Chapter 1

A Systematic Review of the Antioxidant Activity of Apiculture Products in Brazil

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Abstract

The use of substances with antioxidant ability can be very important in the therapeutic prevention of diseases related to increased oxidative stress, such as cancer, heart disease and aging. Brazil has a great diversity of vegetation from which bees can collect resins. This study is a systematic bibliographical review on different electronic scientific databases through descriptors of antioxidant activity of bee products from Brazil. The identification of the articles and their inclusion occurred between the months January to MayW 2016. The bibliographic research was conducted in the following electronic databases: (1) Scientific electronic library online—SciELO; (2) Public library of science—PLOS Medicine; and (3) ScienceDirect. The articles selected were the ones that contained antioxidant activity evaluation of bee products from Brazil during 2011 and 2016. It was possible to observe a large number of articles published in this topic, but a compilation of data from all of these studies was necessary. Given there is a great diversity of vegetation in Brazil, a standardization process of the bee products in Brazil was conducted and by means of this process it was possible to draw a profile of the main antioxidants found in apiculture products in Brazil.

Keywords: phenolic compounds, DPPH, HPLC

1. Introduction

The use of substances with antioxidant ability can be very important in the therapeutic prevention of diseases related to increased oxidative stress, such as cancer, heart disease and aging. Brazil has a great diversity of vegetation from which bees can collect resins. This gives place to large chemical diversity among apiculture products collected in different regions and seasons.

This chemical diversity becomes important due to the derived biological properties. Given that apiculture products have great chemical diversity they must be chemically standardized before use to ensure quality, efficacy and safety and thus it is possible to correlate the type of product with its therapeutic application.

The word propolis is derived from the Greek word pro that means in defense and polis that means city or community; thus propolis means in community defense. It is a material in resin form with complex and varied chemical composition, which is gathered from various species and parts of plants (sprouts and exudates) and results in a substance of different colors and consistencies [1–3]. Propolis production in Brazil is estimated at around 100 tons per year. A large part of this percentage is for export, either in a raw form as in manufactured goods, reaching high prices in foreign trade and representing an important source of income [4, 5]. Among the apiculture products, propolis has been subject of pharmacological studies due to its antimicrobial [6], antiinflammatory [7] and antioxidant properties [8], among others. This biological potential is due to a synergism that occurs amongst its various constituents. Propolis is an important therapeutic alternative from an economic point of view because of its pharmacological efficacy since it is easy to obtain and presents pharmaceutical properties. The use of substances with antioxidant ability can be very important in the prevention and treatment of diseases related to increased oxidative stress, such as cancer, heart disease and aging [9].

Brazil has a great diversity of vegetation from which bees can collect resins. In addition, diversity increases when products are collected in different regions and seasons. Due to this chemical diversity, there has been intense research over the past decades to classify the different types of propolis and bee products found in Brazil [1, 10, 11]. Propolis needs to be chemically standardized before use to ensure quality, efficacy and safety. This way, it is possible to correlate the type of propolis and its therapeutic application, an essential task for a growing market and more demanding throughout the v one of the world's biggest suppliers of bee products [3, 11–13].

In this context, it is important to evaluate the published scientific studies about the antioxidant activity of bee products from Brazil.

2. Research methods

2.1. Study design

Study of systematic literature reviews on different scientific electronic databases through descriptors related to the antioxidant activity of bee products from Brazil. The identification of articles and their inclusion occurred between the months January to May 2016.

2.2. Eletronic databases

The bibliographic research was conducted in the following electronic databases:

1. Scientific Electronic Library Online—SciELO;

2. Public Library Of Science—PLOS Medicine;

3. ScienceDirect.

Additional information was obtained from manual search based on the references listed in the articles included in the review.

2.3. Search strategy

The searches were conducted through cataloged descriptors in Descriptor Health Sciences—DHS and Medical Subject Headings—MeSH, in Portuguese and English, contained in the title or summary of the studies. The combination of terms used together or separately, in the respective databases (SciELO, PLOS Medicine, ScienceDirect) was as follows:

- antioxidants;
- antioxidant response elements;
- phenolic compounds.

2.4. Selection and analysis of publications

For the selection of articles, a personal study was created with the following information: author, year, title, development period of the study, Province, city and research area, study design, descriptor used to locate the publication, objective and main results. An inclusion criterion was used in which the selected articles had to be original, published in international and national journals in English or Portuguese, published between 2011 and 2016 and indexed on one of the databases previously cited. Articles that contained evaluation data of the antioxidant activity of apiculture products from Brazil were selected for review.

2.5. Sampling

About 445.265 scientific articles about antioxidant activity in Brazil and in the world searched in the databases with the descriptors previously mentioned were identified. Of the total number of published articles between the years 2011 and 2016, 77 studies were related to the theme of antioxidant activity of bee products. About 60 articles were excluded for being repeated, in other languages or for not containing evaluation of products from Brazil. For this literature review, 17 studies were selected.

3. Results

For review, only articles containing analyses of evaluation of the antioxidant activity of bee products from Brazil, between 2011 and 2016, were selected. As shown in **Figure 1**, the number of published works on bee products is relevant. However, it is clear that the rate of work on this issue in Brazil is low.

The results obtained with the application of the described search strategy are presented in the logical framework of the study (**Figure 1**).

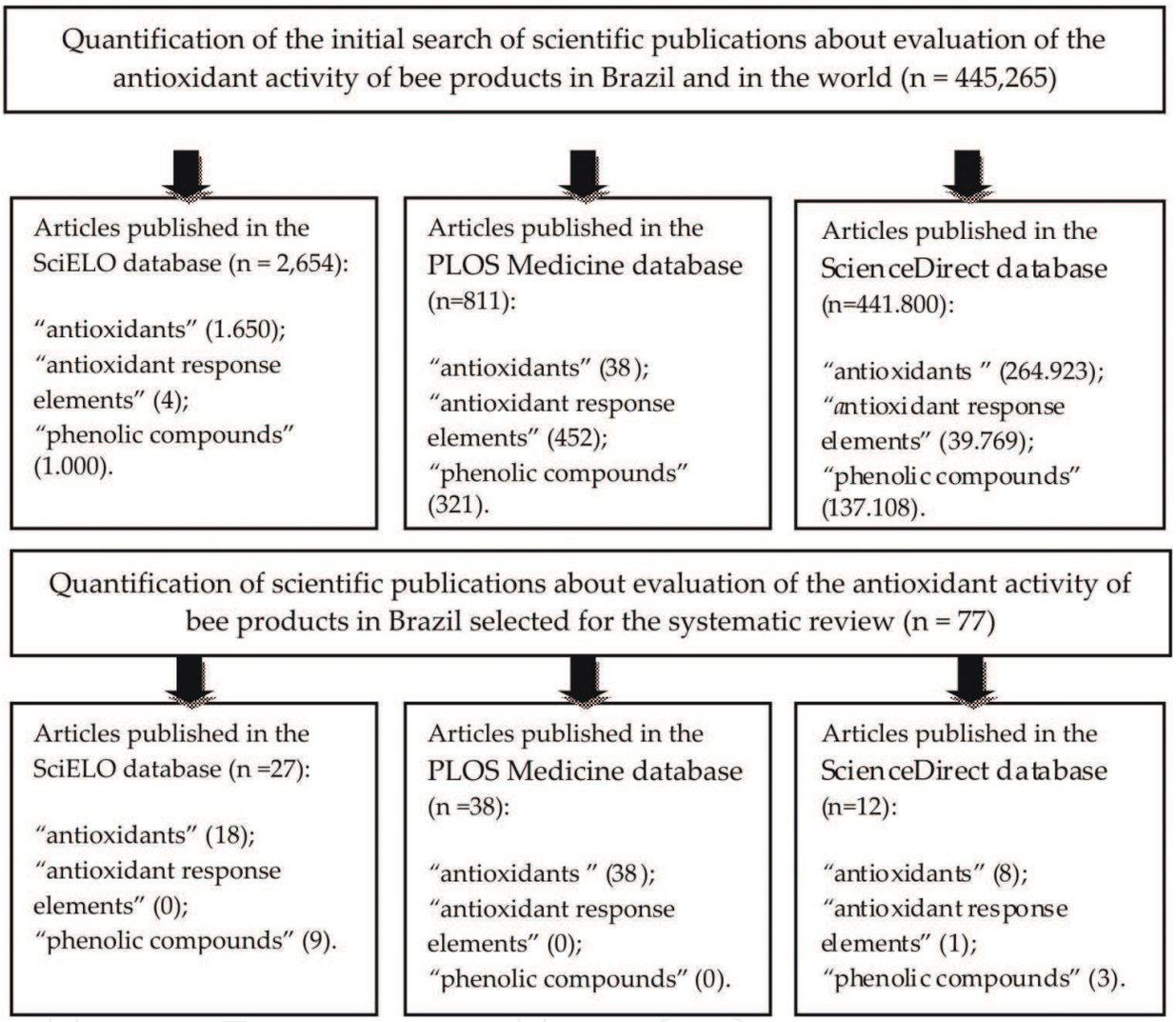

Figure 1. Logical framework of the systematic review, studies about evaluation of the antioxidant activity of bee products in Brazil between the years 2011 and 2016.

Table 1 presents a summary of the studies assessed in this study.

Given the large number of articles published in this topic was observed, a compilation of data from all of these studies was necessary. We can highlight the following identified substances from the main antioxidant substances of apiculture products from Brazil: triterpene compounds of the cycloartane, ursane and oleanane types as the main compounds, in addition to phenolic acids, protocatechuic and gallic acids. It is also important to highlight the high antioxidant potential of geopropolis from Brazil. The works presented here identified the following substances with antioxidant capacity: gallic acid, ellagic acid, catechin, gallocatechin, hesperidin, kaempferol, luteolin, morin, naringin, naringenin and rutin. Analysis of honey also shows the presence of antioxidant substances, especially gallic acid.

No.	Reference	Objectives	Main results/conclusions
01	[14]	Investigate the chemical composition of geopropolis produced by *M. fasciculata* collected from beehives of two phytogeographical regions of Maranhão and evaluate its antioxidant activity.	Geopropolis collected in Palmeirândia contained triterpene compounds of the cycloartane, ursane and oleanane type as the main compounds, in addition to phenolic acids, protocatechuic and gallic acid. In contrast, geopropolis collected in Fernando Falcão contained high concentrations of phenolic acids (gallic acid and ellagic acid) and exhibited high antioxidant activity, suggesting that the high levels of phenolic acids are responsible for the antioxidant property of this geopropolis. The chemical composition and antioxidant activity contribute to the identity and quality of the types of geopropolis produced by *M. fasciculata* collected in two phytogeographical regions of the Maranhão State, northeastern Brazil.
02	[15]	The objective of this study was to determine, among other analyses, the antioxidant capacity of propolis of stingless native bees (Meliponinae) of Tocantins, North of Brazil (State of Tocantins).	Propolis samples collected in the two regions of Tocantins presented physical and chemical characteristics that fit within Brazilian legislation for propolis quality. The flavonoid luteolin was restricted to the samples collected in Santa Maria of Tocantins, while, naringerin and rutin were restricted to the samples collected in Novo Acordo, demonstrating substantial differences between the pasture of the studied regions. And also evidence a high concentration of phenol compounds and good antioxidant capacity.
03	[16]	The objective of this study was to evaluate the effect of cassava starch coatings incorporated with propolis on the content of phytochemicals of nutritional interest and on the antioxidant activity of strawberries stored under refrigeration for 16 days.	The coating with 66% of propolis promoted higher Vitamin C content than fruits submitted to the other treatments at 8 and 12 days of storage.
04	[17]	Identify the antioxidant capacity of propolis samples collected by Apis mellifera L. bees, in four Brazilian regions and compare them using the coupled oxidation method of b-carotene system/linoleic acid.	From the propolis samples collected and analyzed by the coupled oxidation of b-carotene system/linoleic acid, only two showed antioxidant capacity below 60%, both in the northeast. Samples of the southeast had the highest antioxidant capacity, followed by from the midwest, south and northeast. Pollen analysis revealed the predominant presence of Eucalyptus pollen, mainly from the southeast and south and a greater variety of pollen types in northeast samples.
05	[18]	Isolate phenolic substances of the extract in methanol of geopropolis, from species of *M. interrupta*, Mi5, grown in the National Institute of Amazonian Research—INPA by the Bees Research Group—GPA and MI6, grown in Ramal do Brasileirinho community in Manaus.	Analyses of geopropolis extracts of these species showed promising results, presenting antioxidant activities that are important for body maintenance and disease prevention, as well as being used as food (nutraceutical) by local people. From fractionation of the methanol extracts of geopropolis from *M. interrupta* with higher antioxidant activity was possible to isolate four flavonoid.

No.	Reference	Objectives	Main results/conclusions
06	[1]	This study aimed at verifying whether there is positive correlation between the identity and quality criteria for propolis and the biological activities exhibited by the extracts of propolis.	It was established that G12 propolis has a high content of total flavonoid and phenolic compounds, which gives it an excellent quality according to the Brazilian legislation.
07	[19]	Identify the chromatographic profile of phenolic acids and flavonoids using solid phase extraction (SPE) and liquid chromatography reversed-phase (RP-HPLC) and conduct study of antioxidant activity in honeys from three different species of bees *A. mellifera* (Africanized) exotic species, *Melipona flavolineata* (yellow uruçu) and *M. fasciculata* (gray uruçu) native species. The honey samples were obtained from four Pará State municipalities.	From 36 analyzed samples of honey, the presence of 13 phenolic compounds and three unidentified compounds (Ph1, Ph2 and Ph3) was observed. For most of the honeys, major compounds identified as gallic acid and quercetin were analyzed. The AC and ACP methods were able to distinguish the phenolic composition of the analyzed honeys for the species studied. This factor that indicates possible selectivity of the species in relation to the botanical origin of honeys. In general, analyzed honeys showed significant antioxidant activity, especially the darker honeys which also had higher levels of polyphenol.
08	[20]	The objectives of the present study were to produce and evaluate a propolis extract for use in skin care products.	Reasonable stability was noted and the preferred formula was the one that used combination of propolis extract with tocopheryl acetate.
09	[21]	Comparison between the antioxidant activities of ethanolic extracts of propolis prepared using hydrous ethanol with different ethanol/water contents and different propolis concentrations.	It was noted that the DPPH• and FRAP values are dependent on the propolis concentration and the water/ethanol proportion used in the extraction. This correlation was also observed in phenolic contents and flavonoid contents.
10	[22]	The present work aimed at performing a bioassay-guided fractionation of red propolis samples from Igarassu (Pernambuco, Brazil) in order to determine the main constituents associated with its antimicrobial activity, especially against *Candida sp.*	The botanical origin of propolis samples is difficult to ascertain on the basis of only one palynological analysis and a more definite confirmation depends of analysis comparing the chemical profile of the samples with the chemical profile of resins and extracts from plants found near the hives. It should be stressed that red propolis has been suggested as being the only propolis type derived from a plant from the leguminosae family (*D. ecastaphyllum*), rich in inisoflavones such as genistein and formononetin. Although flavonoids exhibit pleiotropic activity affecting several different targets and the synergistic effects cannot be discarded, our results suggest that the isoflavone formononetin is responsible at least partially for the antimicrobial activity of red propolis.
11	[23]	This work aims at optimization the extraction process of bioactive compounds, evaluate antioxidant activity and also conduct the chemical characterization of propolis, using the high performance liquid chromatography technique.	It was concluded that the analyzed propolis has a promising phenolic content and antioxidant activity. Three phenolic acids derived from hydroxycinnamic acid were identified, common in Brazilian propolis.

No.	Reference	Objectives	Main results/conclusions
12	[24]	This paper aims to identify the pollen types and quantify the total phenolic compounds in propolis samples produced in the arid territory of Bahia—Brazil	The levels of the total phenolic compounds found in the propolis samples from the territory of the arid region of Alagoinhas fulfill the standards of the Brazilian legislation. We recommend that more analyses should be conducted in order to obtain more data that corroborate the information contained here.
13	[25]	We evaluated the effects of phenolic compounds from three propolis-based products with different concentrations of propolis and levels of alcohol on feed intake, digestibility (ruminal and intestinal) and blood parameters in lactating dairy cows.	The propolis-based products have positive effects on protein metabolism in the rumen, without interfering with any other parameter evaluated. The propolis concentration and alcoholic level used in this study influences the amounts of flavonoids and phenolic acids in the propolis-based products, which may interfere with the observed effects on ruminal metabolism and digestive parameters.
14	[26]	This study assessed the polyphenolic profile and the antioxidant and antibacterial activities of monofloral honeys produced by Meliponini in the Brazilian semiarid region.	Honeys from Ziziphus joazeiro Mart. (juazeiro) and Croton heliotropiifolius Kunth (white velame) showed the highest total phenolic contents (TPCs) and the greatest antioxidant activity in assays with DPPH and ABTS + radicals. Malícia's honeys showed the greatest quantities of myricetin, quercetin and kaempferol among the studied honeys.
15	[27]	This study aimed to assess the fatty acid composition of milk, the antioxidant quality of milk and blood lipoperoxidation of dairy cows whose diet was supplemented with flaxseed oil containing a propolis-based product (PBP) with or without vitamin E.	Under the studied conditions, the improvements of milk fat quality, of the oxidative properties of milk and the blood's resistance to oxidation, were reached with PBP supplementation and E vitamin.
16	[28]	The aim of this study was to evaluate antioxidant properties of lyophilized bee pollen extract (LBP), to determine the phenolic profile by liquid chromatography and to evaluate the effect of LBP on the oxidative stability of pork meat sausage.	The LBP (lyophilized bee pollen extract) extract exhibited strong antioxidative effects in pork sausage, probably due to high antioxidant activity and the presence of the phenolic compounds in bee pollen; which has potential to be used in pork sausage.
17	[29]	Evaluation of antioxidant activity as well as the determination of phenolic compounds and antimicrobial activity, among other analyses of honey samples from south Brazil.	In the analyzed samples, the bioactive compounds found in a larger amount were the phenolic compounds. With respect to antimicrobial activity, we can highlight the relevance against Gram-positive microorganisms.

Table 1. Reference, objective, main results and conclusions, studies about evaluation of the antioxidant activity of bee products in Brazil between the years 2011 and 2016.

In addition, with respect to methods of extraction and preparation of propolis extracts, the composition of propolis extracts varies with the concentration of propolis, especially with the water/ethanol content of hydrous ethanol used in the extraction. Oldoni et al. observed the influence of standardization of extraction. The results of their study confirmed that the optimization of the extraction conditions is important to obtain extracts that are rich in phenolic compounds and antioxidant activity of propolis [23].

Since propolis has great chemical diversity, it needs to be chemically standardized before use to ensure quality, efficacy and safety and this way, it is possible to correlate the type of propolis and its therapeutic application, an essential task for a growing market and more demanding throughout the world. Brazil is a pioneer in these practices, since it is considered one of the world's biggest suppliers of bee products [3, 11–13].

Brazilian propolis were classified in the most prevalent types, resulting in 12 groups or types based on their geographical origin, chemical composition and plant origin: five in the south, one in the southeast and six in the northeast. A new type of propolis from a mangrove region from the State of Alagoas had its botanical origin identified as Dalbergiaecastophyllum, a species of legume and was ranked as the 13th type of northeast Brazilian propolis. These findings confirm the great Brazilian biodiversity that has become the subject of several scientific research studies throughout the world [3, 12, 13, 30, 31].

In this context, it is important to note that the evaluation of propolis samples from different geographical and climatic regions means that there are variations in the chemical composition and, therefore, biological activities may differ. Thus, when performing pharmacological studies, we should not indiscriminately compare propolis samples from different regions, neither assign a proven activity in a given sample to other samples from different regions. For this reason, publications on evidence of biological activities should include the physical and chemical characterization of the used propolis [32].

Moreover, there is smaller variation in the chemical composition of propolis found in temperate regions of the planet, where its main bioactive compounds are flavonoids: apigenin, quercetin, hesperetin, rutin, luteolin, genistein, daidzein, anthocyanidin, kaempferol, among others. Although flavonoids are the most extensively studied components of propolis, they are not the only ones responsible for their pharmacological properties. Several other compounds have been linked to its medicinal properties [12, 13, 24].

4. Conclusion: key results

In order to assist in the standardization process of apiculture products which are greatly diverse due to the variety of vegetation that exists in Brazil, it was possible to draw a profile of the main antioxidant substances of apiculture products from Brazil. It was possible to identify several substances of antioxidant capacity in bee products from Brazil, mainly in propolis and geopropolis, propolis extracts and honey.

Standardization has not yet been observed in scientific research and legislation in Brazil. Therefore, the types and quantification of substances with antioxidant capacity become too variable, thus, interfering in a more general analysis of the country's propolis. In this review, we observed that the chemical substances in propolis are determined by the flora of the region.

Additionally, since Brazil has great flora diversity, it is complex to follow a standardization process for the substances found in propolis.

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