Apidologie 34 (2003) 291–298

DOI: 10.1051/apido:2003023

Original article

Comparison of propolis from *Apis mellifera* and *Tetragonisca angustula*

Alberto DOS SANTOS PEREIRA[a]*, Beatriz BICALHO[b], Francisco Radler DE AQUINO NETO[a]

[a] LADETEC, Instituto de Química, Universidade Federal do Rio de Janeiro, Ilha do Fundão, CT, Bloco A, Sala 607, Rio de Janeiro, RJ, 21949-900, Brazil
[b] Instituto de Química, Universidade Estadual de Campinas, Campinas, SP, Brazil

(Received 12 August 2002; revised 24 October 2002; accepted 22 November 2002)

Abstract – High-temperature high-resolution gas chromatography (HT-HRGC) and HT-HRGC coupled to mass spectrometry (HT-HRGC-MS) were applied to the study of propolis collected by *Apis mellifera* and by *Tetragonisca angustula*, a stingless bee native to southeastern Brazil. With the exception of amino acids and erytrose/erythritol content, both propolis samples were quite similar in composition and in antimicrobial activity. Triterpenes were the most abundant compounds in the samples, comprising more then 35% of the total amount of each sample.

propolis / *Apis mellifera* / *Tetragonisca angustula*

1. INTRODUCTION

Propolis (CAS No. 9009-62-5) is a complex resinous mixture that honeybees collect from plant exudates for construction, protection and adaptation of their nests (Garcia-Viguera et al., 1992; Marcucci, 1995). Foraging for propolis is secondary in comparison to foraging for nectar or pollen, and the amount of propolis used in the nest may differ considerably among bee colonies (Valcic et al., 1999). Although research has concentrated on *Apis mellifera* L. due to this bee's commercial appeal, about 20 000 bee species are known worldwide, among which around 1 000 species collect propolis. An estimated 350–600 bee species are found in Brazil (Imperatriz-Fonseca et al., 1994), where flora is abundant. The chemistry of propolis depends on the diversity of plants from which the bees collect it. The composition of South American propolis varies considerably, as has recently been shown from Brazilian samples (Bankova et al., 2000; Marcucci, 1995).

In this study, high-temperature high-resolution gas-chromatography coupled to mass-spectrometry (HT-HRGC-MS) (Pereira and Aquino Neto, 1999) was applied to the chemical investigation of propolis collected by *Apis mellifera* L. (Hymenoptera, Apidae) and *Tetragonisca angustula* Illiger (Apidae, Meliponinae) propolis the same geographical location. These bees do not have complete overlap in their foraging preferences. In the city of São Paulo, SP, Brazil, *T. angustula* was reported to visit at least 12 plant species not visited by *A. mellifera* (e.g. *Euphorbia milli* var. *milli*; *Eupatorium* sp.), (Imperatriz-Fonseca et al., 1994).

* Correspondence and reprints
E-mail: ladetec@iq.ufrj.br

Table I. Yields for the extracts obtained from propolis collected from the nests of *Apis mellifera* and *Tetragonisca angustula* from the same geographic area, after sequential extraction with dichloromethane, acetone and methanol.

Propolis	Dichloromethane	Acetone	Methanol	Residue
A. mellifera	1.0 g (33.3%)	0.9 g (30.0%)	0.9 g (30.0%)	0.2 g (6.7%)
T. angustula	1.5 g (50.0%)	0.4 g (13.3%)	0.6 g (20.0%)	0.5 g (16.6%)

2. MATERIALS AND METHODS

2.1. Samples

Propolis from *A. mellifera* and *T. angustula* was collected in October 2000, at the Cantagalo farm, Brotas, São Paulo, Brazil, by apiarists. Their field observations indicated that both bees visited the same plant species, such as *Tipuana tipu*. A rough parallel of propolis productivity by the bee species may be estimated from honey production (kg/year) which, for *A. mellifera*, has been reported to be 10 times greater than that of *T. angustula* (Fabichak, 2000).

2.2. Extracts

The propolis samples (3 g) were sequentially extracted by ultrasonic agitation, at room temperature, with 20 ml of dichloromethane, 20 mL of acetone, and 20 mL of methanol, three times, for 30 min each. The combined extracts for each solvent were concentrated under vacuum, dried on a desiccator over P_2O_5 for weighing (Tab. I), and analyzed by HT-HRGC.

2.3. Derivatization

The acetone and methanol crude extracts were converted to trimethylsilyl esters prior to HT-HRGC and HT-HRGC-MS analyses by reaction with bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma, St. Louis, USA) at 60 °C during 30 min.

2.4. Gas chromatography

2.4.1. Columns

Gas chromatography was performed on fused silica capillary columns (15 m × 0.25 mm i.d.; J&W, Folson, CA, USA) coated with 0.2 µm of DB-5HT (5%-phenyl-95%-methylpolysiloxane).

2.4.2. Chromatographic conditions

An on-column injector (Carlo Erba, Rodano, Italy) was mounted on a Hewlett-Packard (Palo Alto, USA) model 5890-II gas chromatograph. For the dichloromethane extract, the column temperature was maintained at 40 °C during injection, then programmed for 10 °C/min until 390 °C and held for 10 min. For acetone and methanol extracts, the column temperature was maintained at 40 °C during injection, programmed for 25 °C/min until 250 °C and then for 10 °C/min until 390 °C and held for 10 min. The flame ionization detector (FID) and the on-column injector were operated at 400 °C and room temperature, respectively. Hydrogen was used as carrier gas at a linear velocity of 50 cm/s and the sample volume injected was 0.5 µL. GC data were acquired and processed with a HP 3396-II integrator.

2.5. Mass spectrometry conditions

HTHRGC-MS analyses were carried out on a HP 5972A spectrometer (Hewlett Packard, Palo Alto, USA), under electron impact ionization (70 eV). The GC operating conditions were as described above. The on-column injector and the transfer line temperatures were set to 40 °C and 390 °C, respectively and the ion source temperature to 300 °C (MS scan range was 40 to 700 Da). Helium was used as carrier gas at a linear velocity of 38 cm/s.

2.6. Compound characterization

The characterization of the components was based on mass spectra interpretation, compound retention times, and comparison with mass spectra Wiley 275 library data. Accurate quantification of components was difficult due to the complexity of the propolis extracts samples. An estimate was therefore performed using a response factor of 1 (one) for the mass spectrometry detection for all compounds.

2.7. Bioautography

The detection of antimicrobial activity was performed with 5 × 5 cm silicagel 60 chromatoplates (Merck, Darmstadt, Germany). Aliquots containing 180 µg of the propolis extract were spoted and

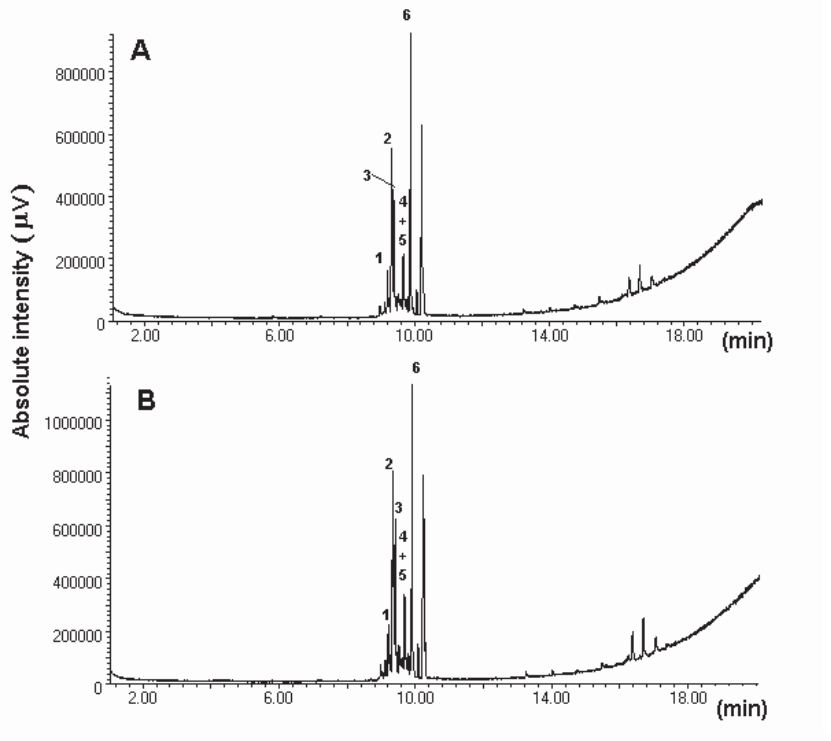

Figure 1. Chromatograms of the dichloromethane crude extracts of the propolis collected by (A) *Apis mellifera* and (B) *Tetragonisca angustula*, from the same geographic area. (1) lupenone; (2) lupeol; (3) cycloartenol; (4) friedours-7-en-3-ol; (5) β-amyrin acetate and (6) lupeol acetate.

developed with hexane-acetate (1:1). After complete evaporation of the organic solvents, the chromatoplates were transferred to Petri dishes, into which were poured 20 mL of Müller Hinton-agar (20 g/L, Merck, Darmstadt, Germany) inoculated with 1% (v/v) aqueous suspension of microorganism (10^7 cell/mL) (*Bacillus subtilis* CCT 0089, *Candida albicans* CCT 0776, *Escherichia coli* CCT 5050 and *Staphylococcus aureus* CCT 4295). As soon as the medium solidified, plates were incubated at 30 °C, for 24 h. The bioactive compounds were detected according to their chromatographic retention factors (R_f) by pouring 15 ml of agar medium (20 g/L) containing 0.05% of 3-(4,5-dimethyl-2-tiazolyl)-2,5-tetrazolium bromide (MTT, Merck, Darmstadt, Germany) onto the plates.

3. RESULTS AND DISCUSSION

A total of 64 compounds were characterized and the extraction yields were similar for the two propolis samples (Tab. I).

The chromatograms of the dichloromethane extracts of the *A. mellifera* and *T. angustula* propolis were practically identical (Fig. 1), suggesting that the bees foraged for exudates from similar available flora (see Materials and Methods).

Both propolis samples were almost entirely comprised of pentacyclic triterpenes, mainly lupeol and lupeol acetate (Tab. II). Triterpenyl alkanoates were not detected in either propolis sample. Other propolis samples collected by Brazilian Meliponinae bees contained high amounts of amyrines (Velikova et al., 2000). In addition, *A. mellifera* propolis from the Brazilian savannah, had a high proportion of α-amyryn, β-amyryn, lupeol, and their respective fatty acid derivatives (Pereira et al., 2002).

On the other hand, polar compounds (acetone and methanol crude extracts, Tab. II) differed in propolis collected by *A. mellifera* and *T. angustula*, as discussed below.

Table II. Determination of compounds from propolis collected from the nests of *Apis mellifera* and *Tetragonisca angustula* after sequential extraction by dichloromethane (DCM), acetone and methanol. Acetone and methanol extracts constituents analyzed after trimethylsilylation with BSTFA[1].

t_R[2] (min)	Compound	*Apis mellifera*			*Tetragonisca angustula*		
		% of crude extracts					
		DCM	Acetone	Methanol	DCM	Acetone	Methanol
3.25	Etylamine		Trace	Trace			Trace
3.35	Etyleneglycol		Trace	0.1			
3.63	Hydroxybutyric acid (isomer)						Trace
4.49	Lactic acid		1.0	0.2		0.2	1.6
4.50	Hydroxybutyric acid (isomer)						Trace
4.51	Glycolic acid		Trace				
4.60	Butanediol					Trace	
4.68	Alanine			0.2			
4.73	Glycine			Trace			
4.79	Hydracrylic acid		Trace			Trace	0.3
4.85	Hydroxymethylbutyric acid						Trace
4.95	Butanetriol (isomer)						8.4
5.05	Butanetriol (isomer)						Trace
5.09	Malonic acid					Trace	Trace
5.10	Dimethylphenol		Trace				
5.12	Valine			0.2			Trace
5.20	Glycerol		7.7	7.7		11.2	9.1
5.23	Phosphoric acid						1.2
5.30	Isoleucine			0.2			
5.34	Leucine			Trace			Trace
5.40	Proline			0.1			
5.45	Succinic acid		0.3	0.4		Trace	4.1
5.61	Diidroxypropanoic acid			Trace			0.2
5.68	Fumaric acid						0.1
6.08	Butanetriol		Trace				
6.08	Threonine			Trace			
6.25	Hydrocinnamic acid		1.1	0.2		0.2	0.7
6.73	Erythritol		1.7	0.4		2.6	6.9
6.88	Malic acid			0.3			1.3
6.97	Hexanedioic acid			Trace			0.1
7.10	Erythrose		4.5	12.1			2.9

[1] bis(trimethylsilyl)trifluoroacetamide, [2] retention time in gas chromatography.

Table II. Continued.

t_R[2] (min)	Compound	*Apis mellifera*			*Tetragonisca angustula*		
		% of crude extracts					
		DCM	Acetone	Methanol	DCM	Acetone	Methanol
7.19	5-oxo-proline			Trace			0.1
7.59	trihydroxybutyric acid			Trace			
8.08	*p*-hydroxybenzoic acid		1.0	0.3		0.5	0.4
8.20	*p*-hydroxyphenylacetic acid			Trace			Trace
9.34	*p*-hydroxy-dihydrocinnamic acid		0.6	0.3		0.3	1.2
9.62	*o*-cumaric acid						0.3
9.96	Dihydroxybenzoic acid						0.7
10.05	Fructose		0.5	7.5			1.3
10.46	Tetradecanoic acid		0.2				
11.13	Inositol			0.9			
11.83	p-coumaric acid		5.3	3.3		2.3	7.8
12.04	Hexadecenoic acid		Trace				
12.98	Hexadecanoic acid		0.9			0.4	1.9
13.15	Ferrulic acid		Trace				
14.14	Caffeic acid		1.1	0.9		0.5	
14.70	Linoleic acid			Trace			Trace
14.90	Oleic acid		1.0	Trace			3.7
14.95	Octadecanoic acid		0.2	0.3			0.2
18.44	Tetracosanoic acid		1.8	0.9		0.7	1.0
19.79	Hexacosanoic acid		1.0			Trace	
20.05	Lanosterol		1.3				
21.07	Octacosanoic acid			0.6		Trace	Trace
22.00	Obtusifoliol	0.5			1.3	1.3	
22.00	α-amirine	0.4					
22.12	β-amirine	1.0	0.6	0.6	1.0	1.2	
22.21	Lupenone	3.4	3.2		3.6	3.4	
22.35	Cycloartenol	8.0		5.0	8.4		7.3
22.50	Lupeol	16.9	12.1		17.3	18.6	
22.65	Friedour-7-en-3-one	1.9	2.5		2.4	0.8	
22.70	Friedour-7-en-3-ol	3.1			3.2		
22.72	β-amirine acetate	3.1		1.3	3.2	1.5	Trace
23.04	Lupeol acetate	26.3	14.9	3.8	23.7	20.0	8.2

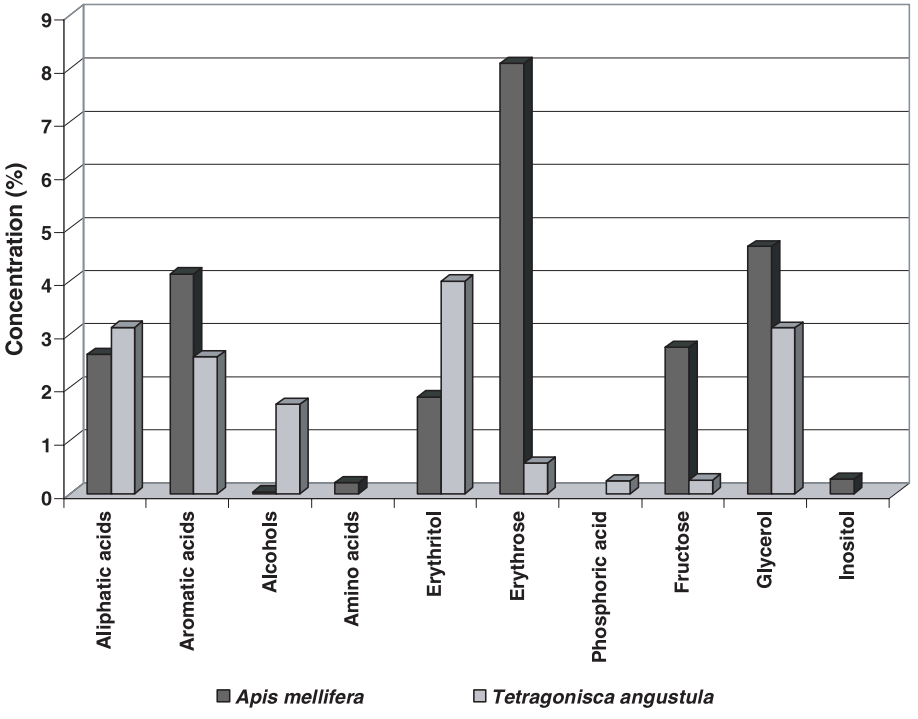

Figure 2. Constituents (with exception of the triterpenes) of crude propolis resins, collected by *Apis mellifera* and *Tetragonisca angustula*. Triterpenes were present in 35% and 41%, respectively.

3.1. Amino acids

Seven amino acids (alanine, glicine, valine, isoleucine, leucine, proline and threonine) were characterized only in propolis from *A. mellifera* (Tab. II and Fig. 2), amounting to 0.2% of the methanol extract. Marcucci et al. (1996) showed that the amino acids present in propolis from *A. mellifera* propolis may come from plant surfaces and/or pollen. However, it may be that these amino acids resulted from bee metabolism during the handling and elaboration of propolis by theses bees. Other possible amino acid sources which cannot be ignored are microorganisms such as bacteria and fungi that normally occur in beehives (Snowdon and Cliver, 1996).

The amino acids detected may be partially responsible for regenerative processes, such as healing and cell growth, attributed to propolis in mammalian tissues (Gabrys et al., 1986).

3.2. Erythrose/erythritol

The main differences between the two propolis samples were the concentrations of an aldotetrol, characterized as erythritol (1.8% *A. mellifera* × 4.0% *T. angustula*), and an aldotetrose, characterized as erythrose (8.1% *A. mellifera* × 0.6% *T. angustula*, Fig. 2 and Tab. II). The distinct concentrations of these two related compounds may reflect metabolic differences between the bees. As another example, butanetriol was characterized only in the methanol extract of propolis from *T. angustula*, and the cyclic polyhydroxy alcohol inositol was found only in the methanol extract of propolis from *A. mellifera*. Park et al. (1998, 2001) isolated microorganisms from pollen of *Mangifera* sp. (Anacardiaceae) and from Brazilian honeys, which were able to convert sucrose, glucose and fructose to erythritol. Erythritol is a non-caloric and non-cariogenic sweetner that is safe for diabetics, and is

Table III. Bioautography of propolis extracts collected from nests of *Apis mellifera* and *Tetragonisca angustula*.

Microorganism	*A. mellifera*			*T. angustula*		
	DCM[1]	Acetone	MeOH[2]	DCM[1]	Acetone	MeOH[2]
S. aureus	0.0–0.7*	0.0–0.7	0.0–0.7	0.0–0.7	0.0–0.7	0.0-0.7
E. coli	nd	0.7	nd	nd	nd	nd
B. subtilis	nd	0.7	nd	nd	nd	nd
C. albicans	nd	nd	nd	nd	nd	nd

[1]DCM = dichloromethane; [2]MeOH = methanol; *TLC R_f of antimicrobial compounds after development of 180 µg of extracts with hexane-ethyl acetate; 1:1. on a silica gel plate; nd: not detected, e.g. absence of inhibitory activity.

70–80% as sweet as sucrose and exists naturally in fruits, fermented food, seaweed and mushrooms (Lin et al., 2001).

3.3. Antimicrobial activity

According to the bioautography assays, both propolis samples showed a similar potential for antimicrobial activity. Gram-positive bacteria (*S. aureus*) were the most sensitive among the microorganisms tested (Tab. III), being inhibed by all propolis fractions of TLC (silica gel; hexane-ethyl acetate, 1:1) R_f = 0.0 to 0.7. Activity against Gram-negative bacteria, *E. coli* and *B. subtilis,* was detected only in TLC fraction of R_f = 0.7 (hexane-ethyl acetate, 1:1) of the acetone extract of propolis from *A. mellifera*. This activity could be due to a higher concentration of aromatic acids (e.g. caffeic and p-coumaric acids, Tab. II), which are known to have antimicrobial and anti-inflammatory activities (Krol et al., 1996).

None of the extracts were active against *C. albicans.*

4. CONCLUSIONS

For the first time, propolis samples from different bee species, *A. mellifera* and *T. angustula*, but from the same geographical origin were chemically characterized. The propolis samples showed similar antimicrobial activity and composition (with the exception of amino acids, some carbohydrates, and poliols). The floral diversity on which the bees foraged provided a predominance of triterpenes, mainly lupeol and lupenone (>35% of total extract composition) in both propolis samples analyzed.

ACKNOWLEDGMENTS

The authors wish to thank FAPERJ, FAPESP, CNPq, FUJB, and FINEP for financial support and fellowships, and Dr. Anita Jocelyne Marsaiolli of the Chemical Institute, UNICAMP, for laboratory facilities used for biautography screening.

Résumé – Comparaison de la propolis d'*Apis mellifera* et de celle de *Tetragonisca angustula*. Dans cette étude la chromatographie en phase gazeuse à haute température et haute résolution couplée à la spectrométrie de masse (HT-HRGC-MS) a été appliquée à l'étude chimique de la propolis récoltée dans la même zone géographique par l'Abeille domestique *Apis mellifera* L. et l'abeille sans dard *Tetragonisca angustula* Illiger (Apidae, Meliponinae). Au total 64 composés ont été caractérisés et les rendements d'extraction ont été similaires pour les deux types de propolis (Tab. I). Les chromatogrammes des extraits au dichlorométhane des deux propolis sont pratiquement semblables (Fig. 1), suggérant que les abeilles butinent les exsudats sur la même flore disponible. Les échantillons de propolis présentent une activité antimicrobienne (Tab. III) et une composition semblables, à l'exception des acides aminés, de quelques sucres et de poliols). La diversité des plantes butinées par les abeilles fournit dans les deux types échantillons de propolis analysés une prédominance de triterpènes, principalement le lupéole et le lupénone, qui représentent plus de 35 % de l'extrait total (Tab. II).

propolis / *Apis mellifera* / *Tetragonisca angustula*

Zusammenfassung – Vergleich des Propolis von *Apis mellifera* und *Tetragonisca angustula.* In dieser Untersuchung wurde mit Massenspektroskopie gekoppelte Hochtemperatur – Hochauflösende Gaschromatografie (HT-HRGC-MS) verwendet, um die chemische Zusammensetzung des von *Apis mellifera* L. und *Tetragonisca angustula* Illiger am selben geografischen Ort gesammelten Propolis zu ermitteln. Es wurden insgesamt 64 verschiedene Komponenten charakterisiert. Die aus der Extraktion erhaltenen Mengen waren für beide Propolisproben ähnlich (Tab. I). Die Chromatogramme der Dichlormethanextrakte von *Apis mellifera* und *Tetragonisca angustula* waren praktisch identisch (Abb. 1). Dies legt den Schluss nahe, dass beide Bienen Abscheidungen von ähnlicher verfügbarer Flora gesammelt haben. Die Propolisproben hatten ähnliche antimikrobielle Aktivität und Zusammensetzung (mit Ausnahme von Aminosäuren, einigen Kohlenwasserstoffen und von Poliolen, Tab. III). Die von den Bienen besammelte Blütendiversität zeigte in beiden analysierten Proben ein Vorherrschen von Triterpenen, in der Hauptsache Lupeol und Lupenon (>35 % der Extraktzusammensetzung, Tab. III).

***Apis mellifera* / *Tetragonisca angustula* / propolis**

REFERENCES

Bankova V., Castro S.L., Marcucci M.C. (2000) Propolis: recent advances and plant origin, Apidologie 31, 3–15.

Fabichak I. (2000) Abelhas indígeneas sem ferrão: Jataí, Ed. Nobel, São Paulo, Brazil, 53 p.

Gabrys J., Konecki J., Krol W., Scheller S., Shani J. (1986) Free amino acids identified and quantified by gas-liquid chromatography, Pharm. Res. Commun. 18, 513–518.

Garcia-Viguera C., Greenaway W., Whatley F.R. (1992) Composition of propolis from two different Spanish regions, Z. Naturforsch. 47c, 634–637.

Imperatriz-Fonseca V.L., Ramalho M., Kleinert-Giovannini A. (1994) Abelhas sociais e flores: Análise polínica como método de estudo, in: Pirani J.R., Cortopassi-Laurino M. (Eds.), Flores e abelhas em São Paulo, Edusp, São Paulo, Brazil, pp. 17–30.

Krol W., Scheller S., Czuba Z., Matsuno T., Zydowicz G., Shani J., Mos M. (1996) Inhibition of neutrophils' chemiluminescence by ethanol extract of propolis (EEP) and its phenolic components, J. Ethnopharm. 55, 19–25.

Lin S.J., Wen C.Y., Liau J.C., Chu W.S. (2001) Screening and production of erythritol by newly isolated osmophilic yeast-like fungi, Proc. Biochem. 36, 1249–1258.

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

Marcucci M.C., Camargo F.A., Lopes C.M.A. (1996) Identification of amino acids in Brazilian propolisogical properties, Z. Naturforsch. 51c, 11–14.

Park J.B., Yook C., Park Y.K. (1998) Production of erythritol by newly isolated osmophilic *Trichosporon* sp., Starch/Starke 50, 120–123.

Park J.B., Suzuki C., Alencar S.M., Aguiar C.L. (2001) Produção de açúcares não-convencionais, Biotec. Ciencia, Desenvolv. 22, 22–24.

Pereira A.S., Aquino Neto F.R. (1999) High temperature high resolution gas chromatography: Breatching the barrier to the analysis of polar and high molecular weight compounds, Trends Anal. Chem. 18, 126–136.

Pereira A.S., Nascimento E.A., Aquino Neto F.R. (2002) Lupeol alkanoates in Brazilian propolis, Z. Naturforsch. 57c, 721–726.

Snowdon J.A., Cliver D.O. (1996) Microorganisms in honey, Int. J. Food Microbiol. 31, 1–26.

Valcic S., Montenegro G., Mujica A.M., Avila G., Franzblau S., Singjh M.P., Maiese W.M., Timmermann B.N. (1999) Phytochemical, morphological and biological investigations from central Chile, Z. Naturforsch. 54C, 406–416.

Velikova M., Bankova V., Marcucci M.C., Tsvetkova I., Kujumgiev A. (2000) Chemical composition and biological activity of propolis from Brazilian Meliponinae, Z. Naturforsch. 55c, 785–789.