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Review

ANTIOXIDANT ACTIVITY OF *APIS MELLIFERA* BEE PROPOLIS: A REVIEW

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Background

Propolis is a natural product manufactured by bees from balsamic materials collected from plants that surround the hive, undergoing subsequent modification by the enzymes of these insects. It has several functions in the hive, such as sealing cracks and antimicrobial action. Folk medicine worldwide has used this resin in their health practices, and modern research turns its eyes to natural materials to become sources of new molecules to treat the most diverse ailments.

Aims

This work collected information on studies that test the antioxidant activity of propolis, produced by *Apis mellifera* bees, using different antioxidant methods available.

Methods

The search for this review was carried out in the following databases: SciELO, Google Scholar, PubMed, MEDLINE, Catalog of Dissertations and Theses of CAPES, BVS, CRD, Embase, Science Direct, Scopus and Cochrane Library. Publications in Portuguese, English and Spanish in the last decade were included.

Results

The 173 articles chosen showed quantitative and qualitative data about the potential of this natural product in the area of interest. Propolis extracts reached amazing values in antioxidant tests; they were as active as isolated substances already recognized as standard patterns. Many studies have brought information about the antioxidant mechanisms of propolis, such as free radical scavenging, metal chelation, and electron donation.

Conclusion

This review brings scientific evidence, *in vitro* and *in vivo*, that supports the idea that propolis is a good candidate for producing new antioxidant pharmaceutical and food formulations in the future.

Keywords: *Apis mellifera*; propolis; antioxidant activity

INTRODUCTION

Propolis is a complex set of balsamic substances arising from resins from different parts of plants (sprouts, flowers, branches, latex, bark) and plant exudates from lesions (1–3) collected by worker bees of the species *Apis mellifera* and stingless bees (Meliponini) (4,5). Although it is an animal product, most of the components of propolis, especially the active ones, come from plants (6). Bees can add pollen, wax, salivary enzymes, among others, increasing their biological activity (2,4,7,8). As it is a lipophilic compound, propolis has a hard and fragile appearance at room temperature, but it is sticky and elastic at higher temperatures (9). Due to its sticky and adhesive characteristics – owing to its great interaction with oils and skin proteins – (10), the expression “bee glue” emerged (11). It has a characteristic odor, bitter and astringent taste. The color varies, from brown to green, passing through red tones, according to its origin; however, dark brown is most common. The color of propolis is closely linked to its commercial value and quality parameters, with green (southeast Brazil) and red (northeast Brazil) being especially valued (6,12).

In hives, propolis plays an important role in covering cracks (1,11), building (10), embalming invaders' bodies (9), promoting thermal insulation (1,4), and, above all, it blocks microbial growth in essential areas of the hive (3). It reflects the origin of its name: from the Greek pro-, in defense, and polis-, city or community, that is, in defense of the community (hive) (1,11).

Although several bee species are capable of producing propolis, *A. mellifera* is recognized as the main producer among all of them. Annually, from each *A. mellifera* hive, 100 to 300 grams of propolis can be extracted, which makes this species a very efficient producer (4,13). Another important point regarding propolis is its high commercial value, with immense appreciation: in 2010, a kilo of propolis cost U$S 84.87, jumping to U$S 129.47 in two years (1). In some cities, such as Tokyo, Japan, the bottle can reach up to US$150, and one gram of propolis produced in Minas Gerais (Brazil) costs around US$200 (14). The propolis market is estimated to grow around USD 40 million from 2020 to 2025, with a CAGR (Compound Annual Growth Rate) of 5,41% (15).

Humankind has used this balsamic product since antiquity, with notes of applying this resin from Mesopotamia, ancient Greece, and Rome (1,16). In the second half of the 20th century, the use of propolis in medicine was disseminated around the world and adopted in integrative and complementary health practices and the scope of production of supplements and foods (17). The rich chemical composition of propolis explained its wide use. This natural product has fixed and volatile portions - some authors claim it has about 500 compounds (18). About 3% of them have some biological activity, which is ordinarily obtained through the synergistic interaction between several substances (19). The most widely known active molecules are phenolic compounds such as flavonoids and phenolic acids (1).

There is evidence of its antimicrobial action against several species of Gram-negative and Gram-positive bacteria, yeasts, and fungi, as well as an anticarcinogenic, anti-inflammatory, antioxidant, anesthetic (3,20), immunostimulant (21), anti-protozoal (1), healing (20), and antimutagenic functions (22). Recent studies have demonstrated the effectiveness of propolis in fighting SARSCoV-2, emphasizing the current importance of this product (23).

Among all biological activities that natural products have, the antioxidant stands out. This fact is due to the impact that oxidizing species, such as free radicals, have on various health problems, like aging, cell degeneration (24), cancer (3,25), immune system dysfunction, arthritis, diabetes, liver and kidney problems, Parkinson, and Alzheimer (4,11).

Antioxidant substances, synthetic or natural, are those present in the medium, even in small quantities, with the power to inhibit the oxidation of substrates (14,22). The mechanism of action of antioxidant substances is based on the inhibition of free radicals and interaction with their biological targets through the donation of electrons or hydrogens (primary antioxidants). Inhibition reactions produce stable, non-reactive molecules, which can later be degraded through other enzymes and processes. They can even act as chelators, scavenge transition metals, as they are generally catalysts for oxidative reactions, absorb ultraviolet radiation (secondary antioxidants), and scavenge oxygen (9,26,27).

For a molecule to be considered a good antioxidant, certain characteristics must be observed. First, it must be nontoxic and have good interaction with other antioxidants. Still, it must have a high scavenging capacity, even at low concentrations in the medium. Its power of action in different media, based on solubility, must be satisfactory (14,28). There should not be modifications of the original organoleptic characteristics of the product, which must be compatible with the formulation and general processes of the product (29). Finally, it must have a long half-life and acceptable bioavailability, with the ability to

cross biological membranes and reach their place of action (27). These characteristics make it difficult to obtain an ideal antioxidant for use in the industry, and although there are various synthetic antioxidant compounds, such as butyl hydroxyanisole (BHA), butylhydroxytoluene (BHT), t-butyl hydroquinone (TBHQ), and propyl gallate (PG), their possible toxic and carcinogenic side effects guide research to natural targets to reduce the risks (1,9).

Furthermore, numerous studies linking the consumption of fruits and other foods with high levels of antioxidants with the low risk of disease have raised population interest in these compounds (1). Consumers seek not only nutritional quality but also foods that promote quality in health (30).Antioxidant substances of natural origin can greatly impact diseases such as diabetes mellitus, obesity,and high blood pressure (31).

In this context, interest in propolis has grown recently. The huge number of studies involving this natural product aims to understand its various biological activities and the properties of each chemical constituent. These studies help build a standardization of reference for producers who use this raw material, increasing its economic value and becoming a source of new drugs (32). Then, this work investigates the antioxidant activity of *Apis mellifera* bee propolis extracts through a literature review to contribute to the field of health and natural products.

METHODS

The design that guided this literature review was based on the following steps: determining the theme, objective, and information to be obtained from each material, the establishment of inclusion and exclusion criteria, choice of databases, sample selection, analysis of materials found, discussion, and presentation of results in the form of a review.

Eligibility Criteria

Inclusion Criteria

Experimental works (articles, theses, and dissertations) that present tests that evaluate the antioxidant activity of *Apis mellifera* propolis, *in vivo* and *in vitro*, were eligible.

Exclusion Criteria

The following works were excluded: (1) Studies outside the specified period (2011 to 2021); (2) Studies with inappropriate format (reviews, books, chapters, conference abstracts and posters, letters, and opinion articles); (3) Studies written in languages other than Portuguese, English, and Spanish; (4) Studies that have not tested the antioxidant activity; (5) Studies that did not use propolis as study material; (6) Studies that did not specify the propolis-producing bee species; (7) Studies that used other bee species, except *Apis mellifera*; (8) Studies that did not specify the extract preparation process or the propolis collection location; (9) Studies that did not use the crude propolis extract, but partitioned, isolated substances, or used co-products and residues; (10) Studies that evaluated the antioxidant activity of propolis in association with other substances.

Information Sources and Search Strategy

The databases chosen for the search were SciELO (Scientific Electronic Library Online), PubMed, BVS (Virtual Health Library – *Biblioteca Virtual em Saúde*), Scopus, Cochrane Library, Embase, Science Direct, CRD (Center for Reviews and Dissemination), and CAPES Theses and Dissertations Catalog, to ensure adequate coverage on the subject.

Two searches were performed in each aforementioned database: the first, with the term in English and the second in Portuguese (Suplemmentary Material – Table S1); although articles in Spanish that eventually appeared in the searches were accepted, there was no direct search for terms in Spanish. Within each database, the exclusion criterion by date has already been performed.

All terms were consulted on health descriptors through the DeCS website (Descriptors in Health Sciences), in addition to the MESH search. The terms "*Apis mellifera*" and “propolis” were duly identified. However, "antioxidant activity" was not found in DeCS/MESH.

Study Selection

This literature review comprised four steps. In the first, a search was performed according to the appropriate terms in each database. Later, in the second stage, such references were added to the EndNote Web citation management program, where duplicate removal was performed. During the third stage, works that did not meet the above criteria were removed by reading the title and abstract. Finally,

the remaining references were analyzed again in the fourth stage, but this time through a full reading of the text.

Information was taken from the materials that make up this review: type of material (article, theses, and dissertation), year of publication, language, type of propolis used, time and place of propolis collection, species and nomenclature of the bee, the type of extract, a type of methodology used to assess the antioxidant activity, and plant source of propolis.

RESULTS

Initially, 1765 publications were identified in the first phase from January 1st, 2011, to April 29th, 2021. After insertion in the manager EndNote Web, 242 duplicates were deleted. Of the 1523 remaining materials, 1239 were eliminated after analyzing the title and abstracts, as it was perceived that they did not meet the pre-established criteria. The remaining 284 articles formed the initial collection, whose later reading would be complete. A full-text analysis of each material eliminated another 111 articles. Finally, this review was based on data obtained from 173 scientific articles, theses, and dissertations, which form the analytical field, as shown in Figure 01.

Study Characteristics

The more specific characteristics, as well as qualitative information (reference, bee race, place and time of collection of propolis and its type, sample extraction process, and possible botanical source) of each work chosen for this literature review, can be found is Supplementary Material (Table S2).

Regarding the year of publication, there is an increase in interest in the topic, with a peak in the bienniums of 2016/17 and 2019/20. 2021 could not be analyzed clearly, because this survey was conducted only with works published until the end of April. However, it is noted that, even if the search is conducted in the first half of 2021, this year still had more results compared to 2012 and 2013 (Figure 02).

One hundred forty-four scientific articles (83.24%) and 29 theses/dissertations (16.76%) were identified, mainly published in English (80.92%), but there were also works in Portuguese (17.34%) and Spanish (1.73%).

DISCUSSION

Antioxidant Activity

The antioxidant activity can be defined as the redox capacity to eliminate oxidizing compounds. Numerous studies prove the antioxidant action of propolis samples – according to Castro*et al*. (2014), it has the highest activity among all hive products (34). This power is mainly due to its chemical composition rich in bioactive compounds, such as flavonoids and other phenolic compounds(1,7).

The quality control of propolis is done based on the extraction yield, number of phenolic compounds, and analysis of the antioxidant activity of this material (12). This essential activity is one of the main factors for propolis to have many other uses, such as neuro and hepatoprotection. One of the most viable applications of this antioxidant power is in the treatment of skin wounds, remembering that the intensified production of free radicals makes it difficult for these inflammatory processes to heal (7). Still, this ability of propolis can be used for treating other illnesses, such as glaucoma, ocular neuropathies resulting from diabetes complications, and cerebral ischemia, since all these cases are aggravated by oxidative stress (35).

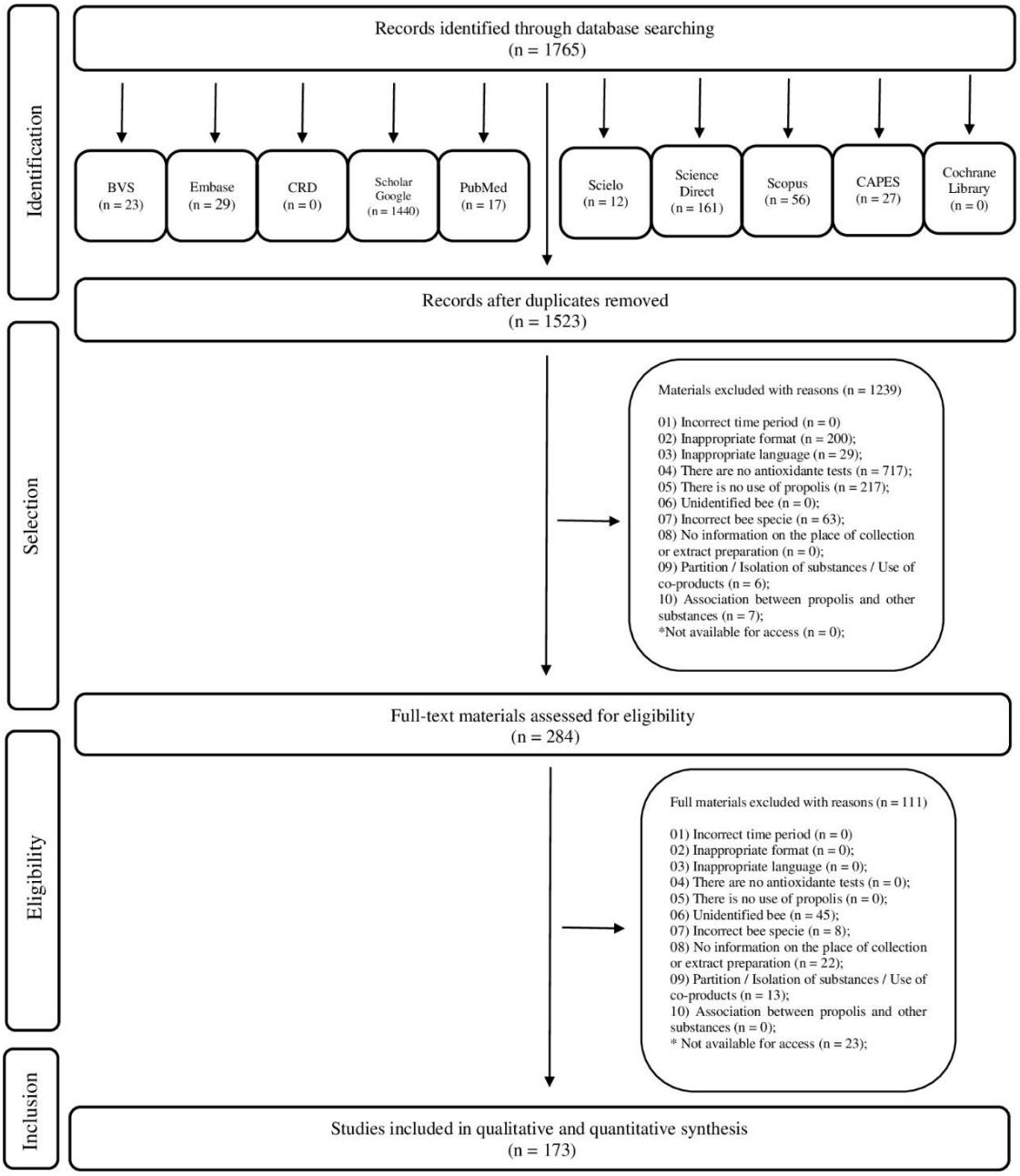

Figure 01 | Flowchart of materials obtained, excluded, duplicated, and used in this literature review. Adapted from PRISMA (33).

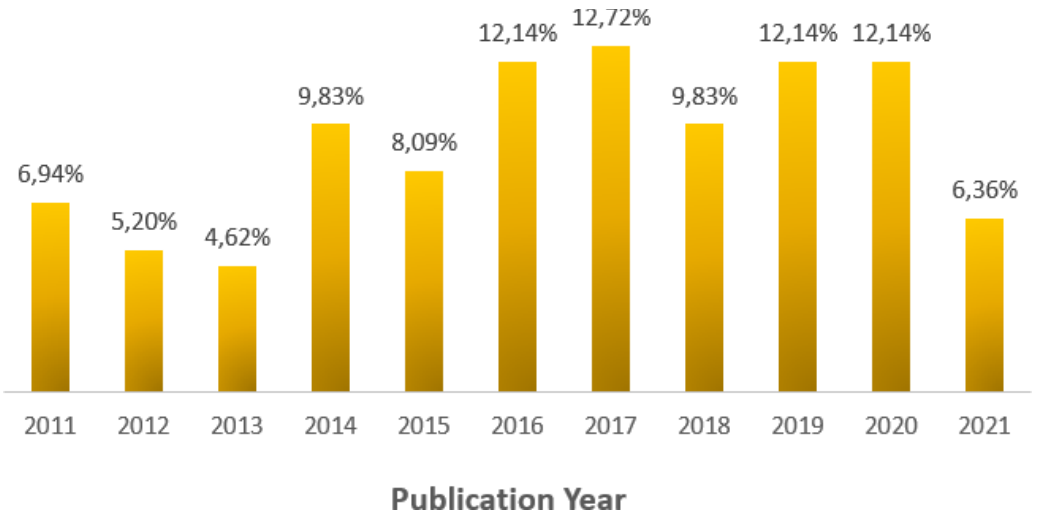

Figure 02 | The year of publication of the materials that make up the collection of this bibliographic review.

There are several methods for evaluating antioxidant capacity, both *in vitro* and *in vivo*, since the samples to be analyzed also differ greatly from each other. Those *in vitro* tests can be performed using instrumental, colorimetric, biological, and electrochemical tests (29). Generally, studies bring more than one method for evaluating the same sample, giving greater credibility to the data. When looking at the results obtained using different methodologies, there are differences. This fact is due to the reaction mechanism and the conditions under which radicals are generated (36). Each methodology has advantages, disadvantages, and limitations, with different reaction mechanisms, complexes, solubility, and compounds. To choose the best method of study, the sample matrix, a form of preparation, and antioxidant solubility should be considered (12,26,35).

This literature review analyzed antioxidant activity by *in vitro* (93.75%), *in vivo* (5.11%), and 1.14% mixed both forms. 29.71% of the materials used only one antioxidant test to evaluate the propolis samples, generally the DPPH; the vast majority (70.29%) opted for more than one analysis methodology. The works that included *in vitro* methodologies analyzed enzymatic activity, protein oxidation levels, lipid peroxidation by the TBARS method (Thiobarbituric Acid Reactive Substances), the production of ROS (Reactive Oxygen Species), cell survival, in addition to tissue histological analysis.

Some studies have obtained remarkable results for the antioxidant activity of propolis samples, with similar or even better response than the used reference compounds, widely recognized as good antioxidants, such as quercetin and BHT.

More detailed results of the works that comprise the collection of this review will be presented below, focusing on the values obtained for the antioxidant activity. The tables were organized to show the material reference and the values of the results of the respective tests. Those that appear together with the term “about” were values taken from graphs whose specific numbers were not reported in the materials.

DPPH• (2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Test

Initially suggested in 1950 by Blois, DPPH is considered an indirect and ancient method for evaluating the antioxidant activity, extremely used, practical and simple, stable in the absence of light, and very sensitive (29,37). It is considered a good method to evaluate bee products (9).

One of the ways to demonstrate the result obtained by this method is through the so-called IC_{50} (Half-inhibitory Concentration), which corresponds to the amount of sample required to reduce 50% of the initial DPPH. The final evaluation can be interpreted considering that the greater the consumption of DPPH, the greater the antioxidant activity of the sample. Another way to express the results is by the percentage of inhibition (9).

Some authors expressed the results in the form of an index of antioxidant activity (IAA), a scale that classifies the action of compounds as weak (IAA<0.50), moderate (IAA between 0.50 and 1.00), strong (IAA between 1.00 and 2.00) and very strong (IAA>2.00) (38). Moreover, this methodology can be performed on a silica plate more simply. In this case, the sample is applied to the plate, followed by spraying a solution of DPPH in methanol. If there is a change in color from purple to yellow, the result is considered positive, demonstrating the antioxidant potential of the analyzed sample (39).

Table 1 shows the values obtained from the collection of chosen works. Generally, the propolis samples showed remarkable antioxidant power; their IC_{50} values approached or were even better than the chosen antioxidant standards, such as ascorbic acid, which already has recognized potency in these tests (13,34,40).

Table 1 | Results of the DPPH radical scavenging test of *Apis mellifera* propolis extracts, obtained from the materials chosen for this literature review.

Author	Percentage of inhibition (%)	Other results
Aguiar, 2015 (10)	97.20% (0.10 mg/mL) and 99.80% (1.00 mg/mL)	IC_{50}: 24.20 µg/mL
Al Naggar *et al*., 2016 (42)	64.64 – 88.15% (25.00 µg/mL); 81.93 – 92.10% (50.00 µg/mL); 91.19 – 93.72% (100.00 µg/mL)	-
Alves, 2018 (5)	EEP: 88.37% Microcapsules: 73.41 – 86.93%	-
Andrade *et al*., 2017 (43)	-	4431.00 – 4663.80 µg Trolox/g
Andrade *et al*., 2018 (36)	82.00 – 89.00%	22843.03 - 24685.82 µmol Trolox/g
Aranguena Salazar, 2019 (44)	-	642.99 – 828.55 µg Trolox/g
Araújo *et al*., 2020 (45)	-	IC_{50}: 100.18 µg/mL
Arruda, 2019 (46)	78.50 – 81.5% (80.00 µg/mL)	IC_{50}: 3.10 – 51.03 µg/mL
Arslan *et al*., 2021 (47)	94.60% and 94.90%	-
Augusto-Obara *et al*., 2019 (48)	-	682.80 – 781.20 µmol Trolox/g
Bakkaloglu, Arici, and Karasu, 2021 (49)	-	0.48 – 835.34 mg Trolox/g
Béji-Srairi *et al*., 2020 (50)	-	IC_{50}: 20.10 – 43.00 µg/mL
Bhargava *et al*., 2014 (51)	-	59.30 and 81.40 g/mL (5.00 and 10.00%)
Bhuyan *et al*., 2021 (52)	4.85 – 75.02% (6.25 to 100.00 µg/mL)	IC_{50}: 52.63 - >100.00 µg/mL
Bonamigo *et al*., 2017 (4)	94.60% (300.00 µg/mL)	IC_{50}: 49.80 µg/mL
Boufadi *et al*., 2014 (53)	-	IC_{50}: 19.40 - >50.00 µg/mL
Cabral *et al*., 2012 (54)	Type 06 propolis: 21.70% Type 12 propolis: 53.00%	-

Calegari, 2018 (26)	-	136.00 – 267.00 µmol Trolox/g
Cao *et al*., 2017 (7)	-	IC_{50}: 47.71 µg/mL
Castro *et al*., 2014 (34)	-	About IC_{50}: 5.00 – 12.00 µg/mL
Cavalaro, Fabricio, and Vieira, 2020 (55)	-	18652.90 µmol Trolox/g
Cécere *et al*., 2021 (56)	-	IC_{50}: 158.15 µg/mL
Ceylan, and Halime, 2020 (57)	-	IC_{50}: 3.94 – 26.33 mg/mL
Coelho, 2013 (58)	-	IC_{50}: 20.00 – 110.00 µg/mL
Coelho *et al*., 2017 (20)	-	IC_{50}: 10.00 – 110.00 µg/mL
Correa *et al*., 2016 (59)	-	IC_{50}: 7.81 and 37.01 µg/mL (some samples were not active)
Cottica *et al*., 2011 (60)	-	IC_{50}: 47.00 – 160.00 µg/mL
Cruz, 2011 (61)	-	50.46 mg of equivalents of gallic acid/g
Da Cruz Almeida *et al*., 2017 (62)	EEP: 16.52 – 98.06% (2.50 – 50.00 µg/mL) Microcapsules: 5.89 – 87.86% (2.50 – 50.00 µg/mL)	IC_{50} EEP: 6.95 and 4.78 µg/mL IC_{50} Microcapsules: 8.89 – 23.36 µg/mL
Da Graça Miguel *et al*., 2014 (63)	-	IC_{50}: 8.00 – 1813.00 mg/mL
Dărăban *et al*., 2019 (64)	-	IC_{50}: 1.30 – 3.70 µL
Da Silva *et al*., 2013 (65)	24.00 – 52.00% (500 and 600 ppm); 62.00 – 81.00% (2000 ppm); 84.00 – 89.00% (5000 ppm)	-
Da Silva *et al*., 2018 (66)	-	11.68 – 275.20 µmol Trolox/g
De Almeida, 2017 (67)	EEP: 74.01 – 96.72% Microcapsules: 53.88 – 92.56%	-
De Francisco *et al*., 2018 (30)	-	IC_{50}: 913.18 µg/mL
De Lima *et al*., 2019a (25)	-	IC_{50}: 25.00 µg/mL
De Lima *et al*., 2019b (68)	-	IC_{50}: 9.35 µg/mL
De Mendonça, 2014 (69)	10.91% (50.00 µg/mL); 13.33% (100.00 µg/mL); 22.10% (150.00 µg/mL); 31.82% (200.00 µg/mL); 40.46% (250.00 µg/mL)	IC_{50}: 372.44 µg/mL

De Oliveira, and Andolfatto, 2014 (70)	34.65 – 57.68%	3.16 – 8.75 µmol Trolox/g
De Souza *et al*., 2018 (71)	-	IC_{50}: 3.74 µg/mL
Devequi-Nunes *et al*., 2018 (72)	-	IC_{50} EEP: 89.90 – 159.74 µg/mL IC_{50} Supercritical: 141.81 – 371.12 µg/mL
Ding *et al*., 2021 (73)	About 80.00 – 95.00% (5.00 mg/mL)	-
Do Nascimento *et al*., 2016 (74)	EEP: 98.00% Nanoparticles: 76.22 – 81.40%	-
Do Nascimento *et al*., 2019 (75)	EEP: 91.07% Microcapsules: 90.60%	-
Duca *et al*., 2019 (76)	84.71 – 92.57 % (10.00 mg/mL); 79.57 – 92.50% (5.00 mg/mL); 72.63 – 92.63% (3.00 mg/mL); 67.58 – 90.66% (1.50 mg/mL); 13.58 – 82.52% (0.50 mg/mL); 7.89 – 78.16% (0.30 mg/mL);	IC_{50}: 70.00 – 932.00 µg/mL
El Meniiy *et al*., 2021 (77)	-	IC_{50}: 21.00 – 1308.00 µg/mL
El Sohaimi, and Masry, 2014 (78)	8.98 – 99.20% (5.00 - 200.00 µg/mL)	IC_{50}: 73.49 and 81.67 µg/mL
Ezzat *et al*., 2019 (79)	12.93 – 91.42% (10.00 – 1280.00 µg/mL)	IC_{50}: 49.70 µg/mL
Falcão, 2013[1] (13)	-	IC_{50}: 8.00 – 93.00 µg/mL
Fangio *et al*., 2019 (80)	-	566.00 – 1477.00 µmol Trolox/g
Farias Azevedo *et al*., 2018 (81)	EEP: 79.10 – 95.70% (2.50 – 80.00 µg/mL); Nanoparticles: 58.90 – 100.00% (2.50 – 80.00 µg/mL)	-
Ferreira, 2015 (2)	4.46 – 17.95% (15.00 µg/mL); 6.85 – 29.66% (30.00 µg/mL); 8.23 – 40.77% (45.00 µg/mL); 9.66 – 52.18% (60.00 µg/mL)	-
Ferreira, 2017 (6)	-	About 120.00 – 310.00 µmol of equivalents of ascorbic acid/g
Ferreira, 2019 (82)	-	IC_{50}: 56.20 and 72.90 µg/mL
Ferreira *et al*., 2017 (83)	-	IC_{50}: 56.20 and 72.90 µg/mL
Fianco, 2014 (8)	-	IC_{50} Green propolis: 0.05 – 0.09 g of extract/g of DPPH IC_{50} Red propolis: 0.08 – 0.10 g of extract/g of DPPH

Galeotti *et al*., 2017 (84)	-	About 28.00 – 57.00 µg Trolox
Gonçalves, Santos, and Srebernich, 2011 (9)	60.93% (0.10%)	505.14 mg/L or 38.13 g of sample/g of DPPH
Guo *et al*., 2011 (85)	-	IAA: 0.28 – 3.29
Hames-Kocabas *et al*., 2013 (86)	-	IC_{50}: 4.95 – 37.09 µg/mL
Ikeda, 2020 (87)	-	EEP: 0.15 µmol Trolox/mg Essential oil: No activity
Irigoiti, Yamul, and Navarro, 2021 (88)	-	About 0.75 – 2.00 µg Trolox/g
Jansen, 2015 (40)	4.55 – 38.32% (25.00 µg/mL); 13.33 – 90.66% (125.00 µg/mL); 36.06 – 91.35% (250.00 µg/mL)	IC_{50}: 50.35 – 108.69 µg/mL (some samples did not reach IC_{50})
Jiang *et al*., 2020 (89)	-	IC_{50}: 71.19 – 432.08 µg/mL
Jug; Končić; Kosalec, 2014 (90)	-	IC_{50}: 29.00 -114.40 µg/mL
Kasote *et al*., 2017 (12)	-	0.29 – 0.38 mmol Trolox/mg
Kumar *et al*., 2011 (91)	-	IC_{50}: 75.00 µg/mL
Kumazawa *et al*., 2013 (21)	23.00 – 85.70%	-
Kumul *et al*., 2020 (92)	4.13 – 67.32%	-
Kunrath *et al*., 2017 (93)	-	IC_{50} EEP: 89.94 g of propolis/g of DPPH IC_{50} Dry extract: 5.86 g of propolis/g of DPPH
Kurek-Górecka *et al*., 2012 (94)	-	IC_{50}: 256.86 and 268.60 mg
Labyad *et al*., 2016 (39)	Plate methodology: positive	-
Lacerda, 2012 (35)	-	4.50 – 148.10 µmol Trolox/g
Luis-Villaroya *et al*., 2015 (95)	-	IC_{50}: 55.00 µg /mL
Machado *et al*., 2016 (96)	-	IC_{50} EEP: 31.80 – 273.46 IC_{50} Supercritical: 85.34 – 373.53
Marcussi, and Gutierrez-Gonçalves, 2013 (97)	-	IC_{50}: 14.83 – 56.29 µg/mL
Masek *et al*., 2019 (98)	About 20.00 – 85.00% (0.50 – 3.00 mg/mL)	-

Mello, and Hubinger, 2012 (99)	About EEP: 52.00 – 88.00% WEP: 25.00 – 45.00%	-
Mendez-Pfeiffer *et al*, 2020 (100)	-	IC_{50}: 58.80 – 98.70 µg/mL
Miguel *et al*., 2014 (101)	Winter: 35.76 – 92.28% Spring: 38.16 – 91.55%	-
Mohdaly *et al*., 2015 (102)	28.50% (5.00 µg/mL) and 85.00% (25.00 µg/mL)	-
Molnár *et al*., 2017 (103)	-	64.10 – 214.20 mg of equivalents of ascorbic acid/g
Moncayo Luján *et al*., 2018 (104)	11.40 – 12.39%	-
Naik, and Vaidya, 2011 (105)	17.10 – 81.09 (0.10 – 2.00 mg/mL)	IC_{50}: 320.00 µg/mL
Nina *et al*., 2015 (106)	-	IC_{50}: 10.29 – 91.84 µg/mL (some samples were inactive)
Nina *et al*., 2016 (107)	-	IC_{50}: 4.54 – 58.71 µg/mL
Nori *et al*., 2011 (108)	15.97 and 21.65% (600.00 ppm); 53.37 and 56.40% (1500.00 ppm); 84.10 and 84.94% (3000.00 ppm)	-
Oldoni *et al*., 2015 (109)	-	31.60 – 87.50 µg Trolox/g
Ozdal *et al*., 2018 (110)	-	391.73 mg Trolox/g
Ozdal *et al*., 2019 (111)	-	13.71– 63.33 mg Trolox/g
Pazin *et al*., 2017 (112)	-	IC_{50} (optical absorbance): 32.4 µg/mL IC_{50} (electronic spin resonance): 9.50 µg/mL
Peixoto *et al*., 2021 (18)	-	IC_{50}: 11.80 – 13.70 µg/mL
Permana *et al*., 2020 (113)	-	IC_{50}: 43.29 – 863.44 µg/mL
Prasniewski, 2015 (114)	-	11.68 – 175.77 µmol Trolox/g
Quintino *et al*., 2020 (115)	-	IC_{50}: 23.48 µg/mL
Ramnath, and Venkataramegowda, 2016 (116)	-	IC_{50}: 333.48 – 600.88 µg/mL
Righi *et al*., 2011 (117)	30.62% (8.00 µg/mg); 32.75% (12.50 µg/mg); 39.12% (25.00 µg/mg);	-

Ristivojević *et al*., 2018 (118)	26.49 – 65.64%	-
Ristivojević *et al*., 2020 (119)	40.51 and 53.21%	-
Rivera-Yañez *et al*., 2018 (120)	-	IC_{50}: 15.75 µg/mL
Rocha *et al*., 2013 (121)	-	IC_{50}: 33.36 and 56.71 µg/mL
Rodríguez *et al*., 2012 (122)	-	65.10 – 190.40 µmol Trolox/g
Rodríguez Pérez *et al*., 2020 (123)	-	IC_{50}: 26.00 – 950.40 µg/mL
Salgueiro, 2016 (37)	-	IC_{50} EEP: 30.65 – 56.71 µg/mL IC_{50} MEP: 6.93 – 23.69 µg/mL IC_{50} Soxhlet: 15.10 – 72.44 µg/mL
Salgueiro, and Castro, 2016 (124)	-	IC_{50}: 30.65 – 56.71 µg/mL
Sánchez *et al*., 2020 (125)	11.50 – 72.30%	-
Sanpa *et al*., 2017 (41)	-	IC_{50}: 19.90 – 67.20 µg/mL
Saral *et al*., 2019 (126)	-	IC_{50}: 20.00 – 130.00 µg/mL
Schmidt *et al*., 2014a (127)	-	IC_{50}: 17.13 – 83.60 µg/mL
Schmidt *et al*., 2014b (128)	-	IC_{50}: 29.00 – 87.83 mg/L
Shahbaz *et al*., 2021 (129)	WEP: 44.73% EEP: 61.26 – 73.18 % MEP: 59.01 – 70.06%	-
Shehata *et al*., 2020 (130)	About 28.00 – 90.00% (1.00 mg/mL)	-
Shubharani, Mahesh, and Yogananda Murti, 2019 (131)	-	IC_{50}: 78.90 – 358.20 µg/mL
Silva *et al*., 2018 (132)	92.40% (250.00 µg/mL); 22.10% (5.00 µg/mL); DPPH in plate: positive	IC_{50}: 3.97 µg/mL
Sime *et al*., 2015 (133)	48.60 – 87.80%	247.00 – 455.00 mg of equivalents of ascorbic acid/g
Sousa *et al*., 2019 (134)	-	IC_{50}: 11.46 – 77.30 µg/mL

Sulaiman *et al*., 2011 (135)	20.00 – 63.30% (1.00 µg/mL); 26.60 – 76.60% (10.00 µg/mL); 40.00 – 83.30% (100.00 µg/mL)	-
Sun *et al*., 2015 (136)	-	IC_{50} WEP: 13798.00 µg/mL IC_{50} EEP: 633.00 – 7129.00 µg/mL
Svečnjak *et al*., 2020 (137)	-	2.60 – 81.60 mg of equivalents of gallic acid/g
Tiveron, 2015 (27)	-	4.47 – 148.06 µmol Trolox/g
Tiveron *et al*., 2016 (138)	-	0.01 – 0.38 mg Trolox/g
Toreti, 2011 (139)	20.59 – 72.84%	1628.00 – 126164.06 µmol Trolox/g
Touzani *et al*., 2018 (140)	-	IC_{50}: 19.00 – 1190.00 µg/mL
Vargas-Sánchez *et al*., 2019 (141)	28.70 – 69.10% (12.50 – 500.00 µg/mL)	-
Vargas-Sánchez; Torrescano-Urrutia; Sánchez Escalante, 2020 (142)	83.20 – 85.10% (250,00 µg/mL)	-
Venegas *et al*., 2016 (143)		IC_{50}: 14.28 – 43.08 µg/mL
Wali *et al*., 2016 (144)	-	IC_{50}: 76.15 – 102.17 µg/mL
Wang *et al*., 2016 (145)	-	IC_{50}: 43.40 – 269.00 µg/mL
Wiwekowati *et al*., 2017 (146)	-	IC_{50}: 35.60 µg/mL
Xavier *et al*., 2017 (147)	14.80 and 44.70%	IC_{50}: 33.10 and 78.50 µg/mL
Yuan *et al*., 2020 (148)	-	IC_{50}: 34.61 and 47.28 µg/mL
Yurteri, 2015 (149)	96.00% (10.00 mg/mL)	IC_{50}: 4101.00 µg/mL
Zeitoun *et al*., 2019 (150)	-	IC_{50}: 122.80 µg/mL
Zhang *et al*., 2015 (151)	-	IC_{50}: 32.35 µg/mL
Zhang *et al*., 2016 (152)	-	IC_{50}: 19.55 – 43.85 µg/mL
Žižić *et al*., 2013 (153)	-	IC_{50}: 55.45 – 118.46 µg/mL

EEP: Ethanol Extract of Propolis; WEP: Water Extract of Propolis; MEP: Methanol Extract of Propolis

ORAC (Oxigen Radical Absorbance Capacity)

The ORAC Index (Oxygen Radical Absorbance Capacity), or evaluation of the antioxidant activity by oxygen radical absorption, is performed, *in vitro*, to measure the capacity antioxidant of a compound or its power to scavenge the peroxide radical through the transfer of a hydrogen atom (154).

Among the chosen works, the results of Castro *et al*. (2014) demonstrate variations between the components and the antioxidant capacity of the samples, although all have been collected in the same

city. These results reinforce the idea that propolis has extremely varied activities and chemical composition, with high dependence on external factors and the genetics of the hive (34).

The other ORAC test results arising from the materials of this review are presented in Table 02.

Table 2 | Results of the ORAC test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Results
Andrade *et al*., 2017 (43)	5343.84 – 6734.87 µmol Trolox/g
Andrade *et al*., 2018 (36)	4332.73 – 6261.96 µmol Trolox/g
Castro *et al*., 2014 (34)	8.90 – 33.10 µmol of equivalents of caffein acid/mg
Cavalaro, 2017[1](29)	2210.20 – 3524.00 µmol Trolox/g
Cavalaro, 2017[2](29)	21.30 – 13244.50 µmol Trolox/g
Correa *et al*., 2016 (59)	1352.16 – 5769.72 µmol Trolox/g (some samples were inactive)
Da Graça: Miguel *et al*., 2014 (63)	1106.42 – 2012.15 µmol Trolox/g
De Morais *et al*., 2021 (154)	4339.61 µmol Trolox/g
Lacerda, 2012 (35)	0.18 - 1.25 µmol Trolox/g
Pandolfo, 2014 (1)	150.00 – 4520.00 µmol Trolox/g
Rodríguez *et al*., 2012 (122)	475.00 – 2211.90 µmol Trolox/g
Silva *et al*., 2011 (155)	1800.00 – 9000.00 µmol Trolox/g
Sun *et al*., 2015 (136)	WEP: 138.50 µmol Trolox/g EEP: 918.00 – 27595.40 µmol Trolox/g
Tiveron, 2015 (27)	500.00 – 1950.00 µmol Trolox/g
Tiveron *et al*., 2016 (138)	500.00 – 1950.00 µmol Trolox/g
Vargas Tapia, 2018 (156)	About 50.00 – 267.48 µmol Trolox/mL
Zhang *et al*., 2015 (151)	9250.00 µmol Trolox/g
Zhang *et al*., 2016 (152)	5600.00 – 9250.00 µmol Trolox/g

EEP: Ethanol Extract of Propolis; WEP: Water Extract of Propolis.

FRAP (Ferric Reducing Antioxidant Power)

This colorimetric test, called the antioxidant test for determining the power of iron ion reduction, has the advantage of being able to use complex samples, biological fluids, and pure substances in aqueous solutions, in addition to being relatively simple, with easy standardization (34,85).

Phenolic compounds are the class of compounds with the greatest biological activity of propolis, especially in terms of antioxidant activity. As far as the FRAP methodology is concerned, the same applies. These molecules have great power to reduce iron ions, due to their ability to donate electrons. The samples that have the highest number of these compounds are those that obtain the best results in this methodology (40).

The results of the studies are shown in Table 03. Again, in most cases, the propolis samples performed similarly or better than the commercial pure antioxidants.

Table 3 | Results of the FRAP test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Results
Abubaker, and Fageer, 2017 (157)	3790.00 and 36530.00 µmol of equivalents of vitamin C/mL
Alves, 2018 (5)	EEP: 815.75 µmol Trolox/g Microcapsules: 34.14 – 139.31 µmol Trolox/g
Andrade *et al*., 2017 (43)	471.51 – 633.18 µmol Trolox/g
Andrade *et al*., 2018 (36)	144.87 – 396.09 µmol Trolox/g
Arslan *et al*., 2021 (47)	3813.20 and 4017.70 µmol Iron/g
Béji-Srairi *et al*., 2020 (50)	IC_{50}: 375.00 – 780.00 µg/mL
Bhargava *et al*., 2014 (51)	1.97 and 3.75 mg/mL (5.00 and 10.00%)
Calegari, 2018 (26)	1571.00 – 1830.00 µmol Iron/g
Calegari *et al.,* 2017 (3)	2013: 89.70 – 286.70 µmol Iron/g 2015: 638.1 – 1041.0 µmol Iron/g
Castro *et al*., 2014 (34)	1700.00 – 3200.00 µmol of equivalents of cafeic acid/g
Cavalaro, Fabricio, and Vieira, 2020 (55)	36231.00 µmol Iron/g
Cottica *et al*., 2011 (60)	528.00 - 1365.00 µmol Iron/g
Dărăban *et al*., 2019 (64)	51.96 – 93.79 µmol Trolox/mL
Da Silva *et al*., 2018 (66)	66.74 – 1164.00 µmol Iron/g
De Francisco *et al*., 2018 (30)	1536.40 µmol Iron/g
De Morais *et al*., 2021 (154)	1472.86 µmol Iron/g
De Oliveira, and Andolfatto, 2014 (70)	60.67 µmol Iron/g
Ding *et al*., 2021 (73)	126.00 – 290.34 µmol Iron/g
Ertürk *et al*., 2016 (158)	361956.00 mmol FRAP
Ferreira, 2017 (6)	About 200.00 – 1200.00 µmol Iron/g
Gokduman, 2019 (159)	247.64 mg Trolox/g
Ikeda, 2020 (87)	EEP: 1386.14 µmol Iron/mg Essential oil: 183.98 µmol Iron/mg
Jansen, 2015 (40)	750.00 – 4670.00 µmol Iron/g
Kasote *et al.,* 2017 (12)	130000.00 – 7340000.00 µmol Trolox/g
Kiziltas, and Erkan, 2020 (160)	1.54– 5.98 µmol Trolox/g
Kunrath *et al.,* 2017 (93)	EEP: 50.98 µmol Iron/g Dry extract: 543.40 µmol Iron/g
Masek *et al*., 2019 (98)	About 0.75 – 2.50 (absorbance difference)
Mello, and Hubinger, 2012 (99)	EEP: 219.04 – 2078.57 µmol Iron WEP: 180.95 – 1308.09 µmol Iron

Nina *et al*., 2015 (106)	667.43 – 1754.03 µmol Trolox/g (some samples were inactive)
Nina *et al*., 2016 (107)	246260.00 – 905950.00 µmol Trolox/kg (some samples were inactive)
Oldoni *et al*., 2015 (109)	259.30 µmol Iron/g
Ozdal *et al*., 2018 (110)	156.59 mg Trolox/g
Prasniewski, 2015 (114)	66.74 – 837.17 µmol Iron/g
Rodríguez *et al*., 2012 (122)	112.10 – 321.30 µmol of equivalents of ascorbic acid/g
Salgueiro, 2016 (37)	EEP: 2607.90 – 6088.50 mmol Iron/g MEP: 4137.90 – 14147.20 mmol Iron/g Soxhlet: 2606.20 – 4907.90 mmol Iron/g
Salgueiro, and Castro, 2016 (124)	2607.90 – 6088.50 mmol Iron/g
Sánchez *et al*., 2020 (125)	1.00 – 7.20 mg Iron/g
Saral *et al*., 2019 (126)	166.91- 1600.25 µmol de Iron/g
Shubharani, Mahesh, and Yogananda Murti, 2019 (131)	IC_{50}: 2.86 – 5.71 µg/mL
Sousa *et al*., 2019 (134)	2075.50 – 3472.00 mmol Iron/g
Sun *et al*., 2015 (136)	WEP: 20.00 µmol Trolox/mg EEP: 16.00 – 233.00 µmol Trolox/mg
Svečnjak *et al*., 2020 (137)	100.00 – 800.00 µmol Iron/g
Touzani *et al*., 2018 (140)	IC_{50}: 39.00 – 1080.00 µg/mL
Vargas Tapia, 2018 (156)	About 18.00 - 49.30 µmol Iron/mL
Xavier *et al*., 2017 (147)	42.00 and 157.60 mg Trolox/g

EEP: Ethanol Extract of Propolis; WEP: Water Extract of Propolis; MEP: Methanol Extract of Propolis.

Reducing Power (RP)

Similar to the FRAP method, the test called “reducing power” (RP) also explores the ability to reduce iron ions through electron donation (13). The results of this methodology found in the works of this literature review are presented below, in Table 04.

Table 4 | Results of the RP test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Results
Cao *et al.,* 2017 (7)	1.73 mmol Trolox/g
Coelho, 2013 (58)	0.07 – 0.99 g of equivalents of caffeic acid: galangin: pinocembrin (1:1:1)/g
Coelho *et al.,* 2017 (20)	0.09 – 0.68 g of equivalents caffeic acid: galangin: pinocembrin (1:1:1)/g
Da Graça Miguel *et al*., 2014 (63)	About; there was an increase in absorbance (0.25 – 2.60) at the concentration of 1.00 mg/mL

Ding *et al*., 2021 (73)	About 0.12 – 0.35 (5.00 mg/mL)
El Meniiy *et al*., 2021 (77)	IC_{50}: 42.00 – 1512.00 µg/mL
Falcão, 2013[1](13)	110.00 – 757.00 mg of equivalents of caffeic acid: galangin: pinocembrin (1:1:1)/g
Guo *et al*., 2011 (85)	1.20 – 3.47 (K value: increase in absorbance)
Mouhoubi-Tafinine, Ouchemoukh, and Tamendjari, 2016 (24)	About 1.00 – 25.00 mg of equivalents of galic acid/g
Rocha *et al*., 2013 (121)	IC_{50}: 270.00 and 282.00 µg/mL
Shehata *et al*., 2020 (130)	About 0.20 – 0.90 (1.00 mg/mL)
Vargas-Sánchez *et al*., 2019 (141)	0.07 – 0.56% (12.50 – 500.00 µg/mL)
Vargas-Sánchez; Torrescano-Urrutia; Sánchez Escalante, 2020 (142)	>50.00% of inhibition (125.00 and 250.00 µg/mL)
Wali *et al*., 2016 (144)	Approximate absorbance from 1.00 to 1.80 (concentration of 150.00 µg/mL)
Zhang *et al*., 2015 (151)	2.08 mmol Trolox/g
Zhang *et al*., 2016 (152)	1.53 – 2.70 mmol Trolox/g

CUPRAC (Cupric Ion Reducing Antioxidant Capacity)

The CUPRAC method is based on measuring the reducing capacities of substances using the reduction of copper ions (23). It is considered cheap, fast (30 minutes), simple and versatile, able to analyze several compounds, such as polyphenols, flavonoids, vitamins, and synthetic antioxidants. Results are generally expressed in Trolox equivalents (161).

Compared to other methods, it is observed that CUPRAC presents higher antioxidant capacity values, mainly due to the difference in solubility between the solvents used in the tests. Additionally, when compared to FRAP, copper has a faster reaction kinetics. Concerning ABTS and DPPH, CUPRAC has more accessible and stable reagents (110).

The values obtained for the CUPRAC test of the works in this review are shown in Table 05.

Table 5 | Results of the CUPRAC test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Results
Bhuyan *et al*., 2021 (52)	Percentage of inhibition: 21.91 – 95.60% (4.19 – 268.30 µg/mL) IC_{50}: 4.84 – 14.21 µg/mL
Dărăban *et al*., 2019 (64)	123.78 – 357.21 µmol Trolox/mL
Masek *et al*., 2019 (98)	About 0.40 -1.8 (absorbance difference)
Ozdal *et al*., 2018 (110)	1184.94 mg Trolox/g
Ozdal *et al*., 2019 (111)	24.62 – 85.80 mg Trolox/g
Özkök *et al*., 2021 (23)	95.35 – 710.43 mg Trolox/g
Saral *et al*., 2019 (126)	270.00 – 400.00 µmol Trolox/g

ABTS ([2,29-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)])

ABTS assay is based on the decolorization reaction, in which a radical in a stable form is produced before meeting an antioxidant (1,21). Generally, the standard used is Trolox, and the results are expressed as a value of TEAC (Trolox Equivalent Antioxidant Capacity) (162). It has as an advantage reliable results both for fat-soluble and water-soluble antioxidants (1) and for pure substances, mixtures, and plant extracts (29). Furthermore, it maintains its stability over a wide pH range (163), it is fast, cheap, and sensitive (1).

Interestingly, the ABTS test radical is more reactive than the DPPH test. Therefore, their values tend to be more representative of the real antioxidant power of the sample (12,26). This fact occurs because ABTS manages to solubilize itself in both aqueous and oily media, reaching a greater number of targets than DPPH, which is usually present in organic media (138).

The data from the literature review materials for this test are presented in Table 06. Corroborating with other methodologies, the *A. mellifera* propolis has shown good antioxidant activity. For example, the article produced by Salas *et al*. (2016), with similar value to pure standards (164).

Table 6 | Results of the ABTS test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Activity Percentage (%)	Other results
Andrade *et al*., 2017 (43)	-	1868.45 – 2913.55 µmol Trolox/g
Andrade *et al*., 2018 (36)	-	10623.48 – 15106.07 µmol Trolox/g
Augusto-Obara *et al*., 2019 (48)	-	8052.00 – 8773.60 µmol Trolox/g
Béji-Srairi *et al*., 2020 (50)	-	IC_{50}: 244.00 – 616.00 µg/mL
Bhuyan *et al*., 2021 (52)	4.52 - 98.74% (0.78 to 50.00 µg/mL)	IC_{50}: 5.60 – 7.77 µg/mL
Boulechfar *et al*., 2019 (165)	Essential oil: 27.51% MEP: 92.03%	Essential oil IC_{50}: 516.05 µg/mL MEP IC_{50}: 10.08 µg/mL
Calegari *et al.,* 2017 (3)	-	2013: 25.50 – 109.20 µmol Trolox/g 2015: 298.10 – 439.20 µmol Trolox/g
Calegari, 2018 (26)	-	1130.00 µmol Trolox/g
Cao *et al.,* 2017 (7)	-	IC_{50}: 110.28 µg/mL
Cavalaro, 2017[2] (29)	-	408.66 – 13412.14 µmol Trolox/g
Cruz, 2011 (61)	-	46.29 mg of equivalents of gallic acid/g
Da Cunha, 2017 (166)	-	253.56 µmol Trolox/mL
Da Graça Miguel *et al*., 2014 (63)	-	IC_{50}: 9.00 – 1009.00 µg/mL
Dărăban *et al*., 2019 (64)	-	IC_{50}: 0.59 – 0.83 µL
Da Silva *et al*., 2018 (66)	-	19.03 – 1077.00 µmol Trolox/g
De Francisco *et al*., 2018 (30)	-	IC_{50}: 2286.16 µg/mL
De Lima *et al.,* 2019a (25)	-	IC_{50}: 30.10 µg/mL
Del Río Del Rosal *et al*., 2017 (167)	-	16.66 – 63.45 µmol Trolox/g
De Morais *et al*., 2021 (154)	-	2700.00- 35508.8 µmol Trolox/g
De Oliveira, and Andolfatto, 2014 (70)	-	52.08 µmol Trolox/g

Ding *et al*., 2021 (73)	-	36.76 – 106.73 µmol Trolox/g
El Meniiy *et al*., 2021 (77)	-	IC_{50}: 26.00 – 1529.00 µg/mL
Fangio *et al*., 2019 (80)	-	843.00 – 1683.00 µmol Trolox/g
Ferreira, 2017 (6)	-	About 10.00 -100.00 µmol of equivalents of ascorbic acid/g
Gargouri *et al*., 2019 (168)	-	109.76 – 252.90 µmol Trolox/g
Ikeda, 2020 (87)	-	About EEP: 3.12 µmol Trolox/mg Essential oil: 0.40 µmol Trolox/mg
Irigoiti, Yamul, and Navarro, 2021 (88)	-	1.00 - 3.00 µmol Trolox/g
Jansen, 2015 (40)	-	2.45 - 18.00 µg Trolox/mL
Kasote *et al.,* 2017 (12)	-	680.00 – 429.00 µmol Trolox/mg
Kumazawa *et al*., 2013 (21)	23.00 – 71.20%	-
Kumul *et al*., 2020 (92)	-	6310.00 – 64290.00 µmol Trolox/g
Kurek-Górecka *et al*., 2012 (94)	-	139380.00- 153520.00 µmol Trolox
Lacerda, 2012 (35)	-	1.01 – 384.60 mg Trolox/g
Machado *et al*., 2016 (96)	EEP: 77.90 – 98.50% Supercritical: 49.60 – 87.60%	-
Masek *et al*., 2019 (98)	About 35.00 – 89.00% (0.50 – 3.00 mg/mL)	-
Miguel *et al*., 2014 (101)	Winter: 30.18 – 78.90% Spring: 24.77 – 64.76%	-
Mohdaly *et al*., 2015 (102)	94.34% (25.00 µg/mL)	-
Naik, and Vaidya, 2011 (105)	-	420.00 – 5150.00 µmol Trolox (0.10 to 2.00 mg/mL) IC_{50}: 180 µg/mL
Nina *et al*., 2015 (106)	-	870.64 – 2328.66 µmol Trolox/g
Nina *et al*., 2016 (107)	-	225.43 – 2666.56 µmol Trolox/g
Oldoni *et al*., 2015 (109)	-	95.88 µmol Trolox/g
Osés *et al*., 2016 (169)	-	1184.66 -1400.86 µmol Trolox/g
OSÉS *et al*., 2020 (170)	-	280.00 – 470.00 µmol Trolox/g
Ozdal *et al*., 2018 (110)	-	422.82 mg Trolox/g
Pandolfo, 2014 (1)	-	120.00 – 2400.00 µmol Trolox/g
Prasniewski, 2015 (114)	-	22.49 – 354.40 µmol Trolox/g
Quintino *et al*., 2020 (115)	-	IC_{50}: 32.18 µg/mL
Ramnath; Venkataramegowda, 2016 (116)	-	IC_{50}: 298.86 – 860.32 µg/mL
Rodríguez *et al*., 2012 (122)	-	739.70 – 1918.40 µmol Trolox/g

Salas *et al*. 2016 (164)	-	IC_{50}: 14.00 µg/mL
Salas *et al*., 2018 (171)	-	IC_{50}: 23.00 – 30.00 µg/mL
Salas *et al*., 2020 (172)	-	IC_{50}: 29.50 – 33.70 µg/mL
Salgueiro, 2016 (37)	-	EEP: 848600.00 – 1576200.00 µmol Trolox/g MEP: 946300.00 – 1163300.00 µmol Trolox/g Soxhlet: 747800.00 – 2139000.00 µmol Trolox/g
Salgueiro, and Castro, 2016 (124)	-	848600.00 – 1576200.00 µmol Trolox/g
Shehata *et al*., 2020 (130)	About 29.00 - 90.00% (1.00 mg/mL)	-
Shubharani, Mahesh, and Yogananda Murti, 2019 (131)	-	IC_{50}: 15.10 – 550.00 µg/mL
Sousa *et al*., 2019 (134)	-	677600.00 -1068200.00 µmol Trolox/g
Sun *et al*., 2015 (136)	-	IC_{50} WEP: 10310.00 µg/mL IC_{50} EEP: 520.00 – 5520.00 µg/mL
Tiveron, 2015 (27)	-	1.01 – 384.62 mg Trolox/g
Tiveron *et al*., 2016 (138)	-	0.29 – 1.05 µmol Trolox/g
Touzani *et al*., 2018 (140)	-	IC_{50}: 21.00 – 983.00 µg/mL
Yurteri, 2015 (149)	-	0.06 – 2.97 µmol Trolox/g (10.00 – 50.00 µg/mL
Zhang *et al*., 2015 (151)	-	IC_{50}: 40.50 µg/mL
Zhang *et al*., 2016 (152)	-	IC_{50}: 20.00 – 40.50 µg/mL

EEP: Ethanol Extract of Propolis; WEP: Water Extract of Propolis; MEP: Methanol Extract of Propolis

β -Carotene/Linoleic Acid

This system is based on the ability to protect a sample against free radical peroxidative degradation of the lipid substrate linoleic acid (35,37). It is widely used, as it is simple and sensitive, including to analyze extracts of plants (90), as β-carotene is very susceptible to free radicals in the environment (16), in addition to not involving the use of higher temperatures, preventing the degradation of thermosensitive compounds (37).

The analysis of lipid peroxidation is often used due to the sensory and nutritional changes it can cause in food and beverages and the lost nutritional factor, which leads to food waste (29,173). Lipid peroxidation also affects cellular levels; plasma membranes are especially sensitive to ROS attack, promoting changes in their permeability and can even lead to cell death due to the extravasation of cytotoxic enzymes (174).

Second Isla *et al*. (2009), one of the typical compounds of propolis that may be involved in the performance of this plant material in this test is galangin, so synergistic with the other components (175). Generally, results are expressed as IC_{50} (90) or are compared to the Trolox standard (37).

Other results of the review materials are listed below in Table 07.

Table 7 | Results of the β-carotene/linoleic acid system test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Inhibition Percentage (%)	Other results
Béji-Srairi *et al*., 2020 (50)	-	IC_{50}:1300.00 – 2000.00 µg/mL
Boulechfar *et al*., 2019 (165)	Essential oil: 48.51 % MEP: 91.98%	Essential oil - IC_{50}: 198.01 µg/mL MEP - IC_{50}: 43.46 µg/mL
Ceylan, and Halime, 2020 (57)	39.21 – 91.10%	-
De-Melo *et al*., 2014 (16)	51.33 – 92.70 %	-
De Souza *et al*., 2018 (71)	75.50%	-
Ferreira, 2015 (2)	16.86 – 54.25% (40.00 µg/mL); 21.92 – 66.30% (80.00 µg/mL); 15.92 – 75.09% (120.00 µg/mL)	-
Ferreira, 2019 (82)	-	IC_{50}: 101.10 and 106.40 µg/mL
Ferreira *et al*., 2017 (83)	-	IC_{50}: 101.10 and 106.40 µg/mL
Jug, Končić, and Kosalec, 2014 (90)	-	IC_{50}: 14.20 – 55.50 µg/mL
Kurek-Górecka *et al*., 2012 (94)	69.64 and 70.30%	-
Righi *et al*., 2011 (117)	84.50% (1.00 mg/mL); 85.30% (1.50 mg/mL); 85.70% (2.00 mg/mL)	-
Salas *et al*, 2016 (164)	-	IC_{50}: 26.00 and 29.00 µg/mL
Shahbaz *et al*., 2021 (129)	WEP: 39.21% EEP: 49.94 – 60.59% MEP: 48.70 – 57.01%	-

EEP: Ethanol Extract of Propolis; WEP: Water Extract of Propolis; MEP: Methanol Extract of Propolis

Other Forms of Lipid Peroxidation Analysis

There are also other methods able to assess lipid peroxidation. This diversity is due to the great importance of lipid degradation reactions in human health and, especially, in the food industry. One of the common tests used to evaluate lipid peroxidation is the TBARS (Thiobarbituric acid reactive substances), which measures mostly the MDA (malondialdehyde). However, this methodology can also evaluate other volatile substances produced in the lipid oxidation processes (28). Results can be expressed in TBA units: weight in mg of MDA per kg of the sample (174); or even, in Antioxidant Index (AI) – a percentage calculated according to the following formula: IA (%) = 1 – (Sample Abs/Control Abs) x 100, where “Abs” refers to the absorbance obtained (176).

Another methodology used to study lipid peroxidation is the Rancimat method. This test is based on the difference of electrical conductivity of distilled water after the formation of volatile molecules produced by lipid peroxidation reactions, under the accelerated conditions of the method, such as high

temperatures (110 - 130°C) and the presence of oxygen-rich air current (102). Generally, the oxidative stability of the medium is assessed over a period under standard conditions (35). In some cases, the results can be expressed as a "protection factor", in which higher values above one indicates inhibition of lipid peroxidation (178).

The iron thiocyanate methodology is based on quantifying peroxides produced in the initial stages of lipid peroxidation. These substances react in the medium, forming ferric ions that unite with the ammonium thiocyanate and produce the red thiocyanate iron, which devices can detect (132). Still, regarding lipid peroxidation, LDL (Low-Density Lipoprotein) oxidation can be evaluated. It is currently known that this process is one of the first steps to develop some diseases, such as atherosclerosis (155).

Among the different substances that make up the complex propolis, phenolic compounds are responsible for their biological activity. About inhibition of lipid peroxidation, the same turns out to be true. The lipophilic groups of phenolic compounds interact with plasma membrane molecules, preventing their damage. Flavonoids also play an important role in this regard, emphasizing quercetin and rutin, which were shown to be more efficient in inhibiting MDA formation than hesperetin and naringenin, due to their great ability to interact with the membrane phospholipids and the possibility of donating hydrogen atoms, present in their hydroxyl groups (141).

The results found in the works of this literature review for lipid assessment methodologies, are shown below, in Table 08.

Table 8 | Results of other methodologies to assess lipid peroxidation of propolis extracts from *Apis mellifera* obtained from the materials chosen for this literature review.

Author	Test methodology	Results
Boufadi et al., 2014 (53)	TBARS	Peroxidation Inhibition percentage (about): 25.00 – 82.00% (100.00 µg/mL)
Boufadi et al., 2014 (53)	LDL	Inhibition percentage: 77.00 – 99.00% (20.00 µg/mL)
Cavalaro, 2017[2](29)	Iron thiocyanate	At the end of the forced lipid peroxidation process, the propolis emulsion maintained the hydroperoxide levels at the same values as the controls with synthetic antioxidants.
Ceylan, and Halime, 2020 (57)	Iron thiocyanate	Inhibition percentage: 34.74 – 51.77%
Da Graça Miguel et al., 2014 (63)	TBARS	IC_{50}: 14.00 – 699.00 µg/mL
Graikou et al., 2016 (178)	Rancimat	Activities ranged from >1, =1 and <1 (protection factor)
Jeong et al., 2012 (179)	TBARS	IC_{50}: 35.65 µg/mL
Mello, and Hubinger, 2012 (99)	Iron thiocyanate	Decreased absorbance indicates the strong antioxidant activity of propolis extracts.
Mohdaly et al., 2015 (102)	Rancimat	Stabilization factor: 13.7
Osés et al., 2020 (170)	TBARS	0.099 – 0.117 mmol of equivalents of uric acid/g
Permana et al., 2020 (113)	Iron thiocyanate	IC_{50}: 65.32 – 1503.00 µg/mL

Shehata et al., 2020 (130)	TBARS	About 25.00 – 90.00% (1.00 mg/mL)
Silva et al., 2011 (155)	LDL	Propolis samples with higher polyphenol concentrations were able to inhibit lipid oxidation.
Silva et al., 2018 (132)	Iron thiocyanate	>86.00% (5.00 – 100.00 µg/mL)
Tiveron, 2015 (27)	Lipid peroxidation by peroxide index	Propolis extracts (100.00 mg/kg) had good protection against soybean oil oxidation (1.70 – 4.90 mmol O_2/kg) when compared to the control (5.52 mmol O_2/kg).
Xavier et al., 2017 (147)	Lipid peroxidation	60.00 - 90.00% protection for lipid membranes

Food Tests

Rancidity analysis helps ensure the quality and safety of food products and the choice ofproducts by the customer, reducing waste (28).

Alves (2018) studied the insertion of microcapsules of ethanolic extract of propolis (2.00 g) into cakes. The antioxidant activity of the ready food was tested by the DPPH method, with results that ranged from 10.32 - 11.71% inhibition. Such values were higher than those found in cakes with the addition of liquid extract (5.26%), demonstrating that the encapsulation protected the bioactive compounds from the oven temperature. Furthermore, research using a sensory panel showed that the encapsulation caused the organoleptic parameters of the cake were not significantly different from the control, which did not show the addition of extracts (5).

Some works that make up the collection of this review evaluated the protection of propolis extract in salami. These meat products have a large amount of fat, which is responsible for many of their pleasant characteristics, such as juiciness and flavor (28).

Kunrath, and Savoldi (2014) and Kunrath *et al*. (2017) studied how the addition of ethanolic extracts of propolis, atomized by a spray dryer, influences the peroxidation lipid from Italian salami stored for 35 days. The results of the TBARS test showed that the presence of the extract (0.01% and 0.05%) reduced the amount of malondialdehyde present in salami (0.46 and 0.22 mg of MDA/kg) when compared to non-treated ones (0.51 mg of MDA/kg). Also, the sensory panel analysis demonstrated that the salami that received the propolis did not differ significantly in sensory parameters compared to the control salami (28,93).

Vargas-Sánchez *et al*. (2019) opted to study the antioxidant activity of propolis in extracts from beef and pork steaks. When compared to the control group, samples that received the extract of propolis had an 88.70% decrease in MDA production by the TBARS method. In addition, this natural product reduced the protein oxidation of the meat sample (141).

Electrochemical Techniques: Voltammetry

The reducing power of a substance can be characterized by its oxidizing potential, both of which are inversely proportional. One way to evaluate the power reducer of antioxidant substances is using electrochemical techniques (180). Such methodologies prove to be useful not only to obtain detailed information about the antioxidant potential but also to observe the number of electrons involved in the process, reaction mechanisms, process reversibility, and equilibrium constants. The most popular electrochemical techniques are differential pulse voltammetry, with high resolution and accuracy, and cyclic voltammetry (98,181).

Through the cyclic voltammetry technique, the oxidative process of a substance is characterized by the generation of a potential difference between the electrodes of the electrochemical cell (work and reference). Then, the anodic peak potential (Ep) and the current intensity magnitude (Ip) are analyzed. The Ep can be associated with the information of electronic transfer energy, providing data on a substance's antioxidant potential. The Ip parameter also predicts an estimate of the antioxidant capacity relating to the electrical charge potentially transferred (181,182).

The differential pulse technique is based on measuring the current immediately after a pulse is released, as well as at the end of it. It uses a solid electrode, which reduces adsorption problems and increases test sensitivity (58).

One way of expressing results is in TEAP (Total Electrochemical Antioxidant Power). This value corresponds to the sum of the current density of each electrochemical process value, obtained at the maximum peak (58).

The fact that they can be used in the analysis of isolated substances or complex extracts and being sensitive, selective, and reproducible, even for samples from biological matrices can be mentioned as an advantage of this methodology. Furthermore, they allow the analysis of antioxidant molecules without the need for the use of additional reagents, being quick and simple processes. However, it is important to emphasize that, concurrently with electrochemical processes, and other chemical reactions may also occur. For this reason, the correlation of parameters of this technique is not always perfectly correct (13,181). The values obtained for the voltametric techniques of the present literature review are shown in Table 09.

Table 9 | Results of the voltammetry test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Results
Coelho, 2013 (58)	0.06 – 0.49 (electrochemical antioxidant power – 0.40 V) or 0.06 - 0.60 mg of p-cumaric acid/mL
Coelho *et al*., 2017 (20)	0.06 – 0.49 (electrochemical antioxidant power – 0.40 V) or 0.06 - 0.60 mg of p-cumaric acid/mL
De Oliveira, 2015 (182)	19.00 – 54.00 mmol of equivalents of galic acid (Ipa)
Falcão, 2013[4](13)	0.14 – 1.14 V or 9.00 – 73.00 TEAP
Masek *et al*., 2019 (98)	1.12 – 1.39 V

Metal Chelation

The role of transition metals in oxidative stress is already well-established. Among them, cobalt, vanadium, arsenic, nickel, and chromium can be mentioned. Also, iron and copper stand out, capable of promoting the production of hydroxyl radicals through the Fenton reaction. There is the interaction of reduced metallic ions with hydrogen peroxide, giving rise to free radicals and oxidized metallic ions. The products of this reaction are capable of degrading cell plasma membranes through oxidative attack to the abundant polyunsaturated fatty acids abundantly present. Also, metals can be electron donors or act as catalysts for chemical reactions (90,183).

Substances able to chelate these ions and thus prevent oxidative damage to biological molecules are classified as secondary antioxidants (Figure 03). By making them less available in the medium, their oxidative potential is reduced, stabilizing them, and reducing the likelihood of interacting with molecular targets (90).

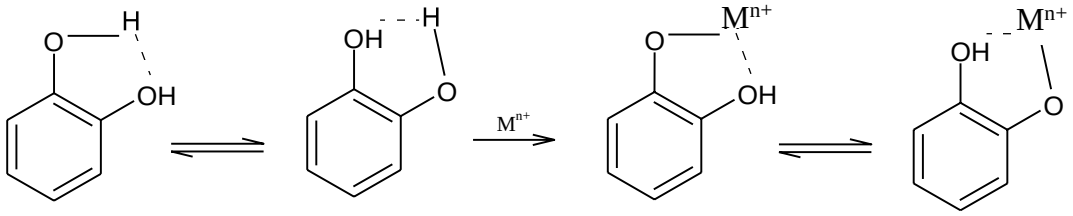

Figure 03 | Schematic process of the chelation mechanism for metals. Source: Adapted from Salgueiro, 2016 (37).

Propolis has compounds capable of helping in this picture described through the chelation of metals in the medium. For example, apigenin, naringenin, and diosmin (101). Through electrochemical measurements, other substances, such as rutin and catechin, have been described as important zinc and copper chelators (181).

Due to its high reactivity, iron is known as a direct and indirect inducer of lipid peroxidation. Due to its great importance in oxidative stress and damage to cell structures, iron is one of the most studied by researchers (157). In this review, all studies evaluated the power of propolis samples in sequestering the iron ion. The results are shown in Table 10.

Table 10 | Results of the iron chelation test of *Apis mellifera* propolis extracts obtained from the materials

chosen for this literature review.

Author	Results
Abubaker, and Fageer, 2017 (157)	8.73 – 43.25% (125.00 – 1000.00 µg/ml)
Jug, Končić, and Kosalec, 2014 (90)	IC_{50}: 324.90 – 1840.40 µg/mL
Miguel *et al*., 2014 (101)	Winter: 43.92 – 82.35% Spring: 41.11 – 71.34%
Wali *et al*., 2016 (144)	IC_{50}: 74.94 – 136.88 µg/mL

Enzymatic Activity

The organism naturally has mechanisms to control oxidizing agents, such as degradation enzymes (catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione S-transferase, among others), and different support molecules, such as reduced glutathione, a cofactor for enzymes, which has thiol groups responsible for scavenging free radicals; if such molecules fail to produce or are inhibited, there is a decrease in the redox potential of the cells and the organism enters oxidative stress, leading to cell damage (184), mitochondrial damage, caspase activation and apoptosis (4).

One of the first lines of antioxidant defense is the metalloenzyme superoxide dismutase (SOD), whose function is to catalyze the transformation of the superoxide radical into hydrogen peroxide or oxygen. Catalase (CAT), in turn, is present in cellular peroxisomes and is responsible for breaking two hydrogen peroxide molecules into molecular oxygen and water, in a reaction of dismutation (184,185).

Glutathione reductase manages to eliminate several harmful compounds, such as singlet oxygen, hydroxyl radicals, and several other electrophiles. Glutathione peroxidase can convert hydroperoxyls and hydrogen peroxide to water (186).

Myeloperoxidase (MPO) is contrary to the enzymes mentioned so far. It is found in neutrophil granules, responsible for catalyzing the reaction between chloride and hydrogen peroxide ions, with the production of hypochlorous acid. This acid plays a crucial role in the destruction of invading pathogens. However, when found in extracellular tissues, or cases of exacerbation of the immune response and chronic diseases, with the so-called “neutrophil net”, there may be an excess of free radicals in the medium, causing damage to various biomolecules (53).

The function of xanthine oxidase is to produce uric acid, with lipoxanthine and xanthine as substrates. After this process, the enzyme is reoxidized, at which point molecular oxygen acts as an electron acceptor, culminating in the production of hydrogen peroxide and superoxide radicals. The lipoxygenase enzyme (LOX) is also noteworthy, essential in the emergence of inflammatory processes and playing a role in oxidative phenomena, with the catalysis of unsaturated acids oxidation reactions. It is responsible for producing inflammatory leukotrienes from arachidonic acid. Furthermore, it promotes the conversion of linoleic acid to 13-hydroperoxide linoleic acid, which can be quantified by a spectrophotometer at 234 nm (63,187).

Due to the great importance that such catalytic biomolecules develop in protecting against oxidative stress, some works strive to verify the activity of these enzymes. In some cases, it is interesting that the enzymes are overactive or over-expressed, which helps the body deal with free radicals such as SOD and catalase. Moreover, in others, enzyme inhibition is more advantageous if the enzyme is a producer of oxidizing species or has a role in some of the cellular damage pathways and tissue, such as MPO.

The propolis compounds generally associated with antioxidant activity with an enzyme modulating mechanism are phenolic compounds and flavonoids, such as galangin. LOX is inhibited by CAPE (caffeic acid phenethyl ester), caffeic acid, quercetin, and naringenin molecules (187).

The results of enzymatic activity after the addition of the propolis extract are listed below, in Table 11. Generally, they were expressed as IC_{50} in the case of enzymes harmful to the organism.

Table 11 | Results of the test to assess the enzymatic modulation of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Test methodology	Results

Boufadi *et al*., 2014 (53)	Inhibition of MPO	IC_{50}: 10.30 – 48.70 µg/mL
Da Graça Miguel *et al*., 2014 (63)	Inhibition of LOX	IC_{50}: 149.00 – 2521.00 µg/mL
El-Guendouz *et al*., 2016 (187)	Inhibition of LOX	IC_{50}: 20.00 – 653.00 µg/mL
El-Guendouz *et al*., 2016 (187)	Inhibition of xanthine oxidase	IC_{50}: 8.00 – 3116.00 µg/mL
Salas *et al*, 2016 (164)	Inhibition of cyclooxygenase 2	IC_{50}: 100.00 and 106.00 µg/mL
Salas *et al*, 2016 (164)	Inhibition of LOX	IC_{50}: 63.90 and 94.90 µg/mL
Salas *et al*., 2018 (171)	Inhibition of LOX	IC_{50}: 90.00 – 100.00 µg/mL
Shubharani, Mahesh, and Yogananda Murti, 2019 (131)	Activity of SOD	IC_{50}: 21.65 – 25.91 µg/mL
Silva *et al*., 2011 (155)	eNOS	Propolis samples increased eNOS expression
Silva *et al*., 2011 (155)	NADPH oxidase	Propolis samples decreased NADPH oxidase activity

ROS and RNS Scavenging

Among the most famous free radicals studied, there are the so-called ROS, or Reactive Oxygen Species, which can be mentioned the hydroxyl radical ($^{\bullet}OH$) the most reactive of them, the superoxide radical ($O2^{\bullet-}$) and hydroperoxyl ($ROO^{\bullet}$). Such compounds can quickly interact with the nitrogenous bases of the DNA, causing mutations, single or double-strand breaks, nitrogenous base modifications, and cross-linking. Furthermore, damage to proteins and lipids, causing poor folding and lipid peroxidation, respectively, are reported (9,90).

The superoxide radical can cross several cell barriers, being transported by anionic channels, and, physiologically, it is thought to be responsible for mediating nerve signals, not being very reactive. The same applies to hydrogen peroxide, produced by enzymes called peroxidases, from oxygen metabolism. It is very soluble and inert, with low oxidizing power; however, this one can produce new free radicals when penetrating cells and reacting with iron or copper ions. Finally, the most reactive oxidizing agent is the hydroxyl radical. It manages to have rapid formation and dissemination through reactions in the body (1,144,157).

Other molecules are important in the context of oxidative attack: singlet oxygen, hypochlorous acid radical - very linked to chronic inflammatory diseases and lipid peroxidation, and molecules from nitrogen, such as nitric oxide (NO), a fat-soluble gaseous radical capable of crossing biological membranes, and the peroxynitrite ($ONOO^{\bullet-}$), a potent vasodilator. Reactive nitrogen species (RNS) are associated with several diseases, including atherosclerosis, cancer, diabetes, and neurodegenerative disorders (1,30).

Regarding damage to the central nervous system, the role of nitric oxide is noted, when reacting with the superoxide radical, producing peroxynitrites. This product is harmful to neurons leading to cell apoptosis (188). Some antioxidant compounds present in propolis extracts compete with oxygen, preventing its reaction with nitric oxide and, consequently, the formation of toxic nitrites and RNS (116).

Several methodologies observe the decrease of free radicals from the reaction medium. Some of them use the Fenton reaction (73), while others observe the decrease in absorbance (144). The superoxide anion measurement assay uses the latter mechanism, by analyzing the decrease in absorbance after reaction between nitrotetrazolium blue and free radicals, generating formazan salt, which can be perceived at 560 nm (101,112). The results of these tests are presented in Table 12.

Table 12 | Results of the ROS and RNS sequestration assessment test of *Apis mellifera* propolis extracts obtained from the materials chosen for this literature review.

Author	Radical	Results
Abubaker, and Fageer, 2017 (157)	Hydrogen peroxide	60.37 – 92.68% (125.00 to 1000.00 µg/mL)
Da Graça Miguel *et al*., 2014 (63)	Hydroxyl radical	IC_{50}: 59.00 – 1389.00 µg/mL
De Francisco *et al*., 2018 (30)	Nitric oxide	IC_{50}: 1.61 µg/mL
De Francisco *et al*., 2018 (30)	Superoxide	IC_{50}: 226.778 µg/mL
De Francisco *et al*., 2018 (30)	Hydrogen peroxide	IC_{50}: 520.29 µg/mL
De Francisco *et al*., 2018 (30)	Hypochlorous acid	IC_{50}: 13.25 µg/mL
De Francisco *et al*., 2018 (30)	Peroxyl radical	IC_{50}: Did not reach IC_{50}
Ding *et al*., 2021 (73)	Hydroxyl radical	60.00 – 77.00% (5.00 mg/mL)
Ding *et al*., 2021 (73)	Superoxide	52.00 – 63.00% (5.00 mg/mL)
Fangio *et al*., 2019 (80)	Hydroxyl radical	0.34 nmol of scavenged hydroxyl radicals (0.04 ng of propolis extract)
Gargouri *et al*., 2019 (168)	Hydroxyl radical	0.052 – 0.068 mmol of equivalents of uric acid/g
Miguel *et al*., 2014 (101)	Superoxide	Winter: 46.02 – 85.72% Spring: 48.18 – 84.79%
Naik, and Vaidya, 2011 (105)	Nitric oxide	5.15 – 53.18% (0.10 – 2.00 mg/mL) IC_{50}: 1650.00 µg/mL
Naik, and Vaidya, 2011 (105)	Superoxide	0.834 – 1.457 nmol Trolox/g
Osés *et al*., 2016 (169)	Hydroxyl radical	0.0012 mmol of equivalents of uric acid/mL
OSÉS *et al*., 2020 (170)	Superoxide	IC_{50}: 20.00 – 440.00 µg/mL
Pazin *et al*., 2017 (112)	Superoxide	IC_{50}: 34.00 µg/mL
Ramnath, and Venkataramegowda, 2016 (116)	Nitric oxide	IC_{50}: 536.19 – 757.75 µg/mL
Ramnath, and Venkataramegowda, 2016 (116)	Hydrogen peroxide	IC_{50}: 325.30 – 765.75 µg/mL
Salas *et al*., 2020 (172)	Hydroxyl radical	IC_{50}: 16.50 – 37.00 µg/mL
Salas *et al*., 2020 (172)	Superoxide	IC_{50}:115.00 – 290.00 µg/mL
Salas *et al*., 2020 (172)	Hydrogen peroxide	IC_{50}: 39.00 – 92.00 µg/mL
Salas *et al*., 2020 (172)	Nitric oxide	IC_{50}: 50.00 – 104.50 µg/mL

Schmidt *et al*., 2014b (128)	Anti-radical activity – Hydrogen peroxide	0.48 – 2.94 mg Trolox/L (2.9×10^{-5} g/mL)
Tiveron, 2015 (27)	Superoxide	IC_{50}: 0.29 – 2.91 µg/mL
Tiveron, 2015 (27)	Hypochlorous acid	IC_{50}: 0.03 – 1.45 µg/mL
Tiveron *et al*., 2016 (138)	Hypochlorous acid	IC_{50}: 0.03 – 1.45 µg/mL
Wali *et al*., 2016 (144)	Hydrogen peroxide	IC_{50}: 109.93 – 145.42 µg/mL
Yuan *et al*., 2020 (148)	Hydroxyl radical	IC_{50}: 54.42 – 59.61 µg/mL
Zhang *et al*., 2015 (151)	Superoxide	1,52 mmol Trolox/g
Zhang *et al*., 2016 (152)	Superoxide	0.96 – 1.65 mmol Trolox/g

Cell Culture Tests

Some authors of the materials in this literature review chose to use cellular assays, among others, to measure the antioxidant activity of extracts in a little more complex environment, which is more similar to reality. It is known that free radicals can be generated by reactions in both the intracellular and extracellular environments. The cells most recognized as producers of such oxidizing species are those that make up the immune system (neutrophils, monocytes, eosinophils, and macrophages), in addition to endothelial cells (44).

One of the methodologies used to measure the antioxidant potential of the ethanol extract of propolis, used by Cao *et al*. (2017), was the evaluation of ROS production by macrophages, *in vitro*, when stimulated by hydrogen peroxide (Fenton reaction). Propolis was able to inhibit the production of ROS in a concentration-dependent manner and increase the survival of these cells (7). Similar tests have been used by Sun *et al*. (2015), who assessed the antioxidant capacity of propolis extracts in HepG2 cells (cellular antioxidant activity). The IC_{50} found ranged from 171.00 - 25738.00 µg/mL, depending on the proportion of water and ethanol in the solvent (136). Gokduman (2019) also worked with HepG2 cells and Hep3B. Treatment with ethanol extract of propolis (10.00 – 100.00 µg/mL) significantly reduced intracellular ROS production (159).

The paper proposed by Bonamigo *et al*. (2017) brought the MDA dosage test, a marker of oxidative damage to membrane lipids. For that purpose, erythrocytes, oxidation inducers, and markers of the desired molecule were used. Afterward, the absorbance of the sample of supernatants was read at 532 nm. As a result, it was noticed that the ascorbic acid control and the propolis sample reduced MDA levels by 65.70% and 38.40%, respectively, at the highest concentration evaluated. The same work also evaluated the antioxidant activity through the inhibition of oxidative hemolysis: the ethanol extract obtained an inhibition of 24,60%, at a concentration of 125.00 µg/mL (4).

Valent *et al*. (2011) also evaluated the protection of the methanolic extract of Portuguese propolis in protecting red blood cells against oxidative lysis processes. As expected, treatment with propolis decreased MDA production levels (71.00% to 78.00%), due to reduced lipid peroxidation reactions in cell membranes, when compared to control groups (IC_{50}: 8.10 and 9.70 µg/mL). In addition, propolis protected cells against hemolysis caused by damage to the membranes, as free radicals disturb surface structures and ion channels (IC_{50}: 6.30 and 10.70 µg/mL) (189).

Working with human breast cancer cells (MCF-7), Arslan *et al*. (2021) measured lipid peroxidation (TBARS) when exposed to mitomycin C. Treatment with propolis extracts (32.50; 65.00; 125.00; 250.00 and 500.00 µg/mL) reduced the levels of lipid peroxidation (0.03 – 0.16 nmol/mL), when compared to untreated control groups (0.40 nmol/mL) (47).

Salas *et al*. (2016) studied the effects of pretreatment with ethanolic extract of propolis on macrophages stimulated with lipopolysaccharide (LPS), a potent pro-inflammatory molecule. It was noted that the treated group had a decrease in the levels of NO production (IC_{50}: 8.40 and 9.40 µg/mL) and its inducible enzyme (iNOS) (IC_{50}: 27.03 and 30.96 µg/mL) (164). The same strategy was used by Bhuyan*et al*. (2021). Propolis treatment also decreased NO production by macrophages, with IC_{50} ranging from2.06 - 22.5 µg/mL (52).

Salgueiro (2016) tested the protective activity of the ethanol extract of propolis against damage to the yeast plasma membrane caused by hydrogen peroxide. The results of the TBARS method verified that

the presence of the treatment with propolis reduced the oxidative damage in the membranes (37).

Also working with yeasts, Cruz (2011) used these microorganisms to study the protective effect of propolis against oxidative damage to DNA and its viability against oxidative stress promoted by hydrogen peroxide. Exposure to pre-treatment and co-treatment with propolis extracts improved yeast cell viability compared to exclusive hydrogen peroxide controls. However, post-treatment with propolis could not reverse the oxidative damage that had already occurred. Also, at concentrations of 25.00 - 300.00 µg/mL, this natural product managed to protect the DNA from oxidative damage. However, in higher concentrations, it also had pro-oxidative and genotoxic effects (61).

Mendez-Pfeiffer *et al*. (2020) studied the effect of treatment with the methanol extract of propolis on M12.C3 cells. F6, when exposed to an environment of oxidative stress caused by the application of hydrogen peroxide. Treatment with propolis (25.00 and 50.00 µg/mL) increased cell survival and decreased damage to the plasma membrane by inhibiting the excess production of intracellular ROS (100).

Jeong *et al*. (2012) analyzed the protective effect of aqueous propolis extract on central nervous system cell survival after oxidative stress induction by hydrogen peroxide. At 25.00 µg/mL, pre-treatment with propolis had a good protective effect, similar to ascorbic acid (200.00 µmol/L), increasing cell survival compared to stress control. Furthermore, the protection against damage to the membrane of PC12 cells was studied, using the enzyme lactate dehydrogenase as a cell lysis marker. At a 100 µg/mL concentration, the extract managed to decrease the enzyme activity from 257.00% (control) to 145.00%, demonstrating protection against cell extravasation (179).

Zeitoun *et al*. (2019) studied the effects of propolis extract on macrophages (RAW 264.7) exposed to LPS, about NO production, and enzyme expression inflammatory drugs such as cyclooxygenase 2 and iNOs. Treatment with propolis (1.00; 5.00; 10.00; 15.00; 30.00; 40.00; 50.00; and 60.00 µg/mL) demonstrated the inhibition of both enzymes, in a dose-dependent way, obtaining almost complete inhibition at a concentration of 60.00 µg/mL. NO production levels also decreased compared to the control groups (150).

Working with human colon cancer cells (HCT-116), Žižić *et al*. (2013) evaluated the intracellular production of superoxide radicals and nitrite. In the presence of propolis extracts (10.00; 50.00; 100.00 and 500.00 µg/mL), there was a decrease in the level of both molecules 24 and 72 hours after the beginning of the tests (153).

Al Naggar *et al*. (2016) followed a different path by evaluating the transcription of Nrf2 (nuclear erythroid factor 2 related to factor 2), a transcription factor activated in cellular stress cases. In such situations, it migrates to the nucleus and binds to promoters, culminating in the transcription of oxidative stress response genes. The stress environment was provided using hydrogen peroxide. Treatment with propolis extract at a concentration of 25.00 µg/mL promoted inhibition of 2.33 - 22.85% of the activation of these, demonstrating that propolis reduces cell stress and, consequently, it reduces the need to transcribe these genes (42).

Similarly, Zhang *et al*. (2015) and Zhang *et al*. (2016) analyzed the expression of antioxidant genes in macrophages. Exposure to propolis extract (2.50; 10.00 and 15.00 µg/mL) significantly increased the expression of these genes, demonstrating the ability of propolis to induce the synthesis of antioxidant mediators. In addition, it was determined that the treatment with propolis extract could reduce intracellular levels of ROS, through the induction of hydrogen peroxide, to amounts smaller than the initial basal levels, in agreement with the results of other studies (151,152).

In vivo **Tests**

It is known that propolis has low innate toxicity (11), since the main chemical constituents, the flavonoids, also have low toxicity (28). Rodents that consumed a daily dose of 1400 mg/kg of this natural product had no side effects. In cats, 100 mg/kg was well tolerated by subcutaneous injections. Studies have inferred that humans can safely ingest up to 1.4 mg/kg per day (about 70 mg/day) (11,14,166). Given this, propolis is considered safe for consumption, having GRAS (Generally Recognized as Safe) status. However, ingesting large amounts of propolis in its raw state can cause discomfort in the gastrointestinal tract, as well as an increase in cases of contact dermatitis in cosmetic formulations (11).

The work of Capucho *et al*. (2012) presented the evaluation of oxidative stress in the epididymis of rats treated with propolis gavage at a concentration of 3.00; 6.00; and 10.00 mg/kg/day, during 56 days. At the end of the process, animals were sacrificed, and epididymis was prepared and evaluated. Treatment with aqueous propolis extract increased the production of reproductive cells. However, it did not increase the oxidative environment that usually accompanies this growth. There was no difference in the TBARS test values and the CAT enzyme activity, only an increase in the thiol levels compared to the control

group (190).

Cécere *et al*. (2021) evaluated oxidative parameters in the blood of lambs supplemented with propolis extract at concentrations of 150.00; 200.00 and 250.00 µL/kg/day for 42 days. The results for the treatment with 250.00 µL/kg/day, on the 42nd day, showed a decrease in ROS levels (243.24 U DCF/mg protein in the control group and 250.94 in the treated group). Unexpectedly, there was a decrease in SOD activity levels (7.17 U SOD/mg protein in the control group and 6.40 in the treated group) and increased NO expression (4.70 mmol NO/mg protein in the control group and 5.34 in the treated group). However, there was an increase in the levels of thiols from proteins (3.11 mmol SH/mL in the control group and 5.37 in the treated group) and from other sources (4.41 mmol/mL in the control group and 6.55 in the group treated) as well as nitrites/nitrates, widely known for their inflammatory capacity (56).

Yonar *et al*. (2012) also performed tests on hematological parameters, in addition to specific organ analyses; however, their specimens were fish (*Ciprinus carpio carpio*) exposed to the pesticide chlorpyrifos. In the control group that received only the pesticide, the parameters worsened, indicating oxidative stress: increased levels of MDA and SOD activity decreased CAT activity and reduced glutathione activity. Treatment with propolis (10.00 mg propolis/kg body weight) resulted in the parameters reverting to normal levels. However, the treatment with propolis only had no significant effect on test values (184). A similar experiment was carried out by Yonar *et al*. (2014). However, this time, the fishes were exposed to the pesticide malathion. Again, the oxidative stress parameters were altered in the group exposed to this substance, and there was a significant improvement when the propolis treatment was started (levels of MDA, SOD, CAT, reduced glutathione, and glutathione peroxidase) (191).

The same group continued the research line by analyzing the effect of the propolis diet on crayfish and their eggs. This natural material supplementation results in reduced lipid peroxidation, decreased MDA levels, and improved activity of antioxidant enzymes (173).

Da Silveira *et al*. (2016) studied the effect of propolis on behavior and hematological parameters of oxidative stress in Wistar rats, right after stressful tests such as forced swimming and open-field anxiety tests. Treatment with yellow propolis EEP (1.00; 3.00; 10.00; and 30.00 mg/kg) was observed to reduce the production of NO and MDA. Still, there was no change in the level of enzymes such as catalase and superoxide dismutase, induced by stress, nor the ABTS test values (188).

The work carried out by Jeong *et al*. (2012) aimed to analyze the neuroprotective effect of aqueous extracts of propolis in the brain of rats exposed to oxidative stress. The results indicated that the treatment reduced cell death when exposed to hydrogen peroxide and decreased intracellular enzyme release (represented by lactate dehydrogenase), indicating that the plasma membrane was intact in most of the cells (179).

Rivera-Yañez *et al*. (2018) evaluated the enzymatic activity present in the pancreas of rats with a model of diabetes-induced by intraperitoneal injection of streptozotocin (130.00 mg/kg). Treatment with propolis was performed for 15 days at a dose of 0.30 g/kg/day. The colorimetric evaluation methods of the enzymatic activity showed an increase in the activity of the enzymes SOD, CAT, and GPx (glutathione peroxidase) compared to the control groups, demonstrating the ability of propolis to fight pathological oxidative stress even in organ systems (120).

Saleh (2012) studied the protective effects of propolis against hepatotoxicity caused by the administration of 4-tertiary-octylphenol (100.00 mg/kg) during six weeks. This generated a decrease in the enzymatic activity of GST (glutathione S-transferase), SOD, and CAT, demonstrating the role of this substance in causing oxidative stress in the evaluated tissues. Treatment with aqueous extracts of propolis (100.00 mg/kg) increased the level of all enzymes, even above the values found in the control groups that did not receive external stress. One of the possible mechanisms of action of this natural product is the presence of trace mineral compounds, such as zinc, magnesium, manganese, and nickel, responsible for the reactivation of such enzymes. In addition, the group treated with propolis also showed a reduction in MDA levels in hepatocytes, performed using the TBARS test (192).

The work developed by Tohamy *et al*. (2014) analyzed the effect, among other activities, of aqueous extracts of Egyptian propolis on the oxidative status of tissues in rats (*Mus musculus*) exposed to cisplatin (2.80 mg/kg twice a week for 2 weeks). Such animals showed an increase in oxidative stress, due to increased levels of lipid peroxidation (TBARS) and decreased activity of antioxidant factors such as catalase and glutathione. The group receiving the propolis treatment (8.40 mg/kg/day, for 14 days) managed to satisfactorily reverse the peroxidation and return to normal enzyme values (CAT and GSH) (193).

Wiwekowati *et al*. (2020) studied the anti-lipid peroxidation effect using an ELISA kit that analyzes the

levels of MDA existing in the medium. The rats (*Rattus norvegicus*) were subjected to placement a foreign body in the jaw (coil spring), and their blood was removed for analysis purposes. The results showed that groups treated with the propolis gel had reduced MDA values (194).

Other Methodologies

In this session, data from tests less frequently used in the selected works were compiled. The Phosphomolybdenum method is used to evaluate the total antioxidant capacity, evaluating both lipophilic and hydrophilic compounds. It is based on reducing molybdenum VI to V in an acidic environment, with subsequent formation of a complex between this molecule and the phosphate, whose color is greenish. Its advantages are low cost, simplicity, plus the fact that it can be used for complex matrices, such as propolis (45,50).

Another method used is the SNPAC (Silver Nanoparticles Antioxidant Capacity). In the presence of antioxidants, there is a reduction of the silver ion, present in silver nitrate, resulting in nanoparticles suspended in solution, stabilized by trisodium citrate. At that moment, the reaction medium changes color, from pale yellow to brown, being sensed at 423 nm (64). The FOX test (Ferrous Oxidation Xilenol Orange) is a spectrophotometric methodology that relies on the ability of hydroperoxides to oxidize iron from its ferrous to ferric form in an acidic medium. A colored complex with xylenol orange is formed when this occurs, perceived at 560 nm (76).

The results of these tests are shown in Table 13.

Table 13 | Results of the test to evaluate the antioxidant activity of *Apis mellifera* bee propolis extracts by different methods obtained from the materials chosen for this literature review.

Author	Test methodology	Results
Araújo *et al*., 2020 (45)	Phosphomolybdenum	22.72% (inhibition percentage when compared to 200 µg/mL ascorbic acid)
Béji-Srairi *et al*., 2020 (50)	Phosphomolybdenum	158.66 – 220.44 mg of equivalents of gallic acid/g
Dărăban *et al*., 2019 (64)	SNPAC	157.31 – 619.53 µmol Trolox/mL
Duca *et al*., 2019 (76)	FOX	About 18.00 - 32.00% inhibition of hydrogen peroxide (5.00 and 0.50 mg/mL)
El Meniiy *et al*., 2021 (77)	Phosphomolybdenum	6.81 – 80.82 mg of equivalents of ascorbic acid/g
Karadal *et al*., 2018 (195)	Total antioxidant Status (TAS) - ELISA	7.30 – 9.45 µmol Trolox/g
Silva *et al*., 2011 (155)	Inhibition of tyrosine nitration	IC_{50}: 50.00 µg/mL
Touzani *et al*., 2018 (140)	Phosphomolybdenum	6.56 – 90.87 mg of equivalents of ascorbic acid/g

CONCLUSION

Given the numerous biological properties attributed to propolis, such as antimicrobial, antioxidant, anti-inflammatory, and anti-cancer, associated with consumers' search for safe (GRAS) and ecological products, this balsamic material is the subject of several types of research around the world, in order of being used by the food and pharmaceutical industry in new formulations, both as an active ingredient and as an adjuvant.

In this literature review, it can be noticed that propolis samples have extreme variations in terms of antioxidant activity. But, overall, this natural product showed great antioxidant power in all reported tests (both *in vitro* and *in vivo*), through multiple mechanisms, such as ROS and RNS sequestration, metal chelation, and inhibition of pro-oxidant enzymes. As already demonstrated in the literature several times, this variation is due to external factors like the botanical source, seasonality, bee species, collection method, extracting, and testing biological activities.

CONFLICT INTEREST

The authors declare no personal or financial conflict of interest related to this work.

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SUPPLEMENTARY MATERIALS
Authors have provided supplementary materials. Table S1: Search strategy in each database chosen for this literature review. Table S2 | Data on the collection methodology and information on samples of the 173 materials used in this literature review.

AUTHOR CONTRIBUTIONS
All authors contributed to Conceptualization, Methodology, Formal analysis, Investigation, Writing. (Y.M.F.B.) Review & Editing.

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