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Chemical compositions and antimicrobial activities of four different Anatolian propolis samples

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Abstract

Propolis means a gum that is gathered by bees from various plants. It is known for its biological properties, having antibacterial, antifungal and healing properties. The aims of this study were to evaluate the antimicrobial activity of four different Anatolian propolis samples on different groups of microorganisms including some oral pathogens and comparison between their chemical compositions. Ethanol extracts of propolis (EEP) were prepared from four different Anatolian propolis samples and examined whether EEP inhibit the growth of the test microorganisms or not. For the antimicrobial activity assays, minimum inhibitory concentrations (MIC) were determined by using macrodilution method. The MIC values of the most effective propolis (TB) were 2 μg/ml for *Streptococcus sobrinus* and *Enterococcus faecalis*, 4 μg/ml for *Micrococcus luteus*, *Candida albicans* and *C. krusei*, 8 μg/ml for *Streptococcus mutans*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterobacter aerogenes*, 16 μg/ml for *Escherichia coli* and *C. tropicalis* and 32 μg/ml for *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The chemical compositions of EEP's were determined by high-temperature high-resolution gas chromatography coupled to mass spectrometry. The main compounds of four Anatolian propolis samples were flavonoids such as pinocembrin, pinostropin, isalpinin, pinobanksin, quercetin, naringenin, galangine and chrysin.

Although propolis samples were collected from different regions of Anatolia all showed significant antimicrobial activity against the Gram positive bacteria and

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yeasts. Propolis can prevent dental caries since it demonstrated significant antimicrobial activity against the microorganisms such as *Streptococcus mutans*, *Streptococcus sobrinus* and *C. albicans*, which involves in oral diseases.

Introduction

Propolis, also known as bee glue and bee propolis, is a brownish resinous substance collected by bees, mainly from plants around their hive, used to reinforce the combs and to keep the hive environment aseptic. The composition of propolis varies according to the plants that can be found in a specific region (Ghisalberti, 1979; Markham et al., 1996). The constituents of propolis vary widely due to climate, season, location and year, and its chemical formula is not stable (Ghisalberti, 1979; Cheng and Wong, 1996). The most important pharmacologically active constituents in propolis are flavonoids (flavones, flavonols, flavononones), phenolics, and aromatics. Flavonoids are thought to account for much of the biologic activity in propolis. The antimicrobial properties of this mixture of natural substances are mainly attributed to the flavonone pinocembrin, to the flavonol galangin and to the caffeic acid phenethyl ester, with a mechanism of action probably based on the inhibition of bacterial RNA-polymerase (Takaisi-Kikuni and Schilcher, 1994). Marcucci (1995) has noted that the compounds in propolis resin (raw, unprocessed propolis) originate from three sources: plant exudates collected by bees, secreted substances from bee metabolism, and materials, which are introduced during propolis elaboration. The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Monti et al., 1983; Cirasino et al., 1987). It has a long history of being used in folk medicine dating back to at least 300 BC (Ghisalberti, 1979) and also has been reported to possess various biological activities, namely anticancer (Burdock, 1998), antioxidant (Sun et al., 2000; Isla et al., 2001), antiinflammatory (Miyataka et al., 1997), antibacterial (Pepeljnjak et al., 1985; Velikova et al., 2000), antifungal (Ota et al., 2001), antihepatotoxic (Banskota et al., 2001) and dental care effects (Koo et al., 2002). Even though propolis may have a great potential against the bacteria related with dental caries such as *Streptococcus mutans*, these types of data are still limited (Santos et al., 2003). The aim of this study is to carry out a comparative analysis of the antimicrobial activity of ethanol extracts obtained from four Anatolian propolis samples against various microorganisms and the comparison between their chemical compositions.

Materials and methods

Propolis samples and standard ethanol extracts

Geographical origin and some other properties of four different Anatolian propolis samples representing hole country are listed in Table 1. Hundred grams of frozen propolis is grained and dissolved in 300 ml ethanol (96%). This mixture was kept in the incubator at 30 °C for 2 weeks in a bottle closed tightly. After incubation procedure, supernatant was filtered twice with Whatman Nos. 4 and 1 filter paper. The final filtered concentrated solution was diluted in 1:10 ratio (v/v) with ethanol (96%). A portion of this final solution called Ethanol Extracts of propolis (EEP) was evaporated to dryness. About 5 mg of residue were mixed with 75 μl of dry pyridine and 50 μl bis (trimethylsilyl) trifluoroacetamide (BSTFA), heated at 80 °C for 20 min and then the final supernatant was analysed by GC-MS.

GC-MS analysis

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of EEP samples. Experimental conditions of GC-MS system were as follows: DB 5MS column (30 m × 0.25 mm and 0.25 μm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 ml/min. In the gas chromatography part, temperature was kept for 1 min at 50 °C and then increased to 150 °C with 10 °C/min heating ramp. After this period, temperature was kept at 150 °C for 2 min. Finally, temperature was increased to 280 with 20 °C/min heating ramp and then kept at 280 °C for 30 min.

Antimicrobial activity test

A total of 13 Gram-positive, Gram-negative bacteria and yeast like fungi were used for

Table 1. Geographical origins and other properties of propolis samples

Phyto-geographical region	Sample location	Collection year	Symbol	Solubility in ethanol (%, w/v)	Yield (%, w/v)
European-Siberian	Bursa-Orhangazi	2002	TB	9.44	31.58
European-Siberian	Bartın	2003	TBA	4.86	44.8
Irano-Turanian	Ankara-Mamak	2003	TA	0.78	20.51
European-Siberian	Trabzon	2003	TT	13.40	36.63

antimicrobial activity studies. Gram-positive bacteria: *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (6538-P), *Streptococcus sobrinus* (ATCC 33478), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212) and *Micrococcus luteus* (ATCC 9341). Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 11230), *Salmonella typhimurium (CCM 5445)* and *Enterobacter aerogenes* (ATCC 13048). Yeast like fungi: *Candida albicans* (ATCC 10231), *C. tropicalis* (ATCC 665) and *C. krusei* (ATCC 6258) were used for antimicrobial activity test.

Stock solutions of all propolis extracts were prepared in 10% dimethylsulphoxide (DMSO). Determination of minimal inhibitory concentration (MIC) by the macrodilution method were performed according to the National Committee of Clinical Laboratory Standard guidelines (National Committee for Clinical Laboratory Standards (NCCLS), 2000) and Koo et al. (2000), followed by subculture. Brain Heart Infusion Broth (Oxoid) (BHIB) was used for *Streptococcus mutans* and *Streptococcus sobrinus* strains, Mueller Hinton Broth (Oxoid) (MHB) used for the rest of the bacteria and RPMI-1640 medium (with glutamine and phenol red, without bicarbonate) (Sigma) for *Candida* species. Individual colonies were isolated from 18- to 24-h cultures of test strains and were suspended in sterile 0.89% NaCl solution. The cell suspensions were properly inoculated in culture media containing a two-fold dilution series of the EEP (concentrations ranging from 0.5 to 1024 μg/ml reaction) or the control (10% DMSO, vol/vol) to achieve an assay concentration of $1–2 \times 10^5$ cfu/ml. The tubes were incubated in 10% CO_2, 37 °C, for 24 h for *Streptococcus mutans* and *Streptococcus sobrinus* strains. Other test bacteria were incubated at 37 °C, for 24 h and *Candida* species incubated for 48 h. MIC was considered the lowest concentration of each extract that yields negative subcultures. All tests were made in triplicate in three different experiments.

Statistical analysis

An exploratory data analysis was performed to determine the most appropriate statistical test. The data obtained from inhibition of microorganisms related to the propolis types were compared by Friedman test using SPSS software10.0. The level of significance for statistical tests was $p < 0.05$.

Results

The yields of dry propolis extracts were; 44.80% (w/v) for Bartın (TBA), 36.63% (w/v) for Trabzon (TT), 31.58% (w/v) for Bursa (TB) and 20.51% (w/v) for Ankara (TA) using 96% ethanol as solvent. The propolis from TBA, gives the best yield of soluble content. For different propolis samples collected from different area showed different solubility in ethanol even if the same amount of propolis samples were tried to be dissolved in the same volume of ethanol. This is because of the different constituents in the propolis sample. If the content of the hydrophilic compounds in the propolis samples were high, the amount of solubility of the propolis samples would be increased. In the other words, it was noted that flavanoid constituent of the propolis sample could be high for the high soluble propolis sample. Table 1 shows the results of the extraction.

All four Anatolian propolis samples evaluated in this study showed antimicrobial activity against to the test microorganisms. The MICs of the EEP samples were obtained using macrodilution method, followed by subculture (Table 2). The MIC of propolis samples ranged from 2.0 to 256 μg/ml. The final concentration of DMSO in the assays did not interfere with the microbial growth. Thus, we may conclude that the antibacterial activity in this assay is exclusively due to propolis components. The MIC values of the most effective propolis (TB)

Table 2. Susceptibility of microbial strains to EEPs prepared with propolis collected from different geographical locations in Turkey

Microorganisms	Propolis extract[a] MIC (μg/ml)			
	TB	TBA	TA	TT
Streptococcus mutans ATCC 25175	8	64	32	64
Streptococcus sobrinus ATCC 33478	2	8	2	8
Staphylococcus aureus 6538-P	8	16	8	16
Staphylococcus epidermidis ATCC 12228	8	32	8	32
Enterococcus faecalis ATCC 29212	2	32	8	32
Micrococcus luteus ATCC 9341	4	16	8	16
Escherichia coli ATCC 11230	16	128	64	128
Enterobacter aerogenes ATCC 13048	8	32	32	64
Salmonella typhimurium CCM 5445	32	64	64	128
Pseudomonas aeruginosa ATCC 27853	32	128	64	256
Candida albicans ATCC 10231	4	32	16	32
Candida tropicalis ATCC 665	16	32	16	64
Candida krusei ATCC 6258	4	16	8	32

were 2 μg/ml for *Streptococcus sobrinus* and *Enterococcus faecalis*, 4 μg/ml for *M. luteus*, *C. albicans* and *C. krusei*, 8 μg/ml for *Streptococcus mutans*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterobacter aerogenes*, 16 μg/ml for *Escherichia coli* and *C. tropicalis* and 32 μg/ml for *Salmonella typhimurium* and *P. aeruginosa*.

The main compounds of four Anatolian propolis samples were flavonoids. All propolis samples showed a similar content of flavonoids but different individual compounds were detected (Table 3). For the qualitative analysis of the compounds available in the propolis samples were obtained used standard Wiley and Nist libraries. However, identification of some unknown compounds not available in the libraries was identified checking the fragments using personal knowledge. In this study, quantitative analysis of the organic compounds was not in exact basis using external and also internal standards. For high number of the compounds in the environmental samples, percent area of each compound could be used in order to measure the concentration of each compound approximately.

Discussion

Several authors have studied the antimicrobial activity of propolis (Kujumgiev et al., 1993; Castro and Higashi, 1995). While some authors found propolis samples active only against Gram-positive bacteria and some fungi (Marcucci, 1995; Kujumgiev et al., 1999; Nieva et al., 1999), others found also weak activity against Gram-negative bacteria (Dobrowolski et al., 1991; Sforcin et al., 2000). In this work, we could verify that Gram-positive bacteria are susceptible to low propolis concentration and Gram-negative bacteria growth was only inhibited in higher propolis concentrations (Table 2). TB was the most active against test microorganisms, followed by TA, TB and TT. We can also verify that there are statistically significant difference between MIC of propolis samples from TB, TA, TB and TT, respectively ($p<0.05$). Our results are in agreement with those of Grange and Davey (1990), who observed a marked action of propolis against Gram-positive bacteria and limited activity against Gram-negative ones.

All EEPs also showed antifungal activity against to the tested *Candida* strains, which corresponds to the literature data (Kujumgiev et al., 1999; Salomao et al., 2004). Consequently, antimicrobial screening clearly indicated that Bursa samples of propolis had much more powerful antifungal activity when compared with other Anatolian propolis samples ($p<0.05$). Since the Anatolian propolis samples inhibit the growth of oral pathogens such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Enterococcus faecalis* and *C. albicans*, propolis appears to be a promising source of new agents that may prevent dental caries and other oral diseases.

GC-MS analysis has been performed as analysis of compounds in propolis samples. With this method only few groups of compounds of propolis could be analysed, because simple fractionation of propolis to obtain compounds is difficult due to its complex composition. The usual manner is to extract the fraction soluble in alcohol, leaving the

Table 3. Chemical compositions of ethanol extract of propolis samples (% of total ion current)

Compounds	TB	TBA	*TA*	TT
Aromatic alcohols				
Benzyl alcohol	0.38	0.57	0.19	0.89
Pheny ethanol	0.66	0.59	0.88	0.83
2-methoxy-4-vinylphenol	–	1.74	–	0.24
2-napthalenemethanol	2.18	1.45	0.87	0.30
5-azulenementhanol	0.80	0.04	–	–
1-naphtlenemethanol	1.20	0.50	–	1.09
Bisabolol-alpha	–	0.20	0.53	0.33
2-phenanthrenol	–	0.41	–	–
Aromatic acids				
Benzoic acid	0.96	1.20	0.53	4.30
Benzenepropanoic acid	–	–	0.04	–
4-pentenoic acid, 5-phenyl	2.40	–	–	0.03
Ferulic acid	–	0.60	–	0.12
Caffeic acid	1.20	0.44	0.05	0.61
2-propenoic Acid,3-phenyl	2.23	0.81	1.06	1.53
2-propenoic acid, 3-(4-methoxyphenyl)	1.21	0.39	0.32	0.16
1-phenanthrenecarboxylic acid	0.30	0.21	0.18	0.41
Aromatic aldehydes				
Benzaldehyde	–	–	0.04	–
Cinnamic acid and its esters				
Cinnamyl cinnamate	5.28	1.32	0.23	0.86
Benzyl cinamate	0.14	0.45	0.12	0.37
Benzyl benzoate	0.32	0.13	0.05	0.02
Cinnamic acids	–	–	–	–
1-3-hydroxy-4-methoxycinnamic acid	0.80	0.80	0.08	0.85
Fatty acids				
Lauric acid	–	0.07	–	–
Myristic acid	–	0.04	–	0.03
Palmitic acid	0.22	0.42	0.20	0.21
Oleic acid	–	1.10	–	0.47
Stearic acid	–	1.26	1.78	0.16
Linoleic acid	0.26	0.37	0.67	0.35
Linear hydrocarbons and their acids				
Cyclohexadecane	0.18	0.75	0.10	2.10
Hexadecane	–	–	–	–
Nonadecane	0.40	0.18	–	–
Octadecane	–	–	0.11	0.20
Octadecanoic acid	0.41	0.41	–	–
Flavanone				
Isalpinin	6.17	5.76	4.97	5.04
Pinocembrin	13.61	14.76	7.01	16.26
Pinostropin	13.06	11.45	4.46	2.26
Naringenin	6.20	1.40	0.90	6.20
4′,5-dihydroxy-7-methoxyflavanone	1.79	–	0.84	0.69
Chrysin	1.45	2.29	3.11	9.86
3,4′,7-trimethoxy flavanone	–	0.31	0.12	0.51
Hexadecanol	–	0.11	–	–
Flavonones				
Pinobanksin and its derivatives	4.3	11.5	8.3	7.6
Quercetin and its derivatives	5.1	6.2	9.1	1.1
Galangine and its derivatives	0.9	3.1	3.4	1.6
Apigenin and its derivatives	0.2	3.2	3.8	2.6

alcohol-insoluble or wax fraction. The composition of propolis depends upon the vegetation of the area from that is collected (Marcucci, 1995). Propolis from temperate zones (Asia, Europe, North America, etc.) contains predominantly phenolic compounds, including several flavonoids, aromatic acids and their esters collected from poplar buds (*Populus* spp.), which appear to be the dominant source of propolis (Tomas-Barberan et al., 1993). In our studies on four Anatolian propolis samples, the major components were flavonoids (pinocembrin, pinostropin, isalpinin, pinobanksin, quercetin, naringenin, galangine and chrysin (Table 3). These results are in agreement with that found by other authors; Kujumgiev et al. (1999) found flavanoids and phenolic acid esters as main constituents in Bulgarian propolis samples. Bosio et al. (2000) examined the antibacterial activity of two Italian propolis samples towards *Streptococcus pyogenes* and found that the activity is mainly due to Pinocembrin and Galangin.

It is apparent that propolis TT contained highest concentrations of pinocembrin and propolis TB contained highest concentrations of pinostropin when compared with the others. However, only propolis TB contains both pinocembrin and pinostropin in higher concentrations. Although caffeic acid esters have been found in different propolis samples and known to be as an antimicrobial substance (Banskota et al., 2001; Miorin et al., 2003), the four Anatolian propolis samples contains caffeic acid only in minor concentrations (Table 3).

Among the most potent microbicidal compounds in propolis are flavonone pinocembrin (5,7-dihydroxyflavanone) and its 3-OH analogue flavonol galangin (3,5,7-trihydroxyflavon) (Koo et al., 2002). Caffeic acid (3,4-dihydroxycinnamic acid) and its esters, volatile fractions with phenols and/or terpenoids and chrysin (5,7-dihydroxyflavone) possess notable antimicrobial activities as well (Kosalec et al., 2003). It is still not known whether antibacterial and antifungal activities of ethanol extracts of propolis depend on the concentration of galangin, pinocembrin and caffeic acid derivates or on synergism of these or other compounds.

Conclusions

All EEPs showed strong antimicrobial activity against Gram-positive bacteria and yeast like fungi, the propolis from Bursa being the most active against test microorganisms ($p<0.05$). It was also found that the four Anatolian propolis samples contained the high concentrations of flavonoids including, pinocembrin, pinostropin, isalpinin, pinobanksin, quercetin, naringenin, galangine and chrysin.

References

Banskota, A.H., Tezuka, Y., Adnyana, I.K., 2001. Hepatoprotective and anti *Helicobacter pylori* activities of constituents from Brazilian propolis. Phytomedicine 8, 16–23.

Bosio, K., Avanzini, C., D'Avolio, A., Ozino, O., Savoia, D., 2000. In vitro activity of propolis against *Streptococcus pyogenes*. Lett. Appl. Microbiol. 31, 174–177.

Burdock, G.A., 1998. Review of the biological properties and toxicity of propolis. Food Chem. Toxicol. 36, 341–363.

Castro, S.L., Higashi, K.O., 1995. Effect of different formulations of propolis on mice infected with *Trypanosoma cruzi*. J. Etnopharmacol. 46, 55–58.

Cheng, P.C., Wong, G., 1996. Honey bee propolis: prospects in medicine. Bee World 77, 8–15.

Cirasino, L., Pisati, A., Fasani, F., 1987. Contact dermatitis from propolis. Contact Dermatitis 16, 110–111.

Dobrowolski, J.W., Vohora, S.B., Sharma, K., Shah, S.A., Naqvi, S.A.H., Dandiya, P.C., 1991. Antibacterial, antifungal, antiamoebic, antiinflammatory and antipyretic studies on propolis bee products. J. Etnopharmacology 35, 77–82.

Ghisalberti, E.L., 1979. Propolis: a review. Bee World 60, 59–84.

Grange, J.M., Davey, R.W., 1990. Antibacterial properties of propolis (bee glue). J. R. Soc. Med. 83, 159–160.

Isla, M.I., Moreno, M.I.N., Sampietro, A.R., Vattuone, M.A., 2001. Antioxidant activity of Argentina propolis extracts. J. Ethnopharmacol. 76, 165–170.

Koo, H., Rosalen, P.L., Cury, J.A., Ambrosano, G.M.B., Murata, R.M., Yatsuda, R., Ikegaki, M., Alencar, S.M., Park, Y.K., 2000. Effect of a new variety of *Apis mellifera* propolis on mutans streptococci. Curr. Microbiol. 41, 192–196.

Koo, H., Rosalen, P.L., Cury, J.A., Park, Y.K., Bowen, W.H., 2002. Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity. Antimicrob. Agents Chemother. 46, 1302–1309.

Kosalec, I., Bakmaz, M., Pepeljnjak, S., 2003. Analysis of propolis from the continental and Adriatic regions of Croatia. Acta Pharm. 53, 275–285.

Kujumgiev, A., Bankova, V., Ignatova, A., Popov, S., 1993. Antibacterial activity of propolis, some of its components and their analogs. Pharmazie 48, 785–786.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Yu., Bankova, V.S., Christov, R., Popov, S., 1999. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. J. Ethnopharmacol. 64, 235–240.

Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 26, 83–99.

Markham, K.R., Mitchell, K.A., Wilkins, A.L., Daldy, J.A., Lu, Y., 1996. HPLC and GC-MS identification of the major organic constituents in New Zealand propolis. Phytochemistry 42, 205–211.

Miorin, P.L., Levy Junior, N.C., Custodio, A.R., Bretz, W.A., Marcucci, M.C., 2003. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. J. Appl. Microbiol. 95, 913–920.

Miyataka, H., Nishiki, M., Matsumoto, H., Fujimoto, T., Matsuka, M., Satoh, T., 1997. Evaluation of Brazilian and Chinese propolis by enzymatic and physicochemical methods. Biol. Pharm. Bull. 20, 496–501.

Monti, M., Berti, E., Carminati, G., Cusini, M., 1983. Occupational and cosmetic dermatitis from propolis. Contact Dermatitis 9, 163.

National Committee for Clinical Laboratory Standards (NCCLS), 2000. Approved Standard M7-A5: Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, fifth edition. NCCLS, Wayne, PA.

Nieva, M.M.I., Isla, M.I., Cudmani, N.G., Vattuone, M.A., Sampietro, A.R., 1999. Screening of antibacterial activity of Amaicha del Valle (Tucuman, Argentina) propolis. J. Ethnopharmacol. 68, 97–102.

Ota, C., Unterkicher, C., Fantinato, V., Shimizu, M.T., 2001. Antifungal activity of propolis on different species of *Candida*. Mycoses 44, 375–378.

Pepeljnjak, S., Jalsenjak, I., Maysinger, D., 1985. Flavonoid content in propolis extracts and growth inhibition of *Bacillus subtilis*. Pharmazie 40, 122–123.

Salomao, K., Dantas, A.P., Borba, C.M., Campos, L.C., Machado, D.G., Aquino, N.F.R., de Castro, S.L., 2004. Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. Lett. Appl. Microbiol. 38, 87–92.

Santos, F.A., Bastos, E.M.A.F., Maia, A.B.R.A., Uzeda, M., Carvalho, M.A.R., Farias, L.M., Moreira, E.S.A., 2003. Brazilian propolis: physicochemical properties, plant origin and antibacterial activity on periodontopathogens. Phytother. Res. 17, 285–289.

Sforcin, J.M., Fernandes Jr., A., Lopes, C.A.M., Bankova, V., Funari, S.R.C., 2000. Seasonal effect on Brazilian propolis antibacterial activity. J. Ethnopharmacol. 73, 243–249.

Sun, F., Hayami, S., Haruna, S., Ogiri, Y., Tanaka, K., Yamada, Y., Ikeda, K., Yamada, H., Sugimoto, H., Kawai, N., Kojo, S., 2000. In vivo antioxidative activity of propolis evaluated by the interaction with vitamin C and vitamin E and the level of lipid hydroperoxides in rats. J. Agric. Food Chem. 48, 1462–1465.

Takaisi-Kikuni, N.B., Schilcher, H., 1994. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. Planta Med. 60, 222–227.

Tomas-Barberan, F.A., Garcia-Viguera, C., Vit-Oliver, P., Ferreres, F., Tomas-Lorente, F., 1993. Phytochemical evidence for botanical origin of tropical propolis from Venezuela. Phytochemistry 34, 191–196.

Velikova, M., Bankova, V., Tsvetkova, I., Kujumgiev, A., Marcucci, M.C., 2000. Antibacterial ent-kaurene from Brazilian propolis of native stingless bees. Fitoterapia 71, 693–696.