Distribution of Quinic Acid Derivatives and Other Phenolic Compounds in Brazilian Propolis

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The quinic acid derivatives (including 4-feruoyl quinic and 5-ferruoyl quinic acids characterized for first time in propolis samples) and other phenolic compounds were quantified in thirteen Brazilian propolis samples by HPLC analysis. For chemometrical analysis, the distribution of quinic acid derivatives and other phenolic compounds were considered. The results suggest that the Brazilian propolis with floral origin from *Citrus* sp. have the highest concentration of the quinic acid derivatives (between 11.0 to 58.4 mg/mg of the dried crude hydroalcoholic extract) and therefore would probably show a more effective hepatoprotective activity.

Key words: Chlorogenic Acid, Phenolic Compounds, Brazilian Propolis

Introduction

In Brazil as in other tropical countries, propolis composition shows significant chemical differences because the high plant biodiversity (Bankova *et al.*, 2000). In Brazilian propolis the main active compounds already characterized are derivatives of aromatic acids (*e.g.* hydroxycinnamic acids) and diterpenoids (Bankova *et al.*, 1996).

A major class of phenolic compounds is the hydroxycinnamic acids, which are found in almost every plant. The major representative of this phenolic class is caffeic acid, which normally occurs in foods mainly as an ester with quinic acid, know as the chlorogenic acid (5-caffeoylquinic acid) (Tapiero *et al.*, 2002). Most of the chlorogenic acid from foods will reach the colon and part will enter into blood circulation (Olthof *et al.*, 2001). Chlorogenic acid and caffeic acid are antioxidants *in vitro*, which protects low-density lipoprotein (LDL) from oxidation and, therefore, is thought to prevent various age-diseases (Robinson, 1998).

Chlorogenic acid also is a competitive inhibitor of glucose-6-phosphatase (Glc-6-Pase) in intact microsomes (Arion *et al.*, 1997). Hepatic GLC-6-Pase may be a key control site in the homeostatic regulation of blood glucose concentration, and the increase of GLC-6-Pase activity is widely held to be a significant factor in the abnormally high rates of hepatic glucose production observed in the diabetic state (Arion *et al.*, 1997). The chlorogenic acid presence can be mitigate cellular damage by the simultaneous formation of peroxynitrite and release of myeloperosidase during chronic infection and inflammation (Grace *et al.*, 1998).

The hepatoprotective activity of an alcoholic extract of propolis against chemically induced liver injury in rats has also been reported (Basnet *et al.*, 1996b), the activity-guided chemical analysis led to the isolation of two compounds, methyl 3,4-di-O-caffeoyl quinate and 3,4-di-O-caffeoyl quinic acid (Basnet *et al.*, 1996a). The dicaffeoylquinic acids are potent and selective inhibidors of human immunodeficiency virus type 1 (HIV-1) integrase, they also inhibit HIV-1 replication at nontoxic concentrations (King *et al.*, 1999).

Due to the important biological activities of quinic acid derivatives, the knowledge of the concentration these compounds in propolis hydroalcoholic extracts is important for research concerning the biological activity of these materials as well as for quality control of this important food supplement.

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Experimental

Material

The propolis samples produced by *Apis mellifera* were obtained with apiarists from different localizations of Southern and Southeastern of Brazil (Table I).

Standards

Caffeic, *p*-coumaric, ferulic and 5-caffeoylquinic acids were obtained by Aldrich, St. Louis, USA.

The standards 3-caffeoylquinic and 4-caffeoylquinic acids were produced in the laboratory by 5-caffeoylquinic acid isomerisation, the procedure is shown in details in Trugo and Macrae, (1984).

Propolis extraction

One gram of propolis samples were extracted with 10 ml of the ethanol 70%, using ultrasonic agitation for 2 h at room temperature. The extracts were concentrated under vacuum.

In 10 ml calibrated flask, 5.00 ml of the propolis sample solutions at 1000 ppm in methanol, 2 ml Carrez I solution [15 g $K_4Fe(CN)_6 \cdot 3H_2O$/100 ml water] and 2 ml Carrez II solution [30 g $ZnSO_4 \cdot 7H_2O$/100 ml water] (Pearson, 1976) were added and the mixture was allowed to stand for 10 min after making up to volume. The precipitate was removed by filtration under gravity (using Whatman No. 1 filter paper) and the filtrate was used directly for liquid chromatography.

Liquid chromatography

A Shimadzu isocratic liquid chromatography (Kyoto, Japan) was used. Injection was performed by means of a fixed-loop (20 μl) Rheodyne injection valve. A Rexchrom (Regis, IL, USA) ODS C18 column (250 mm × 5 mm *i.d.*) was used with the mobile phase 0.01 M tripotassium citrate/methanol (65:35 v/v, pH 2.5) with a flow rate 1 ml/min. HPLC data were acquired and processed with a HP 3396-II integrator.

Multivariate analysis

The chemometrical analyses were realized with the software "Statistica for windows" (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

The characterization of the quinic acid derivatives and other compounds was carried out by comparison of peak retention times with authentical standards, with exception of feruloylquinic acids and dicaffeoylquinic acids which were characterized as previously described (Trugo and Macrae, 1984). The quantification was achieved by peak area measurement and by comparison with a 5-caffeoylquinic acid standard solution allowing corrections due differences in molar absorptivities (Trugo and Macrae, 1984).

The feruloylquinic acids characterized in ten of the thirteen samples studied were not yet reported in Brazilian propolis. The results (Table II) show that in almost all Brazilian propolis samples studied the main quinic derivatives are dicaffeoylquinic acids, with exception of the sample V (Table I). The highest concentration of total diocaffeoylquinic acids was found in sample XII corresponding to 41.6 μg/mg of the dried crude hydroalcoholic extract, the lowest concentration was found in sample X corresponding to 1.2 μg/mg of the dried crude hydroalcoholic extract (Table II). The chlorogenic acid was characterized in all samples; the highest concentration was found in sample IV, corresponding to 9.2 μg/mg in the dried crude hydroalcoholic extract, and the lowest concentration was found in sample X, corresponding to 0.4 μg/mg of the dried crude hydroalcoholic extract. The other phenolic acids (caffeic, coumaric and ferulic) were also characterized in all samples; the highest concentration of such phenolic acids was found in sample XII, corresponding to 21.6 μg/mg of the dried crude hydroalcoholic extract and the lowest concentration was found in sample II, corresponding to 0.3 μg/mg.

Chemometrical analysis has been used for propolis classification; the data matrix used correlated the composition of thirteen propolis studied with the concentration of the all eleven phenolic compounds studied in this present work (Table II).

Cluster analysis provided a dendogram showing chemical affinities among the propolis samples. In relation to geographical origin it was impossible to suggest a correlation. For example, the six samples (I, II, III, IV, V and VI) collected in the Rio de Janeiro State were distributed between the three

Table I. Geographical origin and main flora of Brazilian propolis samples.

Sample	Location (city – state)	Flora[1]	Yield extraction (mg/g, see experimental)
I	Sapucaia – RJ[3]	*Citrus* sp., and *Vermonia polyanthes*	390 ± 10*
II	Nova Friburgo – RJ	*Pinus* sp.	610 ± 18
III	Magé – RJ	Polyfloral	257 ± 22
IV	Itaguaí – RJ	*Citrus* sp.	159 ± 15
V	Nova Friburgo – RJ	Polyfloral	155 ± 15
VI	Sapucaia – RJ	*Citrus* sp.	209 ± 10
VIII	Uberlândia – MG[3]	*Citrus sp.*	517 ± 17
IX	Jaboticabal – SP[3]	*Pinus* sp.	210 ± 13
X	Tubarão – SC[4]	Polyfloral	137 ± 12
XI	Nova Petrópolis – RS[4]	Polyfloral	460 ± 10
XII	Porto Alegre – RS	Unknown	509 ± 11
XIV	Carangola – MG	Unknown	175 ± 17
XV	Commercial[2]	Unknown	140

[1] Apiarist information. [2] Produced by MagisPharma homeopatia, Campinas, SP, Brazil. [3] States of Southeastern Brazil: RJ = Rio de Janeiro State, SP = São Paulo State, MG = Minas Gerais State. [4] States of Southern Brazil: SC = Santa Catarina State, RS = Rio Grande do Sul State. * Average and standard deviation of the extraction in duplicate.

groups; two in group 1, one in group 2 and three in group 3, this fact is probably due to the usual high biodiversity found in Brazil. However, samples of group 1 (II, V, IX and X) are the samples with lowest concentration of the quinic acid derivatives (between 1.7 to 11.0 μg/mg of dried hydroalcoholic extract).

On the other hand, samples of group 3 (I, IV, VI, XII and XIV) presented the highest concentration of quinic acid derivatives (between 36.7 to 58.4 μg/mg of dried hydroalcoholic extract). This result indicates that Brazilian propolis of *Citrus* sp. origin (I, IV and VI) are rich in these compounds. The other sample (VIII) of *Citrus* sp. origin was

Table II. Amount of phenolic compounds characterized in Brazilian propolis.

Sample	Amount of phenolic compounds in Brazilian propolis (μg/mg, based in dry residue of the hydroalcoholic extract)											
	CA	CuA	FA	3CQA[1]	4CQA	5CQA	4FQA	5FQA	3,4diCQA	3,5diCQA	4,5diCQA	Σ of QAD[3]
I	3.3	3.4	1.4	1.4[2]	2.6	5.4	nd[4]	1.6	2.7	12.7	18.5	44.9
II	0.1	0.1	0.1	0.1	nd	0.5	0.3	0.1	0.5	0.3	nd	1.8
III	0.9	2.1	0.5	0.7	1.0	1.7	3.2	2.6	4.4	5.4	4.9	23.9
IV	1.5	12.2	0.6	1.4	0.4	9.2	2.2	1.5	5.4	15.7	22.8	58.6
V	0.2	0,2	0.6	nd	nd	1.7	nd	nd	nd	nd	nd	1.7
VI	1.5	9.0	0.5	1.3	nd	5.3	nd	0.9	3.0	10.5	15.6	36.6
VIII	1.3	4.5	0.9	0.5	0.2	2.5	nd	0.4	1.1	4.6	5.6	14.9
IX	1.5	1.5	0.8	0.7	0.5	1.0	4.5	1.1	1.0	0.9	1.4	11.1
X	0.3	0.4	1.7	0.6	0.5	0.4	nd	3.8	1.2	nd	nd	6.5
XI	3.3	5.5	0.5	0.7	nd	4.2	nd	1.5	7.7	5.3	6.0	25.4
XII	4.4	14.7	2.5	1.6	4.2	5.3	nd	nd	7.4	15.3	18.9	52.7
XIV	3.8	9.4	2.4	1.1	nd	6.8	nd	nd	5.3	10.7	16.5	40.4
XV	2.6	15.3	0.6	0.6	0.4	4.1	nd	4.2	2.4	8.7	10.3	30.7

[1] CA, caffeic acid; CUA, coumaric acid; FA, ferulic acid; 3CQA, 3-caffeoylquinic acid; 4CQA, 4-caffeoylquinic acid; 5CQA, 5-caffeoylquinic acid; 4FQA, 4-feruoylquinic acid; 5FQA, 5-feruoylquinic acid; 3,4diCQA, 3,4-dicaffeoylquinic acid; 3,5diCQA, 3,5-dicaffeoylquinic acid and 4,5diCQA, 4,5-dicaffeoylquinic acid.
[2] Average values of the duplicate analysis, all standard deviation were smaller than 5.0%.
[3] QAD, quinic acid derivatives.
[4] nd, not detected.

classified in group 2 with an intermediate concentration 15.0 μg/mg for quinic acid derivatives in of dried hydroalcoholic extract.

The result suggests that Brazilian propolis with floral origin from *Citrus* sp. have higher concentrations of quinic acid derivatives, and consequently an enchanced hepatoprotective activity and other actions against diseases linked to oxidative process.

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