türkez[a], Mokhtar I. Yousef[b], and Fatime Geyikoglu[a] |
| Link | Food and Chemical Toxicology, Article in Press 2010, Received 28 April 2010; accepted 30 June 2010. Available online 14 July 2010. |
| Research Institute | [a] Department of Biology, Faculty of Sciences, Atatürk University, 25240 Erzurum, Turkey<br>[b] Department of Home Economic, Faculty of Specific Education, Alexandria University, 21529 Alexandria, Egypt |



### Abstract

Aluminium is present in several manufactured foods and medicines and is also used in water purification. Therefore, the present experiment was undertaken to determine the effectiveness of propolis in modulating the aluminium chloride ($AlCl_3$) induced genotoxicity and hepatotoxicity in liver of rats.

Animals were assigned to 1 of 4 groups: control; 34 mg $AlCl_3$/kg bw; 50 mg propolis/kg bw; $AlCl_3$ (34 mg/kg bw) plus propolis (50 mg/kg bw), respectively. Rats were orally administered their respective doses daily for 30 days. At the end of the experiment, rats were anesthetized and hepatocytes (HEP) were isolated for counting the number of micronucleated hepatocytes (MNHEPs). In addition, the levels of serum enzymes and histological alterations in liver were investigated. $AlCl_3$ caused a significant increase in MNHEPs, alkaline phosphatase, transaminases (AST and ALT) and lactate dehydrogenase (LDH). Furthermore, severe pathological damages such as: sinusoidal dilatation, congestion of central vein, lipid accumulation and lymphocyte infiltration were established in liver. On the contrary, treatment with propolis alone did not cause any adverse effect on above parameters.

Moreover, simultaneous treatments with propolis significantly modulated the toxic effects of $AlCl_3$. It can be concluded that propolis has beneficial influences and could be able to antagonize $AlCl_3$ toxicity.

## 2.10. Propolis Helps Protect Liver from Damage by Toxins: Protective Effects of Propolis on Female Rats' Histopathological, Biochemical and Genotoxic Changes During LPS Induced Endotoxemia


Authors: Züleyha Doğanyiğit[a], Fatma Öztürk Küp[b], Sibel Silici[c], Kemal Deniz[d], Birkan Yakan[a], Timucin Atayoglu[e]

Research Institute: [a] Erciyes University, Medical Faculty, Department of Histology and Embryology, Kayseri, Turkey; [b] Erciyes University, Science Faculty, Department of Biology, Kayseri, Turkey; [c] Erciyes University, Agriculture Faculty, Department of Agricultural Biotechnology, Kayseri, Turkey; [d] Erciyes University, Medical Faculty, Department of Pathology, Kayseri, Turkey; [e] American Hospital, Department of Family Medicine, Istanbul, Turkey



Link: Phytomedicine, Available online 27 February 2013



### Abstract

In recent years, propolis has been the object of extensive research for its antibacterial, antioxidant, anti-inflammatory, and antitumoral activities. This study aims to determine the hepatoprotective efficiency of propolis on experimental endotoxemia in rats.

In the current study, fifty adult Sprague Dawley rats (weighing 200–300 g) were randomly divided into five groups of ten rats each. Normal saline solution was administered to the rats in the control group, while in the second group LPS (30 mg/kg), in the third group propolis (250 mg/kg), in the fourth group first propolis and then LPS (30 mg/kg), and in the fifth group, first LPS (30 mg/kg) and then propolis were given. Six hours after the application, biochemical (MDA levels) and histopathological changes as well as global DNA methylation analysis in the liver tissue samples were determined, while in the blood tissue samples Genomic Template Stability (GTS, %) was evaluated using RAPD-PCR profiles.

The results demonstrated that the administration of propolis could have a protective effect against changes of both genomic stability values and methylation profiles, and it minimized the increase in MDA and tissue damage caused by LPS.

In conclusion, the application of propolis prior to LPS-induced endotoxemia has shown to reduce hepatic damage.

## 2.11. Propolis May Help Prevent Kidney Damage: Role of propolis (bee glue) in improving histopathological changes of the kidney of rat treated with aluminum chloride


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|---|---|
| Authors | Ayman EL-meghawry EL-kenawy[1,2], Hosam Eldin Hussein Osman[2,3],* and Maha Hasan Daghestani[4] |
| Link | Environmental Toxicology, Volume 29, Issue 9, pages 1000–1010, September 2014 |
| Research Institute | [1]Department of Molecular Biology, Genetic Engineering and Biotechnology Research Inst., Minufiya University, Egypt, [2]Department of pathology, College of medicine, Taif University, Kingdom of Saudi Arabia, [3]Department of Anatomy, Al Azhar University, Egypt, [4]Department of Zoology, King Saud University, Women Students-Medical Studies and Sciences Section, Kingdom of Saudi Arabia |



### Abstract

Humans are frequently exposed to aluminum from various food additives, therapeutic treatments and the environment, and it can be potentially toxic. This study is aimed to elucidate the protective effects of propolis against aluminum chloride (AlCl3)-induced histopathological and immunohistochemical changes in kidney tissues of rats.

Sixty Wistar Albino male rats (average weight 250–300 g) were divided into three equal groups. The first served as a negative control. The second received AlCl3 (34 mg/kg bw, 1/ 25 LD 50). The third were administered AlCl3 (34 mg/kg bw, 1/ 25 LD 50) plus propolis (50 mg/kg bw). Doses were given once daily via a gavage for 8 weeks every day.

The results showed that shrunken glomeruli, intraglomerular congestion, loss of apical microvilli, degeneration of mitochondria and widened rough endoplasmic reticulum were also observed in the Proximal Convoluted Tubules of these animals.

Treatment with propolis ameliorated the harmful effects of AlCl3; this was also proved histopathologically by the noticeable improvement in the renal tissues. There were also significant variations in the expressed of ki-67 and p53 proteins.

It can be concluded that propolis may be promising as a natural therapeutic agent in AlCl3-induced renal toxicity and oxidative stress in rat kidneys.

# 3. anti-viral

## 3.1. Antibacterial, antifungal and antiviral activity of Propolis of different geographic origin.

| | |
|---|---|
| Authors | Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. |
| Link | J Ethnopharmacol. 1999 Mar;64(3):235-40. |
| Research Institute | Institute of Microbiology, Bulgarian Academy of Sciences, Sofia. |


### Abstract

Propolis samples from different geographic origins were investigated for their antibacterial (against Staphylococcus aureus and Escherichia coli), antifungal (against Candida albicans) and antiviral (against Avian influenza virus) activities. All samples were active against the fungal and Gram-positive bacterial test strains, and most showed antiviral activity. The activities of all samples were similar in spite of the differences in their chemical composition. In samples from the temperate zone, flavonoids and esters of phenolic acids are known to be responsible for the above mentioned activities of bee glue; tropical samples did not contain such substances but showed similar activities. Obviously, in different samples, different substance combinations are essential for the biological activity of the bee glue. It seems that Propolis has general pharmacological value as a natural mixture and not as a source of new powerful antimicrobial, antifungal and antiviral compounds.

## 3.2. Anti-AIDS agents. 48.(1) Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from Brazilian Propolis.

| | |
|---|---|
| Authors | Ito J, Chang FR, Wang HK, Park YK, Ikegaki M, Kilgore N, Lee KH. |
| Link | J Nat Prod. 2001 Oct;64(10):1278-81. |
| Research Institute | Natural Products Laboratory, School of Pharmacy, University of North Carolina, NC 27599, USA. |


### Abstract

A new triterpenoid named melliferone (1), three known triterpenoids, moronic acid (2), anwuweizonic acid (3), and betulonic acid (4), and four known aromatic compounds (5-8) were isolated from Brazilian Propolis and tested for anti-HIV activity in H9 lymphocytes. Moronic acid (2) showed significant anti-HIV activity (EC(50) (0.1 microg/mL, TI >186) and was modified to develop more potent anti-AIDS agents.

## 3.3. The biochemistry and medical significance of the flavonoids.

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|---|---|
| Authors | Havsteen BH. |
| Link | Pharmacol Ther. 2002 Nov-Dec;96(2-3):67-202. |
| Research Institute | Department of Biochemistry, University of Kiel, Olshausenstrasse 40, D-24098, Kiel, Germany. |


### Abstract

Flavonoids are plant pigments that are synthesised from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light, and are ubiquitous to green plant cells. The flavonoids are used by botanists for taxonomical classification. They regulate plant growth by inhibition of the exocytosis of the auxin indolyl acetic acid, as well as by induction of gene expression, and they influence other biological cells in numerous ways. Flavonoids inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Yet, their toxicity to animal cells is low. Flavonoids are major functional components of many herbal and insect preparations for medical use, e.g., Propolis (bee's glue) and honey, which have been used since ancient times. The daily intake of flavonoids with normal food, especially fruit and vegetables, is 1-2 g. Modern authorised physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals.

## 3.4. Anti-influenza virus activity of propolis in vitro and its efficacy against influenza infection in mice.

| | |
|---|---|
| Authors | Shimizu T, Hino A, Tsutsumi A, Park YK, Watanabe W, Kurokawa M. |
| Link | http://www.naturezone.hk/2008_influenza.pdf |
| Research Institute | Department of Biochemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Nobeoka, Miyazaki, Japan. |


Abstract

Background: Propolis has been used worldwide as a dietary supplement to maintain and improve human health. We examined whether ethanol extracts of Brazilian propolis exhibit antiviral activity against influenza virus in vitro and in vivo.

Methods: Among 13 ethanol extracts screened in a plaque reduction assay, four showed anti-influenza virus activity. The anti-influenza efficacy of the four extracts was further examined in a murine influenza virus infection model. The mice were infected intranasally with influenza virus, and the four extracts were orally administered at 10 mg/kg three times daily for seven successive days after infection.

Results: In this infection model, only one extract, AF-08, was significantly effective at 10 mg/kg in reducing the body weight loss of infected mice. The doses of 2 and 10 mg/kg were also effective in prolonging the survival times of infected mice significantly, but 0.4 mg/kg was not. The anti-influenza efficacy of AF-08 at 10 mg/kg was confirmed in a dose-dependent manner in mice. AF-08 at 10 mg/kg significantly reduced virus yields in the bronchoalveolar lavage fluids of lungs in infected mice as compared with the control. The reduction of virus yields by AF-08 at 10 mg/kg significantly corresponded to those induced by oseltamivir at 1 mg/kg twice daily from day 1 to day 4 after infection.

Conclusion: The Brazilian propolis AF-08 was indicated to possess anti-influenza virus activity and to ameliorate influenza symptoms in mice. AF-08 may be a possible candidate for an anti-influenza dietary supplement for humans

## 3.5. Anti-poliovirus activity of *Baccharis dracunculifolia* and propolis by cell viability determination and real-time PCR.

| | |
|---|---|
| Authors | M C Bufalo, A S Figueiredo, J P B de Sousa, J M G Candeias, J K Bastos, and J M Sforcin. |
| Link | J Appl Microbiol. 2009 |
| Research Institute | Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, SP, Brazil. |


Abstract

Abstract Aims: The aim of this work was to evaluate the antiviral activities of *Baccharis dracunculifolia* (extract and essential oil), propolis and some isolated compounds (caffeic and cinnamic acids) against poliovirus type 1 (PV1) replication in HEp-2 cells.

Method: Three different protocols (pre-, simultaneous and post-treatments) were used to verify the effect of addition time of the variables on PV1 replication by crystal violet method and relative viral RNA quantification by real-time PCR for analysing in which step of virus replication the variables could interfere.

Conclusions: Data revealed that the *B. dracunculifolia* showed the best antiviral activity percentage in the simultaneous treatment, as well as lower relative viral quantification by real-time PCR.

Variables might block partially the viral entry within cells, affect the steps of viral cycle replication into cells, or lead to RNA degradation before the virus entry into cells or after their release to the supernatant.

Significance and Impact of the Study: *Baccharis dracunculifolia* is the most important botanical source of the south-eastern Brazilian propolis, and its potential for the development of new phytotherapeutic medicines has been investigated. Propolis is commonly used for its antimicrobial and immunomodulatory activities. Nevertheless, B. *dracunculifolia* and propolis effects on PV1 have not been investigated yet.

## 3.6. Antiviral Activity and Mode of Action of Propolis Extracts and Selected Compounds.

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| Authors | Paul Schnitzler, Annett Neuner, Silke Nolkemper, Christine Zundel, Hans Nowack, Karl Heinz Sensch, and Jurgen Reichling. |
| Link | Phytother Res. May 27, 2009 |
| Research Institute | Department of Virology, Hygiene Institute, University of Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. |


### Abstract

Aqueous and ethanol extracts of propolis were analysed phytochemically and examined for their antiviral activity in vitro. Different polyphenols, flavonoids and phenylcarboxylic acids were identified as major constituents. The antiviral effect of propolis extracts and selected constituents, e.g. caffeic acid (1), p-coumaric acid (2), benzoic acid (3), galangin (4), pinocembrin (5) and chrysin (6) against herpes simplex virus type 1 (HSV-1) was analysed in cell culture. The 50% inhibitory concentration (IC(50)) of aqueous and ethanol propolis extracts for HSV-1 plaque formation was determined at 0.0004% and 0.000035%, respectively.

Both propolis extracts exhibited high levels of antiviral activity against HSV-1 in viral suspension tests, plaque formation was significantly reduced by >98%. In order to determine the mode of antiviral action of propolis, the extracts were added at different times during the viral infection cycle.

Both propolis extracts exhibited high anti-HSV-1 activity when the viruses were pretreated with these drugs prior to infection. Among the analysed compounds, only galangin and chrysin displayed some antiviral activity. However, the extracts containing many different components exhibited significantly higher antiherpetic effects as well as higher selectivity indices than single isolated constituents.

Propolis extracts might be suitable for topical application against herpes infection.

## 3.7. Caffeoylquinic Acids Are Major Constituents with Potent Anti-Influenza Effects in Brazilian Green Propolis Water Extract.


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| Authors | Tomohiko Urushisaki[1], Tomoaki Takemura[1], Shigemi Tazawa[1], Mayuko Fukuoka[2,3], Junji Hosokawa-Muto[4], Yoko Araki[1], and Kazuo Kuwata[2,3,4] |
| Link | Evidence-Based Complementary and Alternative Medicine Volume 2011 (2011), Article ID 254914, 7 pagesdoi:10.1155/2011/254914 |
| Research Institute | [1]Nagaragawa Research Center, API Co., Ltd., 692-3 Nagara, Yamasaki, Gifu 502-0071, Japan<br>[2]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan<br>[3]CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan<br>[4]Center for Emerging Infectious Diseases, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan |



### Abstract

Influenza A viral infections reached pandemic levels in 1918, 1957, 1968, and, most recently, in 2009 with the emergence of the swine-origin H1N1 influenza virus. The development of novel therapeutics or prophylactics for influenza virus infection is urgently needed.

We examined the evaluation of the anti-influenza virus (A/WSN/33 (H1N1)) activity of Brazilian Green Propolis water extract (PWE) and its constituents by cell viability and real-time PCR assays.

Our findings showed strong evidence that PWE has an anti-influenza effect and demonstrate that caffeoylquinic acids are the active anti-influenza components of PWE.

Furthermore, we have found that the amount of viral RNA per cell remained unchanged even in the presence of PWE, suggesting that PWE has no direct impact on the influenza virus but may have a cytoprotective activity by affecting internal cellular process.

These findings indicate that caffeoylquinic acids are the active anti-influenza components of PWE. Above findings might facilitate the prophylactic application of natural products and the realization of novel anti-influenza drugs based on caffeoylquinic acids, as well as further the understanding of cytoprotective intracellular mechanisms in influenza virus-infected cells.

# 4. anti-bacterial

## 4.1. Activity against Paenibacillus sp. In vitro study of the antimicrobial activity of Brazilian Propolis against Paenibacillus larvae.

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|---|---|
| Authors | Esther Margarida A F Bastos, Michael Simone, Daniela Macedo Jorge, Ademilson Espencer Egea Soares, and Marla Spivak. |
| Link | *J Invertebr Pathol.* 2007. [MEDLINE Citation] |
| Research Institute | Research of Diretoria de Pesquisa, Fundação Ezequiel Dias, R. Conde Pereira carneiro 80, B. Gameleira, 30510-010, Belo Horizonte, Minas Gerais, Brazil. |


### Abstract

The honey bee disease American foulbrood (AFB) is a serious problem since its causative agent (Paenibacillus larvae) has become increasingly resistant to conventional antibiotics. The objective of this study was to investigate the in vitro activity of Propolis collected from various states of Brazil against P. larvae. Propolis is derived from plant resins collected by honey bees (Apis mellifera) and is globally known for its antimicrobial properties and particularly valued in tropical regions. Tests on the activity of Propolis against P. larvae were conducted both in Brazil and Minnesota, USA using two resistance assay methods that measured zones of growth inhibition due to treatment exposure. The Propolis extracts from the various states of Brazil showed significant inhibition of P. larvae. Clear dose responses were found for individual Propolis extracts, particularly between the concentrations of 1.7 and 0.12mg Propolis/treatment disk, but the source of the Propolis, rather than the concentration, may be more influential in determining overall activity. Two of the three tested antibiotics (tylosin and terramycin) exhibited a greater level of inhibition compared to most of the Brazilian samples, which could be due to the low concentrations of active compounds present in the Propolis extracts. Additionally, the majority of the Brazilian Propolis samples were more effective than the few collected in MN, USA. Due to the evolution of resistance of P. larvae to conventional antibiotic treatments, this research is an important first step in identifying possible new active compounds to treat AFB in honey bee colonies.

## 4.2. Antibacterial activity of Propolis against MRSA and synergism with topical mupirocin.


| | |
|---|---|
| Authors | Y Onlen, N Duran, E Atik, L Savas, E Altug, S Yakan, and O Aslantas. |
| Link | *J Altern Complement Med.* 2007; 13: 713. [MEDLINE Citation] |
| Research Institute | Department of Infectious Diseases and Clinical Microbiology, Mustafa Kemal University Faculty of Medicine, Hatay, Turkey. |



### Abstract

OBJECTIVES: The aim of the present study was to investigate the activity of the Propolis and its combinations with mupirocin against methicillin-resistant Staphylococcus aureus (MRSA) in nasal carriage.

METHODS: This study was carried out between June and August 2005. To infect nares of the rabbits, MRSA (ATCC 33591) strain was used. Minimum inhibitory concentration was determined according to National Committee for Clinical Laboratory Standards. Each inoculum was prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard (10(5) colony-forming units [cfu]/mL) and diluted 1:100 for the broth microdilution procedure. Ten microliters (10 microL) (10(5) cfu/mL) of the bacterial suspension containing approximately 1000 cfu of MRSA was administered with sterile microsyringe through both nostrils of each rabbit. Ninety-six (96) hours after inoculation, the presence of infection was confirmed by using bacterial cultures. Twenty-six young New Zealand rabbits were randomly divided into 4 groups. Each treatment group (1, 2, and 3) included 7 rabbits and control group (group 4) included 5 rabbits. Group 1 was treated with topical mupirocin + ethanolic extract of Propolis drops, group 2 received topical mupirocin, group 3 was administered ethanolic extract of Propolis drops, and the control group (group 4) was only treated with phosphate-buffered solution drops for 7 days. At the end of study, nasal cultures and smears were obtained for bacterial count and cytologic examination.

RESULTS: The colony numbers of bacteria in group 1 were determined to be significantly lower than in group 2 ($p = 0.0001$), group 3 ($p = 0.0001$), and group 4 ($p = 0.0001$). The mean bacterial cell counts of groups 1-4 were 360.2 +/- 52.4 cfu/mL, 4120.6 +/- 860.4 cfu/mL, 5980.8 +/- 1240.6 cfu/mL, and 11500.0 +/- 2568.4 cfu/mL, respectively. Mupirocin + Propolis administration (group 1) resulted in a significant reduction in the polymorphonuclear leukocyte (PMNL) count in the mucous membranes of rabbits compared with the other treatment groups ($p < 0.05$).

CONCLUSIONS: Propolis addition to mupirocin regimen was found to result in more profound reduction in bacterial cell count and inflammatory response compared with the rest of the treatment modalities.

## 4.3. Propolis reduces bacterial translocation and intestinal villus atrophy in experimental obstructive jaundice.

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| Authors | MZ Sabuncuoglu, K Kismet, SS Kilicoglu, B Kilicoglu, S Erel, S Muratoglu, AE Sunay, E Erdemli, and MA Akkus. |
| Link | *World J Gastroenterol.* 2007; 13: 5226. [MEDLINE Citation] |
| Research Institute | 4th General Surgery Department, Ankara Training and Research Hospital, S B Ankara Egitim ve Arastirma Hastanesi 4th, Cerrahi Klinigi 06340, Ulucanlar, Ankara, Turkey. |


### Abstract

AIM: To investigate the effects of Propolis on bacterial translocation and ultrastructure of intestinal morphology in experimental obstructive jaundice.

METHODS: Thirty Wistar-Albino male rats were randomly divided into three groups, each including 10 animals: group I, sham-operated; group II, ligation and division of the common bile duct (BDL); group III, BDL followed by oral supplementation of Propolis 100 mg/kg per day. Liver, blood, spleen, mesenteric lymph nodes, and ileal samples were taken for microbiological, light and transmission electron microscopic examination on postoperative 7th d after sacrification.

RESULTS: The mean number of villi per centimeter and mean mucosal height of the Propolis group were significantly different in the BDL group (P = 0.001 and 0.012, respectively). The electron microscopic changes were also different between these groups. Sham and BDL + Propolis groups had similar incidence of bacterial translocation (BT). The BDL group had significantly higher rates of BT as compared with sham and BDL + Propolis groups. BT was predominantly detected in MLNs and the most commonly isolated bacteria was Escherichia coli.

CONCLUSION: Propolis showed a significant protective effect on ileal mucosa and reduced bacterial translocation in the experimental obstructive jaundice model. Further studies should be carried out to explain the mechanisms of these effects.

## 4.4. Brazilian Green Propolis on Helicobacter pylori Infection. A Pilot Clinical Study.


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| Authors | LG Vaz Coelho, EM Ferreira Bastos, CC Resende, CM Paula E Silva, BS Fernandes Sanches, FJ de Castro, LD Moretzsohn, WL Dos Santos Vieira, and OR Trindade. |
| Link | *Helicobacter*. 2007; 12: 572. [MEDLINE Citation] |
| Research Institute | Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Fedral de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. lcoelho@gold.com.br. |



### Abstract

Recent in vitro studies suggest that Propolis and some of its phenolic components are able to inhibit Helicobacter pylori growth. To date, there are no clinical studies.

AIMS: To evaluate the effect of Brazilian Green Propolis on H. pylori-infected individuals.

PATIENTS AND METHODS: Eighteen (11 females, 7 males, mean age 47 years) participants were included. Before treatment, all participants were submitted to gastroscopy, and H. pylori infection was confirmed by histology, urease test, and (13)C-urea breath test (UBT). Participants with UBT showing a delta over baseline (DOB) value higher than 4 per thousand were considered positive for H. pylori infection. Twenty drops from an alcoholic preparation of Brazilian Green Propolis were administered three times a day for 7 days. Clinical evaluation and UBT were performed at 1-3 days and at 40 days after the end of therapy to evaluate H. pylori suppression or eradication, respectively.

RESULTS: All participants took all medication and completed the study. Eighty-three percent of the subjects did not succeed in suppressing or eradicating H. pylori. Two participants reached partial suppression after treatment, but became positive again at UBT performed 40 days after treatment. Another participant presented negative at UBT 40 days after treatment, not confirmed by a second UBT performed 100 days after treatment.

CONCLUSIONS: Brazilian Green Propolis used in popular dose showed minimal effect on H. pylori infection. Larger studies with longer duration, larger dose, and different frequency of administration of Propolis extract should be undertaken to define its role on H. pylori therapy.

## 4.5. Antimicrobial activity of the extract and isolated compounds from *Baccharis dracunculifolia* D. C. (Asteraceae).

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|---|---|
| Authors | AA da Silva Filho, JP de Sousa, S Soares, NA Furtado, ML Andrade e Silva, WR Cunha, LE Gregorio, NP Nanayakkara, and JK Bastos. |
| Link | http://highwire.stanford.edu/cgi/medline/pmid;18386486 Z Naturforsch [C], January 1, 2008; 63(1-2): 40-6. |
| Research Institute | Laboratório de Química de Produtos Naturais, Universidade de Franca, Av. Armando Sales de Oliveira, 201, CEP 14404-600, Franca, SP, Brazil. ademar@unifran.br |


### Abstract

*Baccharis dracunculifolia* D.C. (Asteraceae) is the most important plant source of the Brazilian Green Propolis. Since Propolis is known for its antimicrobial activity, the aim of this work was to evaluate the antimicrobial activities of *Baccharis dracunculifolia* and some of its isolated compounds. The results showed that the leaves extract of *Baccharis dracunculifolia* presents antifungal and antibacterial activities, especially against Candida krusei and Cryptococcus neoformans, for which the *Baccharis dracunculifolia* showed IC50 values of 65 microg mL(-1) and 40 microg mL(-1), respectively. In comparison to the *Baccharis dracunculifolia*, it was observed that the Green Propolis extract (GPE) showed better antimicrobial activity, displaying an IC50 value of 9 microg mL(-1) against C. krusei. Also, a phytochemical study of the *Baccharis dracunculifolia* was carried out, affording the isolation of ursolic acid (1), 2a-hydroxy-ursolic acid (2), isosakuranetin (3), aromadendrin-4'-methylether (4), baccharin (5), viscidone (6), hautriwaic acid lactone (7), and the clerodane diterpene 8. This is the first time that the presence of compounds 1, 2, and 8 in *Baccharis dracunculifolia* has been reported. Among the isolated compounds, 1 and 2 showed antibacterial activity against methicillin-resistant Staphylococcus aureus, displaying IC50 values of 5 microg mL(-1) and 3 microg mL(-1), respectively. 3 was active against C. neoformans, showing an IC50 value of 15 microg mL(-1) and a MIC value of 40 microg mL(-1), while compounds 4-8 were inactive against all tested microorganisms. The results showed that the *Baccharis dracunculifolia*, similar to the GPE, displays antimicrobial activity, which may be related to the effect of several compounds present in the crude extract.

## 4.6. Propolis Equals Traditional Treatments for Oral Infection: Antimicrobial Effects of Calcium Hydroxide, Chlorhexidine, and Propolis on Enterococcus faecalis and Candida albicans


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| Authors | Jeison B. Carbajal Mejía* |
| Link | Journal of Investigative and Clinical Dentistry<br>Early View (Online Version of Record published before inclusion in an issue), Article first published online: 4 APR 2013 |
| Research Institute | Department of Endodontics, Faculty of Dentistry, Daniel Alcides Carrión National University, Pasco, Peru |



### Abstract

**Aim:** To evaluate the efficacy of calcium hydroxide (Ca[OH]2), 2% chlorhexidine (CHX) gel, and propolis against both Enterococcus faecalis (E. faecalis) and Candida albicans (C. albicans) using infected dentine models at two different depths (100 and 200 μm) after 14 days of application.

**Methods:** A total of 120 roots of extracted single-rooted human teeth were chemo-mechanically prepared and sterilized. Sixty roots were infected with E. faecalis, and the remaining 60 with C. albicans. Each group was divided into four subgroups (n = 15) to apply intracanal medicaments, namely saline solution (negative control), Ca(OH)2, CHX, and propolis during the 14 days. Dentine shavings were collected and cultivated. Colony-forming units (c.f.u.) were registered. Statistical analysis was done using the Kruskal–Wallis test, followed by Dunn's/Bonferroni multiple comparison test ($P < 0.05$).

**Results:** All experimental agents significantly reduced E. faecalis c.f.u. There was no significant difference between CHX and propolis reducing E. faecalis c.f.u. at 100 and 200 μm. Only CHX had a statistically-significant antifungal efficacy in the C. albicans group at the two depths assessed. CHX was the most potent medicament against both E. faecalis and C. albicans, and Ca(OH)2 was the least.

**Conclusion:** Both CHX and propolis were the most effective against E. faecalis, whereas only CHX had the highest antifungal activity on C. albicans in dentine of extracted teeth.

## 4.7. Propolis Beats Tradition Periodontal Drug: Microparticles Containing Propolis and Metronidazole: in vitro Characterization, Release Study and Antimicrobial Activity Against Periodontal Pathogens


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|---|---|
| Authors | Sabrina Barbosa de Souza Ferreira[1], Bruno Rafael de Assis Dias[1], Clarissa Silva Obregón[1], Carla Carolina Gomes[1], Raphaela Regina de Araújo Pereira[1,2], Janine Silva Ribeiro Godoy[3], Terezinha Inez Estivalet Svidzinski[3], and Marcos Luciano Bruschi[1,2] |
| Link | Pharm Dev Technol, 2013 Jan 28 |
| Research Institute | [1]Department of Pharmacy,<br>[4]Post-Graduate Program in Pharmaceutical Sciences, and<br>[3]Department of Clinical Analysis and Biomedicine, State University of Maringa, Maringa, Parana, Brazil |



### Abstract

Ethylcellulose microparticles containing metronidazole and propolis extractive solution were prepared and evaluated in vitro against periodontal pathogens. Scanning electron microscopy, particle size analysis, drug entrapment efficiency and drug release of microparticles were determined. The antimicrobial activity of microparticles was evaluated against microorganisms of periodontal importance (Enterococcus faecalis, Streptococcus pyogenes, Streptococcus mutans, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli). It was obtained particles with regular morphology, mean diameter of 1.23 µm, and entrapment efficiency for propolis and metronidazole were 91.41% and 22.23%, respectively. In vitro release studies of propolis and metronidazole from microparticles showed prolonged drug release and controlled by Fickian diffusion.

Both propolis and metronidazole displayed activity against the tested strains. Moreover, the results showed that the strains of E. faecalis, S. pyogenes and S. mutans were more susceptible to the propolis and E. faecalis to the metronidazole. It was also observed that the amount of metronidazole to inhibit the microorganism strains in the physical mixture with propolis was smaller than in the metronidazole alone, suggesting potentiation effect between propolis and metronidazole.

These microparticles would be useful for developing intermediary or eventual dosage form to be administered into the periodontal pocket more easily and safely.

## 4.8. Gelatin-Based Propolis Films Kept Antimicrobial Activity for 177 Days: Properties of Gelatin-Based Films with Added Ethanol – Propolis Extract


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| Authors | R.B. Bodini, P.J.A. Sobral, C.S. Favaro-Trindade, R.A. Carvalho |
| Link | LWT - Food Science and Technology, Volume 51, Issue 1, April 2013, Pages 104–110 |
| Research Institute | Food Engineering Department, ZEA-FZEA, Caixa Postal 23, Universidade de São Paulo, USP, CEP 13635-900 Pirassununga, SP, Brazil |



### Abstract

Considering the possibility of using propolis as a natural bioactive compound, and the growing interest in active and biodegradable packaging materials, gelatin-based films plasticized with sorbitol and added of ethanol–propolis extract (EPE) were produced. Four different concentrations of EPE (0, 5, 40 or 200 g/100 g of gelatin) were analyzed. The effect of concentrations of EPE were evaluated on: mechanical properties, solubility, moisture content, water vapor permeability, scanning electron microscopy and infrared spectroscopy characteristics, stability of polyphenol concentrations, and antimicrobial activity against Staphylococcus aureus.

EPE incorporation to the films promoted reduction in rupture tension and water vapor permeability, besides other microstructural changes, when compared with the control films (0 g of EPE/100 g of gelatin).

Activity against S. aureus was observed in films with 40 and 200 g of EPE/100 g of gelatin. These films kept their antimicrobial activity and polyphenol concentration for 177 days of storage.

# 5. anti-fungal

## 5.1. Antibacterial, antifungal and antiviral activity of Propolis of different geographic origin.

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|---|---|
| Authors | Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. |
| Link | J Ethnopharmacol. 1999 Mar;64(3):235-40. |
| Research Institute | Institute of Microbiology, Bulgarian Academy of Sciences, Sofia. |


### Abstract

Propolis samples from different geographic origins were investigated for their antibacterial (against Staphylococcus aureus and Escherichia coli), antifungal (against Candida albicans) and antiviral (against Avian influenza virus) activities. All samples were active against the fungal and Gram-positive bacterial test strains, and most showed antiviral activity. The activities of all samples were similar in spite of the differences in their chemical composition. In samples from the temperate zone, flavonoids and esters of phenolic acids are known to be responsible for the above mentioned activities of bee glue; tropical samples did not contain such substances but showed similar activities. Obviously, in different samples, different substance combinations are essential for the biological activity of the bee glue. It seems that Propolis has general pharmacological value as a natural mixture and not as a source of new powerful antimicrobial, antifungal and antiviral compounds.

## 5.2. Antifungal activity evaluation of different Propolis extracts.


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| Authors | Renata Longhini[1], Sheila M. Raksa[1], Ana Carla P. Oliveira[2], Terezinha I. E. Svidzinski[2], Selma L. Franco[1]. |
| Link | Mem Inst Oswaldo Cruz. 2006 Aug;101(5):493-7. |
| Research Institute | [1] Laboratório de Farmacotécnica, Departamento de Farmácia e Farmacologia, Universidade Estadual deMaringá, Av. Colombo 5790, 87020-900, Maringá, PR, Brasil,<br>2 Laboratório de Micologia, Departamento de Análises Clínicas, Universidade Estadual de Maringá, Av. Colombo5790, 87020-900, Maringá, PR, Brasil. |



### Abstract

Propolis is a resin collected by the bees Apis mellifera L., which contains several substances including the flavonoids. Due to a diversified chemical composition Propolis presents some pharmacological actions, being distinguished the anti-inflammatory, healing, anti-tumoral and antimicrobial properties and, in particular, its antifungal action.

This action has been tested against yeasts obtained from onychomycosis, which are infections of difficult and long treatment and they can manifest intolerable effects on the patient. The Propolis appears as an efficient therapy option, as it has low toxicity and should be of dermal use. The aim of the present study was to evaluate the optimization of the Propolis extractive process through physiochemical parameters and antifungal activity demonstration.

## 5.3. Antifungal Activity of Brazilian Propolis Microparticles against Yeasts Isolated from Vulvovaginal Candidiasis.


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|---|---|
| Authors | Kelen Fátima Dalben Dota[1], Marcia Edilaine Lopes Consolaro[1], Terezinha Inez Estivalet Svidzinski[1] and Marcos Luciano Bruschi[2]. |
| Link | Evid. Based Complement. Altern. Med. published 12 April 2010, 10.1093/ecam/neq029 |
| Research Institute | [1] Graduate Program of Health Sciences and [2] Department of Pharmacy, State University of Maringa, Colombo Avenue, 5790, CEP 87020-900, Maringa, Parana, Brazil |



### Abstract

Propolis, a resinous compound produced by Apis mellifera L. bees, is known to possess a variety of biological activities and is applied in the therapy of various infectious diseases. The aim of this study was to evaluate the in vitro antifungal activity of propolis ethanol extract (PE) and propolis microparticles (PMs) obtained from a sample of Brazilian propolis against clinical yeast isolates of importance in the vulvovaginal candidiasis (VVC).

PE was used to prepare the microparticles. Yeast isolates (n=89), obtained from vaginal exudates of patients with VVC, were exposed to the PE and the PMs. Moreover, the main antifungal drugs used in the treatment of VVC (Fluconazole, Voriconazole, Itraconazole, Ketoconazole, Miconazole and Amphotericin B) were also tested. Minimum inhibitory concentration (MIC) was determined according to the standard broth microdilution method.

Some Candida albicans isolates showed resistance or dose-dependent susceptibility for the azolic drugs and Amphotericin B. Non-C. albicans isolates showed more resistance and dose-dependent susceptibility for the azolic drugs than C. albicans. However, all of them were sensitive or dose-dependent susceptible for Amphotericin B.

All yeasts were inhibited by PE and PMs, with small variation, independent of the species of yeast. The overall results provided important information for the potential application of PMs in the therapy of VVC and the possible prevention of the occurrence of new symptomatic episodes.

## 5.4. Edible Vegetable Oil Extract of Propolis Shows Antifungal Activity


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| Authors | Daiane Finger[1], Christiane Schinieder Machado[1], Yohandra Reyes Torres[1],*, Sueli Percio Quináia[1], Amanda Cristina Godot Thomaz[2], Angélica Rita Gobbo[2], Marta Chagas Monteiro[2], Antonio Gilberto Ferreira[3], Alexandra Christine Helena Frankland Sawaya[4], Marcos Nogueira Eberlin[5] |



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| Link | Journal of Food Quality. Volume 36, Issue 5, pages 291 – 301, October 2013 |



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### Abstract

The study aimed to evaluate the antifungal potential of an extract of propolis obtained with edible vegetable oil (ODEP) and to identify antifungal compounds in this extract. Propolis sample was extracted with canola oil. After filtration, the oily liquid extract was submitted to solvent partition and dried to obtain ODEP, which was tested in vitro against Candida albicans strains. ODEP was fractioned on Sephadex and by high-performance liquid chromatography (HPLC). Active fractions and/or a pure compound were analyzed by nuclear magnetic resonance, liquid chromatographic mass spectrometry (LC – MS) and liquid chromatography-tandem mass spectrometry (LC – MS/MS) to characterize their chemical composition. Bioassay-guided fractionation allowed the isolation of dihydrokaempferide, which was also quantified in ODEP by HPLC together with the total flavonoid content. Kaempferide and isosakuranetin were identified by LC – MS and LC – MS/MS in fractions with a potential activity against C. albicans. In vitro assays showed that some fractions from ODEP produced better inhibition of C. albicans than the propolis extract itself.

## 5.5. Propolis Can Be Used as Natural Antifungal Agent: Inhibitory Effect of Propolis on Patulin Production of Penicillium expansum in Apple Juice


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| Authors | Sibel Silici*, Kevser Karaman |
| Link | Journal of Food Processing and Preservation - Early View (Online Version of Record published before inclusion in an issue) |
| Research Institute | Agricultural Biotechnology, Agricultural Faculty, Erciyes University, Kayseri, Turkey |



### Abstract

In this research, reducing activity of Turkish propolis on patulin production of Penicillium expansum in apple juice was investigated. To compare the antifungal activity of propolis, sodium benzoate was also used as a positive control. Different concentrations of propolis (0.1, 1 and 2 mg/mL) and sodium benzoate (0.35 mg/mL) were added into apple juice after P. expansum inoculation. Apple juice samples were then mixed with propolis, and patulin content was determined in 1st, 24th and 48th hours. High-performance liquid chromatography equipped with diode array detector which is a reliable equipment for the determination of patulin in apple juice, were used after clean-up procedure. Considerable patulin reduction was achieved with the addition of propolis in the apple juice. The best result was obtained at 2 mg/mL propolis added group for 48 h incubation. It was concluded that propolis can be used as a natural antifungal agent for P. expansum inhibition instead of chemical preservatives.

Practical Application: Propolis is a natural honeybee product, and it is rich in functional bioactive compounds. It can be used to inhibit the patulin-producing microorganism. The inhibitory effect of propolis in the apple juice showed that it is an effective natural inhibitor.

## Practical Applications

Aqueous or ethanolic extracts of propolis (EEP) are widely used in alternative homemade medicine products mainly because of their antimicrobial and healing properties. Edible vegetal oils are known to extract bioactive compounds from propolis. The resulting extract (ODEP) has several advantages over the common EEP, such as the possibility of new pharmaceutical presentations for topical or internal applications. Because of the absence of ethanol in its formulation, edible ODEP are expected to be more tolerated. In this study, ODEP was active in vitro against Candida albicans strains. This supports the use of ODEP as an alternative to EEP and also its potential for topical application in combination with antifungal drugs. In addition, the ingestion of ODEP (e.g., contained in gelatin capsules), which have a high percentage of flavonoids, could be of nutritional value, helping to reach the recommended daily amounts of flavonoids and phenolic compounds.

# 6. anti-oxidant

## 6.1. Efficient radical scavenging ability of Artepillin C, a major component of Brazilian Propolis, and the mechanism.

| | |
|---|---|
| Authors | Nakanishi I, Uto Y, Ohkubo K, Miyazaki K, Yakumaru H, Urano S, Okuda H, Ueda J, Ozawa T, Fukuhara K, Fukuzumi S, Nagasawa H, Hori H, Ikota N |
| Link | Org Biomol Chem. 2003 May 7;1(9):1452-4. |
| Research Institute | Redox Regulation Research Group, Research Center for Radiation Safety, National Institute of Radiological Sciences, Inage-ku, Chiba 263-8555, Japan . nakanis@nirs.go.jp |


### Abstract

Hydrogen transfer from Artepillin C to cumylperoxyl radical proceeds via one-step hydrogen atom transfer rather than via electron transfer, the rate constant of which is comparable to that of (+)-catechin, indicating that Artepillin C can act as an efficient antioxidant.

## 6.2. Antioxidative bioavailability of Artepillin C in Brazilian Propolis.

| | |
|---|---|
| Authors | Shimizu K, Ashida H, Matsuura Y, Kanazawa K |
| Link | Arch Biochem Biophys. 2004 Apr 15;424(2):181-8. |
| Research Institute | Department of Life Science, Graduate School of Science and Technology, Kobe University, Rokkodai, Nada-ku, Kobe 657-8501, Japan |


### Abstract

Propolis has strong antioxidative activity. We investigated here whether this activity was available in intestinal Caco-2 and hepatic HepG2 cells. Phenolics in Brazilian Propolis, extracted with ethyl acetate after the removal of resin and wax with 90% methanol, included Artepillin C at 21 mmol/100 g, p-coumaric acid and cinnamic acid relatives 24mmol, kaempferol and its derivatives 9.4 mmol, naringenin 2.8 mmol, isosakuranetin 0.9 mmol, chrysin at 0.8 mmol/100 g, and several minor components. When the extract was added to the apical side of Caco-2 monolayers, Artepillin C was specifically incorporated into the cells and released to the basolateral side mostly without conjugation. Then, Artepillin C was added to HepG2 cells and exposed to reactive oxygens. Artepillin C prevented oxidative damage dose-dependently, and suppressed lipid peroxidation evaluated with thiobarbituric acid reactive substances by 16% and the formation of 8-hydroxy-2'-deoxyguanosine in DNA by 36% at a concentration of 20microM. Artepillin C is a bioavailable antioxidant.

## 6.3. Effect of Brazlian Green Propolis on the production of reactive oxygen species by stimulated neutrophils.

| | |
|---|---|
| Authors | Simoes LM, Gregorio LE, Da Silva Filho AA, de Souza ML, Azzolini AE,Bastos JK, Lucisano-Valim YM. |
| Link | J Ethnopharmacol. 2004 Sep;94(1):59-65. |
| Research Institute | Departamento de Fisica e Quimica, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto-USP. Avenida do Cafe s/no, Monte Alegre, 14040-903, SP, Brazil. |


### Abstract

The activity of a crude ethanol extract of green Propolis and its fractions obtained by partition with hexane, chloroform and n-butanol was assessed on luminol- and lucigenin- enhanced chemiluminescence (CL) produced by rabbit neutrophils (PMNs) stimulated with particles of serum-opsonized zymosan (OZ). The total production of reactive oxygen species (ROS) by PMNs was measured by the luminol-enhanced CL (LumCL) assay and the production of the superoxide anion (O2*-) by the lucigenin-enhanced CL (LucCL) assay. All evaluated Propolis samples had inhibitory effect on the LumCL and LucCL, which was concentration dependent. The n-butanol and chloroform fractions displayed the highest inhibitory effect on the LumCL produced by PMNs stimulated with OZ, in comparison with both the ethanol extract and the hexane fraction. Besides, the hexane fraction was the one which presented the highest effect for the LucCL assay. Some isolated compounds from both n-butanol and chloroform fractions were also assessed, including kaempferide, isosakuranetin, aromadendrine-4'-methyl-ether and 3-prenyl-p-coumaric acid. Kaempferide presented the highest inhibitory effect on the LumCL in comparison with the other compounds. Moreover, under the conditions assessed, the studied green Propolis samples and isolated compounds were not toxic to the rabbit PMNs.

## 6.4. Brazilian Green Propolis protects against retinal damage in vitro and in vivo.

| | |
|---|---|
| Authors | Inokuchi Y, Shimazawa M, Nakajima Y, Suemori S, Mishima S, Hara H. |
| Link | Evid Based Complement Alternat Med. 2006 Mar;3(1):71-7. |
| Research Institute | Department of Biofunctional Molecules, Gifu Pharmaceutical University, Mitahora-Higashi, Japan. |


### Abstract

Propolis, a honeybee product, has gained popularity as a food and alternative medicine. Its constituents have been shown to exert pharmacological (anticancer, antimicrobial and anti-inflammatory) effects. We investigated whether Brazilian green propolis exerts neuroprotective effects in the retina in vitro and/or in vivo. In vitro, retinal damage was induced by 24 h hydrogen peroxide (H2O2) exposure, and cell viability was measured by Hoechst 33342 and YO-PRO-1 staining or by a resazurin-reduction assay. Propolis inhibited the neurotoxicity and apoptosis induced in cultured retinal ganglion cells (RGC-5, a rat ganglion cell line transformed using E1A virus) by 24 h H2O2 exposure. Propolis also inhibited the neurotoxicity induced in RGC-5 cultures by staurosporine. Regarding the possible underlying mechanism, in pig retina homogenates propolis protected against oxidative stress (lipid peroxidation), as also did trolox (water-soluble vitamin E). In mice in vivo, propolis (100 mg kg(-1); intraperitoneally administered four times) reduced the retinal damage (decrease in retinal ganglion cells and in thickness of inner plexiform layer) induced by intravitreal in vivo N-methyl-d-aspartate injection. These findings indicate that Brazilian green propolis has neuroprotective effects against retinal damage both in vitro and in vivo, and that a propolis-induced inhibition of oxidative stress may be partly responsible for these neuroprotective effects.

## 6.5. Brazlian Green Propolis protects against retinal damage in vitro and in vivo.

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|---|---|
| Authors | Inokuchi Y, Shimazawa M, Nakajima Y, Suemori S, Mishima S, Hara H. |
| Link | Evid Based Complement Alternat Med. 2006 Mar;3(1):71-7. |
| Research Institute | Department of Biofunctional Molecules, Gifu Pharmaceutical University, Mitahora-Higashi, Japan. |


### Abstract

Propolis, a honeybee product, has gained popularity as a food and alternative medicine. Its constituents have been shown to exert pharmacological (anticancer, antimicrobial and anti-inflammatory) effects. We investigated whether Brazlian Green Propolis exerts neuroprotective effects in the retina in vitro and/or in vivo. In vitro, retinal damage was induced by 24 h hydrogen peroxide (H2O2) exposure, and cell viability was measured by Hoechst 33342 and YO-PRO-1 staining or by a resazurin-reduction assay. Propolis inhibited the neurotoxicity and apoptosis induced in cultured retinal ganglion cells (RGC-5, a rat ganglion cell line transformed using E1A virus) by 24 h H2O2 exposure. Propolis also inhibited the neurotoxicity induced in RGC-5 cultures by staurosporine. Regarding the possible underlying mechanism, in pig retina homogenates Propolis protected against oxidative stress (lipid peroxidation), as also did trolox (water-soluble vitamin E). In mice in vivo, Propolis (100 mg kg(-1); intraperitoneally administered four times) reduced the retinal damage (decrease in retinal ganglion cells and in thickness of inner plexiform layer) induced by intravitreal in vivo N-methyl-d-aspartate injection. These findings indicate that Brazlian Green Propolis has neuroprotective effects against retinal damage both in vitro and in vivo, and that a Propolis-induced inhibition of oxidative stress may be partly responsible for these neuroprotective effects.

## 6.6. Spray-dried Propolis extract. I: physicochemical and antioxidant properties.

| | |
|---|---|
| Authors | Marquele FD, Stracieri KM, Fonseca MJ, Freitas LA. |
| Link | Pharmazie. 2006 Apr;61(4):325-30. |
| Research Institute | Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Brazil. |


### Abstract

The effect of spray drying on the chemical and biological properties of alcoholic extract of green Propolis was investigated. The total polyphenol and flavonoid contents in spray-dried Propolis extract were determined by the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. The antioxidant activity of the dry extract was assessed by the membrane lipid peroxidation inhibition method, using quercetin as reference. The polyphenol content was shown to depend on the drying air outlet temperature and its square at the 0.5% significance level, while the flavonoid content depended only on the square of the outlet temperature at the 5% significance level. Polyphenol and flavonoid recovery after spray-drying ranged from 45.1 to 54.9% and 30.6 to 40.8%, respectively. The antioxidant activity of the spray-dried Propolis was shown to be affected by the extract feed rate and air outlet temperature at a significance level of 0.1%. The spray-dried Propolis extract showed significant antioxidant activity, with 50% lipid peroxidation inhibition at concentrations ranging from 2.5 to 5.0 microg/ml.

## 6.7. Artepillin C isoprenomics: design and synthesis of Artepillin C isoprene analogues as lipid peroxidation inhibitor having low mitochondrial toxicity.


| | |
|---|---|
| Authors | Uto Y, Ae S, Koyama D, Sakakibara M, Otomo N, Otsuki M, Nagasawa H, Kirk KL, Hori H |
| Link | Bioorg Med Chem. 2006 Aug 15;14(16):5721-8. |
| Research Institute | Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, Japan |



### Abstract

We designed and synthesized isoprene analogues of Artepillin C, a major component of Brazilian Propolis, and investigated the inhibitory activity on lipid peroxidation of rat liver mitochondria (RLM) and RLM toxicity based on isoprenomics. We succeeded in the synthesis of Artepillin C isoprene analogues using regioselective prenylation within the range from 22% to 53% total yield. Reactivity of Artepillin C and its isoprene analogues with ABTS (2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonate)) radical cations showed only a slight difference among the molecules. The isoprene side-chain elongation analogues of Artepillin C showed almost the same inhibitory activity against RLM lipid peroxidation as Artepillin C. Artepillin C and its isoprene analogues had very weak RLM uncoupling activity. Moreover, Artepillin C and its isoprene analogues exhibited a lower inhibitory activity against adenosine 5'-triphosphate (ATP) synthesis by about two orders of magnitude than the effective inhibitory activity against RLM lipid peroxidation. From these results we conclude that Artepillin C isoprene analogues could be potent lipid peroxidation inhibitors having low mitochondrial toxicity. We also conclude that elongation of the isoprene side chain of Artepillin C to increase lipophilicity had little influence on the inhibitory activity toward RLM lipid peroxidation.

## 6.8. Artepillin C isoprenomics: design and synthesis of Artepillin C analogues as antiatherogenic antioxidants.

| | |
|---|---|
| Authors | Uto Y, Ae S, Hotta A, Terao J, Nagasawa H, Hori H |
| Link | Adv Exp Med Biol. 2006;578:113-8. |
| Research Institute | Department of Biological Science & Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjimacho 2, Tokushima, 770-8506, Japan |

### Abstract

[only Japanese]

## 6.9. Water extract of Propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions.


| | |
|---|---|
| Authors | Nakajima Y, Shimazawa M, Mishima S, Hara H. |
| Link | Life Sci. 2007 Jan 2;80(4):370-7. |
| Research Institute | Department of Biofunctional Molecules, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan. |



### Abstract

We investigated whether water extract of Brazlian Green Propolis (WEP) and its main constituents [caffeoylquinic acid derivatives (3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, chlorogenic acid) and cinnamic acid derivatives (p-coumaric acid, Artepillin C, drupanin, baccharin)] exert neuroprotective effects against the retinal damage induced by oxidative stress. Additionally, their neuroprotective effects were compared with their antioxidant effects. WEP, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, chlorogenic acid, and p-coumaric acid (but not Artepillin C, baccharin, or drupanin) concentration-dependently inhibited oxidative stress-induced neurotoxicity [achieved using L-buthionine-(S,R)-sulfoximine (BSO) to deplete glutathione in combination with glutamate to inhibit cystine uptake] in cultured retinal ganglion cells (RGC-5, a rat ganglion cell line transformed using E1A virus). At their effective concentrations against oxidative stress-induced retinal damage, WEP, 3,4-di-caffeoylquinic acid, 3,5-di-caffeoylquinic acid, and chlorogenic acid (but not cinnamic acid derivatives) inhibited lipid peroxidation (LPO) in mouse forebrain homogenates. Thus, the neuroprotective effects of WEP and caffeoylquinic acid derivatives paralleled those against LPO. These findings indicate that WEP and caffeoylquinic acid derivatives have neuroprotective effects against retinal damage in vitro, and that these effects may be partly mediated via antioxidant effects.

## 6.10. Duration-dependent hepatoprotective effects of Propolis extract against carbon tetrachloride-induced acute liver damage in rats.

| | |
|---|---|
| Authors | Bhadauria M, Nirala SK, Shukla S |
| Link | Adv Ther, September 1, 2007; 24(5): 1136-45. |
| Research Institute | School of Studies in Zoology, Jiwaji University, Gwalior 474011. MP, India. |


### Abstract

Propolis is a natural product produced by bees that was discovered through the study of traditional cures and knowledge of indigenous people throughout the world. It is rich in vitamins A, B, C, and E, and in amino acids, copper, iron, manganese, and zinc. The investigators studied the duration-dependent hepatoprotective effects of Propolis extract (200 mg/kg, orally) against carbon tetrachloride (CCl(4); 1.5 mL/kg, intraperitoneally)-induced liver damage in rats. Administration of CCl(4) caused a sharp elevation in the activity of serum transaminases and serum alkaline phosphatase. A significant depletion in hepatically reduced glutathione was observed with significantly enhanced hepatic lipid peroxidation. After CCl(4) administration, glycogen contents and activities of alkaline phosphatase, adenosine triphosphatase, and succinic dehydrogenase were significantly decreased, whereas total protein contents and activity of acid phosphatase were increased in the liver and kidney. Propolis extract reversed alterations in all parameters when administered within 6, 12, and 24 h of toxicant exposure. Propolis therapy produced duration-dependent protection, with maximal protection achieved at 24 h after CCl(4) exposure. It is believed that Propolis in its natural form has general pharmacologic value and marked hepatoprotective potential because of its composition of minerals, flavonoids, and phenolic compounds.

## 6.11. Evaluation of antioxidant and antimicrobial activities and characterization of bioactive components of two Brazilian Propolis samples using a pKa-guided fractionation.


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|---|---|
| Authors | RM Souza, MC de Souza, ML Patitucci, and JF Silva. |
| Link | *Z Naturforsch [C].* 2007; 62: 801. [MEDLINE Citation] |
| Research Institute | Department of Organic Chemistry, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, 21941-590, Brazil. |



### Abstract

The ethanolic extracts of two Brazilian Propolis samples were submitted to a fractionation procedure based on the pKa values of their components. The fractions obtained were evaluated for their antimicrobial activity against Staphylococcus aureus as well as for their antioxidant properties (reduction of DPPH radical). Their phenolic and flavonoid contents were measured spectrophotometrically, in order to establish the correlations between these contents and the measured activities. Further, the most active fractions of both extracts were analyzed by HRGC-MS and about twenty compounds could be characterized. Among them were 3-prenyl-4-hydroxycinnamic acid (drupanin) and 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C), which seem to be the major antioxidant components of the bioactive fractions.

## 6.12. Classification of Green Propolis. Seasonal Variation, Chemical Composition and Antioxidant activity of Brazilian Propolis Samples.


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|---|---|
| Authors | Érica Weinstein Teixeira, Dejair Message, Giuseppina Negri, Antonio Salatino, and Paulo César Stringheta. |
| Link | *Evid. Based Complement. Altern. Med.* published 31 January 2008, 10.1093/ecam/nem177 [Abstract] [Full Text] [PDF] |
| Research Institute | Universidade Federal de Viçosa, Departamento de Tecnologia de Alimentos, 36.571 -000, Viçosa, MG, Brazil. |



### Abstract

Total phenolic contents, antioxidant activity and chemical composition of Propolis samples from three localities of Minas Gerais state (southeast Brazil) were determined. Total phenolic contents were determined by the Folin–Ciocalteau method, antioxidant activity was evaluated by DPPH, using BHT as reference, and chemical composition was analyzed by GC/MS. Propolis from Itapecerica and Paula Cândido municipalities were found to have high phenolic contents and pronounced antioxidant activity. From these extracts, 40 substances were identified, among them were simple phenylpropanoids, prenylated phenylpropanoids, sesqui- and diterpenoids. Quantitatively, the main constituent of both samples was allyl-3-prenylcinnamic acid. A sample from Virginópolis municipality had no detectable phenolic substances and contained mainly triterpenoids, the main constituents being - and β-amyrins. Methanolic extracts from Itapecerica and Paula Cândido exhibited pronounced scavenging activity towards DPPH, indistinguishable from BHT activity. However, extracts from Virginópolis sample exhibited no antioxidant activity. Total phenolic substances, GC/MS analyses and antioxidant activity of samples from Itapecerica collected monthly over a period of 1 year revealed considerable variation. No correlation was observed between antioxidant activity and either total phenolic contents or contents of artepillin C and other phenolic substances, as assayed by CG/MS analysis.

## 6.13. Propolis Shows Cytotoxic and Antioxidative Activities: Chemical Composition, Cytotoxic and Antioxidative Activities of Ethanolic Extracts of propolis on HCT-116 Cell Line


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|---|---|
| Authors | Jovana B Žižić[1],*, Nenad L Vuković[2], Milka B Jadranin[3], Boban D Anđelković[4], Vele V Tešević[4], Miroslava M Kacaniova[5], Slobodan B Sukdolak[2], Snežana D Marković[1] |
| Link | Journal of the Science of Food and Agriculture, Volume 93, Issue 12, pages 3001 – 3009, September 2013 |
| Research Institute | [1]Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia<br>[2]Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia<br>[3]Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia<br>[4]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia<br>[5]Department of Microbiology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Nitra, Slovakia |



### Abstract

Background: Propolis is a complex resinous sticky substance that honeybees collect from buds and exudates of various plants. Due to propolis versatile biological and pharmacological activities, it is widely used in medicine, cosmetics and food industry. The aim of this study was to evaluate cytotoxic and antioxidative effects of various ethanolic extracts of propolis (EEP) on human colon cancer cell line (HCT-116) and compare it with their composition. HPLC-DAD method was used to determine the chemical composition of propolis samples.

Results: The most abundant flavonoids in all samples were chrysin, pinocembrin and galangin (12.697- 40.811 μg mg-1). On the other hand, main phenolic acids were caffeic, ferulic and isoferulic acid. Dose and time-dependent inhibition of cell growth of HCT-116 cells was observed in all propolis samples, with IC50 values ranging from 26.33 to 143.09 μg mL-1. Differences in cytotoxic activity of propolis samples were associated with differences in their composition. Our results showed that all EEP samples reduced both superoxide anion radical and nitrite levels and also had strong DPPH scavenging activity.

Conclusion: All tested propolis samples had pronounced cytotoxic and antioxidative activities.

# 7. anti-cancer

## 7.1. Preferential cytotoxicity to tumor cells of 3,5-diprenyl-4-hydroxycinnamic acid (Artepillin C) isolated from Propolis.

| | |
|---|---|
| Authors | Matsuno T, Jung SK, Matsumoto Y, Saito M, Morikawa J |
| Link | Anticancer Res. 1997 Sep-Oct;17(5A):3565-8. |
| Research Institute | National Institute of Health, Tokyo, Japan |


### Abstract

A tumoricidal substance was isolated from Brazilian Propolis as guided by cytotoxicity assay on HuH 13 (human hepatocellular carcinoma) cell and was characterized to be 3-[4-hydroxy-3,5-bis (3-methyl-2-butenyl) phenyl]-2-propenoic acid (3,5-diprenyl-4-hydroxycinnamic acid (Artepillin C)). It exhibited preferential cytotoxicity to tumor cells cultured in vitro. The cytotoxicity observed seemed to be partly attributable to apoptosis-like DNA fragmentation. The compound showed anti-tumor activity more effective than that of 5-fluorouracil to transplantable human tumor cell lines when tested on histoculture drug response assay system.

## 7.2. Apoptosis and suppression of tumor growth by Artepillin C extracted from Brazilian Propolis.

| | |
|---|---|
| Authors | Kimoto T, Arai S, Kohguchi M, Aga M, Nomura Y, Micallef MJ, Kurimoto M, Mito K |
| Link | Cancer Detect Prev. 1998;22(6):506-15. |
| Research Institute | Fujisaki Institute, Hayashibara Biochemical Laboratories, Okayama, Japan |


### Abstract

Artepillin C was extracted from Brazilian Propolis. Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) has a molecular weight of 300.40 and possesses antibacterial activity. When Artepillin C was applied to human and murine malignant tumor cells in vitro and in vivo, Artepillin C exhibited a cytotoxic effect and the growth of tumor cells was clearly inhibited. The Artepillin C was found to cause significant damage to solid tumor and leukemic cells by the MTT assay, DNA synthesis assay, and morphological observation in vitro. When xenografts of human tumor cells were transplanted into nude mice, the cytotoxic effects of Artepillin C were most noticeable in carcinoma and malignant melanoma. Apoptosis, abortive mitosis, and massive necrosis combined were identified by histological observation after intratumor injection of 500 microg of Artepillin C three times a week. In addition to suppression of tumor growth, there was an increase in the ratio of CD4/CD8 T cells, and in the total number of helper T cells. These findings indicate that Artepillin C activates the immune system, and possesses direct antitumor activity.

## 7.3. Renal carcinogenesis induced by ferric nitrilotriacetate in mice, and protection from it by Brazilian Propolis and Artepillin C.

| | |
|---|---|
| Authors | Kimoto T, Koya S, Hino K, Yamamoto Y, Nomura Y, Micallef MJ, Hanaya T, Arai S, Ikeda M, Kurimoto M |
| Link | Pathol Int. 2000 Sep;50(9):679-89. |
| Research Institute | Hayashibara Biochemical Laboratories Inc., Fujisaki Institute, Fujisaki, Okayama, Japan |


### Abstract

The protective effect of Brazilian Propolis and its extract Artepillin C against ferric nitrilotriacetate (Fe-NTA)-induced renal lipid peroxidation and carcinogenesis was studied in male ddY mice. Fe-NTA-induced renal lipid peroxidation leads to a high incidence of renal cell carcinoma (RCC) in mice. Administration of Propolis by gastric intubation 2 h before or Artepillin C at either the same time, 2 h, or 5 h before the intraperitoneal injection of Fe-NTA (7 mg Fe/kg) effectively inhibited renal lipid peroxidation. This was evaluated from the measurement of renal thiobarbituric acid-reactive substances (TBARS) or histochemical findings of 4-hydroxy-2-nonenal (4-HNE)-modified proteins and 8-hydroxy-2'-deoxyguanosine (8-OHdG). Repeated injection of Fe-NTA (10 mg Fe/kg per day, twice a week for a total of 16 times in 8 weeks) caused subacute nephrotoxicity as revealed by necrosis and pleomorphic large nuclear cells in the renal proximal tubules, and gave rise to RCC 12 months later. A protective effect from carcinogenicity was observed in mice given Propolis or Artepillin C. Furthermore, the mice given Fe-NTA only developed multiple cysts composed of precancerous lesions with multilayered and proliferating large atypical cells. Mice treated with Propolis and Artepillin C also had cysts, but these were dilated and composed of flat cells. These results suggest that Propolis and Artepillin C prevent oxidative renal damage and the carcinogenesis induced by Fe-NTA in mice.

## 7.4. Apoptosis of human leukemia cells induced by Artepillin C, an active ingredient of Brazilian Propolis.


| | |
|---|---|
| Authors | Kimoto T, Aga M, Hino K, Koya-Miyata S, Yamamoto Y, Micallef MJ, Hanaya T, Arai S, Ikeda M, Kurimoto M |
| Link | Anticancer Res. 2001 Jan-Feb;21(1A):221-8. |
| Research Institute | Fujisaki Institute, Hayashibara Biochemical Laboratories Inc., Fujisaki 675-1, Okayama 702-8006, Japan . fujisaki@hayashibara.co.jp |



### Abstract

Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) is an active ingredient of Brazilian Propolis that possesses anti-tumor activity. When Artepillin C was applied to human leukemia cell lines of different phenotypes, namely, lymphocytic leukemia (7 cell lines of T-cell, 5 cell lines of B-cell), myeloid and monocytic leukemia and non-lymphoid non-myeloid leukemia cell lines in vitro, Artepillin C exhibited potent cytocidal effects and induced marked levels of apoptosis in all the cell lines. The most potent effects were observed in the T-cell lines. Apoptotic bodies and DNA fragmentation were induced in the cell lines after exposure to Artepillin C. DNA synthesis in the leukemia cells was clearly inhibited and disintegration of the cells was confirmed microscopically. Apoptosis of the leukemia cells may be partially associated with enhanced Fas antigen expression and loss of mitochondrial membrane potential. In contrast, although Artepillin C inhibited the growth of pokeweed mitogen (PWM)-stimulated normal blood lymphocytes, it was not cytocidal to normal unstimulated lymphocytes. These results suggested that Artepillin C, an active ingredient of Brazilian Propolis, has anti-leukemic effects with limited inhibitory effects on normal lymphocytes.

## 7.5. Pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice and protection from it by Brazilian Propolis and Artepillin C.


| | |
|---|---|
| Authors | Kimoto T, Koya-Miyata S, Hino K, Micallef MJ, Hanaya T, Arai S, Ikeda M, Kurimoto M |
| Link | Virchows Arch. 2001 Mar;438(3):259-70. |
| Research Institute | Hayashibara Biochemical Laboratories Inc., Fujisaki Institute, Fujisaki 675-1, Okayama 702-8006, Japan . fujisaki@hayashibara.co.jp |



### Abstract

In experiments using the renal carcinogen ferric nitrilotriacetate (Fe-NTA) in male ddY mice, primary pulmonary cancers were also induced in bronchiolar and alveolar tissues. 4-Hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), products of oxidative processes, increased in bronchiolar and alveolar cells after administration of Fe-NTA. These substances disappeared after oral administration of Propolis or Artepillin C, as shown histochemically, and correlated with an anticancer prophylactic effect of Propolis and Artepillin C. From our investigation, lipid peroxidation seems to play an important role in pulmonary carcinogenesis. Malignant progression from adenoma of bronchiolar or alveolar origin to malignant tumors has been proposed to involve a stepwise transformation. In our study, adenomas developed into adenocarcinomas and large cell carcinomas after treatment with Fe-NTA. In contrast, after oral administration of Propolis or Artepillin C, adenomas did not progress to carcinomas. Instead of developing into large cell cancers, as induced by Fe-NTA in control mice, adenomas showed remarkable proliferation of macrophages and local anti-oxidant activity after treatment with either Propolis or Artepillin C. Propolis and Artepillin C therefore appear to inhibit lipid peroxidation and the development of pulmonary cancers.

## 7.6. Inhibitory effect of water-soluble derivative of propolis and its polyphenolic compounds on tumor growth and metastasizing ability: a possible mode of antitumor action.


| | |
|---|---|
| Authors | Orsolić N, Sver L, Terzić S, Tadić Z, Basić I. |
| Link | Nutr Cancer. 2003;47(2):156-63. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

Polyphenolic compounds are widely distributed in the plant kingdom and display a variety of biological activities, including chemoprevention and tumor growth inhibition. Propolis is made up of a variety of polyphenolic compounds. We compared how the routes of administration of polyphenolic compounds deriving from propolis and of propolis itself affect the growth and metastatic potential of a transplantable mammary carcinoma (MCa) of the CBA mouse. The influence of tested compounds on local tumor growth was also studied.

Metastases in the lung were generated by 2 x 10(5) tumor cells injected intravenously (IV). A water-soluble derivative of propolis (WSDP) and polyphenolic compounds (caffeic acid, CA, and CA phenethyl ester, CAPE) were given to mice per os (PO) or intraperitoneally (IP) before or after tumor cell inoculation.

Tested compounds significantly decreased the number of lung colonies. When mice were inoculated with 10(5) MCa cells in the exact site of subcutaneous injection of different doses of WSDP, CA, or CAPE, tumor growth was inhibited, and survival of treated mice was prolonged. Antitumor activity, according to the results obtained, is mostly related to the immunomodulatory properties of the compounds and their capacity to induce apoptosis and necrosis.

In conclusion, results presented here indicate that WSDP, CA, and CAPE could be potential useful tools in the control of tumor growth in experimental tumor models when administrated PO; because PO administration is the easiest way of introducing a compound used for prevention and/or cure of any disease, it is likely that this article has reached the goal of the investigation.

## 7.7. Immunomodulation by water-soluble derivative of propolis: a factor of antitumor reactivity.


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| Authors | Orsolić N, Basić I. |
| Link | J Ethnopharmacol. 2003 Feb;84(2-3):265-73. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10 000 Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

The antimetastatic efficacy of a water-soluble derivative of propolis (WSDP) was studied.

Tumor was a transplantable mammary carcinoma of CBA mouse. Metastases in the lung were generated by 2 x 10(5) viable tumor cells i.v. WSDP was given intraperitoneally at doses of 50 or 150 mg/kg before or after tumor cell inoculation.

Therapies reduced the number of metastases in the lung and tumor growth was suppressed significantly by WSDP. It is likely that antimetastatic activity of the WSDP is mainly mediated by immunomodulatory activity.

Changes in several immunological parameters such as production of lymphocyte activating factor by peritoneal macrophages and the efficacy of those macrophages to kill tumor cell in vitro, responses of lymphocytes to mitogen, and weight and cellularity of spleen, respectively, correlated well with antimetastatic properties of the WSDP.

Based on results we postulate that the antimetastatic activity of propolis includes a pronounced immunomodulatory activity mainly toward augmentation of nonspecific antitumor resistance in mice via macrophage activation.

## 7.8. Inhibitory effects of Propolis granular A P C on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice.


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|---|---|
| Authors | Sugimoto Y, Iba Y, Kayasuga R, Kirino Y, Nishiga M, Alejandra Hossen M, Okihara K, Sugimoto H, Yamada H, Kamei C |
| Link | Cancer Lett. 2003 Apr 25;193(2):155-9. |
| Research Institute | Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan |



### Abstract

We examined the effect of Propolis granular A. P. C on lung tumorigenesis in female A/J mice. Lung tumors were induced by the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) administered in drinking water for 7 weeks in mice maintained on an AIN-76A semi-synthetic diet. Propolis granular A. P. C (100 mg/kg body wt.) was administered orally daily for 6 days/week from 1 week before NNK administration and throughout the experiment. Sixteen weeks after the NNK treatment, the mice were killed and the number of surface lung tumors was measured. The number of lung tumors in mice treated with NNK alone for 7 weeks (9.4 mg/mouse) was significantly more than in that observed in control mice. Propolis granular A. P. C significantly decreased the number of lung tumors induced by NNK. These results indicate that Propolis granular A. P. C is effective in suppressing NNK-induced lung tumorigenesis in mice.

## 7.9. Cell growth inhibitory effect of cinnamic acid derivatives from Propolis on human tumor cell lines.


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|---|---|
| Authors | Akao Y, Maruyama H, Matsumoto K, Ohguchi K, Nishizawa K, Sakamoto T, Araki Y, Mishima S, Nozawa Y |
| Link | Biol Pharm Bull. 2003 Jul;26(7):1057-9. |
| Research Institute | Gifu International Institute of Biotechnology, Kakamigahara, Japan. yakao@giib.or.jp |



### Abstract

A cell growth inhibitory effect of drupanin and baccharin, ingredients of Propolis, was found in human cancer cell lines. These compounds induced apoptosis in the cells characterized by morphological and nucleosomal DNA fragmentation analysis. Their effects were less potent compared with that of Artepillin C, which is a known anticancer compound from Propolis. Importantly, HL60 cells were more sensitive to drupanin than were Con A-stimulated peripheral blood lymphocytes, whereas the potency of Artepillin C was the opposite of that of drupanin.

## 7.10. Influence of honey bee products on transplantable murine tumours.

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|---|---|
| Authors | N Orsolic, A Knezevic, L Sver, S Terzic, BK Hackenberger, and I Basic |
| Link | Vet Comp Oncol, December 1, 2003; 1(4): 216-26 |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia |


### Abstract

The effect of propolis [it is a water-soluble derivative (WSDP)] and related polyphenolic compounds of propolis (caffeic acid, caffeic acid phenethyl ester and quercetin), honey, royal jelly and bee venom on tumour growth, metastasizing ability and induction of apoptosis and necrosis in murine tumour models (mammary carcinoma and colon carcinoma) was investigated.

WSDP and related polyphenolic compounds showed significant anti-metastatic effect ($P < 0.01$ and $P < 0.001$) given either before or after tumour-cell inoculation. Oral or systemic application of WSDP or caffeic acid significantly reduced subcutaneous tumour growth and prolonged the survival of mice. Honey also exerted pronounced anti-metastatic effect ($P < 0.05$) when applied before tumour-cell inoculation (peroral 2 g kg(-1) for mice or 1 g kg(-1) for rats, once a day for 10 consecutive days). Royal jelly did not affect metastasis formation when given intraperitoneally or subcutaneously. However, intravenous administration of royal jelly before tumour-cell inoculation significantly ($P < 0.05$) inhibited metastasis formation. When mice were given 10(5) tumour cells intravenously immediately after bee venom injection, the number of tumour nodules in the lung was significantly lower ($P < 0.001$) than in untreated mice or mice treated with bee venom subcutaneously. Local presence of bee venom in the tissue caused significant delay in subcutaneous tumour formation.

These findings clearly demonstrate that anti-tumour and anti-metastatic effects of bee venom are highly dependent on the route of injection and on close contact between components of the bee venom and tumour cells.

These data show that honey bee products given orally or systemically may have an important role in the control of tumour growth and tumour metastasizing ability.

## 7.11. Immunomodulatory and antimetastatic action of propolis and related polyphenolic compounds.


| | |
|---|---|
| Authors | Orsolić N, Knezević AH, Sver L, Terzić S, Basić I. |
| Link | J Ethnopharmacol. 2004 Oct;94(2-3):307-15. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000, Croatia. norsolic@yahoo.com |



### Abstract

The effect of polyphenolic compounds isolated from propolis and propolis itself was investigated on the growth and metastatic potential of a transplantable mammary carcinoma (MCa) of CBA mouse.

Metastases in the lung were generated by intravenous injection of tumor cells (2 x 10(5)). A water-soluble derivative of proplis (WSDP), caffeic acid (CA), caffeic acid phenethyl ester (CAPE) and quercetin (QU) were given to mice per os before tumor cells inoculation.

Tested compounds significantly decreased the number of tumor nodules in the lung.

According to the results obtained the antitumor activity of tested compounds can be related to the immunomodulatory properties of the compounds, their cytotoxicity to tumor cells, and their capacity to induce apoptosis and necrosis. The experimental data support that WSDP, CA, CAPE and QU could be potentially useful in the control of tumor growth in experimental models.

## 7.12. Synergistic antitumor effect of polyphenolic components of water soluble derivative of propolis against Ehrlich ascites tumour.

| | |
|---|---|
| Authors | Orsolić N, Kosalec I, Basić I. |
| Link | Biol Pharm Bull. 2005 Apr;28(4):694-700. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Croatia. |


### Abstract

Effect of two preparation (Croatian and Brazilian) of water-soluble derivative of propolis (WSDP), caffeic acid, quercetin, chrysin, naringenin (components present in WSDP) on the development of Ehrlich ascites tumour (EAT) was evaluated. Test components (50 mg/kg) were given perorally or intraperitoneally 2 h prior the intraperitonel injection of EAT (2 x 10(6)) cells.

It was observed that all test compounds effectively inhibited tumour growth and the proliferation of EAT. The volume of ascitic fluid induced by EAT cells and total number of cells present in the peritoneal cavity was markedly reduced in EAT-bearing mice treated with test components. In treated mice the number of polymorphonuclear (PMN) cells in the peritoneal cavity was increased while the number of macrophages was decreased.

The macrophage spreading activity revealed that WSDP and all test compounds affected the functional state of macrophages increasing their tumorcidal activity; the effect of WSDP was most pronounced indicating synergistic effect of components present in WSDP. Antitumor activity of WSDP may be the result of different specific mechanism(s) of flavonoids present as compared to individual flavonoid given alone.

It is likely that the part of antitumor efficacy of test components against EAT cells was the results of increased activity of macrophages.

## 7.13. Peroral application of water-soluble derivative of propolis (WSDP) and its related polyphenolic compounds and their influence on immunological and antitumour activity.


| | |
|---|---|
| Authors | Orsolic N, Sver L, Terzić S, Basić I |
| Link | Vet Res Commun. 2005 Oct;29(7):575-93. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

Polyphenolic compounds are widely distributed in the plant kingdom and display a variety of biological activities, including chemoprevention and growth inhibition of tumours. Propolis contains a conglomerate of polyphenolic compounds.

We investigated the effect of propolis and polyphenolic compounds, components of propolis, on the growth and metastatic potential of a transplantable mammary carcinoma (MCa) of the mouse.

Metastases in the lung were generated by 2 x 10(5) tumour cells injected intravenously (i.v.). A water-soluble derivative of propolis (WSDP) and the polyphenolic compounds (caffeic acid (CA) and caffeic acid phenethyl ester (CAPE)) were given to mice perorally before or after tumour cell inoculation.

WSDP, CA and CAPE reduced the number of metastases in the lung. This implies that the antitumour activities of the compounds used in these studies are mostly related to the immunomodulatory properties of the compounds, their cytotoxicity to tumour cells, and their ability to induce apoptosis and/or necrosis.

## 7.14. Effects of local administration of propolis and its polyphenolic compounds on tumor formation and growth.


| | |
|---|---|
| Authors | Orsolić N, Terzić S, Mihaljević Z, Sver L, Basić I. |
| Link | Biol Pharm Bull. 2005 Oct;28(10):1928-33. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb; 10000 Zagreb, Rooseveltov trg 6, Croatia. norsolic@yahoo.com |



### Abstract

Many dietary constituents are chemopreventive in animal models, and experiments with cultured cells are revealing various potential mechanisms of action. Compounds classified as blocking agents can prevent, or greatly reduce, initiation of carcinogenesis, or suppressing agents can act on cell proliferation.

Caffeic acid (CA) and caffeic acid phenethyl ester (CAPE), members of the polyphenolic compounds, are present in high concentrations in medicinal plants and propolis, a natural beehive product. A water-soluble extract of propolis (WSDP) and two components of propolis, CA and CAPE were investigated for direct antitumor activity in vivo and in vitro.

The local presence of CA and CAPE in the tissue caused a significant delay in tumor formation and increased life span 29.30 to 51.73%, respectively. CA and CAPE, but not WSDP, significantly suppressed human HeLa cervical carcinoma cell proliferation in vitro.

Based on these results, we postulate that the antitumor activity of polyphenolic compounds includes direct cytotoxic effects on tumor cells.

## 7.15. Water-soluble derivative of propolis and its polyphenolic compounds enhance tumoricidal activity of macrophages.


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|---|---|
| Authors | Orsolić N, Basić I. |
| Link | J Ethnopharmacol. 2005 Oct 31;102(1):37-45. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10 000 Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

Many plants and the plant-derived honeybee propolis have shown biological activities like immunomodulation and antitumor effect. The effect of two water-soluble propolis derivatives (WSDP) from Croatia and Brazil, caffeic acid, quercetin, chrysin and naringenin which are present in WSDP was assessed on the development of Ehrlich ascites tumor (EAT).

The compounds (50 mgkg(-1)) were given by gastric intubations (po) 2 h prior to the intraperitoneal injection of EAT (2x10(6)) cells. It was observed that WSDP and its compounds effectively inhibited tumor growth and proliferation of EAT.

The volume of ascitic fluid induced by EAT cells and total number of cells present in the peritoneal cavity was markedly reduced in EAT-bearing mice treated with test components. Treatment with test components increased the number of polymorphonuclear (PMN) cells and decreased the number of macrophages in the peritoneal cavity of treated animals. The macrophage spreading activity revealed that WSDP and all test compounds affected the functional state of macrophages increasing their tumoricidal activity.

The effect of WSDP was most pronounced suggesting synergistic effect of components present in WSDP. It is likely that part of the antitumor efficacy of the assayed components against EAT cells was the results of increased macrophage activity.

## 7.16. Artepillin C in Brazilian Propolis induces G(0)/G(1) arrest via stimulation of Cip1/p21 expression in human colon cancer cells.


| | |
|---|---|
| Authors | Shimizu K, Das SK, Hashimoto T, Sowa Y, Yoshida T, Sakai T, Matsuura Y, Kanazawa K |
| Link | Mol Carcinog. 2005 Dec;44(4):293-9. |
| Research Institute | Department of Life Science, Graduate School of Science and Technology, Kobe University, Kobe, Japan |



### Abstract

Potential chemopreventive agents exist in foods. Artepillin C in Brazilian Propolis was investigated for its effects on colon carcinogenesis. We had found that Artepillin C was a bioavailable antioxidant, which could be incorporated into intestinal Caco-2 and hepatic HepG2 cells without any conjugation and inhibited the oxidation of intracellular DNA. Artepillin C was then added to human colon cancer WiDr cells. It dose-dependently inhibited cell growth, inducing G(0)/G(1) arrest. The events involved a decrease in the kinase activity of a complex of cyclin D/cyclin-dependent kinase 4 and in the levels of retinoblastoma protein phosphorylated at Ser 780 and 807/811. The inhibitors of the complex, Cip1/p21 and Kip1/p27, increased at the protein level. On the other hand, Northern blotting showed that Artepillin C did not affect the expression of Kip1/p27 mRNA. According to the experiments using isogenic human colorectal carcinoma cell lines, Artepillin C failed to induce G(0)/G(1) arrest in the Cip1/p21-deleted HCT116 cells, but not in the wild-type HCT116 cells. Artepillin C appears to prevent colon cancer through the induction of cell-cycle arrest by stimulating the expression of Cip1/p21 and to be a useful chemopreventing factor in colon carcinogenesis.

## 7.17. Antitumor, hematostimulative and radioprotective action of water-soluble derivative of propolis (WSDP)..


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| Authors | Orsolić N, Basić I. |
| Link | Biomed Pharmacother. 2005 Dec;59(10):561-70. Epub 2005 Aug 10. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, Croatia. norsolic@yahoo.com |



### Abstract

Several studies suggest that dietary supplementation with antioxidant can influence the response to chemotherapy as well as the development of adverse side effects caused by treatment with chemotherapeutic agents. Using CBA mouse model, we investigated a clinically potential use of a water-soluble derivative of propolis (WSDP) in the treatment of various cytopenias induced by radiation and/or chemotherapy. Also, the antimetastatic efficiency of WSDP given intraperitoneally alone or in combination with chemotherapeutic agents and their effects on the blood leukocytes count as well as on hematopoiesis were studied. Tumor was a transplantable mammary carcinoma (MCa) of CBA mouse. Metastases in the lung were generated by injecting viable tumor cells intravenously (iv). WSDP (50 or 150 mg/kg) exerted a significant antimetastatic effect ($P < 0.001$) when given either before or after tumor cell inoculation.

In combined treatment WSDP and Epirubicin profoundly inhibited metastasis formation; this synergistic effect is maximal when Epirubicin and WSDP were administrated after tumor cell inoculation. Positive outcome of combined treatment with WSDP and Epirubicin was also found regarding the number of red and white blood cells in peripheral blood while in mice treated with Epirubicin alone the significant drop in all hematological parameters was noticed on day 13 after tumor cell inoculation. Furthermore, when WSDP (50 mg/kg) was given perorally (po) for 20 consecutive days an increased number of exogenous CFUs was found in treated mice. WSDP given either for 20 or 40 days increased cellularity of hematopoietic tissue and the number of leucocytes in peripheral blood; prolonged treatment with WSDP also elevated myeloid and megakaryocytic types of CFUs.

To conclude, these findings indicate that the combination of WSDP with chemotherapeutics could increase the antimetastatic potential of chemotherapeutic agents; these findings suggest the benefits of potential clinical trials using WSDP combined with chemotherapeutic agents in order to maximize their antitumor activity and minimize postchemotherapeutic or radiotherapeutic deteriorated reactions.

## 7.18. Direct and indirect mechanism(s) of antitumour activity of propolis and its polyphenolic compounds.


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|---|---|
| Authors | Orsolić N, Saranović AB, Basić I. |
| Link | Planta Med. 2006 Jan;72(1):20-7. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

The immunomodulatory actions of a water-soluble derivative of propolis (WSDP) and two components of propolis, caffeic acid (CA) and caffeic acid phenethyl ester (CAPE) were investigated.

Oral administration (50 mg/kg) of WSDP, CA, and CAPE enhanced the weight and cellularity of the spleen ($p<0.05$, $p<0.01$) of treated mice.

The response of spleen cells to polyclonal mitogens (PHA, Con A, PWM) was also increased in mice treated with WSDP as compared to control ($p<0.01$); in contrast, the response of spleen cells of mice treated with CA were significantly suppressed ($p<0.001$).

The colony forming ability of HeLa cells plated on monolayers of macrophages was completely inhibited by peritoneal macrophages from mice receiving either WSDP, CAPE, or CA. Macrophages from treated mice also inhibited [3H]TdR incorporation into HeLa cells in vitro. Testing for the possible presence of NO in the supernatants of 24 hours cultured macrophages activated with either compound revealed that the toxicity of these cells to HeLa cells was in part due to the production of NO.

Tumour growth was suppressed by WSDP and its polyphenolic compounds given orally to mice. Local presence of CA, and CAPE in the tissue, caused a significant delay of tumour formation.

Based on these results, we postulate that the antitumour activity of the test compounds includes pronounced immunomodulatory activity mainly due to the augmentation of non-specific antitumour resistance in mice via macrophage activation and the production of soluble factors by those cells which may interfere with either cells of the immune system or directly by tumour cells.

## 7.19. Dietary Artepillin C suppresses the formation of aberrant crypt foci induced by azoxymethane in mouse colon.


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|---|---|
| Authors | Shimizu K, Das SK, Baba M, Matsuura Y, Kanazawa K |
| Link | Cancer Lett. 2006 Aug 18;240(1):135-42. |
| Research Institute | Department of Life Science, Graduate School of Science and Technology, Kobe University, Rokkodai, Nada-ku, Kobe 657-8501, Japan |



### Abstract

Artepillin C, a prenylated phenylpropanoid found specifically in Brazilian Propolis, has been shown to be a bioavailable antioxidant. In this study, Artepillin C was tested for colon cancer-preventing activity using azoxymethane-challenged ddY mice. Oral doses of 80 and 160 mg/kg body weight of Propolis or 10mg/kg of Artepillin C (equi-amounts to 160 mg Propolis) reduced significantly the frequency of colonic aberrant crypt foci (ACF) by 39.2, 43.7 and 43.4%, respectively. In liver of the mice, glutathione S-transferase and NADPH:quinone reductase activity increased with the doses of Propolis or Artepillin C, and an antioxidant-responsive element (ARE) was found to be activated for binding DNA. Artepillin C is considered to suppress the formation of colonic ACF through the activation of ARE and induction of phase II enzymes in liver.

## 7.20. Assessment by survival analysis of the radioprotective properties of Propolis and its polyphenolic compounds.


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|---|---|
| Authors | Orsolić N, Benković V, Horvat-Knezević A, Kopjar N, Kosalec I, Bakmaz M, Mihaljević Z, Bendelja K, Basić I. |
| Link | Biol Pharm Bull. 2007 May;30(5):946-51. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia. norsolic@yahoo.com |



### Abstract

The radioprotective effects of propolis and polyphenolic compounds from propolis on the radiation-induced mortality of mice exposed to 9 Gy of gamma-irradiation were studied.

Intraperitoneal (i.p.) treatment of mice at doses of 100 mg kg(-1) body weight of propolis (water or ethanolic extract; WSDP or EEP) or its polyphenolic compounds (quercetin, naringin caffeic acid, chrysin) consecutively for 3 d before irradiation, delayed the onset of mortality and reduced the symptoms of radiation sickness.

All test compounds provided protection against hematopoietic death (death within 30 d after irradiation).

The greatest protection was achieved with quercetin; the number of survivors at the termination of the experiment was 63%. According to statistical analyses by the Kaplan-Meier method and the log-rank test, a significant difference between test components and control was found ($p<0.001$). Treatment with test components after lethal irradiation was ineffective.

These results suggest that propolis and its polyphenolic compounds given to mice before irradiation protect mice from the lethal effects of whole-body irradiation.

## 7.21. Enhanced antitumor activity of irinotecan combined with propolis and its polyphenolic compounds on Ehrlich ascites tumor in mice.

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| Authors | Benkovic V, Horvat Knezevic A, Brozovic G, Knezevic F, Dikic D, Bevanda M, Basic I, Orsolic N. |
| Link | Biomed Pharmacother. 2007 Jun;61(5):292-7. Epub 2007 Mar 12. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia. |


### Abstract

The effects of the anticancer drug irinotecan combined with ethanolic extract of propolis (EEP), a water-soluble derivate of propolis (WSDP), quercetin and naringin on the growth of Ehrlich ascites tumor (EAT) and the life span of tumor-bearing Swiss albino mice were studied.

Test components were given to mice intraperitoneally (i.p.) at doses of 100mg kg(-1) for three consecutive days before the i.p. injection of EAT cells (1x10(6)). Irinotecan was administered i.p. at dose of 50mg kg(-1) on days 1, 13, and 19 after tumor cell inoculation.

The results clearly demonstrate the synergistic action of irinotecan and EEP on survival time. These results suggest that clinical trials using a propolis preparation EEP combined with irinotecan may be beneficial in maximizing antitumor activity and minimizing post-chemotherapeutic reactions to the cytostatic drug.

## 7.22. Inhibition of doxorubicin-induced mutagenicity by *Baccharis dracunculifolia.*


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| Authors | Resende FA, Alves JM, Munari CC, Senedese JM, Sousa JP, Bastos JK, Tavares DC |
| Link | Mutat Res. 2007 Jun 30; [Epub ahead of print] |
| Research Institute | Universidade de Franca, Avenida Dr. Armando Salles de Oliveira, 201-Parque Universitário, 14404-600 Franca, São Paulo, Brazil |



### Abstract

*Baccharis dracunculifolia* DC (Asteraceae), a native plant from Brazil, have been used as an antipyretic, stomachic and health tonic in Brazil. The objective of the present study was to investigate the potential mutagenic effect of *B. dracunculifolia* ethyl acetate extract (Bd-EAE) and its influence on the mutagenicity induced by the chemotherapeutic agent doxorubicin (DXR) using the rat bone marrow and peripheral blood micronucleus test. Wistar rats were divided into 10 treatment groups. Five groups received DXR (90mg/kg body weight, b.w., intraperitoneally) to induce mutagenicity and three of these groups received a single oral dose of Bd-EAE at a concentration of 6, 12 or 24mg/kg b.w. prior to DXR administration. A vehicle-treated control group and Bd-EAE control groups were also included. The results showed that Bd-EAE itself was not mutagenic, in the rat micronucleus assay. In animals treated with Bd-EAE and DXR, the number of MNPCEs was significantly decreased compared to animals receiving DXR alone. HPLC analysis of the extract obtained permitted the identification of the following phenolic compounds: caffeic acid, p-coumaric acid, aromadendrin-4'O-methyl ether, 3-prenyl-p-coumaric acid (drupanin), 3,5-diprenyl-p-coumaric acid (Artepillin C) and baccharin. The putative antioxidant activity or the interference of one or more of the active compounds of Bd-EAE with mutagenic metabolic pathways may explain its effect on DXR mutagenicity.

## 7.23. Suppression of tumor-induced angiogenesis by Brazilian Propolis: Major component Artepillin C inhibits in vitro tube formation and endothelial cell proliferation.


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|---|---|
| Authors | Ahn MR, Kunimasa K, Ohta T, Kumazawa S, Kamihira M, Kaji K, Uto Y, Hori H, Nagasawa H, Nakayama T |
| Link | Cancer Lett. 2007 Jul 18;252(2):235-43. Epub 2007 Mar 6. |
| Research Institute | Laboratory of Functional Food Science and COE Program in the 21st Century, School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan |



### Abstract

Propolis, a resinous substance collected by honeybees from various plant sources, possesses various physiological activities such as antitumor effects. We have previously shown that Propolis of Brazilian origin was composed mainly of Artepillin C and that its constituents were quite different from those of Propolis of European origin. In this report, we examined an antiangiogenic effects of Brazilian Propolis and investigated whether Artepillin C was responsible for such effects. In an in vivo angiogenesis assay using ICR mice, we found that the ethanol extract of Brazilian Propolis (EEBP) significantly reduced the number of newly formed vessels. EEBP also showed antiangiogenic effects in an in vitro tube formation assay. When compared with other constituents of EEBP, only Artepillin C was found to significantly inhibit the tube formation of HUVECs in a concentration-dependent manner (3.13-50mug/ml). In addition, Artepillin C significantly suppressed the proliferation of HUVECs in a concentration-dependent manner (3.13-50mug/ml). Furthermore, Artepillin C significantly reduced the number of newly formed vessels in an in vivo angiogenesis assay. Judging from its antiangiogenic activity in vitro and in vivo, we concluded that Artepillin C at least in part is responsible for the antiangiogenic activity of EEBP in vivo. Artepillin C may prove useful in the development of agents and foods with therapeutic or preventive activity against tumor angiogenesis.

## 7.24. Antiproliferation of human prostate cancer cells by ethanolic extracts of Brazilian Propolis and its botanical origin.


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| Authors | Li H, Kapur A, Yang JX, Srivastava S, McLeod DG, Paredes-Guzman JF, Daugsch A, Park YK, Rhim JS |
| Link | Int J Oncol. 2007 Sep;31(3):601-6. |
| Research Institute | Center for Prostate Disease Research, Uniformed Services University of the Health Sciences, Bethesda, MD, USA |



### Abstract

Propolis is a resinous substance collected by bees (Apis mellifera) from various tree buds which they then use to coat hive parts and to seal cracks and crevices in the hive. Propolis, a known ancient folk medicine, has been extensively used in diet to improve health and to prevent disease. In the present study, we have evaluated the effects of ethanolic extracts of Brazilian Propolis group I2 and bud resins of botanical origin (B. dracunculifolia), and Propolis group 3 on proliferation of metastasis (DU145 and PC-3) and primary malignant tumor (RC58T/h/SA#4)-derived human prostate cancer cells. The strongest inhibition was observed in Propolis group 3 (sample #3) extracts whereas moderate growth inhibition was observed in human prostate epithelial cells. In the RC58T/h/SA#4 cells, resins of botanical origin of Propolis group 12 (sample #1) and Propolis group 12 (sample #2) induced growth inhibition that was associated with S phase arrest whereas Propolis group 3 (sample #3) induced growth inhibition that was associated with G2 arrest. The mechanisms of cell cycle effects of Propolis were investigated. The resins of botanical origin of Propolis group 12 and Propolis group 12 showed similar inhibition of cyclin D1, CDK4 and cyclin B1 expression. Propolis group 3 showed higher induction of p21 expression but no inhibition of cyclin D1, CDK4 and cyclin B1 expression. The results obtained here demonstrate that the Brazilian Propolis extracts have significant inhibitory effect on proliferation of human prostate cancer cells. Inhibition was achieved through regulation of protein expression of cyclin D1, B1 and cyclin dependent kinase (CDK) as well as p21. Our results indicate that the Brazilian Propolis extracts show promise as chemotherapeutic agents as well as preventive agents against prostate cancer.

## 7.25. In vitro Cytotoxic Effect of Brazilian Green Propolis on Human Laryngeal Epidermoid Carcinoma (HEp-2) Cells.


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| Authors | Michelle C. Búfalo, João M. G. Candeias, and José Maurício Sforcin. |
| Link | *Evid. Based Complement. Altern. Med.* published 22 October 2007, 10.1093/ecam/nem147 [Abstract] [Full Text] [PDF] |
| Research Institute | Department of Microbiology and Immunology, Biosciences Institute, UNESP, 18618-000 Botucatu, S.P., Brazil. |



### Abstract

Propolis is a sticky dark-colored material showing a very complex chemical composition that honeybees collect from plants. It has been used in folk medicine since ancient times, due to several biological properties, such as antimicrobial, anti-inflammatory, antioxidant and immunomodulatory activities, among others. Its antitumor action in vivo and in vitro has also been reported, using Propolis extracts or its isolated compounds. The goal of this work was to evaluate Propolis's cytotoxic action in vitro on human laryngeal epidermoid carcinoma (Hep-2) cells. These cells were incubated with different concentrations of this bee product for different time periods, and morphology and the number of viable HEp-2 cells analyzed. Data showed that Propolis exhibited a cytotoxic effect in vitro against HEp-2 cells, in a dose- and time-dependent way. Propolis solvent had no effects on morphology and number of viable cells, proving that the cytotoxic effects were exclusively due to Propolis components. Since humans have been using Propolis for a long time, further assays will provide a better comprehension of Propolis's antitumor action.

## 7.26. Biological Therapy Using Propolis as Nutritional Supplement in Cancer Treatment.

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|---|---|
| Authors | Galvao J, Abreu JA, Cruz T, Machado GAS, Niraldo P, Daugsch A, Moraes CS, Fort P, Park YK. |
| Link | International Journal of Cancer Research 2007;3(1):43-53. |
| Research Institute | Department of Food Science, College of Food Engineering, State University of Campinas, P.O. Box 6177, Campinas, SP, Brazil. |


### Abstract

Neoplasia cause several disorders in the affected body, such as suppression of immune function besides emotional and social impairments. Therefore, handling patients can be extremely difficult, offering several challenges in choosing the appropriate treatment option to be used. The right selection of treatment demands special attention. Chemotherapy is usually the standard treatment, although there are many other different therapeutic modalities also used in medicine. The present study is a literature review focusing on the pharmacological properties of Propolis, the resinous product collected by the honeybee from different plant sources, which represents a secure and efficient option for biological therapy and cancer prevention.

## 7.27. Evaluation of the radioprotective effects of propolis and flavonoids in gamma-irradiated mice: the alkaline comet assay study.


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| Authors | Benković V, Orsolić N, Knezević AH, Ramić S, Dikić D, Basić I, Kopjar N. |
| Link | Biol Pharm Bull. 2008 Jan;31(1):167-72. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb,Croatia. vesna@pmf.biol.hr |



### Abstract

The radioprotective effects of water-soluble derivate of propolis (WSDP) collected in Croatia, and single flavonoids, caffeic acid, chrysin and naringin in the whole-body irradiated CBA mice were investigated.

Irradiation was performed using a gamma-ray source ((60)Co), and absorbed doses were 4 and 9 Gy. The efficiency of test components was evaluated when given intraperitoneally (i.p.) at dose of 100 mg kg(-1) for 3 consecutive days before and/or after irradiation. Moreover, possible genotoxic effects of all test components were assessed on non-irradiated animals.

The higher efficiency of test components was observed when given preventively.

The results suggest that propolis and related flavonoids given to mice before irradiation protected mice from lethal effects of whole-body irradiation and diminish primary DNA damage in their white blood cells as detected by the alkaline comet assay.

## 7.28. Cytotoxic constituents from Brazilian red propolis and their structure-activity relationship.

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| Authors | Feng Li, Suresh Awale, Yasuhiro Tezuka, Shigetoshi Kadota |
| Link | Bioorg. Med. Chem. 2008, article in press |
| Research Institute | Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan |


### Abstract

Several classes of flavonoids [flavanoids (1-10), flavonol (11), isoflavones (12-18), isoflavanones (19-22), isoflavans (23-26), chalcones (27-30), auronol (31), pterocarpans (32-37), 2-arylbenzofuran (38), and neoflavonoid (39)] and lignans (40-42) isolated from the MeOH extract of Brazilian red propolis were investigated for their cytotoxic activity against a panel of six different cancer cell lines including murine colon 26-L5 carcinoma, murine B16-BL6 melanoma, murine Lewis lung carcinoma, human lung A549 adenocarcinoma, human cervix HeLa adenocarcinoma, and human HT-1080 fibrosarcoma cell lines. Based on the observed results, structure-activity relationships were discussed. Among the tested compounds, 7-hydroxy-6-methoxyflavanone (3) exhibited the most potent activity against B16-BL6 (IC50, 6.66 μM), LLC (IC50, 9.29 μM), A549 (IC50, 8.63 μM), and HT-1080 (IC50, 7.94 μM) cancer cell lines, and mucronulatol (26) against LLC (IC50, 8.38 μM) and A549 (IC50, 9.9 μM) cancer cell lines. These activity data were comparable to those of the clinically used anticancer drugs, 5-fluorouracil and doxorubicin, against the tested cell lines, suggesting that 3 and 26 are the good candidates for future anticancer drug development.

## 7.29. Mutagenicity and Antimutagenicity of *Baccharis dracunculifolia* Extract in Chromosomal Aberration Assays in Chinese Hamster Ovary Cells.

| | |
|---|---|
| Authors | Carla Carolina Munari, Flavia Aparecida Resende, Jacqueline Morais Alves, Joao Paulo de Sousa, Jairo Kenupp Bastos, and Denise Crispim Tavares |
| Link | Planta Med, August 5, 2008 |
| Research Institute | Universidade de Franca, Franca, São Paulo, Brazil |


### Abstract

*Baccharis Dracunculifolia* De Candole (Asteraceae), a native plant from the Brazilian "cerrado", is widely used in folk medicine as an anti-inflammatory agent and for the treatment of gastrointestinal diseases. *B. dracunculifolia* has been described as the most important plant source of Propolis in southeastern Brazil, which is called Green Propolis due to its color.

The aim of the present study was to evaluate the mutagenic and antimutagenic effects of the ethyl acetate extract of *B. dracunculifolia* leaves (Bd-EAE) on Chinese hamster ovary cells.

On one hand, the results showed a significant increase in the frequencies of chromosome aberrations at the highest Bd-EAE concentration tested (100 mug/mL). On the other hand, the lowest Bd-EAE concentration tested (12.5 mu/mL) significantly reduced the chromosome damage induced by the chemotherapeutic agent doxorubicin.

The present results indicate that Bd-EAE has the characteristics of a so-called Janus compound, that is, Bd-EAE is mutagenic at higher concentrations, whereas it displays a chemopreventive effect on doxorubicin-induced mutagenicity at lower concentrations. The constituents of *B. dracunculifolia* responsible for its mutagenic and antimutagenic effects are probably flavonoids and phenylpropanoids, since these compounds can act either as pro-oxidants or as free radical scavengers depending on their concentration.

## 7.30. Artepillin C (ARC) in Brazilian green propolis selectively and suppresses the growth of NF tumors in mice.

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| Authors | Shanta M Messerli, Mok-Ryeon Ahn, Kazuhiro Kunimasa, Miyako Yanagihara, Tomoki Tatefuji, Ken Hashimoto, Victor Mautner, Yoshihiro Uto, Hitoshi Hori, Shigenori Kumazawa, Kazuhiko Kaji, Toshiro Ohta, and Hiroshi Maruta |
| Link | Phytother Res. 2008. [MEDLINE Citation] |
| Research Institute | Marine Biological Laboratory, Woods Hole, USA. |


### Abstract

There are mainly three types of propolis whose major anticancer ingredients are entirely different: (1) CAPE (caffeic acid phenethyl ester)-based propolis in Europe, Far East and New Zealand, (2) artepillin C (ARC)-based Brazilian green propolis and (3) Brazilian red propolis.

It was shown previously that NF (neurofibromatosis)-associated tumors require the kinase PAK1 for their growth, and CAPE-based propolis extracts such as Bio 30 suppress completely the growth of NF tumors in vivo by blocking PAK1 signaling.

Also it was demonstrated that ARC suppresses angiogenesis, suggesting the possibility that ARC also blocks oncogenic PAK1 signaling. Here it is shown for the first time that both ARC and Green Propolis Extract (GPE) indeed block the PAK1 signaling selectively, without affecting another kinase known as AKT.

Furthermore, it was confirmed that ARC as well as GPE suppress almost completely the growth of human NF tumor xenografts in mice, as does Bio 30. These results suggest that both CAPE-based and ARC-based Propolis extracts are natural anti-PAK1 remedies and could be among the first effective NF therapeutics available on the market.

Since more than 70% of human cancers such as breast and prostate cancers require the kinase PAK1 for their growth, it is quite possible that GPE could be potentially useful for the treatment of these cancers, as is Bio 30.

## 7.31. Radioprotective effects of propolis and quercetin in gamma-irradiated mice evaluated by the alkaline comet assay.


| | |
|---|---|
| Authors | Benkovic V, Knezevic AH, Dikic D, Lisicic D, Orsolic N, Basic I, Kosalec I, Kopjar N. |
| Link | Phytomedicine. 2008 Oct;15(10):851-8. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Rooseveltov trg 6, Croatia. vesna@biol.pmf.hr |



### Abstract

The radioprotective effects of ethanolic extract of propolis (EEP) and quercetin on the white blood cells of the whole-body irradiated CBA mice were investigated.

Irradiation was performed using a gamma-ray source ((60)Co), and absorbed dose was 9 Gy. The efficiency of test components was evaluated when given intraperitoneally (ip) at a dose of 100 mg kg(-1) for 3 consecutive days before and/or after irradiation. Moreover, possible genotoxic effects of test components were also assessed on non-irradiated animals.

For each experimental group leukocyte count was determined and the primary DNA damage in leukocytes was assessed using the alkaline comet assay. The higher efficiency of EEP and quercetin was observed when given preventively.

The results suggest that propolis and quercetin given to mice before irradiation protect their white blood cells from lethal effects of irradiation and diminish primary DNA damage as confirmed by the alkaline comet assay.

Positive results obtained on gamma-irradiated mice given EEP and quercetin, complementary with our earlier observations on survival of irradiated mice, indicate that these compounds could be considered effective non-toxic radioprotectors. The exact mechanisms of radioprotection by these compounds and their effects on DNA repair processes are still to be elucidated.

## 7.32. Cytotoxicity of Polyphenolic/Flavonoid Compounds in a Leukaemia Cell Culture.

| | |
|---|---|
| Authors | Pavle Josipovic and Nada Orsolic |
| Link | Arh Hig Rada Toksikol, December 1, 2008; 59(4): 299-308. |
| Research Institute | Zavod za animalnu fiziologiju Bioloski odsjek, Prirodoslovno-matematicki fakultet, Sveuciliste u Zagrebu, Zagreb. |


### Abstract

Flavonoid components of propolis are biologically active substances with antioxidative, immunostimulative, immunomodulative, and anti-inflamatory properties.

The aim of the study was to investigate their cytotoxic effect on different leukaemia cell lines. For this purpose we used five different flavonoids (quercetin, caffeic acid, chrysin, naringenin, and naringin) and five types of leukemia cell lines (MOLT, JURKAT, HL-60, RAJI and U937). Cells were cultured at 37 degrees C in the RPMI-1640 medium supplemented with 10% FCS in humified atmosphere with 5% of CO2. Flavonoids were added in the following concentrations: 100 mug mL-1, 50 mug mL-1, 25 mug mL-1, or 12.5 mug mL-1.

The results show different dose- and cell-type-dependent cytotoxicity. Among the flavonoids, quercetin showed the strongest cytotoxic effect in all cell lines. Caffeic acid and chrisyn also expressed a high level of cytotoxicty. Treatment of U937 and HL-60 cell lines with low concentrations of chrisyn or naringenin stimulated cell proliferation.

These results suggest a biphase effect of the tested compounds on monocyte cell lines. Cytotoxicity and growth stimulation mechanisms caused directly by flavonoids should further be investigated on the molecular level.

## 7.33. Correlation between antiangiogenic activity and antioxidant activity of various components from propolis.

| | |
|---|---|
| Authors | Mok-Ryeon Ahn, Kazuhiro Kunimasa, Shigenori Kumazawa, Tsutomu Nakayama, Kazuhiko Kaji, Yoshihiro Uto, Hitoshi Hori, Hideko Nagasawa, and Toshiro Ohta. |
| Link | Mol Nutr Food Res, December 8, 2008 |
| Research Institute | Graduate School of Nutritional and Environmental Sciences and Global COE Program, University of Shizuoka, Shizuoka, Japan. Fax: +81-54-264-5571. |


### Abstract

Propolis possesses various physiological activities. In this study, we examined the antiangiogenic and antioxidant activities of various components from propolis: acacetin, apigenin, artepillin C, caffeic acid phenethyl ester, chrysin, p-coumaric acid, galangin, kaempferol, pinocembrin, and quercetin.

The effects of these components were tested on in vitro models of angiogenesis, tube formation and growth of human umbilical vein endothelial cells (HUVECs). Furthermore, these components were evaluated for their antioxidant activities by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging and ferric reducing/antioxidant power (FRAP) assays.

Two propolis components, caffeic acid phenethyl ester, and quercetin, possessed strong inhibitory effects on tube formation and on endothelial cell proliferation and, coincidentally, showed strong antioxidant activity. Artepillin C, galangin, and kaempferol also possessed strong antiangiogenic and antioxidant activities to a slightly less degree. In contrast, acacetin, apigenin, and pinocembrin possessed a considerable degree of antiangiogenic activities, although they showed very low antioxidant activities. From these results, we propose that components from propolis such as artepillin C, caffeic acid phenethyl ester, galangin, kaempferol, and quercetin might represent a new class of dietary-derived antioxidative compounds with antiangiogenic activities.

These propolis components may have the potential to be developed into pharmaceutical drugs for the treatment of angiogenesis-dependent human diseases such as tumors.

## 7.34. Ethanolic Extract of Propolis (EEP) Enhances the Apoptosis-Inducing Potential of TRAIL in Cancer Cells.

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| Authors | Ewelina Szliszka, Zenon P. Czuba, Maciej Domino, Bogdan Mazur, Grzegorz Zydowicz and Wojciech Krol |
| Link | Molecules. 2009 Feb 13;14(2):738-754 |
| Research Institute | Chair and Department of Microbiology and Immunology, Jordana 19, 41 808 Zabrze Medical University of Silesia in Katowice, Poland. |


### Abstract

Ethanolic extract of propolis (EEP) is one of the richest sources of phenolic acids and flavonoids. EEP and its phenolic compounds have been known for various biological activities including immunopotentiation, chemopreventive and antitumor effects.

Tumor necrosis factor related apoptosis inducing ligand (TRAIL) is a naturally occurring anticancer agent that preferentially induces apoptosis in cancer cells and is not toxic toward normal cells.

We examined the cytotoxic and apoptotic effect of EEP and phenolic compounds identified in propolis in combination with TRAIL on HeLa cancer cells. HeLa cells were resistant to TRAIL-induced apoptosis.

Our study demonstrated that EEP and its components significantly sensitize to TRAIL induced death in cancer cells. The percentage of the apoptotic cell after exposure to 50 μg/mL EEP and 100 ng/mL TRAIL increased to 71.10±1.16%. The strongest cytotoxic effect in combination with TRAIL on HeLa cells exhibited apigenin and CAPE at the concentration of 50 μM (58.87±0.75% and 49.59±0.39%, respectively).

In this report, we show for the first time that EEP markedly augmented TRAIL mediated apoptosis in cancer cells and confirmed the importance of propolis in chemoprevention of malignant tumors.

## 7.35. Brazilian Propolis Suppresses Angiogenesis by Inducing Apoptosis in Tube-forming Endothelial Cells through Inactivation of Survival Signal ERK1/2.


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| Authors | Kazuhiro Kunimasa[1,*], Mok-Ryeon Ahn[1],, Tomomi Kobayashi[1], Ryoji Eguchi[2], Shigenori Kumazawa[1], Yoshihiro Fujimori[3], Takashi Nakano[2], Tsutomu Nakayama[1], Kazuhiko Kaji[1] and Toshiro Ohta[1,*] |
| Link | Evid. Based Complement. Altern. Med. published 7 April 2009, 10.1093/ecam/nep024 |
| Research Institute | [1]Graduate School of Nutritional and Environmental Sciences and Global COE Program, University of Shizuoka, Shizuoka 422-8526, [2]Department of Thoracic Oncology, Hyogo College of Medicine and [3]Cancer Center, Hyogo College of Medicine, Hyogo 663-8501, Japan. |



### Abstract

We recently reported that propolis suppresses tumor-induced angiogenesis through tube formation inhibition and apoptosis induction in endothelial cells. However, molecular mechanisms underlying such angiogenesis suppression by propolis have not been fully elucidated.

The aim of this study was to investigate the effects of ethanol extract of Brazilian propolis (EEBP) on two major survival signals, extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, and to elucidate whether changes in these signals were actually involved in antiangiogenic effects of the propolis.

Detection by western blotting revealed that EEBP suppressed phosphorylation of ERK1/2, but not that of Akt. Pharmacological inhibition by U0126 demonstrated that ERK1/2 inactivation alone was enough to inhibit tube formation and induce apoptosis. It was also shown that EEBP and U0126 similarly induced activation of caspase-3 and cleavage of poly ADP-ribose polymerase (PARP) and lamin A/C, all of which are molecular markers of apoptosis.

These results indicate that inhibition of survival signal ERK1/2, and subsequent induction of apoptosis, is a critical mechanism of angiogenesis suppression by EEBP.

## 7.36. Radioprotective effects of quercetin and ethanolic extract of propolis in gamma-irradiated mice.


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|---|---|
| Authors | V Benkovic, AH Knezevic, D Dikic, D Lisicic, N Orsolic, I Basic, and N Kopjar |
| Link | Arh Hig Rada Toksikol, June 1, 2009; 60(2): 129-38. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia. vesna@pmf.biol.hr |



### Abstract

The aim of this study was to assess radioprotective effects of quercetin and the ethanolic extract of Propolis (EEP) in CBA mice exposed to a single radiation dose 4 Gy (60Co).

The mice were treated with 100 mg kg(-1) quercetin or EEP a day for three consecutive days either before (pre-treatment) or after gamma-irradiation (therapy). Leukocyte count was determined in blood drawn from the tail vein, and DNA damage in leukocytes was assessed using the alkaline comet assay. Genotoxic effects of the test compounds were also evaluated in non-irradiated mice. The levels of radioprotection provided by both test compounds were compared with those established in mice that were given chemical radioprotector S-(2-aminoethy1) isothiouronium bromide hydrobromide (AET).

Mice that received pre-treatment were less sensitive to irradiation. Mice given the post-irradiation therapy showed a slight but not significant increase in total leukocyte count over irradiated negative control. Quercetin showed better protective properties than EEP in both pre-treatment and therapy, and activated a higher number of leukocytes in non-irradiated mice.

The alkaline comet assay suggests that both natural compounds, especially when given as pre-treatment, protect against primary leukocyte DNA damage in mice. At tested concentrations, EEP and quercetin were not genotoxic to non-irradiated mice. AET, however, caused a slight but not significant increase in DNA damage.

Although the results of this study show the radioprotective potential of the test compounds, further investigation is needed to clarify the underlying protection mechanisms.

## 7.37. Growth inhibitory activity of ethanol extracts of Chinese and Brazilian Propolis in four human colon carcinoma cell lines.

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| Authors | M Ishihara, K Naoi, M Hashita, Y Itoh, and M Suzui |
| Link | Oncol Rep, August 1, 2009; 22(2): 349-54 |
| Research Institute | Department of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University, Gifu 502-8585, Japan |


### Abstract

More than 300 bio-active compounds have been identified from bee Propolis in various regions of the world. The objective of this study was to examine whether the ethanol extracts of Chinese and Brazilian Propolis may exert anticancer activities in four human colon carcinoma cell lines, namely CaCo2, HCT116, HT29 and SW480.

Propolis samples were extracted with ethanol, and the crude extracts were dissolved in dimethylsulfoxide and used for the experiments.

In HCT116, HT29 and SW480 cell lines, the extracts of both Chinese and Brazilian Propolis caused a marked dose-dependent growth inhibition, with IC50 values in the range of 4-41 microg/ml. In HCT116 cell line, Chinese Propolis extract induced apoptosis in the cells after 72 h of treatment. In addition, Chinese Propolis extract caused a dose-dependent increase in the cellular mRNA levels of p21CIP1 and p53 in the HCT116 cell line.

These findings indicate that the ethanol extracts of Propolis contain components that may have anticancer activity. Thus, Propolis and related products may provide a novel approach to the chemoprevention and treatment of human colon carcinoma.

## 7.38. Ethanolic Extract of Propolis Augments TRAIL-Induced Apoptotic Death in Prostate Cancer Cells


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|---|---|
| Authors | Ewelina Szliszka[1], Zenon P. Czuba[1], Joanna Bronikowska[1], Anna Mertas[1], Andrzej Paradysz[2] and Wojciech Krol[1] |
| Link | Evid. Based Complement. Altern. Med., 5 November 2009, 10.1093/ecam/nep180 |
| Research Institute | [1]Chair and Department of Microbiology and Immunology, Jordana 19, 41808 Zabrze and [2]Chair and Department of Urology, 3-go Maja 13, 41 800 Zabrze, Medical University of Silesia in Katowice, Poland |



### Abstract

Prostate cancer is a commonly diagnosed cancer in men. The ethanolic extract of Propolis (EEP) and its phenolic compounds possess immunomodulatory, chemopreventive and antitumor effects.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/APO2L) is a naturally occurring anticancer agent that preferentially induces apoptosis in cancer cells and is not toxic to normal cells.

We examined the cytotoxic and apoptotic effects of EEP and phenolic compounds isolated from Propolis in combination with TRAIL on two prostate cancer cell lines, hormone-sensitivity LNCaP and hormone-refractory DU145. The cytotoxicity was evaluated by MTT and LDH assays. The apoptosis was determined using flow cytometry with annexin V-FITC/propidium iodide.

The prostate cancer cell lines were proved to be resistant to TRAIL-induced apoptosis. Our study demonstrated that EEP and its components significantly sensitize to TRAIL-induced death in prostate cancer cells. The percentage of the apoptotic cells after cotreatment with 50 μg ml−1 EEP and 100 ng ml−1 TRAIL increased to 74.9 ± 0.7% for LNCaP and 57.4 ± 0.7% for DU145 cells. The strongest cytotoxic effect on LNCaP cells was exhibited by apigenin, kaempferid, galangin and caffeic acid phenylethyl ester (CAPE) in combination with TRAIL (53.51 ± 0.68 − 66.06 ± 0.62% death cells).

In this work, we showed that EEP markedly augmented TRAIL-mediated apoptosis in prostate cancer cells and suggested the significant role of Propolis in chemoprevention of prostate cancer.

## 7.39. In vitro Cytotoxic Effect of Brazilian Green Propolis on Human Laryngeal Epidermoid Carcinoma (HEp-2) Cells.

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|---|---|
| Authors | Michelle C. Búfalo, João M. G. Candeias and José Maurício Sforcin |
| Link | eCAM, in press |
| Research Institute | Department of Microbiology and Immunology, Biosciences Institute, UNESP, 18618-000 Botucatu, S.P., Brazil |


### Abstract

Propolis is a sticky dark-colored material showing a very complex chemical composition that honeybees collect from plants. It has been used in folk medicine since ancient times, due to several biological properties, such as antimicrobial, anti-inflammatory, antioxidant and immunomodulatory activities, among others.

Its antitumor action in vivo and in vitro has also been reported, using Propolis extracts or its isolated compounds.

The goal of this work was to evaluate propolis's cytotoxic action in vitro on human laryngeal epidermoid carcinoma (Hep-2) cells. These cells were incubated with different concentrations of this bee product for different time periods, and morphology and the number of viable HEp-2 cells analyzed.

Data showed that Propolis exhibited a cytotoxic effect in vitro against HEp-2 cells, in a dose- and timedependent way. Propolis solvent had no effects on morphology and number of viable cells, proving that the cytotoxic effects were exclusively due to Propolis components.

Since humans have been using Propolis for a long time, further assays will provide a better comprehension of Propolis's antitumor action.

## 7.40. Angiostatic effects of Brazilian green propolis and its chemical constituents.

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| Authors | Yuichi Chikaraishi, Hiroshi Izuta, Masamitsu Shimazawa, Satoshi Mishima, and Hideaki Hara |
| Link | Mol Nutr Food Res, December 3, 2009 |
| Research Institute | Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, Gifu, Japan |


### Abstract

Propolis, a resinous substance collected by honeybees from various plant sources, has several pharmacological actions, such as anti-tumor and anti-inflammatory effects. The aim of this study was to evaluate the anti-angiogenic effects of a water extract of Brazilian green Propolis (WEP) and its constituents, caffeoylquinic acid derivatives, against angiogenic processes in human umbilical vein endothelial cells (HUVECs) in vitro.

We also examined the anti-angiogenic effects of WEP against retinal neovascularization in a murine oxygen-induced retinopathy model in vivo. WEP and its constituents significantly suppressed vascular endothelial growth factor (VEGF)-induced HUVEC proliferation, migration, and tube formation in vitro. WEP and its caffeoylquinic acid derivatives suppressed VEGF-stimulated phosphorylation of mitogen-activated protein kinase in HUVECs (versus VEGF alone). Moreover, WEP (300 mg/kg/day, subcutaneously for 5 days) significantly suppressed retinal neovascularization in the murine oxygen-induced retinopathy model.

These data indicate that (i) WEP has angiostatic effects against angiogenic processes in vitro and in an in vivo model of murine oxygen-induced retinopathy and (ii) the inhibitory effects of WEP against in vitro angiogenesis are chiefly derived from its caffeoylquinic acid derivatives.

Judging from these findings, WEP and its caffeoylquinic acid derivatives may represent candidates for preventive or therapeutic agents against diseases caused by angiogenesis.

## 7.41. Scientific Evidences to Pharmacological Anticancer Action of *Baccharis dracunculifolia* Brazilian Propolis.


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| Authors | [1]Paulino, N., [2]Abreu, S.R.L, [3]Machado, G., [4]Silveira, E |
| Link | Rev. Pesq. Inov. Farm. 1(1):15-26, ago-dez, 2009 |
| Research Institute | [1] Grupo de Pesquisa e Desenvolvimento de Biomedicamentos.<br>[2] Programa de Mestrado Profissional em Farmácia. – UNIBAN.<br>[2] Nectar Pharmaceutica Ltda,<br>[3] Verdente Nutraceutical, Bristol, United Kingdom<br>[4] Santa Casa de Misericórdia de Belo Horizonte Centro de Pesquisa em Cardiologia e Doenças Crônico-degenerativas |



### Abstract

Propolis is a traditional nutritional supplement produced by bees and widely used as a folk remedy around the world. Its chemical composition and biological activities varies depending on the geographical location, botanical origin, season and the type of bees. In Brazil, the most popular and the most researched grade is "green" propolis, largely produced in the State of Minas Gerais, from the plant *Baccharis dracunculifolia*.

This specific grade of propolis when properly processed shows remarkable activities as an antitumoral (cytotoxic, anti-proliferative and antimetastasic), analgesic and anti-inflammatory, immune-modulator, antioxidant, antimicrobial and healing agent. It has been safely used either by doctors or as a folk remedy in Brazil, Japan and many other countries as supportive nutrition along with orthodox cancer treatment, or supporting biological therapy of cancer.

Paulino et al., here reviews his own propolis studies and many other peer reviewed publications on its pharmacological inter-and intracellular pathways. He will demonstrate that Baccharis dracunculifolia propolis can modulate interesting cellular targets in different cancer cells, such as nuclear transcription factor (NFκB); post translational prenylation in Ras-GTPase signalling; p38-MAPK, PI3K/Akt/PKB pathway; COX-2 and prostaglandin E2 pathway; and iNOS or e-NOS expression and respective nitric oxide production. In addition, it modulates DNA fragmentation induced by cytochrome-C pathway; p53-protein signalling; releasing of pro-apoptotic proteins Bax and Bak; inhibition of neoangiogenesis by modulation of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) expression; control of the cell differentiation by modulating p21(Waf1/Cip1) protein in association with CDK2 and cyclin E.

Finally, Baccharis dracunculifolia propolis has been used in associative therapy to improve the efficacy of chemotherapy drugs (e.g. combination with paclitaxel, resveratrol, vinorelbine, etc) while shortening treatment time and reducing treatment side-effects (with both synthetic chemotherapy and radiotherapy).

Recently published results suggest that Baccharis dracunculifolia propolis can be used for supporting biological therapy or in association with chemotherapy drugs or

radiotherapy, as a natural food supplement to help to treat or prevent cancer. Clinical and pharmacokinetics trials with animals and humans will be mentioned.

## 7.42. Caffeic Acid Phenetyl Ester, a Brazilian-Green-Propolis Derivative, Induces apoptosis in AML Cells, Promotes up Regulation of G-Protein Signaling and Hyper Secretion of IL-8.


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| Authors | Priscila S Scheucher*[1], Guilherme A dos Santos*[1], Hamilton L Gimenes Teixeira*[1], Carolina H Thomé*[2], Antonio R Lucena-Araujo*[1], Roberto P. Falcao, MD, PhD[1], Eduardo M. Rego, MD, PhD[1] |
| Link | Blood (ASH Annual Meeting Abstracts) 2010; 116:3274. |
| Research Institute | [1]Clinical Medicine, Ribeirao Preto Medical School of University of Sao Paulo, Ribeirao Preto, Brazil, [2]Biochemistry, UNIFESP Medical School, Sao Paulo, Brazil |



Abstract 3274

Propolis is a generic name for an adhesive resin collected, processed and used by bees to plug gaps, smooth internal walls and protect the entrance of the hive from intruders. Chemically is a complex blend of resin and fragments of plant tissues, volatile substances and wax. It contains over 300 constituents including benzoic acids, flavonoids and cinnamic acid derivatives.

The Brazilian green propolis (BGP) has been shown to have immunomodulatory and antitumor properties in vitro and in vivo. We selected the caffeic acid phenethyl ester (CAPE), which is one of the components of BGP, as a candidate molecule for further studies regarding the antineoplastic activity.

Cytotoxicity induced by Brazilian green propolis alcoholic extract (BGPAE) and CAPE in the cell lineages of acute promyelocytic leukemia (APL) (NB4 and NB4R2), and bone marrow cells from patients with acute myeloid leukemia (AML) at diagnosis (primary cells) was evaluated by annexin V/propidium iodide staining and analyzed by flow cytometry.

BGPAE and CAPE showed antiproliferative and apoptotic activity and this effect was dose and time dependent (Table 1 and 2). Accordingly, in primary cells, CAPE (32µg/ml) for 24 hours significantly increased the percentage of apoptotic cells compared to control. The median (25 and 75 percentiles) for the percentage of apoptotic cells was 17.32% (13-27%) in control and 37% (24-52%) in CAPE, p-value = 0.0008 The cell cycle was analysed by flow cytometry using propidium iodide staining and demonstrated that CAPE blocked the cell cycle at G2/M. In addition, CAPE induced activation of caspases 3 and 9, as determined by Western blot, thus suggesting the involvement of the intrinsic pathway of apoptosis. The modulation of gene expression induced by CAPE in NB4 was determined by microarray using the CodeLink Uniset Human I bioarray (Amersham/GE) and analyzing 10,000 genes. Adopting a False Discovery Rate of 4.72% and using the Significance Analysis of Miroarray (SAM) software, we detected an increased expression of: negative mediators of cell cycle, including the dual specific phosphatases CDC14A, CDC14B and the cyclin-dependent kinase (CDK) inhibitor CDKN1A (p21/CIP1); protein phosphatases; chemokines and molecules associated with signaling by G protein. Furthermore, CAPE induced a decrease in gene expression of: positive mediators of

cell cycle (including CDK4 and CCNA2); genes related to the "spliceosome" and protein translation.

The most important differences in gene expression were confirmed by real time PCR using Taqman® technology (Applied Biosystems). Based on the microarray analysis, we decided to further study the production of IL-8 induced by CAPE in NB4 cells. Using an ELISA assay, it was detected a time dependent increase in the production of IL-8 at CAPE concentrations of 16 and 32µg/ml that was significantly higher than in controls at 12 and 24h of treatment.

In conclusion, our results demonstrate that CAPE was able to block the cell cycle and induce apoptosis in APL cells, and induce the production of IL-8 ALP cells.

## 7.43. Kaempferide Targets Side Population, the Putative Cancer Stem Cell, In Myeloma and Induced Apoptosis In Dose-Dependant Manner


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| Link | Blood (ASH Annual Meeting Abstracts) 2010 116: Abstract 5029. |
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Abstract 5029

Multiple myeloma (MM), cancer of the plasma cells, remains incurable despite advancement in therapeutic regiments. Studies showed that the 'side population (SP)', a subpopulation enriched with CSC in various cancers, has higher drug transporter activity than the bulk tumor; suggesting the reason why these cells survived despite post-chemotherapy. The increasing evidence of cancer stem cells (CSC) suggests that anti-cancer drugs targeting this subpopulation may lead to eradication of the root of cancer recurrence.

In this study, we explored the capability of kaempferide (KFD), a flavanoid in propolis (bee glue) that can reverse drug transporter activity, to combat SP cells in myeloma. KFD was one of the compounds in Brazilian propolis that reduced SP percentage in myeloma cell lines. We report for the first time that KFD is able to induce apoptosis in the SP cells in myeloma. The ability of KFD to inhibit growth of unfractionated KMS-11 cells was first investigated. Parthenolide (PAR), a natural product shown to inhibit growth of putative CSC in acute and chronic myelogenous leukemia was included as control. Unfractionated cells were seeded at 20000 cells per well in a 96 well plate. After 24h of incubation with various concentration of KFD, PAR, and DMSO (vehicle control), MTS solution was added and absorbance at 490 nm was determined. The IC50 of KFD and PAR in KMS-11 cells was 26 μM and 5 μM with 95% confidence intervals between 17.7 to 33.3 μM and 4.0 to 5.8 μM respectively. This is shown in the dose-response curve in figure 1A. No significant growth inhibition was observed in DMSO (0.1% to 0.5 % v/v) treated cells. We then examined if the KFD causes apoptosis in the sorted-SP cells. Sorted-SP cells were treated with 26 μM KFD, 5 μM PAR, or 0.2% v/v DMSO as mentioned above. After 1, 3, and 6 hour of incubation, cells were harvested and stained with Annexin V and propidium iodide and then analyzed using FACS Calibur.

Result showed that percentage of Annexin V+ apoptotic cells in KFD-treated cells increased in a time series manner (1, 3, 6 h) (figure 1B). Because KFD was reported to be capable to reverse the activity of drug efflux transporter, further studies to investigate the synergistic effect of KFD with conventional drugs to treat myeloma will be carried out.

Alternative medicines employing natural products have become increasingly sought after when conventional drugs cause immense side effects. Component in propolis that was reported to have anti-cancer properties, has low toxicity at high concentration, and spare normal cells, appeals to be a potential compound to combat myeloma. Exploitation of KFD that has the dual effect to induce apoptosis in putative CSC in myeloma and reverse drug transporter activity offers great

opportunity in cancer drug development and future clinical trials in patients with myeloma.

## 7.44. Human Head and Neck Squamous Cell Carcinoma Cell Lines are Differentially Radiosensitised by the Honeybee Product Propolis


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| Link | Int J Radiat Biol, 2010 Dec 10 |
| Research Institute | [1]OncoRay – Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden<br>[2]Department of Radiation Oncology, University Hospital and Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden<br>[3]Zentrum für Biosystemanalyse, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany |



### Abstract

**Purpose:** Propolis, a product of honeybees, has anti-tumoural, cytotoxic, anti-metastatic and anti-inflammatory properties. The aim of this study was the evaluation of the radiosensitising capacity of Propolis in human head and neck squamous cell carcinoma (HNSCC) cells.

**Materials and methods:** HNSCC cell lines (FaDu, UT-SCC15, UT-SCC45), fibroblasts (HSF2) and keratinocytes (HaCaT) were treated with Propolis (0–250µg/ml; 1, 4, 24h) without and in combination with X-rays (0–6 Gy, single dose). Clonogenic survival, proliferation, apoptosis, expression and phosphorylation of different signalling proteins were determined. Liquid chromatography-mass spectrometry (LC-MS) was performed on Propolis.

**Results:** Propolis significantly ($P<0.01$) reduced cell growth and clonogenic survival in a time- and concentration-dependent manner. Propolis-induced apoptosis and Caspase 3 cleavage, increased phosphorylation of Extracellular signal Regulated Kinase 1/2 (ERK1/2), protein kinase B/Akt1 (Akt1) and Focal adhesion kinase (FAK). While a 1-h Propolis pretreatment was ineffective, a 3-h pretreatment significantly ($P<0.05$) radiosensitised FaDu cells. LC-MS analysis identified 14 compounds of Propolis.

**Conclusions:** Our data show that Propolis exerts cytotoxicity in a concentration- and time-dependent manner. In one out of three HNSCC cell lines, Propolis also caused an enhancement of radiosensitivity. Future studies on Propolis will shed further light on its potential as an adjuvant to radiotherapy.

## 7.45. Cytotoxic constituents of Propolis inducing anticancer effects: a review


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|---|---|
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| Link | Journal of Pharmacy and Pharmacology, 63: 1378–1386, DOI: 10.1111/j.2042-7158.2011.01331.x |
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### Abstract

**Objectives:** Propolis is a honeybee product used extensively in traditional medicine for its antioxidant, anti-inflammatory, immunomodulatory and anticancer effects. Propolis exhibits a broad spectrum of biological activities because it is a complex mixture of natural substances. In this review, the antitumour effects of propolis extracts and its constituents (e.g. flavonoids, terpenes and caffeic acid phenethyl ester) are discussed.

**Key findings**: The effect of Propolis on experimental carcinogenesis is discussed, as well as its possible mechanisms of action against tumours, involving apoptosis, cell cycle arrest and interference on metabolic pathways. Propolis seems to be efficient against different tumour cells both in vitro and in vivo, which suggests its potential in the development of new anticancer drugs.

**Summary:** Propolis extracts may be important economically and would allow a relatively inexpensive cancer treatment. Preclinical investigations are needed to further elucidate the benefits of propolis and its antitumour properties.

## 7.46. Propolis May Help Prevent Colorectal Cancer - Propolis Augments Apoptosis Induced by Butyrate via Targeting Cell Survival Pathways


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| Link | PLoS One, 2013 Sep 4;8(9):e73151 |
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### Abstract

Diet is one of the major lifestyle factors affecting incidence of colorectal cancer (CC), and despite accumulating evidence that numerous diet-derived compounds modulate CC incidence, definitive dietary recommendations are not available. We propose a strategy that could facilitate the design of dietary supplements with CC-preventive properties. Thus, nutrient combinations that are a source of apoptosis-inducers and inhibitors of compensatory cell proliferation pathways (e.g., AKT signaling) may produce high levels of programmed death in CC cells. Here we report the combined effect of butyrate, an apoptosis inducer that is produced through fermentation of fiber in the colon, and propolis, a honeybee product, on CC cells. We established that propolis increases the apoptosis of CC cells exposed to butyrate through suppression of cell survival pathways such as the AKT signaling. The programmed death of CC cells by combined exposure to butyrate and propolis is further augmented by inhibition of the JNK signaling pathway. Analyses on the contribution of the downstream targets of JNK signaling, c-JUN and JAK/STAT, to the apoptosis of butyrate/propolis-treated CC cells ascertained that JAK/STAT signaling has an anti-apoptotic role; whereas, the role of cJUN might be dependent upon regulatory cell factors. Thus, our studies ascertained that propolis augments apoptosis of butyrate-sensitive CC cells and re-sensitizes butyrate-resistant CC cells to apoptosis by suppressing AKT signaling and downregulating the JAK/STAT pathway. Future in vivo studies should evaluate the CC-preventive potential of a dietary supplement that produces high levels of colonic butyrate, propolis, and diet-derived JAK/STAT inhibitors.

## 7.47. Apimedica: High Doses of Propolis Non-Toxic, Acute Toxicological Test of Propolis

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|---|---|
| Authors | ZHUO Bi-rong, SHI Pei-ying, WU Zhen-hong |
| Link | Apimondia Apimedica-Apiquality International Forum, 22-25 Oct 2012 |
| Research Institute | Bee Science College of Fujian Agriculture and Forestry University, State and Local Joint Engineering Laboratory of Natural Biotoxi, Fuzhou 350002, China, Apimondia Apimedica-Apiquality International Forum, Zhenjiang, China |


### Abstract

This project used Fixed-dose procedure to study acute toxicity reaction of mice to ethanol extract of propolis.

The result showed that mice, which have been lavaged with ethanol extract of propolis were all live during 0~14 days. In addition, the tested mice shown no-affected locomotor activity and had no abnormal symptoms. The mice showed no abnormal secretion in mouths, noses and ears. Also the mice showed other normal symptoms such as eyelid without prolapse, clear eye pupil, normal excretions, and smooth hair. Autopsy results showed no obvious abnormity characterization of the organs. The organs index has no obvious difference with control group (The max dose is 2000mg/kg).

In conclusion, mice had no acute toxicity reaction to ethanol extract of propolis.

Since 2000mg/kg is 100 times of the human body recommended dose, people can also use propolis with this dose.

## 7.48. Propolis May Help Prevent Oral Cancer: Caffeic Acid phenethyl Ester Inhibits Oral Cancer Cell Metastasis by Regulating Matrix Metalloproteinase-2 and the Mitogen-Activated Protein Kinase Pathway


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| Link | Evidence-Based Complementary and Alternative Medicine<br>Volume 2012 (2012), Article ID 732578, 10 pages |
| Research Institute | [1]School of Dentistry, Chung Shan Medical University, Taichung 40201<br>[2]Department of Dentistry, Chung Shan Medical University Hospital, Taichung 40201<br>[3]Institute of Medicine, Chung Shan Medical University, Taichung 402<br>[4]School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung 40201<br>[5]Department of Pathology, Changhua Christian Hospital, Changhua 50006<br>[6]Department of Medical Research, Chung Shan Medical University Hospital, Taichung 40201<br>[7]Institute of Oral Sciences, Chung Shan Medical University, Taichung 40201<br>All in Taiwan |



### Abstract

Caffeic acid phenethyl ester (CAPE), an active component extracted from honeybee hives, exhibits anti-inflammatory and anticancer activities. However, the molecular mechanism by which CAPE affects oral cancer cell metastasis has yet to be elucidated.

In this study, we investigated the potential mechanisms underlying the effects of CAPE on the invasive ability of SCC-9 oral cancer cells.

Results showed that CAPE attenuated SCC-9 cell migration and invasion at noncytotoxic concentrations (0 μM to 40 μM). Western blot and gelatin zymography analysis findings further indicated that CAPE downregulated matrix metalloproteinase-2 (MMP-2) protein expression and inhibited its enzymatic activity. CAPE exerted its inhibitory effects on MMP-2 expression and activity by upregulating tissue inhibitor of metalloproteinase-2 (TIMP-2) and potently decreased migration by reducing focal adhesion kinase (FAK) phosphorylation and the activation of its downstream signaling molecules p38/MAPK and JNK.

These data indicate that CAPE could potentially be used as a chemoagent to prevent oral cancer metastasis.

## 7.49. Honey, Propolis Component a Promising Anticancer Drug: Anti-tumor activity evaluation of novel chrysin–organogermanium(IV) complex in MCF-7 cells


| | |
|---|---|
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| Link | Bioorganic & Medicinal Chemistry Letters, Volume 23, Issue 20, 15 October 2013, Pages 5544–5551 |
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### Abstract

Chrysin (5,7-dihydroxylflavone, Chry) is a natural product extracted from plants, honey, and propolis. In this work, a novel chrysin–organogermanium(IV) complex (Chry–Ge) with enhanced anticancer activities was synthesized, and its potential anticancer effects against cancer cells were measured using various methods. MTT results showed that Chry–Ge had significant inhibition effects on the proliferation of MCF-7, HepG2 and Colo205 human cancer cell lines in a dose-dependent manner while had little cytotoxic effects on MCF-10A human normal cells (MCF-10A cells) with the same treatment of Chry–Ge. These results suggested that Chry–Ge possessed enhanced anticancer effects and high selectivity between cancer cells and normal cells. The immuno-staining results showed that the nuclei of MCF-7 cells represented a total fragmented morphology and a disorganized cytoskeletal network in MCF-7 cells after Chry–Ge treatment. Besides, atomic force microscopy (AFM) was applied to detect the changes of ultrastructural and biomechanical properties of MCF-7 cellular membrane induced by Chry–Ge.

The AFM data indicated that Chry–Ge treatment directly caused the decrease of cell rigidity and adhesion force of MCF-7 cells, suggesting that membrane toxicity might be one of the targets for Chry–Ge in MCF-7 cells.

Moreover, the fluorescence-based flow cytometric analysis demonstrated that Chry–Ge could induce apoptosis in MCF-7 cells in ROS-dependent mitochondrial pathway.

All results collectively showed that Chry–Ge could be as a promising anticancer drug for cancer therapy.

## 7.50. The Immunomodulatory and Anticancer Properties of Propolis


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|---|---|
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| Link | Clinical Reviews in Allergy & Immunology<br>June 2013, Volume 44, Issue 3, pp 262-273 |
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### Abstract

Propolis, a waxy substance produced by the honeybee, has been adopted as a form of folk medicine since ancient times. It has a wide spectrum of alleged applications including potential anti-infection and anticancer effects. Many of the therapeutic effects can be attributed to its immunomodulatory functions. The composition of propolis can vary according to the geographic locations from where the bees obtained the ingredients.

Two main immunopotent chemicals have been identified as caffeic acid phenethyl ester (CAPE) and artepillin C. Propolis, CAPE, and artepillin C have been shown to exert summative immunosuppressive function on T lymphocyte subsets but paradoxically activate macrophage function. On the other hand, they also have potential antitumor properties by different postulated mechanisms such as suppressing cancer cells proliferation via its anti-inflammatory effects; decreasing the cancer stem cell populations; blocking specific oncogene signaling pathways; exerting antiangiogenic effects; and modulating the tumor microenvironment.

The good bioavailability by the oral route and good historical safety profile makes propolis an ideal adjuvant agent for future immunomodulatory or anticancer regimens. However, standardized quality controls and good design clinical trials are essential before either propolis or its active ingredients can be adopted routinely in our future therapeutic armamentarium.

## 7.51. Propolis Component May Help Boost Cancer Cell Death: Chrysin Overcomes TRAIL Resistance of Cancer Cells Through Mcl-1 Downregulation by Inhibiting STAT3 Phosphorylation


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|---|---|
| Authors | Kriengsak Lirdprapamongkol, Hiroaki Sakurai, Sherif Abdelhamed, Satoru Yokoyama, Sirivan Athikomkulchai, Amornrat Viriyaroj, Suresh Awale, Somsak Ruchirawat, Jisnuson Svasti, Ikuo Saiki |



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| Link | Int J Oncol, 2013 May 1, pages 329-337 |



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### Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively kills various types of cancer cells without harming normal cells, but TRAIL resistance has been frequently observed in cancer cells. Propolis (bee glue) is a material collected from various plants by honeybees and is a rich source of bioactive compounds, including the natural flavonoid chrysin, which possesses multiple anticancer effects. We investigated the mechanism underlying the TRAIL sensitization effect of chrysin, which is a major constituent of Thai propolis, in human lung and cervical cancer cell lines. Propolis extract and chrysin sensitizes A549 and HeLa human cancer cell lines to TRAIL-induced apoptosis. The TRAIL sensitization effect of chrysin is not mediated by inhibition of TRAIL-induced NF-κB activation or by glutathione depletion. Immunoblot analysis using a panel of anti-apoptotic proteins revealed that chrysin selectively decreases the levels of Mcl-1 protein, by downregulating Mcl-1 gene expression as determined by qRT-PCR. The contribution of Mcl-1 in TRAIL resistance was confirmed by si-Mcl-1 knockdown. Among signaling pathways that regulate Mcl-1 gene expression, only constitutive STAT3 phosphorylation was suppressed by chrysin. The proposed action of chrysin in TRAIL sensitization by inhibiting STAT3 and downregulating Mcl-1 was supported by using a STAT3-specific inhibitor, cucurbitacin-I, which decreased Mcl-1 levels and enhanced TRAIL-induced cell death, similar to that observed with chrysin treatment. In conclusion, we show the potential of chrysin in overcoming TRAIL resistance of cancer cells and elucidate its mechanism of action.

## 7.52. Propolis Component May Help Treat Oral Squamous Cell Carcinoma: Caffeic Acid Phenethyl Ester Suppresses Proliferation and Survival of TW2.6 Human Oral Cancer Cells via Inhibition of Akt Signaling


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Link: Int J Mol Sci, 2013 Apr 24;14(5):8801-17



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### Abstract

Caffeic acid phenethyl ester (CAPE) is a bioactive component extracted from honeybee hive propolis. Our observations indicated that CAPE treatment suppressed cell proliferation and colony formation of TW2.6 human oral squamous cell carcinoma (OSCC) cells dose-dependently.

CAPE treatment decreased G1 phase cell population, increased G2/M phase cell population, and induced apoptosis in TW2.6 cells. Treatment with CAPE decreased protein abundance of Akt, Akt1, Akt2, Akt3, phospho-Akt Ser473, phospho-Akt Thr 308, GSK3β, FOXO1, FOXO3a, phospho-FOXO1 Thr24, phospho-FoxO3a Thr32, NF-κB, phospho-NF-κB Ser536, Rb, phospho-Rb Ser807/811, Skp2, and cyclin D1, but increased cell cycle inhibitor p27Kip. Overexpression of Akt1 or Akt2 in TW2.6 cells rescued growth inhibition caused by CAPE treatment. Co-treating TW2.6 cells with CAPE and 5-fluorouracil, a commonly used chemotherapeutic drug for oral cancers, exhibited additive cell proliferation inhibition.

Our study suggested that administration of CAPE is a potential adjuvant therapy for patients with OSCC oral cancer.

## 7.53. Propolis Component Inhibits Cancer-Causing Enzymes: The Flavonoid Chrysin Attenuates Colorectal Pathological Remodeling Reducing the Number and Severity of Pre-Neoplastic Lesions In Rats Exposed to the Carcinogen 1,2-dimethylhydrazine


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Link: Cell Tissue Res, 2013 Mar 7



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### Abstract

Phenolic compounds are naturally occurring, bioactive substances with marked antioxidant and anti-inflammatory potential. The flavonoid chrysin, found in high levels in honey bee propolis, inhibits the activity of enzymes involved in carcinogenesis. We have investigated the effect of chrysin on pre-neoplastic colorectal lesions (ACF, aberrant crypt foci) in a rat model of chemical carcinogenesis induced by 1,2-dimethylhydrazine (DMH). Female Wistar rats weighing 137.2 ± 24.3 g received weekly one subcutaneous injection of DMH (20 mg/kg) for 10 weeks.

The animals were divided into five groups each with seven animals: Group 1, 0.9% saline; Group 2, DMH+0.9% saline; Group 3, DMH+chrysin (10 mg/kg); Group 4, DMH+chrysin (100 mg/kg); Group 5, DMH+chrysin (200 mg/kg). Groups 2 and 3 showed a significant increase in ACF number, nucleolus organizer regions per enterocyte nucleus and nitrite/nitrate serum levels compared with Group 1. Groups 4 and 5 presented a significant reduction in all these parameters compared with Group 2. The levels of antioxidant minerals (copper, magnesium, selenium, zinc) and the number of enteroendocrine and mucin-producing cells were significantly reduced in Groups 2 and 3 but were similar in Groups 4 and 5 compared with Group 1.

Chrysin, at 100 mg/kg and 200 mg/kg, was effective in attenuating pathological colorectal remodeling, reducing the number of pre-neoplastic lesions in rats exposed to DMH. Some of these effects might be attributable to the recovery of antioxidant mineral levels, a reduction in systemic nitrosative stress and an inhibition of the cellular proliferation induced by this flavonoid.

## 7.54. Propolis Component May Help Treat Advanced Prostate Cancer: Caffeic Acid Phenethyl Ester as an Adjuvant Therapy for Advanced Prostate Cancer


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|---|---|
| Authors | Chun-Chieh Liu[1], Jong-Ming Hsu[2], Li-Kuo Kuo[3], Chih-Pin Chuu[4] |
| Link | Med Hypotheses, 2013 Feb 22. pii: S0306-9877(13)00075-3 |
| Research Institute | [1.] Division of Cardiology, Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan, Mackay Medical College, Taipei, Taiwan, Mackay Medicine, Nursing and Management College, Taipei, Taiwan<br>[2] Department of Urology, Mackay Memorial Hospital, Taipei, Taiwan<br>[3] Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Mackay Memorial Hospital, Taipei, Taiwan<br>[4] Institute of Cellular and System Medicine, National Health Research Institutes, Miaoli, Taiwan, Translational Center for Glandular Malignancies, National Health Research Institutes, Miaoli, Taiwan, Graduate Program for Aging, China Medical University, Taichung, Taiwan |



### Abstract

Prostate cancer is the second most frequently diagnosed cancer of men. Androgen ablation therapy is the primary treatment for metastatic prostate cancer. However, the majority of prostate cancer patients receiving the androgen ablation therapy will ultimately develop recurrent castration-resistant tumors within 3years.

Chemotherapy shows little effect on prolonging survival for patients with metastatic hormone-refractory prostate cancer. More than 80% of prostate tumors acquire mutation or deletion of tumor suppressor phosphatase and tensin homolog (PTEN), a negative regulator of PI3K/Akt signaling. Caffeic acid phenethyl ester (CAPE) is a strong antioxidant extracted from honeybee hive propolis. Recent studies indicate that CAPE treatment suppresses tumor growth and Akt signaling in human prostate cancer cells. Combined treatments of CAPE with chemotherapeutic drugs exhibit synergistic suppression effects. Pharmacokinetic studies suggest that intraperitoneal injection of CAPE at concentration of 10mg/kg is not toxic.

CAPE treatment sensitizes cancer cells to chemotherapy and radiation treatments. In addition, CAPE treatment protects therapy-associated toxicities in animal models. We therefore propose that administration of CAPE is a potential adjuvant therapy for patients with castration-resistant prostate cancer.

## 7.55. Propolis Can Help Prevent Skin Cancer: 10 ways you can prevent and even reverse Skin Cancer

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| Authors | Stasia Bliss |
| Link | The Guardian, 5/21/2013 |
| Sources | Propolis treatments; Alternative medicine; Fitness Magazine; Fox59 vaccine story; Medical News Today; Ehow Natural sunscreen; Natural News 8/11; TransformationsNet; losaltosonline.com; Yoga and Cancer; umm.edu; NetworkNature; Skin cancer foods; E-news; Examiner on-line; Fox News report on Frankincense |


### Abstract

May is skin cancer awareness month, with this Friday, May 24th being "Don't Fry Day", supported by the EPA, the FDA and the National Cancer Association. How can we take measures to avert over-exposure to damaging rays? A recent article on Fox suggests that researchers are looking for a vaccine to help prevent skin cancer, but is a vaccine really what we need? Another recent article states "holidaymakers are at risk…because their sunscreen is out of date", claiming that people are lathering up with expired sunscreen that isn't working.

Statistics say skin cancer is the most common form of cancer these days with 1 in 6 Americans diagnosed. What can we do to remain healthy and cancer-free?

Here are 10 healthy suggestions to prevent and even reverse possible skin cancer…

Bee Propolis – With excellent results, more than 20 studies in experimental medicine and hospital settings used Bee Propolis internally and externally to successfully prevent malignant cell growth, increase the aspects of the immune system that wards off cancer, bringing balance back into the body of one previously ravaged with cancer. Bee Propolis in amounts of only 30-50 drops of a tincture 4x a day had these amazing results, reducing different types of cancer, including malignant melanoma. (Other types of cancer that received benefits were breast, colon and genital cancer along with lung and liver metastases). In Yugoslavia, the use of Propolis was found to reduce harmful side-effects of radiation therapy…

## 7.56. Propolis Component Inhibits Growth of Pancreatic Cancer Cells: Caffeic Acid Phenethyl Ester Inhibits Epithelial-Mesenchymal Transition of Human Pancreatic Cancer Cells


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|---|---|
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| Link | Evid Based Complement Alternat Med, 2013;2013:270906, Epub 2013 Apr 4 |
| Research Institute | [1] Division of Gastroenterology, Department of Internal Medicine, Mackay Memorial Hospital, Taiwan<br>[2] Mackay Medicine, Nursing and Management College, Taipei, Taiwan<br>[3] Department of Radiation Oncology, Mackay Memorial Hospital, No. 92, Sec. 2, Chungshan North Road, Taipei, Taiwan |



### Abstract

Background. This study aimed to investigate the effect of propolis component caffeic acid phenethyl ester (CAPE) on epithelial-mesenchymal transition (EMT) of human pancreatic cancer cells and the molecular mechanisms underlying these effects.

Methods. The transforming growth factor β (TGF-β-) induced EMT in human pancreatic PANC-1 cancer cells was characterized by observation of morphology and the expression of E-cadherin and vimentin by western blotting. The migration potential was estimated with wound closure assay. The expression of transcriptional factors was measured by quantitative RT-PCR and immunocytochemistry staining. The orthotopic pancreatic cancer xenograft model was used for in vivo assessment.

Results. The overexpression of vimentin was attenuated by CAPE, and the alteration in morphology from polygonal to spindle shape was partially reversed by CAPE. Furthermore, CAPE delayed the TGF-β-stimulated migration potential. CAPE treatment did not reduce the expression levels of Smad 2/3, Snail 1, and Zeb 1 but inhibited the expression of transcriptional factor Twist 2. By using an orthotopic pancreatic cancer model, CAPE suppressed the expression of Twist 2 and growth of PANC-1 xenografts without significant toxicity.

Conclusion. CAPE could inhibit the orthotopic growth and EMT of pancreatic cancer PANC-1 cells accompanied by downregulation of vimentin and Twist 2 expression.

# 8. anti-inflammatory

## 8.1. Xanthine oxidase inhibitory activity and hypouricemia effect of Propolis in rats.


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|---|---|
| Authors | Yoshizumi K, Nishioka N, Tsuji T |
| Link | Yakugaku Zasshi. 2005 Mar;125(3):315-21. |
| Research Institute | Fancl Corporation Central Research Laboratory, Yokohama 244-0806, Japan. kayoshizu@fancl.co.jp |



### Abstract

The xanthine oxidase (XOD) inhibitory activity of Propolis from China and Brazil was measured. The Propolis from both place were seen to have XOD inhibitory activity. However, a stronger tendency was shown in the Propolis from China. The compounds in each the Propolis were measured quantitatively. A great deal of chrysin, galangin, and caffeic acid phenetyl ester were found in the Propolis from China, an abundance of p-coumaric acid and Artepillin C in the Propolis from Brazil. Therefore it was revealed that the Propolis compounds are very different depending on their place of origin. The XOD inhibitory activity of these five compounds was measured. Caffeic acid phenetyl ester had the strongest activity, with chrysin and galangin next; p-coumaric acid and Artepillin C showed weak XOD inhibitory activity. We evaluated the hypouricemic effect of Propolis from China on hyperuricemia induced by the uricase inhibitor, oxonic acid (500 mg/kg p.o., 1 h before the test drugs), and measured plasma uric acid values in rats. Oral Propolis had a hypouricemic effect 2 h after its administration to oxonate-pretreated rats. These results suggested that a continuous intake of Propolis may be effective for the prevention and the treatment of gout and hyperuricemia.

## 8.2. Evaluation of the analgesic and anti-inflammatory effects of a Brazlian Green Propolis.

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| Authors | Paulino N, Teixeira C, Martins R, Scremin A, Dirsch VM, Vollmar AM, Abreu SR, de Castro SL, Marcucci MC. |
| Link | Planta Med. 2006 Aug;72(10):899-906. |
| Research Institute | Grupo de Pesquisa e Desenvolvimento de Biofarmacos BIOFAR, Universidade do Sul de Santa Catarina, Tubarao/SC, Brazil. |


### Abstract

Phamacological activities of a standard ethanol extract G1 from Brazlian Green Propolis, typified as BRP1, was evaluated in mouse models of pain and inflammation. Intraperitoneal injection ( I. P.) of G1 inhibited acetic acid-induced abdominal constrictions with an ID (50) = 0.75 +/- 0.05 mg/kg, and in the formalin test the ID (50) values were 0.85 +/- 0.07 mg/kg and 13.88 +/- 1.12 mg/kg, respectively, for the neurogenic and inflammatory phases. The extract was ineffective when assessed in the hot-plate assay. In serotonin-induced paw edema, G1 led to a maximal inhibition (MI) of 51.6 % after 120 min when administered I. P. and of 36 % after 15 min by the oral route ( O. R.). When the inflammatory agent was complete Freund's adjuvant, inhibition of paw edema was also observed after administration of the extract by both routes. In the capsaicin-induced ear edema the ID (50) values were 1.09 +/- 0.08 mg/kg ( I. P.) and 10.00 +/- 0.90 mg/kg ( O. R.). In the acute carrageenan-induced inflammatory reaction induced by carrageenan, G1 reduced the number of neutrophils in the peritoneal cavity with IC (50) values of 0.72 +/- 0.08 mg/kg and 4.17 +/- 0.50 mg/kg, by I. P. or O. R. administration, with a preferential migration of polymorphonuclear neutrophils. IN VITRO, G1 decreased nitric oxide production in LPS-stimulated RAW 264.7 cells (IC (50) = 41.60 microg/mL), and also the luciferase activity in TNF-alpha-stimulated HEK 293 cells transfected with NF-kappaB-luciferase reporter gene driven by the nuclear factor kappaB (NF-kappaB) (IC (50) = 200 microg/mL). This extract, which at low concentrations induces anti-inflammatory and analgesic effects in mouse models, presents a high content of flavonoids, known to inhibit inducible NOS (iNOS) activity.

These data taken together led us to reinforce the hypothesis in the literature that the anti-inflammatory effect of Propolis may be a due to inhibition of iNOS gene expression, through interference with NF-kappaB sites in the iNOS promoter.

## 8.3. Effectiveness of mesalamine and Propolis in experimental colitis.


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|---|---|
| Authors | A Aslan, M Temiz, E Atik, G Polat, N Sahinler, E Besirov, N Aban, and CK Parsak. |
| Link | *Adv Ther.* 2007; 24: 1085. [MEDLINE Citation] |
| Research Institute | Department of General Surgery, Mustafa Kemal University, Antakya-Hatay, Turkey. |



### Abstract

This study was conducted to investigate the effects of Propolis and mesalamine on experimental colitis in rats. Distal colitis was induced in rats by intracolonic instillation of 2 mL of 4% acetic acid. The animals were randomly assigned to 5 groups: group 1, control, (n=8); group 2, colitis, received no treatment (n=8); group 3, colitis+mesalamine, 2 mL once a day via an enema (n=8); group 4, colitis+Propolis, 600 mg/kg once a day via intragastric lavage (n=8); and group 5, colitis+mesalamine+Propolis for 1 wk (n=8). Levels of nitric oxide were statistically significantly different in comparisons between groups 1 and 2, groups 2 and 3, and groups 4 and 5. Malondialdehyde levels were significantly different when group 2 was compared with groups 3, 4, and 5. A significant difference was observed when group 3 was compared with group 4 for myeloperoxidase. Most Propolis-treated rats had normal histology; mesalamine-treated and Propolis+mesalamine-treated rats had inflammatory cell infiltration at rates of 50% and 33%, respectively. The investigators concluded that Propolis and mesalamine are efficient independently and in combination, but that their combined effect was not observed to be additive in experimental colitis.

## 8.4. The effect of Propolis in experimental Acanthamoeba keratitis.


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| Authors | A Vural, ZA Polat, A Topalkara, MI Toker, H Erdogan, MK Arici, and A Cetin. |
| Link | *Clin Experiment Ophthalmol.* 2007; 35: 749. [MEDLINE Citation] |
| Research Institute | Department of Ophthalmology, Cumhuriyet University School of Medicine, Sivas, Turkey. vural.ayse@gmail.com |



### Abstract

PURPOSE: To examine the effect of Propolis in a rat model of Acanthamoeba keratitis and to determine its in vitro cytotoxicity in cultured corneal epithelial cells.

METHODS: Eighteen Wistar albino rats were used. Cultured corneal epithelial cells obtained from two healthy rats for in vitro cytotoxicity of Propolis. Corneal stromal inoculation was performed in 16 rats with amoebic culture containing 1 x 10(6) amoeba/mL. Rats with Acanthamoeba keratitis 5 days later after the inoculation were divided randomly into four groups, and eight eyes of each group were treated with study drugs. The Propolis, chlorhexidine (CHX), Propolis plus CHX and control eyes were treated with topical Propolis, 0.002% CHX, Propolis plus 0.002% CHX and lubricant eye drops, respectively. The study drugs were instilled every one hour for 10 days. All eyes were examined and keratitis graded by slit-lamp biomicroscopy on days 2, 5 and 10 during the administration of the study drugs. After the completion of keratitis grading, all the 16 rats were humanely killed and their corneas were excised and used for Acanthamoeba culture to evaluate presence of Acanthamoeba growth after treatment 14 days later.

RESULTS: Concentrations of Propolis higher than 7.81 mg/mL cause damage to corneal epithelial cells in the experiment of in vitro cytotoxicity of Propolis on corneal epithelial cells. The keratitis grade on day 2 in the CHX eyes was significantly lower than that in the control eyes ($P < 0.05$). The keratitis grades on days 5 and 10 in the Propolis, CHX and Propolis plus CHX eyes were significantly lower compared with those on days 5 and 10 in the control eyes ($P < 0.05$). In the Propolis eyes, the keratitis grade on day 5 was significantly lower than that on day 2 ($P < 0.05$), and it was significantly lower on day 10 compared with that on day 5 ($P < 0.05$). In the CHX and Propolis plus CHX eyes, the keratitis grade on day 10 was significantly lower compared with that on days 2 and 5 ($P < 0.05$). In the control eyes, there was no significant difference in the keratitis grades on days 2, 5 and 10 ($P > 0.05$). The culture positivity at Acanthamoeba growth after treatment experiment in the Propolis, CHX and Propolis plus CHX eyes was significantly lower than that in the control eyes ($P < 0.05$).

CONCLUSIONS: We suggest that Propolis had amoebicidal properties in this rat model of Acanthamoeba keratitis. Further investigations to evaluate the antimicrobial activity of the individual fractions of the resin could yield more information about its mechanism of action in treating this disease.

## 8.5. Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian Propolis.

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| Authors | N Paulino, SR Abreu, Y Uto, D Koyama, H Nagasawa, H Hori, VM Dirsch, AM Vollmar, A Scremin, and WA Bretz. |
| Link | Eur J Pharmacol, June 10, 2008; 587(1-3): 296-301 |
| Research Institute | Programa de Pós-graduação em Farmácia, Universidade Bandeirante de São Paulo, São Paulo, Brazil; Pharmacy Department, Centro Universitário Barriga Verde, Orleans, Brazil |


### Abstract

Artepillin C is the major compound in the Brazilian Green Propolis from *Baccharis dracunculifolia*.

Our aim in this study was to investigate the anti-inflammatory effects, absorption, and bioavailability of Artepillin C in mice. The animals used were male Swiss mice subjected to: paw oedema by carrageenan (300 mug/paw), carrageenan-induced peritonitis, and prostaglandin E(2) determination. We also measured in vitro nitric oxide production by RAW 264.7 cells and NF-kappaB activity in HEK 293 cells. Finally, we measured the absorption and bioavailability of Artepillin C in plasma from mice by means of GC-MS after a single oral dose (10 mg/kg).

In vivo, Artepillin C produced a maximal inhibition of 38% after 360 min on paw oedema. Artepillin C also decreased the number of neutrophils during peritonitis (IC(50): 0.9 (0.5-1.4) mg/kg). Treatment with Artepillin C decreased prostaglandin E(2) by 29+/-3% and 58+/-5% at 1 and 10 mg/kg, respectively, with a mean ID(50) of 8.5 (8.0-8.7)mg/kg). Similarly, in in vitro models, Artepillin C (3, 10, or 100 muM) decreased nitric oxide production by RAW 264.7 cells with a mean IC(50) of 8.5 (7.8-9.2) muM. In HEK 293 cells, Artepillin C reduced NF-kappaB activity with a mean IC(50) of 26 (22-30) mug/ml), suggesting anti-inflammatory activity, particularly during acute inflammation. Lastly, Artepillin C was absorbed after an oral dose (10 mg/kg) with maximal peaks found at 1 h (22 mug/ml).

Collectively, Artepillin C showed anti-inflammatory effects mediated, at least in part, by prostaglandin E(2) and nitric oxide inhibition through NF-kappaB modulation, and exhibited bioavailability by oral administration.

## 8.6. The beneficial effect of Propolis on cerulein-induced experimental acute pancreatitis in rats.


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| Authors | M Buyukberber, MC Savas, C Bagci, M Koruk, MT Gulsen, E Tutar, T Bilgic, R Deveci, and C Kucuk |
| Link | Turk J Gastroenterol. June 1, 2009; 20(2): 122-8 |
| Research Institute | Department of Gastroenterology, Gaziantep University, School of Medicine, Gaziantep, Turkey. mehmetbuyukberber@yahoo.com |



Abstract

BACKGROUND/AIMS: Inflammatory cytokines and oxidative stress have a central role in the pathogenesis of acute pancreatitis. Propolis is a resinous hive product collected by honeybees from various plant sources and has anti-inflammatory and anti-oxidant effects. The present work aimed to investigate the therapeutic role of ethanolic extract of Propolis on a cerulein-induced acute pancreatitis model in rats.

METHODS: Seventy male Wistar albino rats were used in the study. Acute edematous pancreatitis was induced by subcutaneous cerulein injection (20 microg/kg) four times at one-hour intervals. Ethanolic extract of propolis 300 mg/kg was given subcutaneously at the beginning of the procedure (ethanolic extract of propolis-1 group) or 12 h after the last cerulein injection (ethanolic extract of propolis-2 group). Serum amylase and lipase levels, white blood cell count and serum tumor necrosis factor-alpha levels were measured and pancreatic tissue was evaluated histologically.

RESULTS: In the acute pancreatitis group, serum amylase and lipase levels were found to be elevated and the histopathological evaluation of the tissue revealed massive edema and inflammation with less fatty necrosis when compared to the sham and control groups. Serum amylase and lipase levels and edema formation were significantly decreased in the ethanolic extract of propolis-treated groups ($p<0.001$). In the ethanolic extract of propolis-2 group, in particular, tissue edema was improved markedly ($p=0.001$). Tissue inflammation and fatty necrosis were decreased with ethanolic extract of propolis treatment; however, the improvement was not statistically significant.

CONCLUSIONS: Treatment with ethanolic extract of Propolis improved the biochemical and histopathological findings in a rat model of experimental pancreatitis. Although our findings suggest that ethanolic extract of Propolis might be considered an effective agent for the treatment of acute pancreatitis, this notion should be supported with further experimental and clinical investigations.

## 8.7. The influence of irradiation on the potential chondroprotective effect of aqueous extract of propolis in rats.


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| Authors | Mona A. El-ghazaly[1], Doaa H. Abd el-naby[1] & Mohamed T. Khayyal[2] |
| Link | International Journal of Radiation Biology, Posted online on November 19, 2010. (doi:10.3109/09553002.2011.530337) |
| Research Institute | [1]Department of Drug Radiation Research, National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt, [2]Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt |



### Abstract

**Purpose:** Cartilage degradation usually results as a consequence of inflammatory processes in the joints. To study this phenomenon experimentally, adjuvant-induced arthritis (AIA) was used as a model of chronic inflammation under the influence of irradiation. The potential chondroprotective effect of 13% aqueous extract of propolis (AEP) in arthritic rats was investigated.

**Materials and methods:** The influence of whole body irradiation on the arthritic inflammatory response was investigated by subjecting rats to a Gamma source before the induction of arthritis. 13% AEP was injected intraperitoneally in a dose of 5 ml/kg and diclofenac was used as reference non-steroidal anti-inflammatory drug (NSAID) in a dose of 3 mg/kg. The chosen parameters for cartilage integrity were glycosaminoglycan (GAG), hydroxyproline contents in cartilage and cartilage oligomeric matrix protein (COMP) in serum. The serum levels of tumour necrosis factor-alpha (TNF-α), nitric oxide (NO) and the oxidative stress biomarkers such as blood glutathione (GSH) and plasma malondialdehyde (MDA) levels.

**Results:** Induction of arthritis led to a reduction in GAG and hydroxyproline content of femoral cartilage and a corresponding rise in COMP in serum. Previous exposure to irradiation resulted in a milder reduction of GAG and hydroxyproline and a lesser rise in COMP. Treatment of arthritic irradiated and non-irradiated rats with 13% AEP markedly prevented the breakdown of cartilage in a much more effective manner than diclofenac. Both AEP and diclofenac were equipotent in reducing the level of TNF-α and were able to normalize NO and the oxidative stress biomarkers in non-irradiated and irradiated arthritic rats.

**Conclusion:** The ability of propolis to protect cartilage degradation could therefore prove of value in the treatment of chronic arthritic diseases, offering an advantage over some NSAID, particularly those with a potential detrimental effect on cartilage integrity.

## 8.8. Brazilian Red Propolis Isoflavonoids Have Strong Anti-Inflammatory and Antimicrobial Properties: Anti-Inflammatory and Antimicrobial Evaluation of Neovestitol and Vestitol Isolated from Brazilian Red Propolis


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| Authors | Bruno Bueno-Silva †, Severino M. Alencar *‡, Hyun Koo §, Masaharu Ikegaki , Gil V. J. Silva , Marcelo H. Napimoga #, and Pedro L. Rosalen † |
| Link | J. Agric. Food Chem, 2013, 61 (19), pp 4546−4550, 23 April 2013 |
| Research Institute | † Department of Physiological Science, Piracicaba Dental School, University of Campinas (UNICAMP), C.P. 52, 13414-903 Piracicaba, São Paulo, Brazil, ‡ College of Agriculture "Luiz de Queiroz" (ESALQ), University of São Paulo (USP), C.P. 9, 13418-900 Piracicaba, São Paulo, Brazil, § Center for Oral Biology, Eastman Department of Dentistry, and Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York 14642, United States, Federal University of Alfenas, 37130-000 Alfenas, Minas Gerais, Brazil, Department of Chemistry, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), USP, 14040-901 Ribeirão Preto, São Paulo, Brazil, # Laboratory of Immunology and Molecular Biology, São Leopoldo Mandic Institute and Research Center, Campinas, São Paulo, Brazil |



### Abstract

The objective of this study was to evaluate anti-inflammatory and antimicrobial activities of neovestitol and vestitol isolated from Brazilian red propolis (BRP).

BRP ethanolic extract (EEP), neovestitol, and vestitol were evaluated by anti-inflammatory properties using a neutrophil migration assay. The antimicrobial activity was evaluated by minimal inhibitory and bactericidal concentrations (MIC and MBC) against Streptococcus mutans, Streptococcus sobrinus, Staphylococcus aureus, and Actinomyces naeslundii. Neovestitol, vestitol, and EEP inhibited neutrophil migration at a dose of 10 mg/kg. Regarding antimicrobial activity, neovestitol showed MICs ranging from < 6.25 to 25−50 μg/mL and MBCs ranging from 25−50 to 50−100 μg/mL, while vestitol showed MICs ranging from 25−50 to 50−100 μg/mL and MBCs ranging from 25−50 to 50−100 μg/mL.

Both isoflavonoids neovestitol and vestitol are consistent bioactive compounds displaying anti-inflammatory and antimicrobial activities that can strongly act in a low dose and concentration and have a promising potential to be applied in the pharmaceutical and food industries.

## 8.9. Propolis Component Shows Anti-Inflammatory, Neuroprotective; Hepatoprotective and Cardioprotective Activities: Caffeic Acid Phenethyl Ester, a Promising Component of Propolis with a Plethora of Biological Activities: A Review On Its Anti-Inflammatory, Neuroprotective, Hepatoprotective, and Cardioprotective Effects


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| Authors | Mai F. Tolba[1,2], Samar S. Azab[1], Amani E. Khalifa[1], Sherif Z. Abdel-Rahman[2], Ashraf B. Abdel-Naim[1,3] |
| Link | IUBMB Life, 2013 Jul 11 |
| Research Institute | [1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt<br>[2]Department of Obstetrics and gynecology, The University of Texas Medical Branch, Galveston, TX, USA<br>[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia |



### Abstract

Caffeic acid phenethyl ester (CAPE) is an important active component of honey bee propolis that possesses a plethora of biological activities. Propolis is used safely in traditional medicine as a dietary supplement for its therapeutic benefits.

This review highlights the recently published data about CAPE bioavailability, anti-inflammatory, neuroprotective; hepatoprotective and cardioprotective activities.

CAPE showed promising efficacy both in vitro and in vivo studies in animal models with minimum adverse effects. Its effectiveness was demonstrated in multiple target organs. Despite this fact, it has not been yet investigated as a protective agent or a potential therapy in humans.

Investigation of CAPE efficacy in clinical trials is strongly encouraged to elucidate its therapeutic benefit for different human diseases after performing full preclinical toxicological studies and gaining more insights into its pharmacokinetics.

# 9. anti-ulcer

## 9.1. *Baccharis dracunculifolia*, the main botanical source of Brazlian Green Propolis, displays antiulcer activity.

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| Authors | Lemos M, de Barros MP, Sousa JP, da Silva Filho AA, Bastos JK, de Andrade SF. |
| Link | J Pharm Pharmacol. 2007 Apr;59(4):603-8. |
| Research Institute | Nucleo de Ciencia e Tecnologia, Area de Ciencias Biologicas e da Saude, Universidade do Oeste de Santa Catarina, Campus de Videira, Rua Paese, 198, Bairro das Torres, Videira-SC, 89560-000, SC, Brazil. |


### Abstract

*Baccharis dracunculifolia* is the most important botanical source of Southeastern Brazilian Propolis, known as green Propolis for its colour. In a previous study, we described the gastric protective effect of the hydroalcoholic extract of Brazlian Green Propolis. We therefore wanted to investigate the possibility of using *B. dracunculifolia* extract for antiulcer treatment. This study was undertaken to evaluate the anti-ulcerogenic property of hydroalcoholic extract of *B. dracunculifolia* aerial parts. The HPLC analysis of the chemical composition of *B. dracunculifolia* extract used in this study revealed the presence mainly of cinnamic acid derivates and flavonoids. Doses of 50, 250 and 500 mg/kg of *B. dracunculifolia* crude extract and positive controls (omeprazole or cimetidine) significantly diminished the lesion index, the total lesion area and the percentage of lesion compared with negative control groups. The percentage of ulcer inhibition was significantly higher in groups treated with *B. dracunculifolia*, cimetidine or omeprazole, with all protocols used, compared with negative control groups. Regarding the model of gastric secretion, reductions in the volume of gastric juice and total acidity were observed, as well as an increase in the gastric pH. These results were similar to results from studies carried out with green Propolis extract. Although more investigations are required, our results suggest that *B. dracunculifolia* has potential to be used as a phytotherapic preparation for the treatment of gastric ulcer.

## 9.2. Effect of Brazlian Green Propolis on experimental gastric ulcers in rats.

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| Authors | de Barros MP, Sousa JP, Bastos JK, de Andrade SF. |
| Link | J Ethnopharmacol. 2007 Apr 4;110(3):567-71. |
| Research Institute | Nucleo de Ciencia e Tecnologia, Area de Ciencias Biologicas e da Saude, Universidade do Oeste de Santa Catarina, Campus de Videira, SC, Brazil. |


### Abstract

Propolis is a resinous hive product collected by honeybees from plants. The Propolis produced in Southeastern of Brazil is known as green Propolis because of its color. Modern herbalists recommend its use because it displays antibacterial, antifungal, antiviral, hepatoprotective, anti-inflammatory, immunomodulatory and anti-ulcer properties. The anti-ulcer activity of green Propolis hydroalcoholic crude extract was evaluated by using models of acute gastric lesions induced by ethanol, indomethacin and stress in rats. Moreover, the effects of extract on gastric content volume, pH and total acidity, using pylorus ligated model were evaluated. Animals pretreated with Propolis hydroalcoholic crude extract (50, 250 and 500 mg/kg) showed a significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group ($p=0.05$) in the ethanol-induced ulcer model. Green Propolis extract, at a higher dose (500 mg/kg), displayed a significant protection by reducing ($p=0.05$) the evaluated parameters in the gastric ulceration induced by indomethacin. In the stress-induced ulcer model it was observed a significant reduction ($p=0.05$) in those parameters in animals treated with green Propolis extract (250 and 500 mg/kg). Regarding the pylorus ligated model it was observed that green Propolis extract (250 and 500 mg/kg) displayed an anti-secretory activity, which lead to a reduction in the gastric juice volume, total acidity and pH. These findings indicate that Brazlian Green Propolis displays good anti-ulcer activity, corroborating the folk use of Propolis preparations, and contributing for its pharmacological validation.

## 9.3. Antiulcerogenic activity of the essential oil of *Baccharis dracunculifolia* on different experimental models in rats.


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|---|---|
| Authors | Juliane Jose Massignani, Marivane Lemos, Edson Luis Maistro, Hamilton Pedro Schaphauser, Renata Fabiane Jorge, Joao Paulo Barreto Sousa, Jairo Kennup Bastos, and Sergio Faloni de Andrade |
| Link | Phytother Res. March 9, 2009 |
| Research Institute | Núcleo de Ciência e Tecnologia, Area de Ciências Biológicas e da Saúde, Universidade do Oeste de Santa Catarina, Campus de Videira, Rua Paese, 198, CEP: 89560-000, Videira, SC, Brazil. |



### Abstract

*Baccharis dracunculifolia* DC (Asteraceae), a native plant from Brazil, commonly known as 'Alecrimdo-campo' is widely used in folk medicine to treat inflammation, hepatic disorders and stomach ulcers, and it is the most important botanical source of Southeastern Brazilian propolis, known as Green Propolis. Its essential oil is composed of non-oxygenated and oxygenated terpenes. In this work, the effects of the essential oil obtained from the aerial parts of *B. dracunculifolia* on gastric ulcers were evaluated.

The antiulcer assays were undertaken using the following protocols in rats: nonsteroidal antiinflammatory drug (NSAID)-induced ulcer, ethanol-induced ulcer, stress-induced ulcer, and determination of gastric secretion using ligated pylorus.

The treatment in the doses of 50, 250 and 500 mg/kg of *B. dracunculifolia* essential oil significantly diminished the lesion index, the total lesion area and the percentage of lesions in comparison with both positive and negative control groups. With regard to the model of gastric secretion a reduction of gastric juice volume and total acidity was observed, as well as an increase in the gastric pH. No sign of toxicity was observed in the acute toxicity study.

Considering the results, it is suggested that the essential oil of *B. dracunculifolia* could probably be a good therapeutic agent for the development of new phytotherapeutic medicine for the treatment of gastric ulcer.

# 10. anti-caries

## 10.1. Antimicrobial activity of Propolis on oral microorganisms.

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| Authors | Park YK, Koo MH, Abreu JA, Ikegaki M, Cury JA, Rosalen PL. |
| Link | Curr Microbiol. 1998 Jan;36(1):24-8. |
| Research Institute | College of Food Engineering, State University of Campinas (UNICAMP), 13081-970, Caixa Postal 6177, Campinas, SP, Brazil. |


### Abstract

Formation of dental caries is caused by the colonization and accumulation of oral microorganisms and extracellular polysaccharides that are synthesized from sucrose by glucosyltransferase of *Streptococcus mutans*. The production of glucosyltransferase from oral microorganisms was attempted, and it was found that *Streptococcus mutans* produced highest activity of the enzyme. Ethanolic extracts of Propolis (EEP) were examined whether EEP inhibit the enzyme activity and growth of the bacteria or not. All EEP from various regions in Brazil inhibited both glucosyltransferase activity and growth of *S. mutans*, but one of the Propolis from Rio Grande do Sul (RS2) demonstrated the highest inhibition of the enzyme activity and growth of the bacteria. It was also found that Propolis (RS2) contained the highest concentrations of pinocembrin and galangin.

## 10.2. Effects of Apis mellifera Propolis on the activities of streptococcal glucosyltransferases in solution and adsorbed onto saliva-coated hydroxyapatite.


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|---|---|
| Authors | Koo H, Vacca Smith AM, Bowen WH, Rosalen PL, Cury JA, Park YK. |
| Link | Caries Res. 2000 Sep-Oct;34(5):418-26. |
| Research Institute | Faculty of Dentistry of Piracicaba, State University of Campinas, Piracicaba, Brazil. |



### Abstract

Propolis, a resinous hive product collected by *Apis mellifera* bees, has been used for thousands of years in folk medicine. Ethanolic extracts of Propolis (EEP) have been shown to inhibit the activity of a mixture of crude glucosyltransferase (Gtf) enzymes in solution. These enzymes synthesize glucans from sucrose, which are important for the formation of pathogenic dental plaque. In the present study, the effects of Propolis from two different regions of Brazil on the activity of separate, purified Gtf enzymes in solution and on the surface of saliva-coated hydroxyapatite (sHA) beads were evaluated. The EEP from Minas Gerais (MG; Southeastern Brazil) and Rio Grande do Sul (RS; Southern Brazil) were tested for their ability to inhibit the enzymes GtfB (synthesis of insoluble glucan), GtfC (insoluble/soluble glucan) and GtfD (soluble glucan). The effects of Propolis on Gtf from *Streptococcus sanguis* (soluble glucan synthesis) was also explored. The EEP from both regions effectively inhibited the activity of all Gtfs in solution (75-95%) and on the surface of sHA beads (45-95%) at concentrations between 0.75 and 3.0 mg of Propolis/ml. However, the two samples of Propolis showed different levels of inhibition on each of the enzymes tested. In general, EEP RS demonstrated a significantly higher inhibitory activity on GtfB and C activities (both solution and surface assays) than EEP MG at concentrations between 0.047 and 0.187 mg/ml ($p=0.05$). EEP MG, on the other hand, exhibited a greater inhibitory effect on the activities of surface GtfD (at 0.375, 0.75 and 1.5 mg/ml) and *S. sanguis* Gtf (at 1.5 and 3.0 mg/ml; $p=0.05$). These data indicate that EEP is a potent inhibitor of Gtf enzymes in solution and adsorbed on an experimental pellicle; however, its effect on Gtf activity is variable depending on the geographical origin of the Propolis samples. There is a need to identify the active compounds of Propolis.

## 10.3. Effects of compounds found in Propolis on *Streptococcus mutans* growth and on glucosyltransferase activity.


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| Authors | Koo H, Rosalen PL, Cury JA, Park YK, Bowen WH. |
| Link | Antimicrob Agents Chemother. 2002 May;46(5):1302-9. |
| Research Institute | Center for Oral Biology and Eastman Department of Dentistry, University of Rochester Medical Center, Rochester, New York 14642, USA. |



### Abstract

Propolis, a resinous bee product, has been shown to inhibit the growth of oral microorganisms and the activity of bacterium-derived glucosyltransferases (GTFs). Several compounds, mainly polyphenolics, have been identified in this natural product. The present study evaluated the effects of distinct chemical groups found in Propolis on the activity of GTF enzymes in solution and on the surface of saliva-coated hydroxyapatite (sHA) beads. Thirty compounds, including flavonoids, cinnamic acid derivatives, and terpenoids, were tested for the ability to inhibit GTFs B, C, and D from *Streptococcus mutans* and GTF from S. sanguinis (GTF Ss). Flavones and flavonols were potent inhibitors of GTF activity in solution; lesser effects were noted on insolubilized enzymes. Apigenin, a 4',5,7-trihydroxyflavone, was the most effective inhibitor of GTFs, both in solution (90.5 to 95% inhibition at a concentration of 135 microg/ml) and on the surface of sHA beads (30 to 60% at 135 microg/ml). Antibacterial activity was determined by using MICs, minimum bactericidal concentrations (MBCs), and time-kill studies. Flavanones and some dihydroflavonols, as well as the sesquiterpene tt-farnesol, inhibited the growth of *S. mutans* and S. sobrinus; tt-farnesol was the most effective antibacterial compound (MICs of 14 to 28 microg/ml and MBCs of 56 to 112 microg/ml). tt-Farnesol (56 to 112 microg/ml) produced a 3-log-fold reduction in the bacterial population after 4 h of incubation. Cinnamic acid derivatives had negligible biological activities. Several of the compounds identified in Propolis inhibit GTF activities and bacterial growth. Apigenin is a novel and potent inhibitor of GTF activity, and tt-farnesol was found to be an effective antibacterial agent.

## 10.4. Effects of compounds found in Propolis on *Streptococcus mutans* growth and on glucosyltransferase activity.


| | |
|---|---|
| Authors | Koo H, Rosalen PL, Cury JA, Park YK, Bowen WH. |
| Link | Antimicrob Agents Chemother. 2002 May;46(5):1302-9. |
| Research Institute | Center for Oral Biology and Eastman Department of Dentistry, University of Rochester Medical Center, Rochester, New York 14642, USA. |



### Abstract

Propolis, a resinous bee product, has been shown to inhibit the growth of oral microorganisms and the activity of bacterium-derived glucosyltransferases (GTFs). Several compounds, mainly polyphenolics, have been identified in this natural product. The present study evaluated the effects of distinct chemical groups found in Propolis on the activity of GTF enzymes in solution and on the surface of saliva-coated hydroxyapatite (sHA) beads. Thirty compounds, including flavonoids, cinnamic acid derivatives, and terpenoids, were tested for the ability to inhibit GTFs B, C, and D from *Streptococcus mutans* and GTF from S. sanguinis (GTF Ss). Flavones and flavonols were potent inhibitors of GTF activity in solution; lesser effects were noted on insolubilized enzymes. Apigenin, a 4',5,7-trihydroxyflavone, was the most effective inhibitor of GTFs, both in solution (90.5 to 95% inhibition at a concentration of 135 microg/ml) and on the surface of sHA beads (30 to 60% at 135 microg/ml). Antibacterial activity was determined by using MICs, minimum bactericidal concentrations (MBCs), and time-kill studies. Flavanones and some dihydroflavonols, as well as the sesquiterpene tt-farnesol, inhibited the growth of *S. mutans* and S. sobrinus; tt-farnesol was the most effective antibacterial compound (MICs of 14 to 28 microg/ml and MBCs of 56 to 112 microg/ml). tt-Farnesol (56 to 112 microg/ml) produced a 3-log-fold reduction in the bacterial population after 4 h of incubation. Cinnamic acid derivatives had negligible biological activities. Several of the compounds identified in Propolis inhibit GTF activities and bacterial growth. Apigenin is a novel and potent inhibitor of GTF activity, and tt-farnesol was found to be an effective antibacterial agent.

## 10.5. Effect of a mouth rinse containing selected Propolis on 3-day dental plaque accumulation and polysaccharide formation.


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|---|---|
| Authors | Koo H, Cury JA, Rosalen PL, Ambrosano GM, Ikegaki M, Park YK. |
| Link | Caries Res. 2002 Nov-Dec;36(6):445-8. |
| Research Institute | Department of Dentistry, University of Rochester Medical Center, Rochester, NY, USA. |



### Abstract

The aim of this study was to evaluate the effect of a mouthrinse containing Propolis SNB-RS on 3-day dental plaque accumulation. Six volunteers took part in a double-blind crossover study performed in two phases of 3 days. During each phase the volunteers refrained from all oral hygiene and rinsed with 20% sucrose solution 5 times a day to enhance dental plaque formation and with mouthrinse (placebo or experimental) twice a day. On the 4th day, the plaque index (PI) of the volunteers was scored and the supragingival dental plaque was analyzed for insoluble polysaccharide (IP). The PI (SD) for the experimental group was 0.78 (0.17), significantly less than for the placebo group, 1.41 (0.14). The experimental mouthrinse reduced the IP concentration in dental plaque by 61.7% compared to placebo ($p < 0.05$). An experimental mouthrinse containing Propolis SNB-RS was thus efficient in reducing supragingival plaque formation and IP formation under conditions of high plaque accumulation.

## 10.6. Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats.


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|---|---|
| Authors | Koo H, Pearson SK, Scott-Anne K, Abranches J, Cury JA, Rosalen PL, Park YK, Marquis RE, Bowen WH. |
| Link | Oral Microbiol Immunol. 2002 Dec;17(6):337-43. |
| Research Institute | Center for Oral Biology and Eastman Department of Dentistry, University of Rochester Medical Center, NY 14642, USA. |



### Abstract

Propolis, a resinous hive product secreted by *Apis mellifera* bees, has been shown to reduce the incidence of dental caries in rats. Several compounds, mainly polyphenolics, have been identified in Propolis. Apigenin and tt-farnesol demonstrated biological activity against mutans streptococci. We determined here their effects, alone or in combination, on glucosyltransferase activity, biofilm viability, and development of caries in rats. Sprague-Dawley rats were infected with Streptococcus sobrinus 6715 and treated topically twice daily as follows: (1) tt-farnesol, (2) apigenin, (3) vehicle control, (4) fluoride, (5) apigenin +tt-farnesol, and (6) chlorhexidine. Apigenin (1.33 mM) inhibited the activity of glucosyltransferases in solution (90-95%) and on the surface of saliva-coated hydroxyapatite beads (35-58%); it was devoid of antibacterial activity. tt-Farnesol (1.33 mM) showed modest antibacterial activity against biofilms and its effects on glucosyltransferases were minimal. The incidence of smooth-surface caries was significantly reduced by apigenin +tt-farnesol (60%), fluoride (70%), and chlorhexidine (72%) treatments compared to control (P = 0.05).

## 10.7. Comparative evaluation of in-vitro effects of Brazlian Green Propolis and *Baccharis dracunculifolia* extracts on cariogenic factors of *Streptococcus mutans*.


| | |
|---|---|
| Authors | Leitao DP, Filho AA, Polizello AC, Bastos JK, Spadaro AC. |
| Link | Biol Pharm Bull. 2004 Nov;27(11):1834-9. |
| Research Institute | Laboratorio de Bioquimica, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto-Universidade de Sao Paulo, Brazil |



### Abstract

*Streptococcus mutans* triggers dental caries establishment by two major factors: synthesis of organic acids, which demineralize dental enamel, and synthesis of glucans, which mediate the attachment of bacteria to the tooth surface. Propolis is a natural product that may prevent dental caries. *Baccharis dracunculifolia* DC (Asteraceae), a native plant from Brazil, is the most important botanical origin for the production of green Propolis (Brazilian Propolis) by honeybees. However, whether *B. dracunculifolia* (Bd) has an anticariogenic effect, like green Propolis, remains unknown. Herein, we have made a comparative evaluation of the effects of extracts from green Propolis and Bd on the glucan synthesis and acidogenic potential of *S. mutans*. The inhibitory effects of the extracts on bacterial acid production were evaluated through the potentiometric measurement of pH from bacterial suspensions treated with serial concentrations of both extracts. Besides presenting close inhibitory values at the same concentration range, Bd leaf rinse and green Propolis extracts had similar IC(50) values (0.41 and 0.34 mg/ml, respectively). Both extracts produced a bacteriostatic effect on *S. mutans* cultures at a concentration of 0.40 mg/ml. Estimated inhibitory values of green Propolis and Bd leaf rinse extracts on the synthesis of insoluble glucans (IC(50)=12.9 and 25.0 microg/ml, respectively) and soluble glucans (IC(50)=50.4 and 49.1 microg/ml, respectively) were not significantly different from each other at p=0.05. The results demonstrate that Bd leaf rinse and green Propolis extracts have similar inhibitory effects on the *S. mutans* cariogenic factors evaluated herein, and allowed us to suggest that Bd leaves may be a potential source for pharmaceutical products employed for this purpose.

## 10.8. Antimicrobial effect of Propolis and other substances against selected endodontic pathogens.


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|---|---|
| Authors | FB Ferreira, SA Torres, OP Rosa, CM Ferreira, RB Garcia, MC Marcucci, and BP Gomes. |
| Link | *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007; 104: 709. [MEDLINE Citation] |
| Research Institute | North of Paraná University, Londrina, Brazil. flavianaferreira@uol.com.br. |



Abstract

OBJECTIVE: The aim of this study was to determine the antimicrobial effect of ethanol extract of Propolis (EEP) and intracanal medicaments calcium hydroxide, camphorated paramonochlorophenol, and formocresol by means of the macrodilution method using the reinforced clostridial medium (RCM) and brucella and brain heart infusion media.

STUDY DESIGN: The antimicrobial agents were sequentially diluted and tested against anaerobic bacteria Prevotella nigrescens, Fusobacterium nucleatum, Actinomyces israelii, and Clostridium perfringens and against Enterococcus faecalis, with the 5 x 10(5) CFU/mL standardized inocula. The tubes were anaerobically incubated and the minimum inhibitory concentration was detected. Blood agar RCM subcultures were performed to provide minimum bactericidal concentration. The results were analyzed by analysis of variance test.

RESULTS: All drugs were effective against all tested strains, without statistical differences. E. faecalis was the less susceptible strain, and RCM broth promoted faster bacterial growth, but there were no significant differences in these results. Ethanol did not influence the antimicrobial effect of EEP.

## 10.9. Propolis Mouthwash: A New Beginning


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|---|---|
| Authors | Vidya Dodwad, Bhavna Jha Kukreja |
| Link | J Indian Soc Periodontol. 2011 Apr;15(2):121-5 |
| Research Institute | Department of Periodontology and Oral Implantology, I.T.S-centre for Dental Studies and Research, Muradnagar, Ghaziabad, Uttar Pradesh, India |



### Abstract

**Background:** This study was carried out to investigate the effectiveness of a Propolis-containing mouthrinse in inhibition of plaque formation and improvement of gingival health.

**Materials and Methods:** Thirty subjects were selected and randomly assigned into three groups of ten subjects each, which received a Propolis-containing mouthrinse, or a negative control (Saline) or a positive control (Chlorhexidine 0.2%). Plaque index and gingival index were assessed at baseline and at a five-day interval.

**Results:** Chlorhexidine mouthwash was found to be better than Propolis and saline in inhibiting plaque formation. Propolis was found to be only marginally better than chlorhexidine in improving gingival scores.

**Conclusion:** The present study suggests that Propolis might be used as a natural mouthwash, an alternative to chemical mouthwashes, e.g., chlorhexidine. Further, long term trials are required for more accurate data and any conclusive evidence.

## 10.10. Influence of Hygienic Preparations with a 3% Content of Ethanol Extract of Brazilian Propolis on the State of the Oral Cavity


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|---|---|
| Authors | Tanasiewicz M, Skucha-Nowak M, Dawiec M, Król W, Skaba D, Twardawa H., |
| Link | Adv Clin Exp Med. *2012 Jan-Feb;21(1):81-92* |
| Research Institute | Department of Conservative Dentistry with Endodontics, Medical University of Silesia, Bytom, Poland. martatanasiewicz@sum.edu.pl |



Abstract

BACKGROUND: One of the most important measures to be undertaken in order to fight gingivitis and periodontitis is maintenance of proper hygiene of the oral cavity. The research to improve the content of toothpaste has continued for many years so that they should become better in terms of therapeutic abilities.

OBJECTIVES: The aim of this work was to determine and investigate the influence of the application of toothpaste and gel with 3% ethanol propolis extract on the state of the oral cavity.

MATERIAL AND METHODS: The research group comprised 80 adult patients divided into two subgroups: Group I, which comprised 40 patients without pathological changes within the boundaries of the periodontium, and Group II, also 40 patients endangered with the occurrence of periodontitis caused by dental plaque and lack of proper hygiene of the oral cavity. Qualification for both groups was based on an interview and analysis of clinical documentation and assessment of adequate indices such as API, OHI and SBI. The patients underwent three examinations: initial, follow-up after 7 days and after 8 weeks since the beginning of the program. Moreover, the patients were instructed about hygienic procedures of the oral cavity. Four groups (T, G, CT, CG), 20 patients each, were created from research groups I and II. They used the following preparations: T--Dental Polis DX toothpaste with propolis content, G--Dental Polis DX toothpaste without propolis content, CT--Carepolis gel with propolis content, CG--Carepolis gel without propolis content. The patients were informed about the type of hygienic preparation they were given to use (whether it contained propolis or not). Moreover, they were interviewed for their subjective evaluation of the product received.

RESULTS AND CONCLUSION: Results of the research show the effectiveness of hygienic preparations with 3% content of ethanol propolis extract in both groups of patients: without pathological changes within the boundaries of the periodontium and in the case of patients endangered with the occurrence of gingivitis caused by dental plaque.

# 11. Diabetes

## 11.1. Experimental diabetic nephropathy can be prevented by propolis: Effect on metabolic disturbances and renal oxidative parameters.


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|---|---|
| Authors | OM Abo-Salem, RH El-Edel, GE Harisa, N El-Halawany, and MM Ghonaim |
| Link | Pak J Pharm Sci, April 1, 2009; 22(2): 205-10 |
| Research Institute | Phamacology and Toxicology Departments, Faculty of Pharmacy, Al-Azahar University, Cairo, Egypt. |



### Abstract

Oxidative stress may play a key role in the pathogenesis of diabetic nephropathy. Propolis and its extract have antioxidant properties. The effect of ethanolic extract of propolis against experimental diabetes mellitus-associated changes was examined.

Diabetes was induced experimentally in rats by i.p. injection of streptozotocin (STZ) in a dose of 60 mg/kg bwt for 3 successive days. Blood urea nitrogen (BNU), creatinine, glucose, lipid profile, malondialdehyde (MDA) and urinary albumin were measured. Superoxide dimutase (SOD), glutathione (GSH), catalase (CAT) and MDA were measured in the renal tissue.

The results showed decreased body weight and increased kidney weight in diabetic animals. Compared to the control normal rats, diabetic rats had higher blood glucose, BNU, creatinine, total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C), MDA and urinary albumin and lower high-density lipoprotein-cholesterol (HDL-C) levels.

Moreover, renal tissue MDA was markedly increased while SOD, GSH and CAT were significantly decreased. Oral administration of propolis extract in doses of 100,200 & 300 mg/kg bwt improved the body and kidney weights, serum glucose, lipid profile, MDA and renal function tests. Renal GSH, SOD and CAT were significantly increased while MDA was markedly reduced.

These results may suggest a strong antioxidant effect of propolis which can ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetes mellitus.

## 11.2. Potential antidiabetic and hypolipidemic effects of propolis extract in streptozotocin-induced diabetic rats.


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|---|---|
| Authors | el-SM El-Sayed, OM Abo-Salem, HA Aly, and AM Mansour |
| Link | Pak J Pharm Sci, April 1, 2009; 22(2): 168-74. |
| Research Institute | Pharmacology & Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Nasr-City, Cairo, Egypt. |



### Abstract

Free radicals have been implicated in the pathogenesis of diabetes mellitus leading to various complications including atherosclerosis. Propolis was reported to have oxygen radical scavenging activity. The present study was designed to investigate the possible antidiabetic, hypolipidemic and antioxidant effects of ethanolic extract of propolis (EEP).

Type capital I, Ukrainian diabetes was induced in rats by injection of streptozotocin (STZ) in a dose of 60 mg/kg bwt, i.p. for 3 consecutive days. After 5 weeks of STZ injection, there were an apparent reduction in the animal body weight amounting to 21% and significant increases in serum glucose (184%), triglycerides (63%), total cholesterol (43%) and low density lipoprotein-cholesterol (LDL-C) (148%) with a concomitant decrease in serum high density lipoprotein-cholesterol (HDL-C) (51%) as compared to the control normal group.

In addition, there was significant elevation in pancreatic lipid peroxides measured as malondialdehyde (MDA) and serum nitric oxide (NO) amounting to 185% and 224%, respectively with marked reduction in serum reduced glutathione (GSH) andcatalase (CAT) (66% and 31%, respectively) and pancreatic superoxide dismutase (SOD) (54%) in STZ-treated rats. On the other hand, oral daily treatment of animals with EEP in a dose of 200mg/kg bwt for a period of 5 weeks ameliorated STZ-induced alterations in the animal body weight as well as in serum glucose, lipids, lipoproteins, NO, GSH & CAT and pancreatic MDA & SOD.

In conclusion, propolis extract offers promising antidiabetic and hypolipidemic effects that may be mainly attributed to its potent antioxidant potential. Further studies will be needed in future in order to determine which one (or more) of its active constituents has the main antidiabetic and hypolipidemic effects.

## 11.3. Biological Activities of Chinese Propolis and Brazilian Propolis on Streptozotocin-Induced Type 1 Diabetes Mellitus in Rats


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|---|---|
| Authors | Wei Zhu[1], Minli Chen[2], Qiyang Shou[2], Yinghua Li[3] and Fuliang Hu[1] |
| Link | Evid. Based Complement. Altern. Med. published 5 April 2010, 10 |
| Research Institute | [1]College of Animal Sciences, Zhejiang University, Hangzhou 310029, [2]Zhejiang Traditional Chinese Medicine University, Hangzhou 310053 and [3]Zhejiang Economic & Trade Polytechnic, Hangzhou 310018, China |



### Abstract

Propolis is a bee-collected natural product and has been proven to have various bioactivities. This study tested the effects of Chinese Propolis and Brazilian Propolis on streptozotocin-induced type 1 diabetes mellitus in Sprague–Dawley rats.

The results showed that Chinese Propolis and Brazilian Propolis significantly inhibited body weight loss and blood glucose increase in diabetic rats. In addition, Chinese Propolis-treated rats showed an 8.4% reduction of glycated hemoglobin levels compared with untreated diabetic rats. Measurement of blood lipid metabolism showed dyslipidemia in diabetic rats and Chinese Propolis helped to reduce total cholesterol level by 16.6%. Moreover, oxidative stress in blood, liver and kidney was improved to various degrees by both Chinese Propolis and Brazilian Propolis.

An apparent reduction in levels of alanine transaminase, aspartate transaminase, blood urea nitrogen and urine microalbuminuria-excretion rate demonstrated the beneficial effects of Propolis in hepatorenal function.

All these results suggested that Chinese Propolis and Brazilian Propolis can alleviate symptoms of diabetes mellitus in rats and these effects may partially be due to their antioxidant ability.

## 11.4. Artepillin C, as a PPARγ Ligand, Enhances Adipocyte Differentiation and Glucose Uptake in 3T3-L1 Cells


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|---|---|
| Authors | Sun-Sil Choi[a], Byung-Yoon Cha[a], Kagami Iida[a], Young-Sil Lee[a], Takayuki Yonezawa[a], Toshiaki Teruya[a], Kazuo Nagai[a, b] and Je-Tae Woo[a, b, c,] |



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| Link | Chem Pharmacol, Received 11 October 2010; accepted 4 January 2011. Available online 8 January 2011 |



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| Research Institute | [a] Research Institute for Biological Functions, Chubu University, 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan<br>[b] Department of Biological Chemistry, Chubu University, 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan<br>[c] Department of Research and Development, Erina Co., Inc., 1-9-2 Higashi-Shinbashi, Minato-ku, Tokyo 105-0021, Japan |



### Abstract

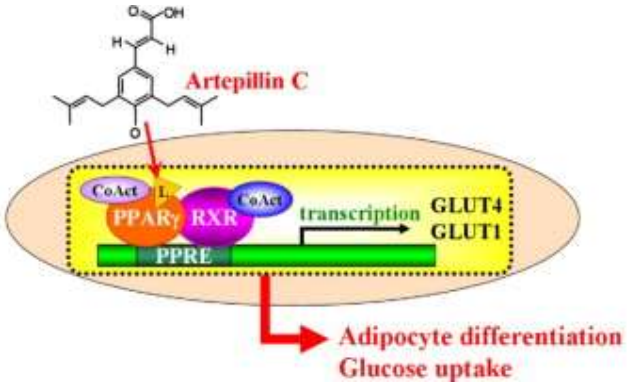
The nuclear receptor peroxisome proliferator-activated receptor (PPAR) γ plays an important role in adipocyte differentiation. Its ligands, including thiazolidinediones, improve insulin sensitivity in type 2 diabetes. We investigated the effects of Artepillin C, an ingredient of Baccharis Dracunculifolia, on adipogenesis and glucose uptake using 3T3-L1 cells. In PPARγ ligand-binding assays, artepillin C exhibited binding affinity toward PPARγ. Artepillin C dose-dependently enhanced adipocyte differentiation of 3T3-L1 cells.

As a result of the artepillin C-induced adipocyte differentiation, the gene expression of PPARγ and its target genes, such as aP2, adiponectin and glucose transporter (GLUT) 4, was increased. These increases were abolished by cotreatment with GW9662, a PPARγ antagonist. In mature 3T3-L1 adipocytes, artepillin C significantly enhanced the basal and insulin-stimulated glucose uptake. These effects were decreased by cotreatment with a PI3K inhibitor. Although Artepillin C had no effects on the insulin signaling cascade, Artepillin C enhanced the expression and plasma membrane translocation of GLUT1 and GLUT4 in mature adipocytes.

In conclusion, these findings suggest that Artepillin C promotes adipocyte differentiation and glucose uptake in part by direct binding to PPARγ, which could be the basis of the pharmacological benefits of green propolis intake in reducing the risk of type 2 diabetes.


### Graphical abstract

Artepillin C promotes adipocyte differentiation and glucose uptake in part by direct binding to PPARγ


Artepillin C
CoAct
L
PPARγ
RXR
CoAct
transcription
GLUT4
GLUT1
PPRE
Adipocyte differentiation
Glucose uptake

## 11.5. Propolis Component May Help Prevent Blood Clots: Caffeic Acid Phenethyl Ester Inhibits Endothelial Tissue Factor Expression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|---|---|
| Authors | Cathérine Gebhard[1) 2) 3)], Barbara Elisabeth Stähli[1) 2) 3)], Stephanie Largiadèr[1) 2)], Erik Walter Holy[1) 2) 3)], Alexander Akhmedov[1) 2)], Giovanni Guido Camici[1) 2)], Thomas Felix Lüscher[1) 2) 3)], Felix Christoph Tanner[1) 2) 3)] |
| Link | Biol Pharm Bull, 2013;36(6):1032-1035 |
| Research Institute | [1)] Cardiovascular Research, Physiology Institute, University of Zürich<br>[2)] Center for Integrative Human Physiology, University of Zürich<br>[3)] Cardiology, Cardiovascular Center, University Hospital Zürich |



### Abstract

Caffeic acid phenethyl ester (CAPE) is a component of honeybee hives with various beneficial properties. Tissue factor (TF), the key trigger of thrombosis, is expressed in human endothelial cells.

This study was designed to investigate whether CAPE modulates TF expression in human aortic endothelial cells (HAECs).

Western blots and real-time polymerase chain reactions were performed. CAPE (10-7-10-5 m) inhibited tumor necrosis factor (TNF)-α induced endothelial TF protein expression by 2.1-fold at 10-5 m ($p < 0.0001$). Similarly, TF surface activity was reduced ($p < 0.02$). In contrast, TF mRNA expression, TF promoter activity, and mitogen-activated protein (MAP) kinase activation remained unaltered.

In conclusion, CAPE inhibits TF protein expression and activity at the posttranscriptional level thereby exhibiting anti-thrombotic potential.

## 11.6. Propolis Helps Reduce Glucose and Cholesterol Levels Associated with Aging: Positive Influence of a Natural Product as Propolis on antioxidant status and Lipid Peroxidation in Senescent Rats


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|---|---|
| Authors | Cristina Lisbona [1], Javier Díaz-Castro [2], María J. M. Alférez [2], Isabel M. Guisado [3], Rafael Guisado [3], Inmaculada López-Aliaga[2] [4] |
| Link | J Physiol Biochem, 2013 Jun 28 |
| Research Institute | 1. Department of Physiotherapy, Faculty of Health Sciences, University of Granada, 18071, Granada, Spain<br>2. Department of Physiology, Faculty of Pharmacy, University of Granada and Institute of Nutrition and Food Technology "José Mataix", 18071, Granada, Spain<br>3. Department of Nursing, Faculty of Health Sciences, University of Granada, 18071, Granada, Spain<br>4. Department of Physiology, Faculty of Pharmacy, Campus Universitario de Cartuja, University of Granada, 18071, Granada, Spain |



### Abstract

Given the importance of oxidative stress associated to aging, it would be interesting to assess the effect of oral supplementation with antioxidant substances capable of diminishing oxidative aggression and free radicals generation associated to this condition.

This study investigated the effects of AIN-93 M diet supplemented either with 2 % of propolis, or with 4 % of a natural product obtained from lyophilizate vegetables, selected by its antioxidant properties, in senescent healthy Wistar rats fed ad libitum over 3 months.

Propolis supplementation leads to a lower level of glucose and cholesterol concentrations together with a reduction in protein oxidation. Plasma thiobarbituric acid-reactive substance levels were lower in the rats consuming the natural vegetable product and propolis possibly due to its antioxidant components, neutralizing the free radical produced, and thus preventing cellular damage.

The results of the present study suggest a synergic effect of overall propolis compounds reducing the oxidative stress and glucose and cholesterol plasma levels associated with aging.

## 11.7. Propolis May Help Treat Hypertension: Propolis Reduces Oxidative Stress in l-NAME-Induced Hypertension Rats


| | |
|---|---|
| Authors | Zeliha Selamoglu Talas |
| Link | Cell Biochemistry and Function, Early View, Article first published online: 21 JUN 2013 |
| Research Institute | Department of Biology, Faculty of Arts and Science, Nigde University, Nigde, Turkey |



### Abstract

The inhibition in the synthesis or bioavailability of nitric oxide (NO) has an important role in progress of hypertension. The blocking of nitric oxide synthase activity may cause vasoconstriction with the formation of reactive oxygen species (ROS). Propolis is a resinous substance collected by honey bees from various plants. Propolis has biological and pharmacological properties. The aim of this study was to examine the effect of propolis on catalase (CAT) activity, malondialdehyde (MDA) and NO levels in the testis tissues of hypertensive rats by Nω-nitro-l-arginine methyl ester (l-NAME).

Rats have received nitric oxide synthase inhibitor (l-NAME, 40 mg kg–1, intraperitoneally) for 15 days to produce hypertension and propolis (200 mg kg–1, by gavage) during the last 5 days. MDA level in l-NAME-treated group significantly increased compared with control group ($P < 0.01$). MDA level of l-NAME + propolis-treated rats significantly reduced ($P < 0.01$) compared with l-NAME-treated group. CAT activity and NO level significantly reduced ($P < 0.01$) in l-NAME group compared with control group. There were no statistically significant increases in the CAT activity and NO level of the l-NAME + propolis group compared with the l-NAME-treated group ($P > 0.01$).

These results suggest that propolis changes CAT activity, NO and MDA levels in testis of l-NAME-treated animals, and so it may modulate the antioxidant system.

## 11.8. Propolis Component May Help Treat Thrombosis: Caffeic Acid Phenethyl Ester Inhibits Endothelial Tissue Factor Expression.


Authors: Cathérine Gebhard[1) 2) 3)], Barbara Elisabeth Stähli[1) 2) 3)], Stephanie Largiadèr[1) 2)], Erik Walter Holy[1) 2) 3)], Alexander Akhmedov[1) 2)], Giovanni Guido Camici[1) 2)], Thomas Felix Lüscher[1) 2) 3)], Felix Christoph Tanner[1) 2) 3)]



Link: Biol Pharm Bull, 2013;36(6):1032-5



Research Institute:
[1)] Cardiovascular Research, Physiology Institute, University of Zürich
[2)] Center for Integrative Human Physiology, University of Zürich
[3)] Cardiology, Cardiovascular Center, University Hospital Zürich



### Abstract

Caffeic acid phenethyl ester (CAPE) is a component of honeybee hives with various beneficial properties. Tissue factor (TF), the key trigger of thrombosis, is expressed in human endothelial cells.

This study was designed to investigate whether CAPE modulates TF expression in human aortic endothelial cells (HAECs). Western blots and real-time polymerase chain reactions were performed. CAPE (10(-7)-10(-5) m) inhibited tumor necrosis factor (TNF)-α induced endothelial TF protein expression by 2.1-fold at 10(-5) m ($p < 0.0001$). Similarly, TF surface activity was reduced ($p < 0.02$). In contrast, TF mRNA expression, TF promoter activity, and mitogen-activated protein (MAP) kinase activation remained unaltered.

## 11.9. Dietary Propolis Improves Insulin Sensitivity: Improvement of Insulin Resistance, Blood Pressure and Interstitial pH in Early Developmental Stage of Insulin Resistance in OLETF Rats by Intake of Propolis Extracts


Authors: Wataru Aoi[a], Shigekuni Hosogi[b, d], Naomi Niisato[b, d], Noriko Yokoyama[b], Hiroki Hayata[b], Hiroaki Miyazaki[b, d], Katsuyuki Kusuzaki[b, d], Takuya Fukuda[c], Michiaki Fukui[c], Naoto Nakamura[c], Yoshinori Marunaka[b, d]



Link: Biochem Biophys Res Commun, 2013 Feb 14



Research Institute: [a] Laboratory of Health Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan; [b] Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan; [c] Department of Endocrinology and Metabolism, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan; [d] Japan Institute for Food Education and Health, Heian Jogakuin (St. Agnes') University, Kyoto 602-8013, Japan



### Abstract

Propolis, a resinous mixture collected from plants by the Apis mellifera bee, contains high level nutrient factors including vitamins, polyphenols, and amino acids that would be expected to improve insulin sensitivity. Insulin resistance would secondarily cause elevation of blood pressure and increase the risk of cardiovascular diseases. The purpose of this study is to investigate the effect of propolis extracts on blood glucose levels and blood pressures in an early developmental stage of insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. OLETF rats (10 weeks old) were divided into 3 different groups: normal diet, 0.1% propolis diet, and 0.5% propolis diet. After 8 weeks, blood glucose levels, blood pressures, plasma metabolic factors and hormones, and interstitial fluid pH were measured. Casual blood glucose levels were decreased associated with a reduction of plasma insulin levels in both proplois diet groups compared with normal diet group. Propolis decreased systolic blood pressure with no significant changes in plasma aldosterone levels. We also found that interstitial fluid pH in ascites, liver, and skeletal muscle was higher in rats fed propolis diet than rats fed normal diet.
These data suggests that dietary propolis improves insulin sensitivity and blood pressures in the early stage of the process in development of insulin resistance, which may be mediated by suppression of metabolic acidosis.

## 11.10. Propolis Component Exhibits Cardioprotective, Antiarrhythmic Effects: Electrophysiological and Mechanical Effects of Caffeic Acid Phenethyl Ester, a Novel Cardioprotective Agent with Antiarrhythmic Activity, in Guinea-Pig Heart


| | |
|---|---|
| Authors | Gwo-Jyh Chang[a], Chi-Jen Chang[b], Wei-Jan Chen[b], Yung-Hsin Yeh[b], Hsiao-Yu Lee[a] |
| Link | Eur J Pharmacol, 2013 Feb 5 |
| Research Institute | [a] Graduate Institute of Clinical Medicinal Sciences, College of Medicine, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan<br>[b] First Cardiovascular Division of Medicine, Chang Gung Memorial Hospital, 5 Fu-Shing St, Kwei-Shan, Tao-Yuan 333, Taiwan |



### Abstract

Caffeic acid phenethyl ester (CAPE) is an active component of propolis that exhibits cardioprotective and antiarrhythmic effects. The detailed mechanisms underlying these effects, however, are not entirely understood.

The aim of this study was to elucidate the electromechanical effects of CAPE in guinea-pig cardiac preparations. Intracardiac electrograms, left ventricular (LV) pressure, and the anti-arrhythmic efficacy were determined using isolated hearts. Action potentials of papillary muscles were assessed with microelectrodes, Ca(2+) transients were measured by fluorescence, and ion fluxes were measured by patch-clamp techniques. In a perfused heart model, CAPE prolonged the atrio-ventricular conduction interval, the Wenckebach cycle length, and the refractory periods of the AV node and His-Purkinje system, while shortening the QT interval. CAPE reduced the occurrence of reperfusion-induced ventricular fibrillation and decreased LV pressure in isolated hearts. In papillary muscles, CAPE shortened the action potential duration and reduced both the maximum upstroke velocity and contractile force. In fura-2-loaded single ventricular myocytes, CAPE decreased cell shortening and the Ca(2+) transient amplitude. Patch-clamp experiments revealed that CAPE produced a use-dependent decrease in L-type Ca(2+) current (I(Ca,L)) (IC(50)=1.1μM) and Na(+) current (I(Na)) (IC(50)=0.43μM), caused a negative-shift of the voltage-dependent inactivation and a delay of recovery from inactivation. CAPE decreased the delayed outward K(+) current (I(K)) slightly, without affecting the inward rectifier K(+) current (I(K1)).

These results suggest that the preferential inhibition of Ca(2+) inward and Na(+) inward currents by CAPE may induce major electromechanical alterations in guinea-pig cardiac preparations, which may underlie its antiarrhythmic action.

# 12. WOUND HEALING

## 12.1. Effect of Propolis on healing in experimental colon anastomosis in rats.


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|---|---|
| Authors | Muhyittin Temiz, Ahmet Aslan, Elif Canbolant, Sibel Hakverdi, Gurbuz Polat, Semire Uzun, Abdulkerim Temiz, and Ramazan Gonenci. |
| Link | Adv Ther. 2008 [MEDLINE Citation] |
| Research Institute | Department of General Surgery, Mustafa Kemal University, Faculty of Medicine, Antakya, Hatay, Turkey, mhytemiz@yahoo.com . |



### Abstract

Introduction: Propolis is the generic name for the resinous substance collected by honeybees, which is known to have antioxidant, antiinflammatory, apoptosis-inducible effects. Anastomotic dehiscence after colorectal surgery is an important cause of morbidity and mortality. We aimed to assess the effect of Propolis on healing in an experimental colon anastomosis in rats.

Methods: Forty adult male Wistar albino rats were randomly assigned into 5 treatment groups with 8 rats in each: Group I, anastomosis+no treatment; Group II, anastomosis+oral Propolis (600 mg/kg/d); Group III, anastomosis+oral ethyl alcohol (1 cc/d); Group IV, anastomosis+rectal Propolis (600 mg/kg/d); Group V, anastomosis+rectal ethyl alcohol (1 cc/d). The bursting pressures, hydroxiproline levels and histopathological changes in each group were measured.

Results: When bursting pressures were compared between groups, we observed that they were increased in the groups treated with Propolis in contrast to all other groups. Hydroxiproline levels in the Propolis groups were also significantly increased in contrast to the other groups. There was also a statistically significant difference in histopathological changes between the treatment types. When Propolis administration methods were compared, we did not observe a statistically significant difference.

Conclusion: Propolis has a significantly favourable effect on healing in experimental colon anastomosis, independent from the method of administration.

## 12.2. Green Propolis Better than Red in Helping to Heal Wounds: Comparative Study of Topical Green and Red Propolis in the Repair of Wounds Induced in Rats


| | |
|---|---|
| Authors | Lara Lívia Valença Batista, UFAL[I]; Eliane Aparecida Campesatto[II]; Maria Lysete Bastos de Assis[III]; Ana Paula Fernandes Barbosa[IV]; Luciano Aparecido Meireles Grillo[V]; Camila Braga Dornelas[V] |
| Link | Rev Col Bras Cir. 2012 Dec;39(6):515-520 |
| Research Institute | [I]Undergraduate, Faculty of Pharmacy, School of Pharmacy and Nursing, Federal University of Alagoas; [II]Associate Professor, Institute of Biological and Health Sciences, Federal University of Alagoas; [III]Associate Professor, Faculty of Nursing, School of Nursing and Pharmacy, Federal University of Alagoas; [IV]Associate Professor, Faculty of Medicine, Federal University of Alagoas; [V]Associate Professor, Faculty of Pharmacy, School of Pharmacy and Nursing, Federal University of Alagoas |



### Abstract

OBJECTIVE: To evaluate the healing action of green and red propolis, correlating it with the content of flavonoids.

METHODS: We performed quantification of total flavonoids of green and red propolis ethanol extracts for subsequent incorporation in ointment base to 20%. We used 20 Wistar rats divided into four groups: 0.9% saline (S), ointment base (B), green propolis ointment (G) and red propolis ointment (R). All animals were submitted to excisional lesions in the midian back region. The rats were treated daily for 15 days. During this period we observed weight, body temperature and diameters of the wounds. For histological analysis, samples were collected from wounds. At the end of the experiment we performed blood collection and removal of the kidney and liver for biochemical and histological analyzes.

RESULTS: The levels of total flavonoids of green (4.50%) and red (5.92%) propolis were high (> 2%), but, while the latter showed a content larger than the former, the evolution of green propolis was better in the repair of wounds, both macroscopically and histologically. There were no nephrotoxicity or hepatotoxicity, a result confirmed by biochemical tests (ALT and albumin). Propolis influenced the reduction of total cholesterol, triglycerides and glucose.

CONCLUSION: There was no correlation between total flavonoid contents and the healing action of propolis. This reveals the need for elucidation of the flavonoids found in each class of propolis to unravel which one(s) would be important for the healing process.

## 12.3. Review of Propolis Use on Poor Healing and Chronic Non-Healing Wounds: Stan Scheller: The Forerunner of Clinical Studies on Using Propolis for Poor and Chronic Nonhealing Wounds


| | |
|---|---|
| Authors | M. Kucharzewski,[1,2] S. Kubacka,[3] T. Urbanek,[4] K. Wilemska-Kucharzewska,[2] and T. Morawiec[5] |
| Link | Evidence-Based Complementary and Alternative Medicine, Volume 2013 (2013), Article ID 456859, 5 pages |
| Research Institute | [1]Department of Descriptive and Topographic Anatomy, Medical University of Silesia, Ulica Jordana 19, 41-808 Zabrze, Poland<br>[2]Outpatient Surgery Center No. 2, Specialist Hospital No. 2, Ulica Batorego 15, 41-902 Bytom, Poland<br>[3]WZOZ Mother and Children, Ulica Sobieskiego 7A, 42-200 Częstochowa, Poland<br>[4]Department of General and Vascular Surgery, Medical University of Silesia, Ulica Ziołowa 45/47, 40-635 Katowice, Poland<br>[5]Department of Oral Surgery, Faculty of Medicine and Dentistry, Medical University of Silesia, Plac Akademicki 7, 41-902 Bytom, Poland |



### Abstract

For hundreds of years poor and chronic nonhealing wounds have constituted a serious problem to medicine. What is more, treating such wounds is an expensive let alone a long-lasting process. The following paper describes Professor Scheller's achievements in using propolis for poor and chronic non-healing wounds. The authors' intention was to present the results connected with the use of the ethanolic extract propolis, in the treatment of patients suffering from burns, venous crural ulceration, local sacral bone pressure ulcers, suppurative osteitis and arthritis, suppurative postoperative local wound complications, and infected traumatic wounds…

As previously mentioned and clinically confirmed, propolis is said to have several therapeutic properties, such as antibacterial, anti-inflammatory, healing, anesthetic, anticarcinogenic, antifungal, antiprotozoan, and antiviral activities. Added to that, propolis contains copper 26.5 mg/kg, manganese 40 mg/kg, and the ash residue contains iron, calcium, aluminum, vanadium, strontium, and silicon, vitamins such as B1, B2, B6, C, and E, and a number of fatty acids [18]. In addition, it also includes some enzymes such as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase…

## 12.4. Propolis Ointment Boosts Healing of Leg Ulcers: Topical Treatment of Nonhealing Venous Leg Ulcer with Propolis Ointment


| | |
|---|---|
| Authors | M. Kucharzewski,[1,2] M. Kózka,[3] and T. Urbanek[4] |
| Link | Evid Based Complement Alternat Med, 2013;2013:254017 |
| Research Institute | [1]Department of Descriptive and Topographic Anatomy, Medical University of Silesia, ul. Jordana 19, 41-808 Zabrze, Poland<br>[2]Outpatient Surgery Center no. 2, Specialist Hospital no. 2, ul. Batorego 15, 41-902 Bytom, Poland<br>[3]5th Military Hospital, ul. Wrocławska 1-3, 30-901 Cracow, Poland<br>[4]Department of General and Vascular Surgery, Medical University of Silesia, ul. Ziołowa 45/47, 40-635 Katowice, Poland |



### Abstract

An investigation of effectiveness of topical treatment of nonhealing chronic venous leg ulcers with propolis ointment was conducted. 56 patients were included in the study and randomized into two groups.

In group 1, there were 28 patients (ulceration area: 6.9-9.78 cm(2)) treated by means of topical propolis ointment application and short stretch bandage compression. In group 2, there were 29 patients (ulceration area: 7.2-9.4 cm(2)) treated by means of Unna boot leg compression without topical propolis treatment. In the study, the efficacy of both treatment methods in patients with resistive venous leg ulcers was compared.

The ulceration of patients from group 1 healed completely after 6 weeks of therapy in all cases. In all patients from group 2, the process of healing was longer but successfully completed after 16 weeks of the therapy.

We found that an adjunctive propolis ointment treatment increases the efficacy of the short stretch bandage compression stocking, and this combined treatment is more effective than Unna's boot compression alone.

# 13. Others

## 13.1. Botanical origin and chemical composition of Brazilian Propolis.

| | |
|---|---|
| Authors | Park YK, Alencar SM, Aguiar CL. |
| Link | J Agric Food Chem. 2002 Apr 24;50(9):2502-6. |
| Research Institute | Department of Food Science, College of Food Engineering, State University of Campinas, 13081-970 Campinas, Sao Paulo, Brazil. |


### Abstract

Brazilian Propolis has been classified into 12 groups based on physicochemical characteristics: five in the southern Brazil group (group 3), one in the southeastern Brazil group (group 12), and six in the northeastern Brazil group (group 6). The plant origins of these groups were investigated using reversed-phase high-performance thin-layer chromatography (RPHPTLC), reversed-phase high-performance liquid chromatography (RPHPLC), and gas chromatography-mass spectrometry (GC-MS). It was concluded that the origins of Propolis group 3, group 6, and group 12 are resins of the poplar tree, Hyptis divaricata, and *Baccharis dracunculifolia*, respectively.

## 13.2. In-vitro trypanocidal activity evaluation of crude extract and isolated compounds from *Baccharis dracunculifolia* D.C. (Asteraceae).


| | |
|---|---|
| Authors | da Silva Filho AA, Pires Bueno PC, Gregorio LE, Andrade e Silva ML, Albuquerque S, Bastos JK. |
| Link | J Pharm Pharmacol. 2004 Sep;56(9):1195-9. |
| Research Institute | Departamento de Ciencias Farmaceuticas, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Avenida do cafe s/n, 14040-903 Ribeirao Preto, SP, Brazil. |



### Abstract

We have performed a trypanocidal bioactivity-guided study of *Baccharis dracunculifolia* (Asteraceae), the main botanical origin of Brazlian Green Propolis. The leaf rinse extract of *B. dracunculifolia* , at a concentration of 3.0 mg mL(-1), displayed 100% lysis of trypomastigote forms of the Y strain of Trypanosoma cruzi (2 x 10(6) parasites mL(-1)). The chromatographic fractionation of the leaf rinse, using several techniques, afforded the isolation of the compounds isosakuranetin (1), aromadendrin-4'-methylether (2), baccharis oxide (3), ferulic acid (4), dihydrocinnamic acid (5), 3-prenyl-4-(dihydrocinnamoyloxy)-cinnamic acid (6), and friedelanol (7). The chemical structures of all compounds were established by UV-vis, (1)H and (13)CNMR data analysis in comparison with the literature. Compounds 1 and 3 were the most active in the trypanocidal assay, showing IC50 values (inhibitory concentration required for 50% inhibition) of 247.6 and 249.8 microM, respectively. Compounds 2, 4, and 6 displayed moderate activity, whilst compounds 5 and 7 were inactive.

## 13.3. Plant Origin of Green Propolis: Bee Behavior, Plant Anatomy and Chemistry.

| | |
|---|---|
| Authors | Teixeira EW, Negri G, Meira RM, Message D, Salatino A. |
| Link | Evid Based Complement Alternat Med. 2005 Mar;2(1):85-92. |
| Research Institute | University of São Paulo, Institute of Biosciences, Department of Botany, C. Postal. 11461, 05422-970, São Paulo, SP, Brazil. |


### Abstract

Propolis, a honeybee product, has gained popularity as a food and alternative medicine. Its constituents have been shown to exert pharmacological effects, such as anti-microbial, anti-inflammatory and anticancer. Shoot apices of *Baccharis dracunculifolia* (alecrim plant, Asteraceae) have been pointed out as sources of resin for green Propolis. The present work aimed (i) to observe the collecting behavior of bees, (ii) to test the efficacy of histological analysis in studies of Propolis botanical origin and (iii) to compare the chemistries of alecrim apices, resin masses and green Propolis. Bee behavior was observed, and resin and Propolis were microscopically analyzed by inclusion in methacrylate. Ethanol extracts of shoot apices, resin and Propolis were analyzed by gas chromatography/mass spectroscopy. Bees cut small fragments from alecrim apices, manipulate and place the resulting mass in the corbiculae. Fragments were detected in Propolis and identified as alecrim vestiges by detection of alecrim structures. Prenylated and non-prenylated phenylpropanoids, terpenoids and compounds from other classes were identified. Compounds so far unreported for Propolis were identified, including anthracene derivatives. Some compounds were found in Propolis and resin mass, but not in shoot apices. Differences were detected between male and female apices and, among apices, resin and Propolis. Alecrim apices are resin sources for green Propolis. Chemical composition of alecrim apices seems to vary independently of season and phenology. Probably, green Propolis composition is more complex and unpredictable than previously assumed.

## 13.4. Absorption and bioavailability of Artepillin C in rats after oral administration.


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|---|---|
| Authors | Konishi Y, Hitomi Y, Yoshida M, Yoshioka E |
| Link | J Agric Food Chem. 2005 Dec 28;53(26):9928-33. |
| Research Institute | Central Laboratories for Frontier Technology, Kirin Brewery Co., Ltd., 1-13-5, Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236-0004, Japan. konishiy@kirin.co.jp |



### Abstract

Artepillin C (AC), an active ingredient of Brazilian Propolis, permeates intact across Caco-2 cells by transcellular passive diffusion. The permeation of AC across Caco-2 cells is as efficient as that of phenolic acids and the microbial metabolites of poorly absorbed polyphenols, which are actively absorbed by the monocarboxylic acid transporter (MCT) (Biochim. Biophys. Acta 2005, 1713, 138-144). Here, the absorption of orally administered AC in rats has been studied to evaluate its pharmacokinetics and bioavailability in vivo in comparison with those of p-coumaric acid (CA), a substrate of MCT. Rats were given 100 micromol/kg of body weight of AC or CA, and blood was subsequently collected from the portal vein and abdominal artery. AC, CA, and their metabolites were quantified by coulometric detection using HPLC-ECD. The serum concentration of intact AC and CA in the portal vein peaked at 5-10 min after administration, with a C(max) of 19.7 micromol/L for AC and 74.8 micromol/L for CA. The area under the curve (AUC) for intact AC and CA in the portal vein was calculated from the serum concentration as 182.6 and 3057.3 micromol.min.L(-1), respectively. The absorption efficiency of CA was about 17-fold higher than that of AC. Furthermore, the bioavailability of CA was about 278-fold higher than that of AC, and the ratio of AUC in the abdominal artery to AUC in the portal vein was 0.04 and 0.70, for AC and CA, respectively. Thus, AC is likely to be more susceptible to hepatic elimination than is CA. The bioactive compound of AC in vivo should be investigated further.

## 13.5. Brazilian Red Propolis—Chemical Composition and Botanical Origin.


| | |
|---|---|
| Authors | Andreas Daugsch, Cleber S. Moraes, Patricia Fort and Yong K. Park |
| Link | Evid. Based Complement. Altern. Med. 2008; 5:435-441. [Abstract] [Full Text] [PDF] |
| Research Institute | Department of Food Science, College of Food Engineering, State University of Campinas, PO Box 6177, Campinas, SP, Brazil |



### Abstract

Propolis contains resinous substances collected by honey bees from various plant sources and has been used as a traditional folk medicine since ca 300 BC. Nowadays, the use of evidence-based complementary and alternative medicine (CAM) is increasing rapidly and so is the use of Propolis in order to treat or support the treatment of various diseases.

Much attention has been focused on propolis from *Populus sp*. (Salicaceae) and *Baccharis dracunculifolia* (Asteracea), but scientific information about the numerous other types of Propolis is still sparse. We gathered six samples of red Propolis in five states of Northeastern Brazil. The beehives were located near woody perennial shrubs along the sea and river shores. The bees were observed to collect red resinous exudates on *Dalbergia ecastophyllum (L) Taub. (Leguminosae)* to make Propolis. The flavonoids of propolis and red resinous exudates were investigated using reversed-phase high-performance liquid chromatography and reversed-phase high-performance thin-layer chromatography.

We conclude that the botanical origin of the reddish Propolis is *D. ecastophyllum*. In areas where this source (*D. ecastophyllum*) was scarce or missing, bees were collecting resinous material from other plants. Propolis, which contained the chemical constituents from the main botanical origin, showed higher antimicrobial activity.

## 13.6. Evaluation of anti-allergic properties of caffeic acid phenethyl ester in a murine model of systemic anaphylaxis.


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|---|---|
| Authors | Sae-Gwang Park, Da-Young Lee, Su-Kil Seo, Soo-Woong Lee, Se-Kwon Kim, Won-Kyo Jung, Mi-Seon Kang, Yung Hyun Choi, Sung Su Yea, Inhak Choi, and Il-Whan Choi. |
| Link | *Toxicol Appl Pharmacol.* 2007. [MEDLINE Citation] |
| Research Institute | Department of Microbiology, College of Medicine and Center for Viral Disease Research, Inje University, Busan 614-735, South Korea. |



### Abstract

Caffeic acid phenethyl ester (CAPE) is an active component of honeybee Propolis extracts. It has several positive effects, including anti-inflammatory, anti-oxidation, anti-cancer, anti-bacterial, anti-viral, anti-fungal, and immunomodulatory effects. In particular, the suppressive effect of NF-kappaB may disrupt a component of allergic induction. The principal objective of this experimental study was to evaluate the effects of CAPE on the active systemic anaphylaxis induced by ovalbumin (OVA) challenge in mice. Mice were intraperitoneally sensitized and intravenously challenged with OVA. Histopathological analysis, nuclear factor (NF)-kappaB activation, and the plasma levels of histamine and total IgE after allergen challenge were evaluated. After challenges, all of the sham-treated mice developed anaphylactic symptoms, increased plasma levels of histamine and OVA-specific IgE, marked vascular leakage, NF-kappaB activation, platelet-activating factor (PAF) production, and histological changes including pulmonary edema and hemorrhage in the renal medullae within 20 min. By way of contrast, a reduction in the plasma levels of histamine and OVA-specific IgE and an inhibition of NF-kappaB activation and PAF release were observed in the CAPE-treated mice. In addition, a significant prevention of hemoconcentration and OVA-induced pathological changes were noted. These results indicate that CAPE demonstrates an anti-allergic effect, which may be the result of its protective effects against IgE-mediated allergy.

## 13.7. Effect of Propolis versus metronidazole and their combined use in treatment of acute experimental giardiasis.


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|---|---|
| Authors | NS Abdel-Fattah and OH Nada. |
| Link | *J Egypt Soc Parasitol.* 2007; 37: 691. [MEDLINE Citation] |
| Research Institute | Department of Parasitology, Faculty of Medicine, Ain-Shams University, Cairo 11566, Egypt. |



### Abstract

Propolis, a honey bee product, gained popularity in alternative medicine. Its prophylactic and therapeutic effects were experimentally evaluated. One hundred and fifty immunocompetent mice were orally infected by 5 x 10(5) axenically cultivated Giardia lamblia trophozoites. The trophozoite count in intestine, interferon-gamma serum level, histopathological examination of duodenal and jejunal sections were assessed for evaluation of Propolis and metronidazole (MTZ) effect after 6 & 12 days post infection (p.i). Also, T-lymphocyte profile in blood was investigated 12 days p.i using flow cytometry (FCM). Propolis as prophylaxis showed a significant decrease in intensity of infection, together with a significant increase in IF-gamma serum level and increase in CD4+: CD8+T-cell ratio. In treatment it gave a highly significant decrease in trophozoite count than that obtained by MTZ 6 days after infection but the efficacy was almost equal after 12 days. The mice treated with Propolis alone showed a reversed CD4+: CD8+ T-lymphocyte ratio, such strong immune enhancing effect resulted in an undesirable increase in inflammatory response at intestinal level. The combined therapy showed a stronger efficacy in reducing the parasite count than that gained by each drug alone. Their combined use caused an immunological balance as shown by the T-lymphocyte profile that saved the intestinal homeostasis and histological architecture.

## 13.8. Dietary supplement usage among elementary school children in Taiwan: their school performance and emotional status.


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|---|---|
| Authors | SY Chen, JR Lin, MD Kao, CM Hang, L Cheng, and WH Pan. |
| Link | *Asia Pac J Clin Nutr.* 2007; 16 Suppl 2: 554. [MEDLINE Citation] |
| Research Institute | Department of Health and Nutrition, Chia Nan University of Pharmacy and Science, Jen-Te Hsiang, Tainan Hsien, Taiwan, ROC. shihying@mail.chna.edu.tw |



### Abstract

Dietary supplement consumption practices among 2417 children (1295 boys and 1122 girls) aged 6 to 12 years in Taiwan were derived from the Nutrition and Health Survey in Taiwan Elementary School Children (NAHSIT 2001-2002). The proportion (22%) of boys and girls using supplements was equivalent. Some 77% of the child supplement takers took only one type of supplement. The top five supplements consumed were: multivitamins and minerals, calcium, vitamin C, cod-liver oil and bee Propolis in that order. Children in the most urbanized southern Taiwan had the highest usage (33%), but prevalence was lowest in the mountainous areas (5%). Higher parental education level and household monthly income were associated with higher intakes. Supplement users were more competent at school; however, the frequency and number of supplement types were not related to competence.

## 13.9. Spray-dried Propolis extract, II: prenylated components of Green Propolis.


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|---|---|
| Authors | JP Souza, LA Tacon, CC Correia, JK Bastos, and LA Freitas. |
| Link | *Pharmazie.* 2007; 62: 488. [MEDLINE Citation] |
| Research Institute | Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Brazil. |



### Abstract

The effect of spray drying conditions on the chemical composition of Brazilian Green Propolis extract was investigated using a factorial design and high performance liquid chromatography. The raw and dried extract contents of caffeic acid, p-coumaric acid, drupanin, isosakuranetin, artepillin C, baccharin and 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran were quantified using veratraldehyde (3,4-dimethoxybenzaldehyde) as internal standard. The baccharin content in spray-dried Propolis was affected by the drying temperature with a 5% significance level, while the coumaric acid and drupanin contents were dependent on drying temperature at a 15% significance level. The other chemical markers, caffeic acid, isosakuranetin, artepillin C and 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran, showed to be independent of drying conditions. However, all the chemical markers showed some loss on drying, which varied from 30 to 50%. The results showed that prenylated compounds are sensitive to drying, but their losses may be considerably reduced under low temperatures, around 40 degrees C. The antioxidant activity of the spray dried Propolis was determined by the diphenylpicrylhydrazyl (DPPH) method and showed a quadratic dependency on the temperature; extract feed rate and the interaction between them. However, spray dried Propolis extracts presented antioxidant activities similar to the original Propolis tincturae.

## 13.10. Photoprotective activity of Propolis.

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|---|---|
| Authors | C Couteau, M Pommier, E Paparis, and LJ Coiffard. |
| Link | Nat Prod Res. *2008;* 22: 264. [MEDLINE Citation] |
| Research Institute | Université de Nantes, Nantes Atlantique Universités, Nantes, F-44000 France. |


### Abstract

The purpose of this study was to determine the photoprotective properties of Propolis. The sun protection factor (SPF) of ethanol extract of Propolis was evaluated by an in vitro method, using homosalate as control. This determination is based on the physical determination of the reduction of the energy in the UV range, through a film of product which has previously been spread on an adequate substrate. About 15 mg of O/W emulsion containing Propolis at various concentrations were applied on roughened Polymethylmethacrylate (PMMA) plates and the transmission measurements were carried out using a spectrophotometer equipped with integrating sphere. The results may justify their use as a natural sunscreen agent.

## 13.11. Apitherapy: Usage And Experience In German Beekeepers.


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|---|---|
| Authors | Markus Hellner[1], Daniel Winter[2], Richard von Georgi[3,4] and Karsten Münstedt[1] |
| Link | Evid. Based Complement. Altern. Med. 2008; 5:475-479. [Abstract] [Full Text] [PDF] |
| Research Institute | [1]Department of Obstetrics and Gynecology, University Hospital Giessen and Marburg, Justus Liebig University, Klinikstrasse 32, 35385 Giessen, [2]Medical Clinic and Policlinic 3, University Hospital Giessen and Marburg, Justus Liebig University, Rodthohl 6, 35385 Giessen, [3]Institute of Medical Psychology and Sociology of the Justus-Liebig University, Giessen and [4]Institute of Music Science, Justus-Liebig-University, Giessen, Germany |



### Abstract

This study aimed to investigate the practice of apitherapy - using bee products such as honey, pollen, propolis, royal jelly and bee venom to prevent or treat illness and promote healing - among German beekeepers and to evaluate their experiences with these therapies. A questionnaire incorporating two instruments on beekeepers' physical and mental health and working practice was included in three German beekeeping journals and readers were asked to complete it. The instrument included questions on the use of apitherapy. Simple descriptive methods, bivariate correlation, cross-tabulation and one-way ANOVA were used to analyze the data. Altogether 1059 completed questionnaires were received.

The beekeepers reported the most effective and favorable therapeutic effects with honey, followed by propolis, pollen and royal jelly. The factors associated with successful experiences were: age, number of hives tended, health consciousness, positive experiences with one product and self-administration of treatment. Beekeepers were asked for which condition they would employ propolis and pollen. They reported that they used propolis most frequently to treat colds, wounds and burns, sore throats, gum disorders and also as a general prophylactic, while pollen was most commonly used as a general prophylactic and, less frequently, in treating prostate diseases. No adverse experiences were reported. The potential benefit of bee products is supported by the positive experiences of a large group of beekeepers who use some of these products to treat a wide range of conditions. The indications and treatments given here may be important in selecting bee products and designing future trials.

## 13.12. Lack of clastogenic/genotoxic effects of *Baccharis dracunculifolia* extract on Swiss mouse peripheral blood cells.


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| Authors | Andrade NS, Perazzo FF, Maistro EL |
| Link | Genet Mol Res. 2008 Dec 23;7(4):1414-21 |
| Research Institute | Laboratório de Genética, Universidade José do Rosário Vellano, Alfenas, MG, Brasil. |



### Abstract

*Baccharis dracunculifolia* De Candole (DC) (Asteraceae) is indigenous throughout southeastern Latin America and is used by local people in traditional medicine. This plant is known to be the source of resin for the highly valued Brazilian Green Propolis.

As no information is available on the safety of high doses of *B. dracunculifolia* extract, we evaluated the mutagenic potential of high doses of this plant extract in vivo on peripheral blood cells of Swiss mice using the comet assay and the micronucleus test.

The extract was administered by gavage at doses of 1000, 1500 and 2000 mg/kg body weight. Peripheral blood cell samples were collected 4 and 24 h after treatment for the comet assay (genotoxicity assay), and at 48 and 72 h for the micronucleus test (clastogenicity assay).

The *B. dracunculifolia* extract was devoid of clastogenic/genotoxic activity at all doses.

## 13.13. Comparison of Thymus vulgaris (Thyme), Achillea millefolium (Yarrow) and propolis hydroalcoholic extracts versus systemic glucantime in the treatment of cutaneous leishmaniasis in balb/c mice


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| Authors | MA Nilforoushzadeh, L Shirani-Bidabadi, A Zolfaghari-Baghbaderani, S Saberi, AH Siadat, and M Mahmoudi |
| Link | J Vector Borne Dis, December 1, 2008; 45(4): 301-6. |
| Research Institute | Skin Diseases and Leishmaniasis Research Center (Sedigheh Tahereh), Isfahan University of Medical Sciences, Isfahan, Iran. |



### Abstract

Background & Objectives: Leishmaniasis is a parasitic disease transmitted by sand flies. Many investigations are performed to find an effective and safe treatment for leishmaniasis. In this study, we evaluated the efficacy of herbal extracts of Thymus vulgaris (Thyme) and Achillea millefolium (Yarrow), propolis hydroalcoholic extract and systemic glucantime against cutaneous leishmaniasis in Balb/c mice.

Methods: A total of 45 mice were randomised into five groups each including nine mice. They were treated with pure ethanol 70 degrees, systemic glucantime, Achillea millefolium hydroalcoholic extract, Thymus vulgaris hydroalcoholic extract and propolis hydroalcoholic extract for six weeks. The statistical tests including student t-test were used for analysis. Data were analyzed by SPSS software, ver 13.00.

Results: Mean of ulcer size reduction were -17.66, -22.57, 43.29, 36.09 and 43.77% for the alcohol, glucantime, yarrow, thyme and propolis groups, respectively. The results were suggestive that Thymus vulgaris, Achillea millefolium and propolis hydroalcoholic extracts were significantly more effective in reduction of ulcer size as compared with glucantime ($p = 0.006$, 0.002 and 0.008, respectively).

Interpretation & Conclusion: Our results are suggestive that Thymus vulgaris, Achillea millefolium and propolis extracts are effective for treatment of cutaneous leishmaniasis in mice. Regarding these results, we suggest that efficacy of these extracts alone or in combination are evaluated against human cutaneous leishmaniasis as a randomized clinical trial.

## 13.14. Neuroprotective effects of Brazilian green propolis and its main constituents against oxygen-glucose deprivation Stress, with a gene-expression analysis.


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| Authors | Yoshimi Nakajima, Masamitsu Shimazawa, Satoshi Mishima, and Hideaki Hara |
| Link | Phytother Res, March 10, 2009 |
| Research Institute | Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan. |



### Abstract

Our purpose was to investigate the neuroprotective effects (and the underlying mechanism) exerted by water extract of Brazilian green propolis (WEP) and its main constituents against the neuronal damage induced by oxygen-glucose deprivation (OGD)/reoxygenation in retinal ganglion cells (RGC-5, a rat ganglion cell-line transformed using E1A virus).

Cell damage was induced by OGD 4 h plus reoxygenation 18 h exposure. In RGC-5, and also in PC12 (rat pheochromocytoma, neuronal cells), WEP and some of its main constituents attenuated the cell damage. At the end of the period of OGD/reoxygenation, RNA was extracted and DNA microarray analysis was performed to examine the gene-expression profile in RGC-5. Expression of casein kinase 2 (CK2) was down-regulated and that of Bcl-2-related ovarian killer protein (Bok) was up-regulated following OGD stress, results that were confirmed by quantitative reverse transcriptase-PCR (qRT-PCR). These effects were normalized by WEP.

Our findings indicate that WEP has neuroprotective effects against OGD/reoxygenation-induced cell damage and that certain constituents of WEP (caffeoylquinic acid derivatives, artepillin C, and p-coumaric acid) may be partly responsible for its neuroprotective effects.

Furthermore, the protective mechanism may involve normalization of the expressions of antioxidant- and apoptosis-related genes (such as CK2 and Bok, respectively).

## 13.15. Protective effects of propolis on inorganic mercury induced oxidative stress in mice.


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| Authors | JQ Zhao, YF Wen, M Bhadauria, SK Nirala, A Sharma, S Shrivastava, S Shukla, OP Agrawal, and R Mathur |
| Link | Indian J Exp Biol, April 1, 2009; 47(4): 264-9 |
| Research Institute | College of Animal Science and Technology, Yunnan Agricultural University, Kunming 650 201, PR China. |



### Abstract

Protective potential of propolis was evaluated against mercury induced oxidative stress and antioxidant enzymatic alterations in mice liver.

Exposure to mercuric chloride (HgCl2; 5 mg/kg; ip) induced oxidative stress by increasing lipid peroxidation and oxidized glutathione level along with concomitant decrease in glutathione and various antioxidant enzymes. Mercury intoxication deviated the activity of liver marker enzymes in serum.

Conjoint treatment of propolis (200 mg/kg; po) inhibited lipid peroxidation and oxidized glutathione level, whereas increased glutathione level. Activities of antioxidants enzymes, i.e., superoxide dismutase, catalase, glutathione-S-transferase and glucose-6-phosphate dehydrogenase were also restored concomitantly towards control after propolis administration. Release of serum transaminases, alkaline phosphatase, lactate dehydrogenase and y-glutamyl transpeptidase were significantly restored towards control after propolis treatment.

Results suggest that propolis augments the antioxidants defense against mercury induced toxicity and provides evidence that it has therapeutic potential as hepatoprotective agent.

## 13.16. The vasorelaxant effect of caffeic acid phenethyl ester on porcine coronary artery ring segments.


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| Authors | Yuan Long, Min Han, Juan Chen, Xiao-Zhu Tian, Qiang Chen, and Rui Wang |
| Link | Vascul Pharmacol, April 2, 2009 |
| Research Institute | Institute of Biochemistry and Molecular Biology, School of Life Sciences, Lanzhou University, 222 Tianshui South Road, Lanzhou, 730000, P.R.China. |



### Abstract

Caffeic acid phenethyl ester (CAPE) is a naturally occurring compound isolated from honeybee propolis whose cardiovascular properties remain uncertain. The purpose of this study was to investigate the possible mechanisms of CAPE-induced vasorelaxation in porcine coronary artery rings.

It was found that both the quiescent and pre-contracted coronary artery ring segments were relaxed by CAPE (10(-7)~10(-4) M). N(omega)-nitro-L-arginine (L-NNA), methylene blue and removal of endothelium significantly attenuated CAPE-induced relaxation of both quiescent and pre-contracted artery rings. This relaxing effect of CAPE on coronary arteries was also significantly reduced by propranolol, and SQ22536, but not by indomethacin. In addition, the dose-response curves of KCl (2.5~100 mM) and CaCl(2) (10(-5)~10(-2) M) were displaced downwards in the presence of CAPE.

These results suggest that the relaxant effect of CAPE on porcine coronary artery rings might involve the action of nitric oxide (NO) and adrenergic beta-receptor, together with their second messenger, cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP), respectively, but not involve the synthesis of prostaglandin.

## 13.17. Radioprotective effects of quercetin and ethanolic extract of propolis in gamma-irradiated mice.


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|---|---|
| Authors | V Benkovic, AH Knezevic, D Dikic, D Lisicic, N Orsolic, I Basic, and N Kopjar |
| Link | Arh Hig Rada Toksikol, June 1, 2009; 60(2): 129-38. |
| Research Institute | Department of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia. vesna@pmf.biol.hr |



### Abstract

The aim of this study was to assess radioprotective effects of quercetin and the ethanolic extract of Propolis (EEP) in CBA mice exposed to a single radiation dose 4 Gy (60Co).

The mice were treated with 100 mg kg(-1) quercetin or EEP a day for three consecutive days either before (pre-treatment) or after gamma-irradiation (therapy). Leukocyte count was determined in blood drawn from the tail vein, and DNA damage in leukocytes was assessed using the alkaline comet assay. Genotoxic effects of the test compounds were also evaluated in non-irradiated mice. The levels of radioprotection provided by both test compounds were compared with those established in mice that were given chemical radioprotector S-(2-aminoethy1) isothiouronium bromide hydrobromide (AET).

Mice that received pre-treatment were less sensitive to irradiation. Mice given the post-irradiation therapy showed a slight but not significant increase in total leukocyte count over irradiated negative control. Quercetin showed better protective properties than EEP in both pre-treatment and therapy, and activated a higher number of leukocytes in non-irradiated mice.

The alkaline comet assay suggests that both natural compounds, especially when given as pre-treatment, protect against primary leukocyte DNA damage in mice. At tested concentrations, EEP and quercetin were not genotoxic to non-irradiated mice. AET, however, caused a slight but not significant increase in DNA damage.

Although the results of this study show the radioprotective potential of the test compounds, further investigation is needed to clarify the underlying protection mechanisms.

## 13.18. Antihypertensive effects of flavonoids isolated from Brazilian Green Propolis in spontaneously hypertensive rats.


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| Authors | H Maruyama, Y Sumitou, T Sakamoto, Y Araki, and H Hara |
| Link | Biol Pharm Bull, July 1, 2009; 32(7): 1244-50 |
| Research Institute | Nagaragawa Research Center, API Co., Ltd. |



### Abstract

Propolis, a honeybee product, has become popular as a food and alternative medicine. Its constituents have been shown to exert pharmacological effects, such as anticancer, antimicrobial, and anti-inflammatory effects. The present study was performed to investigate whether Brazilian green Propolis exerts antihypertensive effects in spontaneously hypertensive rats (SHR) and which constituents are involved in its effects.

Brazilian green Propolis was extracted with ethanol and subjected to LH-20 column chromatography eluted with ethanol. The ethanol-eluted fractions at 10 mg/kg were administered orally to SHR for 14 d. Significant decreases in blood pressure were observed in fractions 6 and 7. The active constituents were purified and identified to be four flavonoids: dihydrokaempferide and isosakuranetin in fraction 6 and betuletol and kaempferide in fraction 7. These flavonoids at 10 mg/kg were administered orally to SHR for 28 d, and as a result, isosakuranetin, dihydrokaempferide and betuletol produced significant decrease in blood pressure, especially marked were the effects observed in the group that received isosakuranetin. Brazilian green Propolis, fractions 6 and 7, and the 4 active constituents relaxed isolated SHR aorta in a concentration-dependent manner. Therefore, these finding suggest that the vasodilating action may be partly involved in the mechanism of antihypertensive effect. Hence, the ethanol extract of Brazilian green Propolis and its main constituents may be useful for prevention of hypertension.

## 13.19. Effect of Brazilian propolis on sneezing and nasal rubbing in experimental allergic rhinitis of mice.


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| Authors | Yoshifumi Shinmei, Haruna Yano, Yoto Kagawa, Kana Izawa, Masaaki Akagi, Toshio Inoue, and Chiaki Kamei |
| Link | Immunopharmacol Immunotoxicol, July 17, 2009 |
| Research Institute | Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan. |



### Abstract

We studied the effect of Brazilian Propolis on sneezing and nasal rubbing in experimental allergic rhinitis of mice.

A single administration of Propolis caused no significant effect on both antigen-induced nasal rubbing and sneezing at a dose of 1000 mg/kg, but a significant inhibition was observed after repeated administration for 2 weeks at this dose.

Propolis caused no significant inhibitory effect on the production of total IgE level after repeated administration of 1000 mg/kg. The drug also caused no significant inhibition of histamine-induced nasal rubbing and sneezing at a dose of 1000 mg/kg.

On the other hand, Propolis significantly inhibited histamine release from rat mast cells induced by antigen and compound 48/80 at a concentration of more than 10 mug/ml.

These results clearly demonstrated that Propolis may be effective in the relief of symptoms of allergic rhinitis through inhibition of histamine release.

## 13.20. Inhibitory activity of Brazilian green propolis components and their derivatives on the release of cys-leukotrienes.

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| Authors | Hiroko Tani, Keiko Hasumi, Tomoki Tatefuji, Ken Hashimoto, Hiroyuki Koshino, and Shunya Takahashi |
| Link | Bioorg Med Chem, November 10, 2009 |
| Research Institute | Institute for Bee Products and Health Science, Yamada Apiculture Center, Inc., 194 Ichiba, Kagamino 708-0393, Japan. |


### Abstract

The effects of Brazilian Green Propolis ethanol extract on Cry j1-induced cys-leukotrienes and histamine release from peripheral leukocytes of patients with allergic rhinitis were investigated.

One of the key mechanisms for the anti-allergic properties of the extract was revealed to be the suppression of cys-LTs release. Furthermore, a series of Propolis components and their phenethyl esters were synthesized and evaluated as inhibitors of cys-LTs release.

Artepillin C, baccharin, and kaempferide were the major active components of the ethanol extract.

The inhibitory activity of artepillin C phenethyl ester was comparable to that of existing LT synthesis inhibitors.

## 13.21. Propolis prevents diet-induced hyperlipidemia and mitigates weight gain in diet-induced obesity in mice


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| Authors | S Koya-Miyata, N Arai, A Mizote, Y Taniguchi, S Ushio, K Iwaki, and S Fukuda |
| Link | Biol Pharm Bull, December 1, 2009; 32(12): 2022-8. |
| Research Institute | Biomedical Institute, Research Center, Hayashibara Biochemical Laboratories, Inc., 675-1 Fujisaki, Naka-ku, Okayama 702-8006, Japan. fujisaki@hayashibara.co.jp. |



### Abstract

We examined the hypolipidemic effect of Propolis in a mouse obesity model induced by a high fat-diet. C57BL/6N mice were fed a high-fat diet ad libitum and given Propolis extract intragastrically at 0 mg/kg (control), 5 mg/kg or 50 mg/kg twice daily for 10 d. Compared with mice in the control group, mice in the Propolis extract-administrated groups displayed a reduction in all of the following parameters: body weight gain, weight of visceral adipose tissue, liver and serum triglycerides, cholesterol, and non-esterified fatty acids.

Real-time polymerase chain reaction analysis of the liver showed down-regulation of mRNA expression associated with fatty acid biosynthesis, including fatty acid synthase, acetyl-CoA carboxylase alpha, and sterol regulatory element binding protein in the propolis-administrated mice.

Subsequently, obese C57BL/6N mice that had been administered a high-fat diet were given Propolis extract at 0 mg/kg (control), 2.5 mg/kg or 25 mg/kg for 4 weeks. The Propolis extract treated mice showed a decrease in weight gain, a reduction of serum non-esterified fatty acids, and lipid accumulation in the liver.

These results suggest that Propolis extract prevented and mitigated high-fat diet-induced hyperlipidemia by down-regulating the expression of genes associated with lipid metabolism.

## 13.22. Ameliorative Effect of Propolis on Insulin Resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) Rats


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|---|---|
| Authors | Yoshito Zamami [1], Hiroki Fujiwara [1], Miho Hosoda [1], Hayato Hino [1], Kazuhiro Hirai [1], Kazuaki Okamoto [1], Xin Jin [1], Shingo Takatori [2], Shima Doi-Takaki [3], and Hiromu Kawasaki*[1] |
| Link | Yakugaku Zasshi. 2010 Jun;130(6):833-40 |
| Research Institute | [1]Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530 Japan [2]Research Laboratories Kyoto, Nippon Shinyaku Co., Ltd. , 14 Nishinosho-Monguchi-cho, Minami-ku, Kyoto 601-8550 Japan and [3]Department of Research and Development, Yamada Apiculture Center, Inc. 194 Ichiba, Kagamino-cho, Tomata-gun, Okayama 708-0393, Japan |



### Abstract

Propolis is known to have abundant bioactive constituents and a variety of biological activities.

To investigate the effect of Brazilian Propolis on insulin resistance, 10-week-old Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a non-insulin-dependent type 2 diabetic model, were treated for 4 weeks with Propolis (100 and 300 mg/kg, p.o.) or vehicle (control).

Propolis treatment significantly decreased the plasma levels of insulin and insulin resistance index (Homeostasis Model Assessment-Insulin Resistance; HOM-IR), without affecting blood glucose levels and tended to lower systolic blood pressure compared with the control.

In isolated and perfused mesenteric vascular beds of OLETF rats, Propolis treatment resulted in significant reduction of sympathetic nerve-mediated vasoconstrictor response to periarterial nerve stimulation (PNS) and tended to increase calcitonin gene-related peptide (CGRP) nerve-mediated vasodilator response to PNS compared with in vehicle-treated OLETF rats.

However, Propolis treatment did not significantly affect the vasoconstrictor and vasodilator response to noradrenaline, CGRP, acetylcholine, and sodium nitroprusside.

These results suggest that Propolis could be an effective and functional food to prevent development of insulin resistance.

## 13.23. Antigenotoxicity of Artepillin C in vivo Evaluated by the Micronucleus and Comet Assays.


Authors: Moacir de Azevedo Bentes Monteiro Neto[1], Ildercílio Mota de Souza Lima[1], Ricardo Andrade Furtado[2], Jairo Kenupp Bastos[2], Ademar Alves da Silva Filho[3], Denise Crispim Tavares[1]



Link: J Appl Toxicol. 2011 Jan 24



Research Institute: [1]Universidade de Franca, Franca, São Paulo, Brazil
[2]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil
[3]Faculdade de Farmácia, Departamento Farmacêutico, Universidade Federal de Juiz de Fora, Campus Universitário Martelos, Juiz de Fora, Minas Gerais, Brazil,
EMail: Denise Crispim Tavares (denisecrispim2001@yahoo.com)



### Abstract

Artepillin C (3,5-diprenyl-p-coumaric acid), a major compound found in Brazilian Green Propolis and Baccharis Dracunculifolia, shows anti-inflammatory, antibacterial, antiviral, antioxidant and antitumoral activities, among others.

The aim of this study was to evaluate the genotoxic potential of artepillin C and its ability to prevent the chemically induced chromosome breakage or loss and the primary DNA damage using the micronucleus and comet assays in male Swiss mice, respectively.

The animals were treated by gavage with different doses of Artepillin C (0.4, 0.8 and 1.6 mg kg(-1) b.w.). For the antigenotoxicity assays, the different doses of Artepillin C were administered simultaneously to doxorubicin (DXR; micronucleus test; 15 mg kg(-1) b.w.) and to methyl methanesulfonate (MMS; comet assay; 40 mg kg(-1) b.w.).

The results showed that Artepillin C itself was not genotoxic in the mouse micronucleus and comet assays. In the animals treated with artepillin C and DXR, the number of micronucleated reticulocytes was significantly lower in comparison with the animals treated only with DXR.

Regarding antigenotoxicity, Artepillin C at the tested doses significantly reduced the extent of DNA damage in liver cells induced by MMS.

## 13.24. Propolis May Help Prevent Kidney Damage: Role of Propolis (Bee Glue) in Improving Histopathological Changes of the Kidney of Rat Treated with Aluminum Chloride.


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| Authors | El-Kenawy AE, Hussein Osman HE, Daghestani MH |
| Link | Environmental Toxicology, Article first published online: 22 NOV 2012 |
| Research Institute | Department of Molecular Biology, Genetic Engineering and Biotechnology Research Inst., Minufiya University, Egypt; Department of pathology, College of medicine, Taif University, Kingdom of Saudi Arabia. |



### Abstract

Humans are frequently exposed to aluminum from various food additives, therapeutic treatments and the environment, and it can be potentially toxic. This study is aimed to elucidate the protective effects of Propolis against aluminum chloride (AlCl3)-induced histopathological and immunohistochemical changes in kidney tissues of rats.

Sixty Wistar Albino male rats (average weight 250–300 g) were divided into three equal groups. The first served as a negative control. The second received AlCl3 (34 mg/kg bw, 1/ 25 LD 50). The third were administered AlCl3 (34 mg/kg bw, 1/ 25 LD 50) plus propolis (50 mg/kg bw). Doses were given once daily via a gavage for 8 weeks every day.

The results showed that shrunken glomeruli, intraglomerular congestion, loss of apical microvilli, degeneration of mitochondria and widened rough endoplasmic reticulum were also observed in the Proximal Convoluted Tubules of these animals. Treatment with Propolis ameliorated the harmful effects of AlCl3; this was also proved histopathologically by the noticeable improvement in the renal tissues. There were also significant variations in the expressed of ki-67 and p53 proteins.

It can be concluded that Propolis may be promising as a natural therapeutic agent in AlCl3-induced renal toxicity and oxidative stress in rat kidneys.

## 13.25. A comparative multi-centre study of the efficacy of propolis, acyclovir and placebo in the treatment of genital herpes (HSV).


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| Authors | Vynograd N, Vynograd I, Sosnowski Z. |
| Link | Phytomedicine. 2000 Mar;7(1):1-6. |
| Research Institute | Institute of Epidemiology, Lvov State Medical University, Ukraine. |



### Abstract

Ninety men and women with recurrent genital HSV type 2 participated in a randomized, single-blind, masked investigator, controlled multi-centre study comparing the efficacy of ointment of Canadian Propolis containing natural flavonoids with ointments of acyclovir and placebo (vehicle) on healing ability and capacity to remedy symptoms.

Thirty individuals were randomized to each group. Treatment was intended to start in the blister phase. All participants had HSV type 2 isolated, confirmed by serum immunoglobulin levels. The participants were examined on the 3rd, 7th and 10th days of treatment by gynaecologists, dermatovenerologists or urologists at seven different medical centres. Apart from clinical symptoms the number and size of the herpetic lesions were noted. At each examination the lesions were classified into four stages: vesicular, ulcerated, crusted and healed. The study ointments were applied to affected areas four times daily. In women with vaginal or cervical lesions a tampon with the appropriate ointment was inserted four times daily for 10 days. Endpoint variables were healing time and time until loss of symptoms.

RESULTS: On Day 10, 24 out of 30 individuals in the Propolis group had healed. In the acyclovir group 14 out of 30 and in the placebo group 12 out of 30 had healed. ($p = 0.0015$). The healing process appeared to be faster in the Propolis group. In the Propolis group 15 individuals had crusted lesions on Day 3 compared to 8 individuals in the acyclovir group and none in the placebo group ($p = 0.0006$). On Day 7, 10 participants in the Propolis group, 4 in the acyclovir group and 3 in the placebo group had healed. At the initial examination all patients had local symptoms and 28% general symptoms. At Day 3, 3 patients in the propolis group had local symptoms compared to 8 and 9 in the acyclovir and placebo groups respectively. Of the women, 66% had vaginal superinfections of microbial pathogens at the initial examination. In the acyclovir and placebo groups no change in the vaginal flora was found following treatment whereas in the propolis group the incidence of superinfection was reduced by 55%. ($p = 0.10$ n.s.).

CONCLUSION: An ointment containing flavonoids appeared to be more effective than both acyclovir and placebo ointments in healing genital herpetic lesions, and in reducing local symptoms.

## 13.26. Propolis Compoenent May Help Treat Pulmonary Fibrosis (Regulatory effect of caffeic acid phenethyl ester on type I collagen and interferon-gamma in bleomycin-induced pulmonary fibrosis in rat

Authors A Larki, AA Hemmati, A Arzi, M Ghafurian Borujerdnia, S Esmaeilzadeh,MR Zad Karami

Link Res Pharm Sci. 2013 Oct;8(4):243-252

Research Institute

### Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease of unknown etiology. Recent investigations have demonstrated that the impaired immune response is a common characteristic feature of IPF. Unfortunately, no definitive and effective drug treatment is available that could improve or at least inhibit the progressive course of this fatal disease. That is why one of the main priorities of pulmonary fibrosis investigations is to identify novel and effective molecular targets for preventive and therapeutic interventions. Caffeic acid phenethyl ester (CAPE) is one of the most interesting bioactive compounds extracted from bee propolis. It has been shown that CAPE has an antioxidant activity and modulatory impact on immune system. Accordingly, the aim of the present study was to investigate the regulatory effects of CAPE on the levels of type I collagen (COL-1) and Interferon-gamma (IFN-γ) in bleomycin (BLM)-induced pulmonary fibrosis. Immunohistochemistry procedure was employed to assess the effects of CAPE on lung tissue.

In this study, male Sprague-Dawley rats were divided into 5 groups (n=8) included 1: Positive control group: bleomycin (BLM). 2: Negative (saline) control group. 3, 4: Treatment groups of 1 and 2: BLM+CAPE (5 and 10 μmol/kg/day, respectively). (5: Sham group: CAPE (10 μmol/kg/day). BLM application resulted in significant changes in the level of studied parameters as compared to the controls. CAPE could decrease type I collagen concentration, modulate IFN-γ level, increase the animals' body weight and decrease the lung index dose-dependently, compared with model group.

In conclusion, CAPE may provide a novel therapeutic target for treating pulmonary fibrosis.

## 13.27. Caffeic acid phenethyl ester prevents apoptotic cell death in the developing rat brain after pentylenetetrazole-induced status epilepticus


Authors: Uluç Yiş[a], Yasemin Topçu[a], Seda Özbal[b], Kazım Tuğyan[b], Erhan Bayram[a], Pakize Karakaya[a], Osman Yilmaz[c], Semra Hız Kurul[a]



Link: Epilepsy Behav 2013 Sep 5. pii: S1525-5050(13)00407-1



Research Institute: Dokuz Eylül University, School of Medicine, İzmir, Turkey
[a] Department of Pediatrics, Division of Pediatric Neurology
[b] Department of Histology and Embryology
[c] Department of Laboratory of Animal Science



### Abstract

Population-based studies suggest that seizure incidence is highest during the first year of life, and early-life seizures frequently result in the development of epilepsy and behavioral alterations later in life. The early-life insults like status epilepticus often lead to epileptogenesis, a process in which initial brain injury triggers cascades of molecular, cellular, and network changes and eventually spontaneous seizures. Caffeic acid phenethyl ester is an active component of propolis obtained from honeybees and has neuroprotective properties. The aim of this study was to investigate whether caffeic acid phenethyl ester exerts neuroprotective effects on the developing rat brain after status epilepticus. Twenty-one dams reared Wistar male rats, and 21-day-old rats were divided into three groups: control group, pentylenetetrazole-induced status epilepticus group, and caffeic acid phenethyl ester-treated group. Status epilepticus was induced on the first day of experiment. Caffeic acid phenethyl ester injections (30 mg/kg intraperitoneally) started 40 min after the tonic phase of status epilepticus was reached, and the injections of caffeic acid phenethyl ester were repeated over 5 days. Rats were sacrificed, and brain tissues were collected on the 5th day of experiment after the last injection of caffeic acid phenethyl ester. Apoptotic cell death was evaluated. Histopathological examination showed that caffeic acid phenethyl ester significantly preserved the number of neurons in the CA1, CA3, and dentate gyrus regions of the hippocampus and the prefrontal cortex. It also diminished apoptosis in the hippocampus and the prefrontal cortex. In conclusion, this experimental study suggests that caffeic acid phenethyl ester administration may be neuroprotective in status epilepticus in the developing rat brain.

## 13.28. Ask The Pharmacist: Propolis is Perfect for Immunity, Blood Sugar and Cholesterol

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| Authors | By Suzy Cohen |
| Link | Naples News, 9/19/2013 |
| Research Institute | |


### Abstract

Question: I take a dozen antibiotics per year due to frequent infections. What else can I take? Don't worry, I take probiotics, what I want is a natural antibiotic. M.W., Santa Barbara, California

Answer: For millions of years, honeybees have protected themselves with a sticky substance called propolis to coat and clean their hives. Call it "bee glue" this compound has exceptional medicinal benefits just like other tree saps such as Frankincense and Myrrh.

Propolis has over 200 active ingredients including cinnamic acid derivatives which cause cancer cells inside you to kill themselves (even leukemia). It has antibacterial, antiviral, antiseptic, anti-fungal and antimicrobial effects. I'm stocking up now before cough and cold season rolls in. The Brazilian species, as in Brazilian green propolis has higher amounts of these healing compounds, sold at health food stores and online. When combined with vitamin D, probiotics, Matcha tea, Maitake mushrooms and prescribed low-dose naltrexone (LDN about 4.5mg at night), I am confident you will ramp up your immune system. Talk to your doctor about these options. Now, I'd like to focus on how propolis can rapidly clear the body of dangerous pathogens, improve blood sugar and cholesterol, all the while reducing pain-causing cytokines.

Artepillin C, a compound in propolis shuts down NFKB, a metabolic pathway in your body that churns inflammatory compounds that make you hurt. So propolis is an anti-inflammatory…

## 13.29. Propolis May Help Treat Sleeping Sickness: In Vitro Evaluation of Portuguese Propolis and Floral Sources for Antiprotozoal, Antibacterial and Antifungal Activity


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|---|---|
| Authors | Soraia I. Falcão[1,2], Nuno Vale[3], Paul Cos[4], Paula Gomes[3], Cristina Freire[2], Louis Maes[4], Miguel Vilas-Boas[1] |
| Link | Phytotherapy Research, Early View |
| Research Institute | [1]CIMO - Escola Superior Agrária, Instituto Politécnico de Bragança, Bragança, Portugal<br>[2]REQUIMTE - Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Porto, Portugal<br>[3]CIQ-UP, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Porto, Portugal<br>[4]Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium |



### Abstract

Propolis is a beehive product with a very complex chemical composition, used since ancient times in several therapeutic treatments. As a contribution to the improvement of drugs against several tropical diseases caused by protozoa, we screened Portuguese propolis and its potential floral sources Populus x Canadensis and Cistus ladanifer against Plasmodium falciparum, Leishmania infantum, Trypanosoma brucei and Trypanosoma cruzi. The toxicity against MRC-5 fibroblast cells was evaluated to assess selectivity. The in vitro assays were performed following the recommendations of WHO Special Programme for Research and Training in Tropical Diseases (TDR) and revealed moderate activity, with the propolis extracts presenting the relatively highest inhibitory effect against T. brucei.

Additionally, the antimicrobial activity against Staphylococcus aureus, Candida albicans, Trichophyton rubrum and Aspergillus fumigatus was also verified with the better results observed against T. rubrum.

The quality of the extracts was controlled by evaluating the phenolic content and antioxidant activity.

The observed biological activity variations are associated with the variable chemical composition of the propolis and the potential floral sources under study.

## 13.30. First Report of Prenylated Flavonoids in Brazilian Propolis and Schaftoside in Green Propolis: Comparative Chemistry of Propolis from Eight Brazilian Localities


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Link Evid Based Complement Alternat Med, 2013;2013:267878



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### Abstract

Propolis is a complex honeybee product with resinous aspect, containing plant exudates and beeswax. Their color, texture, and chemical composition vary, depending on the location of the hives and local flora. The most studied Brazilian propolis is the green (alecrim-do-campo) type, which contains mainly prenylated phenylpropanoids and caffeoylquinic acids. Other types of propolis are produced in Brazil, some with red color, others brown, grey, or black. The aim of the present work was to determine the chemical profiles of alcohol and chloroform extracts of eight samples of propolis, corresponding to six Brazilian regions. Methanol and chloroform extracts were obtained and analyzed by HPLC/DAD/ESI/MS and GC/MS.

Two chemical profiles were recognized among the samples analyzed: (1) black Brazilian propolis, characterized chiefly by flavanones and glycosyl flavones, stemming from Picos (Piauí state) and Pirenópolis (Goiás state); (2) green Brazilian propolis, characterized by prenylated phenylpropanoids and caffeoylquinic acids, stemming from Cabo Verde (Bahia state), Lavras and Mira Bela (Minas Gerais state), Pariquera-Açu and Bauru (São Paulo state), and Ponta Grossa (Paraná state).

The present work represents the first report of prenylated flavonoids in Brazilian propolis and schaftoside (apigenin-8-C-glucosyl-6-C-arabinose) in green propolis.

## 13.31. Video: A Talk About the Medicinal Properties of Propolis by James Fearnley

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|---|---|
| Authors | James Fearnley |
| Link | Apitherapy News, 6 March 2013 |