



Brazilian Brown Propolis: an Overview About Its Chemical Composition, Botanical Sources, Quality Control, and Pharmacological Properties

Victor Pena Ribeiro¹ · Jennyfer Andrea Aldana Mejia² · Debora Munhoz Rodrigues² · Gabriel Rocha Alves² · Ana Maria de Freitas Pinheiro² · Matheus Hikaru Tanimoto² · Jairo Kenupp Bastos² · Sérgio Ricardo Ambrósio¹

Received: 22 November 2022 / Accepted: 7 February 2023 / Published online: 24 February 2023
© The Author(s) under exclusive licence to Sociedade Brasileira de Farmacognosia 2023

Abstract

Brazil is one of the largest propolis producers in the world. Propolis is produced by bees from plant exudates and tissues, leading to many variations in the types of propolis. Generally, Brazilian propolis types are green, brown, and red. Despite not being the main research focus as the green and red propolis, brown propolis is the second most produced propolis type in Brazil and has tremendous economic and medicinal importance. Propolis has drawn attention with the rise in the search for healthier lifestyles, functional foods, biocosmetics, and natural products as therapeutic sources. This review covers the main chemical constituents identified in different types of Brazilian brown propolis, and their botanical sources, chemistry, and biological activities. The economic aspect of brown propolis is also presented. There are many gaps to be filled for brown propolis regarding the development of analytical methods, and quality control to allow its standardization, limiting its applicability in the food and pharmaceutical industries. Future perspectives regarding brown propolis research were discussed, especially biological activities, to support the medicinal uses of different types of brown propolis.

Keywords Propolis · Chemical composition · Botanical source · Biological activity

Introduction

Propolis is a resinous and complex product produced by honeybees (*Apis mellifera*) through the collection of different parts of plants and exudates mixed with bees' wax and salivary secretions in the hive (Salas et al. 2016). This mixture forms a robust adhesive material used for structural repairs to maintain sealing, humidity, and internal hive temperature. It is also important as a defense against invading microorganisms that may threaten the community, essential for the hive's survival (Simone-Finstrom et al. 2017; Bankova et al. 2018).

Propolis has been used since ancient times in different civilizations in traditional medicine, either alone or in combination

with other medicinal plants, playing an important role in maintaining community health for disease prevention and treatment (Rivera-Yañez et al. 2020). These medicinal properties reported for Brazilian brown propolis have become the target of intense research mainly due to their activities, such as, antioxidant (Sartori et al. 2012; Calegari et al. 2017), antibacterial (Fernandes et al. 2015; Pimenta et al. 2015), anti-inflammatory (Sartori et al. 2012; Ribeiro et al. 2018), cytotoxic (Lima et al. 2019), anti-leishmanial (Santana et al. 2014), antigenotoxicity (Fernandes et al. 2014, 2019), and antimycoplasma (Araújo et al. 2020).

The chemical composition of propolis is directly linked to the botanical sources surrounding beehives in each region (Simões-Ambrosio et al. 2010). Considering Brazil's continental dimension and its conspicuous plant diversity, several Brazilian types of propolis have been classified, including color, botanical source, and phytochemical profile. Green, red, and brown propolis are the main types (Park et al. 2002).

The green propolis has one primary botanical source, *Baccharis dracunculifolia* DC., Asteraceae (Arruda et al. 2020a, b). Red propolis has two botanical sources: *Dalbergia ecastaphyllum* (L.) Taub., Fabaceae, rich in isoflavonoids,

✉ Jairo Kenupp Bastos
jkbastos@fcrfp.usp.br

¹ Núcleo de Pesquisa Em Ciências Exatas E Tecnológicas, Universidade de Franca, Franca, SP 14404-600, Brazil

² Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. Do Café, Ribeirão Preto, SP 14040-930, Brazil

pterocarpan, and chalcones; and *Symphonia globulifera* L.f., Clusiaceae, rich in polyprenylated benzophenone (Ccana-Ccapatinta et al. 2020).

The different types of Brazilian brown propolis (BBP) bear different chemical profiles, leading to different chemical compositions and, consequently, different biological activities. This represents a great challenge in the case of BBP, since it is produced in different regions of Brazil, from the northeast to the south of the country, thus having several biomes and vast plant diversity as possible botanical sources. (Tazawa et al. 2016).

In this review, we discuss botanical sources, the importance of analytical methods development, quality control, pharmacological properties, and the economic aspects.

Search Strategies

A search in the literature was undertaken by using the following platforms: Web of Science, SciFinder, Pub-Med, Science Direct, and Google Scholar. The terms “Brown Propolis” and “Brazilian propolis” were used as keywords in search engines. The inclusion criteria of the articles were that propolis must be classified as brown and contained at least one of the following points: chemical composition, identification of the botanical origin, and/or evaluation of some biological activity. Brown propolis articles from regions other than Brazil were not considered in this review. No time filter was used. Articles published in Portuguese, Spanish, and English were considered.

Discussion

Chemical Composition

The chemical composition of propolis is highly dependent on the conditions of the location and the chemical constituents of the botanical source (Bankova et al. 2018). One production center of BP is the southern region of Brazil. Forty-four samples of BP from Paraná and the Santa Catarina States were analyzed to identify the regional identity of these locations. Their compounds were identified and quantified by comparing their chemical profile with authentic standards (Machado et al. 2021). According to this study, the BP from Southern Brazil is chemically characterized by caffeoyl-quinic acids (range, 11.14 – 21.45 mg/g), *p*-coumaric acid derivatives (6.27 – 12.17 mg/g), flavonols (9.35 – 23.55 mg/g), followed by benzoic acid derivatives (3.18 – 7.45 mg/g), and dihydroflavonols (0.17 – 4.25 mg/g).

Phenolic compounds already reported in Brazilian green propolis have been found on standardized BP extracts, like *p*-coumaric acid (1), drupanin (2), artepillin C (3), and baccharin (4), revealing similarity on phenolic profile between

these two propolis types (Fonseca et al. 2011). According to Rodrigues et al. (2016), besides those traditional green propolis chemical markers, BP can also present lower concentrations of propenoic and cinnamic acid. Other phenolic compounds like chrysin, pinocembrin, galangin, caffeic acid phenylethyl ester, and pinobanksin-3-*O*-acetate have also been detected by HPLC–UV–ESI–MS analysis in Brazilian propolis samples (Fabio et al. 2019).

An RP–HPLC–DAD–ESI–MS/MS was used to chemically characterize a BP sample from Paraná (Araújo et al. 2020). The raw material was submitted to extraction and partition with different solvents in a Soxhlet system, revealing the prevalence of some classes of compounds in the fractions. In all fractions, artepillin C, baccharin, and 3-hydroxy-2,2-dimethyl-8-prenyl chromane-6-propenoic acid were identified, although the hexane fraction was enriched with propenoic and cinnamic acid derivatives like drupanin; the hexane:ethyl acetate fraction presented propenoic and cinnamic acid derivatives, and flavonoids as kaempferol and quercetin. In comparison, the ethyl acetate fraction was rich in flavonoids, chlorogenic acids, and quinic acid esterified by one or more units of cinnamic, *p*-coumaric, caffeic, or ferulic acids. The methanol and aqueous fractions contained quinic and caffeoyl acid derivatives.

Furthermore, diterpenes as isocupressic acid (5), (*E*)-communic acid (6), (*Z*)-communic acid (7), and abietic acid (8) have been isolated from samples of BP from Paraná State (Tazawa et al. 2016). The presence of the 15-acetoxyisocupressic acid and an unreported diterpene, the *rel*-(5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-epoxy-2-oxocleroda-3,12(*E*),14-triene-6,18,19-triol 18,19-diacetate 6-benzoate, were reported on BP ethanolic extract (Santos et al. 2021).

Capillartemisin A and caffeoylquinic acid derivatives like 3,4-di-*O*-*E*-caffeoylquinic acid, the 3,5-di-*O*-*E*-caffeoylquinic acid, *O*-hexosyl-caffeoyl dihydrocaffeate, 4,5-di-*O*-*E*-caffeoylquinic acid, and *O*-*E*-coumaroyl-caffeoylquinic acid were detected in Minas Gerais (Southeast region) BP by LC–DAD–MS analysis (Dembogurski et al. 2018). Brown propolis from Mato Grosso also furnished acetylisocupressic acid, dihydro-*p*-coumaric acid (9), caffeic acid (10), and aromadendrin (Fernandes et al. 2019). Analysis by UPLC–MS of the hydroalcoholic extract of BP from Rio Grande do Sul (South region) revealed the presence of rutin, chlorogenic acid, ferulic acid, and caffeic acid (Waller et al. 2017).

Another area of production of BP is the northeast region. A sample from Bahia State was submitted to extraction with hexane, methanol, and dichloromethane, and the chemical profile was assessed by CG–MS (Santos et al. 2017). The hexane fraction was composed of pentadecane, hexadecane, heptadecane, and tricosane. Methyl cinnamate and sitosterol cinnamate were isolated from the hexane fraction, and anixinanthone was isolated from the dichloromethane fraction.

The GC–MS chromatographic analysis of BP from Piauí (Northeast region) displayed the triterpenes lupeol, germanicol,

β -amyrin, hop-22-(29)-*en*-3-one, and lupenone for hexanic fraction, and for dichloromethane fraction, the compounds 2,3-dihydroxybenzofurane, lupeol, and dodecanoic acid. In ethyl acetate fraction, *p*-coumaric acid and 3,5-dihydroxybenzoic acid were identified (Santana et al. 2014).

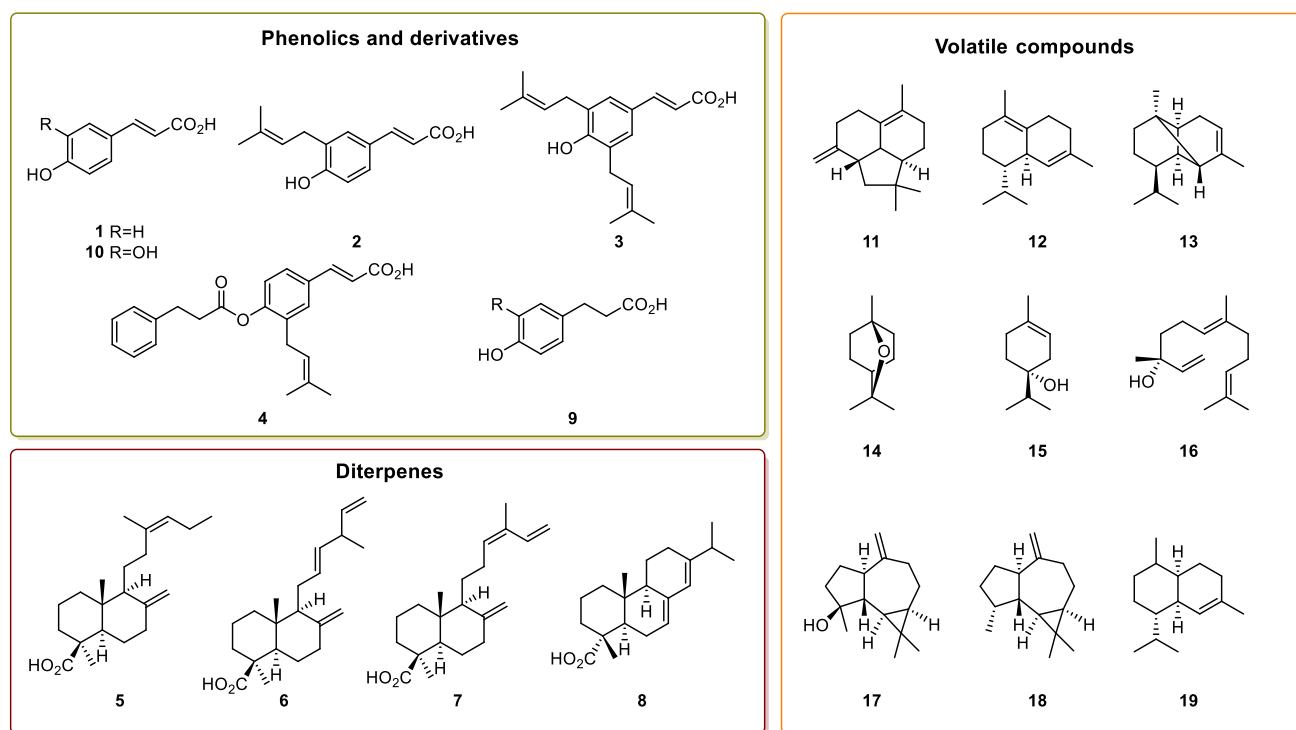
Samples from Paraná (South) and Ceará (Northeast) state were submitted to the acid/*n*-butanol hydrolysis method to detect proanthocyanidins and their quantification by precipitation with BSA (bovine serum albumin). All the samples had a positive reaction for proanthocyanidins with low tannin content values (between 0.6 and 1%) but without a complete chemical characterization (Mayworm et al. 2014).

The volatile oil from BP has been characterized by several studies, showing differences in the chemical profile of propolis from different locations. Headspace solid-phase micro-extraction (HS-SPME) and GF-MS analysis helped identify more than 315 volatile compounds in BP from Bahia, Minas Gerais, Paraná, and Sergipe States (Olegário et al. 2019). Terpenes were the predominant class of compounds in all samples, followed by the aldehydes.

The sesquiterpenes β -caryophyllene (**11**) and humulene were the most abundant compounds in the BP sample from Bahia state (Olegário et al. 2019); acetophenone, (*R*)- α -pinene, and (+)- δ -cadinene (**12**) were predominant in the Paraná sample; *D*-limonene and nonanal were the major compounds in the Sergipe state sample (Northeast region).

Brown propolis from Minas Gerais sample displayed sesquiterpenes (33.62%), oxygenated sesquiterpenes (26.98%), and oxygenated monoterpenes (18.99%) (Ribeiro et al. 2021a, b, c). Quantification differences were observed for samples from this state, although the sesquiterpenes β -caryophyllene and α -copaene (**13**) were predominant. 1,8-Cineol (**14**), terpineol-4-ol (**15**), nerolidol (**16**), spathulenol (**17**), δ -cadinene, aromadendrene (**18**), γ -muurolene (**19**), and the alkyl-phenylketone, acetophenone have also been reported (Lima et al. 2019; Olegário et al. 2019; Ribeiro et al. 2021a, b, c; Símaro, et al. 2021).

The volatile oil of BP from Mato Grosso do Sul displayed (*E*)-caryophyllene, δ -cadinene, spathulenol, α -copaene, (*E*)-nerolidol, and aromadendrene, with the prevalence of viridiflorene and *trans*- α -bergamotene (Fernandes et al. 2015).



Botanical Sources

One of the major bottlenecks in propolis studies is the elucidation of its botanical sources. The botanical source visited by bees is directly related to the chemical composition of propolis, impacting its pharmacological properties. Many publications on propolis do not describe its type, botanical

sources, and even its chemical composition, making it difficult to standardize this medicinal product.

Several approaches are aiming to find the probable botanical source of propolis under study in the literature. One of the approaches is to observe bees in the field, as *Apis mellifera* bees collect red exudates from *Dalbergia ecastaphylum* to produce red propolis, which was confirmed by the

chemical similarity between the plant exudate and propolis (Daugusch et al. 2008). Another approach is the identification of chemotaxonomic markers and correlating them with botanical species near the hives, which led Ccana-Ccpatinta et al. (2020) to describe *Symphonia globulifera* as the source of benzophenones in Brazilian red propolis. Some researchers use palynology for botanical identification of propolis, but bees visit many plants for nectar collection and a few plants for resin collection, making it challenging to identify the botanical source for propolis production (Freitas et al. 2011). Currently, the metabolomics associated with techniques such as UPLC-MS/MS has shown to be an essential tool in propolis's chemical prospection to identify its origin.

The botanical origins of some Brazilian propolis were well-established, as *Baccharis dracunculifolia* for green propolis and *Dalbergia ecastaphyllum* and *Symphonia globulifera* for red propolis. Several plants have been described as responsible for their composition regarding brown propolis, as *Pinus* spp., *B. dracunculifolia*, *Eucalyptus* spp., and *Araucaria angustifolia* (Freitas et al. 2011; Frota et al. 2021; Ribeiro et al. 2021a, b, c; Santos et al. 2021; Serafim et al. 2022).

Baccharis dracunculifolia, popularly known as “alecrim-do-campo,” is largely distributed in South America from Southeastern Brazil to Argentina and Uruguay (Ribeiro et al. 2018). It is the primary plant source of Southeastern Brazilian propolis, called green propolis, because of its color. Green propolis contains high levels of prenylated *p*-coumaric acids, mainly artepillin C and baccharin, and the volatile compounds nerolidol and spathulenol, all found in *B. dracunculifolia* (Beserra et al. 2021; Bernardes et al. 2022).

Due to its geographic location, *B. dracunculifolia* is found mainly in BP samples collected in the southeastern region of Brazil, and there are other possible associated botanical sources, making it brown. The phenolic acids of *B. dracunculifolia* and its volatile components nerolidol and spathulenol are described in several BP (Dembogurski et al. 2018; Araújo et al. 2020; Ribeiro et al. 2022). Compounds not described for *Baccharis* are usually identified in phytochemical studies, thus evidencing the participation of other plants in the production of brown propolis (Beserra et al. 2021).

Diterpenic acids reported in Brazilian Southeast BP are also found in conifers species as *Araucaria angustifolia*. Some phytochemical studies of Brazilian brown propolis confirmed *A. angustifolia* as the primary plant source (Sartori et al. 2021; Ribeiro et al. 2021a, b, c; Santos et al. 2021). In a previous work published by our research group, we reported the isolation of diterpenes from *Araucaria* sp. Brazilian brown propolis. During the collection of propolis samples in the field, bees collected the exudates from the *A. angustifolia* trunk for propolis production (Santos et al. 2021). The bees collect *A. angustifolia* exudate, store it in the corbicula of the left leg, and take it to the hive (Fig. 1).



Fig. 1 Collection of exudates of *Araucaria angustifolia* by *Apis mellifera* bee in União da Vitória (Paraná state, Brazil)

Araucaria's participation was confirmed later through the phytochemical study of this propolis (Santos et al. 2021).

The participation of *A. angustifolia* in the chemical composition of propolis is mainly due to the presence of acid diterpenes, such as 13-*epi*-cupressic acid, abietic acid, and communic acid (Santos et al. 2021; Tazawa et al. 2016). From volatile compounds, *A. angustifolia* presents the sesquiterpene germacrene-D and the diterpenes hibaene and phyllocladene as significant components of its volatile oil (Brophy et al. 2000). The anti-inflammatory and antimicrobial biological properties are attributed to diterpenes. Many diterpenes isolated from BP and *A. angustifolia* possessed antimicrobial activity (Bankova et al. 1999; Ribeiro et al. 2021a, b, c).

Diterpenes from *Pinus* spp. and *Eucalyptus* spp. are also found in BP. Almost all isolated diterpenes from a Brazilian Southeast BP sample were reported in *Pinus* resin, as 19-acetoxy-13-hydroxyabda-8(17),14-diene, totarol, 7-oxodehydroabietic acid, dehydroabietic acid, communic acid, and isopimaric acid. Pinoresinol and matairesinol lignans were also isolated from the same propolis sample (Ribeiro et al. 2021a, b, c). These lignans are described as major compounds in *A. angustifolia* knots resin and are also described as major compounds in *Pinus taeda* resin (Eberhardt et al. 1993).

Many BBP contain α -pinene and β -pinene as major compounds of their volatile fraction. These compounds are chemical markers of *Pinus* volatile oil, corroborating this plant as a botanical source for some BBP (Ioannou et al. 2014). *Eucalyptus* spp. also contributes with flavonoids and glucopyranoside compounds for BP composition (Freitas et al. 2008).

Analytical Methods and Quality Control

Determination of total phenolic and flavonoid contents in propolis samples has been widely used to determine

biological properties (Sawaya et al. 2011), mainly using spectrophotometric assays. Usually, total phenolic content is measured by Folin–Ciocalteu's method, and flavonoid content is measured by the AlCl_3 complexation method (Machado et al. 2021). However, these methods do not specify each compound in the class of phenolic and flavonoids, being not selective. It is crucial to identify the compounds responsible for the biological activities to guarantee the quality of propolis and its products. The development of validated analytical methods is mandatory to guarantee selectivity, accuracy, and precision in quantifying compounds (Machado et al. 2021).

Several analytical methods have been developed to analyze raw propolis and its commercial products. Many of them aim to identify the chemical components with biological activities, mainly phenolic compounds used as biomarkers/standards (Fabio et al. 2019). It is challenging to develop analytical methods for propolis analysis because it bears a complex matrix demanding different methods' approaches to analyze all the compound classes present in propolis (Pavlovic et al. 2020). For propolis' polyphenol analysis, thin-layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) are the most used methods. HPLC coupled with photodiode array detector (DAD) is beneficial for polyphenol analysis, but HPLC coupled with a mass spectrometer (HPLC–MS) has gained space in propolis analysis, and it allows the identification of compounds in complex matrices (Fabio et al. 2019).

Besides phenolic analysis, propolis volatile compound analysis is essential, and solid-phase microextraction (SPME) with GC coupled to the mass spectrometer (GC–MS) is a good choice for this class of compounds (Pavlovic et al. 2020). Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (HS-SPME GC–MS) allows the study of several samples. Furthermore, HS-SPME has the advantage of avoiding using solvents, except when the matrix effect interferes in analysis (Burzynski-Chang et al. 2018).

There are some qualitative and quantitative methods reported in the literature for BP analysis, using the above-cited techniques. The qualitative methods are used only for chemical characterization, and there are reported quantifying method samples, even without validation. Most of the methods are not validated and usually show incomplete information about compound quantification. This review did not include the qualitative and classical spectrophotometric methods for phenolic and flavonoid analysis.

Quantitative Methods

HPLC has been the most used equipment for quantitative method development, varying the type of detector and mass

analyzer apparatus to determine the chemical composition of ethanol extracts from different types of Brazilian propolis, considering their predominant botanical origin (Salomão et al. 2008). Fonseca et al. (2011) quantified phenolic compounds by HPLC–MS in brown and green Brazilian propolis from São Paulo. Tazawa et al. (2016) discovered a novel diterpene in BP from the state of Paraná, using 1D- and 2D-NMR analyses and identified five more diterpenes. All the six compounds were quantified in the sample by ultra-performance liquid chromatography (UPLC) using calibration curves without validation.

Rodrigues et al. (2016) used HPLC–DAD to quantify prenylated phenolic acids and phenolic acids in brown and green Brazilian propolis samples from Paraná and Minas Gerais, respectively. Waller et al. (2017) detected 17 compounds in a BP sample from the state of Rio Grande Sul using HPLC–MS, in which the compounds were characterized by their UV and mass spectra. They used external standards calibration curves to quantify *p*-coumaric acid, rutin, chlorogenic acid, ferulic acid, and caffeic acid.

Mayworm et al. (2014) quantified tannins in different types of Brazilian propolis. Tannic acid was used as a reference for determining tannin content using the precipitation method with bovine serum albumin (BSA). Brown propolis samples contained tannins in a low quantity compared with other propolis types.

Validated Methods

Currently, there are few articles with details reporting validated methods for BP analysis. Machado et al. (2016a, b) identified and quantified phenolic compounds in different types of propolis, including samples of BBP from the southern states Santa Catarina, Rio Grande do Sul, and Paraná. The analyses were made in HPLC–DAD, and the article mentions that the method was validated based on National Health Surveillance Agency (Anvisa 2017) and National Institute of Metrology, Standardization, and Industrial Quality (Inmetro) guidelines.

Ribeiro et al. (2021a, b, c) validated a method to quantify volatile compounds from Brazilian southeast brown propolis using GC-FID. The authors identified 56 compounds by GC–MS in a volatile oil extracted from BP. Eight major compounds were isolated and used in the validated method. The chemical structures were determined by GC–MS and NMR analyses. The method was validated following International Conference on Harmonization (ICH) and Anvisa guidelines and can be used for quality control to quantify these compounds in different types of propolis.

Santos et al. (2021) also validated a method for BP diterpene analysis using HPLC–DAD. The major diterpenes from a BBP from Paraná state were isolated and identified

by ^1H and ^{13}C -NMR. The method was validated based on Anvisa guidelines, and all the parameters were analyzed to guarantee the method's reliability to identify and quantify the six diterpenes in BP samples. Machado et al. (2021) analyzed 44 BBP samples from Paraná by HPLC–DAD. Analytical standards were used to quantify the total content of caffeoylquinic acid, phenylpropanoids (*p*-coumaric acid derivatives), benzoic acids, flavonols, and dihydroflavonols in the samples.

Based on the literature review, developing more validated analytical methods is still necessary for BP analysis around the world. It is still indispensable to develop validated methods using HPLC–MS and GC–MS, which are already broadly used in quality control as they give structural information and allow the quantification of many compounds. HPLC–MS is a powerful technique and should be more explored as it provides a complete characterization of the biomarker compounds of propolis. BP composition varies depending on several factors, and it is increasingly important to construct a database with the already characterized biomarkers and develop validated methods to analyze them.

Pharmacological Properties

Several diseases do not have available medicines in the market for their treatment, and sometimes the available medicines present relevant side effects. Therefore, novel drugs must be developed for treating these illnesses. Propolis has been used since ancient times as a healing agent. Its isolated compounds have been tested against many pathologies and parasites. The biological effects of BBP and its chemical composition are displayed in Table S1.

Cytotoxicity Assays

The cytotoxicity studies on BBP are still scarce; the literature reports especially the cytotoxicity of the ethanolic brown propolis extract from the different geographic origins of Brazil, such as the northeastern (Frota et al. 2021), the southeastern (Ribeiro et al. 2021a, b, c), and the southern (Machado et al. 2021) regions. As propolis activities are related to the environmental conditions prevailing in each region, the cytotoxicity reported from BP of different regions in Brazil also shows variations in the antiproliferative activities.

The ethanol extract of BP from Ceará inhibited more than 75% of the proliferation of human cancer cells, such as colorectal (HCT116), leukemia (HL60), prostate (PC3), and murine melanoma cancer cells (B16F10). Despite observing an excellent inhibition against these cancer cell lines, inhibition values in normal cells also exhibited remarkable cytotoxicity, demonstrating the non-selectivity of the tested extract. This study also evaluated the hexane extract of BP against the same human and murine cancer cells reported

above, with inhibition values between 50 and 100% of cell growth and with low cytotoxicity against normal cells, showing excellent activity and selectivity against PC3 and HL60 cancer cells (Frota et al. 2021).

The BP from Paraná also showed moderate activity against human ovarian cancer cells (OVCAR-8) and colorectal carcinoma (HCT-116), with 75 and 50% of cell growth inhibition, respectively. It is interesting to report that Machado et al. (2021) tested 44 types of BP from the southern region with different levels of antitumor activities, ranging from inactive extracts to highly active extracts against three human cancer cells (OVCAR-8, HCT-116, and SF-295).

Other BP samples from Santa Catarina and the Rio Grande do Sul were also evaluated against different cancer cell lines, but they did not show *in vitro* antiproliferative effect against the tested cells (Silva et al. 2017).

Overall, little is known about the antitumor activities of brown propolis since there is a wide range of possibilities to be tested. Furthermore, chemical variations directly influence biological results, and each Brazilian region has specific BP that could display antitumor activities. Scientific studies on BBP should be encouraged to discover new molecules with antiproliferative actions.

Mutagenic and Antimutagenic Activity

A series of *in vivo* experiments with *Drosophila melanogaster* were performed to evaluate the mutagenic potential of Brazilian brown propolis. In this test, the genotoxicity was evaluated through the somatic mutation and recombination test (SMART), in which different mutant strains of *D. melanogaster* are used to be crossed and exposed to the samples (propolis extract, isolated compounds, or volatile oil). The offspring obtained from the emerging adults of the nonlethal concentrations are analyzed, observing spots in the wings that indicate mutagenic treatment activity.

Ethanolic extract of brown propolis from Paraná state in concentrations between 0.5 and 7.5 mg/ml showed no induction of genetic and chromosomal mutations or the somatic recombination indicating safety in use at low concentrations (Rodrigues et al. 2016). This was confirmed by another study, where BP from Mato Grosso do Sul State, at 1, 2, and 4 mg/ml, did not indicate mutagenicity (Fernandes et al. 2019). However, the isolated compounds caffeic acid and *p*-coumaric acid presented toxicity against *D. melanogaster* larvae at 40 mmol/l. Similarly, the acetyliscoupressic acid was mutagenic at 2.76 mmol/l on the individuals of the HB cross (crossing with the P450 cytochrome bioactivation), indicating a promutagen effect. On the other hand, the essential oil of BP from this location showed a lack of mutagenic activity and somatic mutations at concentrations of 0.05, 0.1, and 0.2% (Fernandes et al. 2015).

The ethanolic extract of Mato Grosso do Sul state propolis was tested *in vivo* on *D. melanogaster* larvae. Concentrations of 0.375, 0.75, and 1.5 mg/ml, administered with doxorubicin hydrochloride (DRX), a mutagenic agent, indicated inhibition of mutation frequencies in ST descendants, between 32.5 and 51.8%. Despite the HB crosses, with metabolic activation of procarcinogens and promutagens by CYP450 enzymes, no inhibition of mutation was observed. Instead, the highest concentration presented a marked co-mutagenic activity between the extract and the DXR. The authors attribute this result to the presence of compounds like flavonoids that can potentiate the effect of some chemotherapeutic agents. Considering that BBP did not show a mutagenic effect, its potential antigenotoxicity effect was assessed (Fernandes et al. 2014).

Fractions from the ethanolic extract showed no genotoxicity against *D. melanogaster* larvae. Instead, treatment with butanolic (*n*-BuOH) fraction decreased the mutations caused by DXR on the adults of the crosses, with inhibition of the mutagenic effect higher than 90% on the HB descendants in a dose–response manner. It indicates a possible metabolic activation of *n*-BuOH fraction constituents and CYP450 enzymes (Fernandes et al. 2014).

Antioxidant Effect

Several researchers have tested the antioxidant potential of BP from different locations. Compared to other propolis, brown types showed low values of flavonoids and phenolic compounds, decreasing their antioxidant potential (Machado et al. 2016a, b; Silva et al. 2017). However, some papers reported an excellent antioxidant capacity of ethanolic extracts, fractions, and essential oil of brown propolis.

According to Mohafez et al. (2010), the BP ethanolic extract at 50 µg/ml displayed antioxidant activity comparable to the hydroxyl ammonium chloride, an antioxidant compound. By increasing the reaction time and the concentration, the scavenging effect reached 47.5% at 100 µg/ml on the DPPH assay. A Mato Grosso do Sul propolis ethanolic extract and its fractions were tested on the DPPH assay to calculate the IC₅₀ value (Fernandes et al. 2014). The extract displayed an IC₅₀ of 532.2 µg/ml, while their ethyl acetate and *n*-butanol fractions presented the lowest IC₅₀ values (109.3 and 38.8 µg/ml, respectively), which were the most potent ones. Even with potential, these fractions did not display the caffeic acid antioxidant potential (IC₅₀ 3.47 µg/ml).

Hexane and dichloromethane fractions presented a poor antioxidant potential due to the significant presence of non-polar compounds (Fernandes et al. 2014; Santos et al. 2017). It revealed that the solvent and the extraction method have a remarkable influence on the biological effect. The antioxidant capacities of propolis samples from different locations

(Paraná, Minas Gerais, and Mato Grosso do Sul states), extracted with ethanol 96%–water (7:3, v/v) and ethanol 96%, were previously submitted to wax and non-polar component removal prior to being tested on the DPPH assay (Dembogurski et al. 2018).

BP potential of preventing oxidative stress in the skin was tested using a UVB-induced oxidative model (Fonseca et al. 2011). Previously, the extract displayed significant antioxidant activity against superoxide radicals on the xanthine/luminol/XOD system, with an IC₅₀ of free radicals' inhibition value of 0.005 µl/ml in an *in vivo* topical pretreatment (2.5%) and oral treatment (100 mg/kg) of BP extract, tested on hairless mice of the HRS/J strain exposed to UV irradiation. The oral treatment showed recovery of glutathione (GSH) levels. Furthermore, topical pretreatment and oral treatment inhibited the cutaneous metalloproteinase activity induced by UV irradiation exposure. However, the treatments did not exhibit inhibition of metalloproteinases (MMP-2 and MMP-9) in the irradiated animals. These enzymes are involved in elastin degradation and aging processes.

The antioxidant capacity of the essential oil of BP from Minas Gerais was also tested on the DPPH and ABTS assays by a spectrophotometric method (Lima et al. 2019). The volatile oil displayed IC₅₀ of 30.1 µg/ml on the ABTS assays and 25 µg/ml on the DPPH test. The last value was close to the positive control, butylated hydroxytoluene (BHT), indicating an antioxidant potential of the volatile constituents of BBP.

Toxicity

The toxic potential of BBP is poorly studied, and as mentioned, it was focused on the mutagenic activity. Only one study focuses on the acute toxicity assays of BBP extracts on *Artemia salina* (Santos et al. 2017). According to their results, the hexane extract enriched with hydrocarbons did not exhibit toxicity with a DL₅₀ of 1000 mg/ml, although the dichloromethane extract enriched with ananixathone presented DL₅₀ toxicity value of 68.99 mg/ml. The methanolic extract without a chemical characterization presented a DL₅₀ value of 118.1 mg/ml.

Antifungal Activity

The antifungal activity of BP was evaluated by Waller et al. (2017) using *Sporothrix brasiliensis*. The BP could inhibit the growth of 100% of the *S. brasiliensis* and presented a minimum inhibitory concentration (MIC) range from 0.19 to 1.56 mg/ml. The minimum fungicidal concentration (MFC) was also evaluated, and the values ranged from 0.78 to 3.125 mg/ml. More than 70% of the strains

used in this study were itraconazole resistant. Deegan et al. (2019) compared the activity of BP ethanolic extract against *Malassezia pachydermatis* and some commercially antifungal agents. BP displayed a MIC value > 16 mg/ml, but it did not exhibit lethality (MFC) at the highest tested concentrations. The MIC and MFC values of amphotericin B, itraconazole, and ketoconazole ranged from 0.0625 to 16 µg/ml.

Candida is a genus comprising more than 300 species, and some can cause human infections that significantly affect immunocompromised people (Ruhnke 2006). Different types of Brazilian BP were not active against *Candida albicans* (Silva et al. 2017). Salomão et al. (2008) reported the results of BP extract against *C. albicans* and *Paracoccidioides brasiliensis*, and it was more active against *P. brasiliensis*, but still with weak activity. The volatile oil of BP (VOBP) was tested against three strains of *Candida*: *C. krusei*, *C. glabrata*, and *C. parapsilosis*. The best activity was against *C. parapsilosis* with MIC of 100 µg/ml (Ribeiro et al. 2021a, b, c).

Antiparasitical Activity

There are 20 neglected tropical diseases (NTDs) listed by the World Health Organization (WHO 2019) including *Leishmaniasis* and Chagas disease. These NTDs affect people who live in great poverty, corresponding to 1.4 billion people worldwide. (Holmes et al. 2017).

Trypanosoma cruzi is a protist hemoflagellate responsible for causing Chagas disease. Salomão et al. (2008) evaluated the *in vitro* activity of some BP extracts against tripomastigote forms of *Trypanosoma cruzi*. Crystal violet, the positive control, displayed IC₅₀ 187 µg/ml, and BP samples displayed IC₅₀'s ranging from 200 to 2000 µg/ml. BP containing 24.74% of benzoic acid displayed the smaller IC₅₀. Another 96 h *in vitro* study with different extracts of BP against epimastigote forms of *T. cruzi* with concentrations between 75 and 300 mg/ inhibited the growth of *T. cruzi* by 90% (Silva et al. 2017).

Considering other studies where BP showed potential against *Plasmodium*, *Trypanosoma*, and *Leishmania* genus, it became an exciting alternative for discovering new compounds with antileishmanial activities. Santana et al. (2014) reported the activity of BP against promastigote and amastigote forms of *L. amazonensis*, comparing different fractions obtained by the partition of the crude extract. The *in vitro* assay of growth inhibition of *L. amazonensis* promastigote form after 72 h with dichloromethane fraction containing 2,3-dihydroxybenzofurane as its major compound displayed the best result (IC₅₀ 3.22 µg/ml). The hydroalcoholic fraction (IC₅₀ 4.64 µg/ml), hexane fraction (IC₅₀ 4.79 µg/ml), and ethyl acetate fraction (IC₅₀ 8.83 µg/ml) were also active. The activity was evaluated by the 50% cytotoxicity

concentration (CC₅₀) to macrophages for the amastigote forms. The hydroalcoholic fraction displayed the highest activity (CC₅₀ 21.69 µg/ml), and the ethyl acetate fraction, which contains *p*-coumaric acid as the major compound (17.16%), displayed the weaker activity (CC₅₀ > 400 µg/ml).

The antileishmanial activity of VOBP was reported by Ribeiro et al. (2021a, b, c). The *in vitro* assay was accomplished with promastigote and amastigote forms of the parasite, and it was noticed that VOBP inhibited flagellar motility in the promastigote forms in a dose-dependent manner with IC₅₀ 21.3 µg/ml and also increased the death of amastigote forms with IC₅₀ 25.1 µg/ml. Amphotericin B was the positive control used at 50 µg/ml, and VOBP was more active against amastigote forms than the positive control. Nerolidol was the major compound of VOBP, which has already demonstrated leishmanicidal activity against *L. braziliensis* (Ceole et al. 2017).

One study reported the activity of BP extract and its fractions against trophozoite forms of *T. vaginalis* with MIC of 400 µg/ml. Furthermore, one dichloromethane fraction and one ethyl acetate fraction inhibit 100% of the trophozoite viability at 500 µg/ml (Dembogurski et al. 2018). Different types of BP extract were used, and the ethanolic extracts were the most active.

Antiviral Activity

Hocchein et al. (2019) reported the antiviral activity of BP hydroalcoholic extract and its dichloromethane and aqueous fractions against herpes simplex virus type-1 (HSV-1). Dichloromethane fraction displayed cytotoxicity activity over the cell with CC₅₀ 88.4 µg/ml, followed by the crude hydroalcoholic extract (CC₅₀ 143.7 µg/ml), and aqueous fraction with weak activity (CC₅₀ 1240 µg/ml). They also evaluated the percentage of an inhibitory concentration of the fractions and extract, finding a range of IC₅₀ from 58.5 µg/ml (dichloromethane fraction) to 294 µg/ml (butanol fraction). Likewise, two fractions inhibited 90% of the virus replication at concentrations above 200 µg/ml.

Antibacterial Activity

The antimicrobial activity of BP has been studied worldwide against gram-positive and gram-negative bacteria, drawing the attention of pharmaceutical and food companies. Moreover, the need to develop new drugs with antimicrobial potential has increased annually due to the high number of new resistance against the usual treatment (Morrill et al. 2015).

A good activity of BP extract was observed against gram-positive bacteria *Staphylococcus aureus* and *Enterococcus* spp. (MIC 31.3–500 µg/ml). However, no activity was found against gram-negative bacteria, like *Klebsiella* spp. and *Escherichia coli* (Silva et al. 2017). Salomão et al. (2008) reported a similar

result, by describing the activity of BP extract against *Streptococcus pneumonia* (MIC 0.2–0.8 µg/ml) and *Staphylococcus aureus* (MIC 1.6–52.4 µg/ml). On the other hand, it was inactive against gram-negative *Klebsiella pneumoniae*.

Other studies reported the inactivity of BP extracts against strains of *Salmonella* spp., *P. aeruginosa*, *S. aureus*, and *E. coli*, including resistant strains (Bastos et al. 2011). Furthermore, forty samples of BP from different origins were tested against *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*. A few of them displayed weak MIC values (156–1250 µg/ml), and the majority were inactive. The same occurred for the minimum bactericidal concentration (MBC), with no extract being active at any concentration (Machado et al. 2021).

Dembogurski et al. (2018) evaluated the antibacterial activity of BP against planktonic cells of *Staphylococcus aureus* and biofilm cells. Five different fractions of BP extract were tested on seven strains of the gram-positive *Mycoplasma* genera, and the less polar fractions displayed the best MIC value (3.9 µg/ml) (Araújo et al. 2020). The result seemed to be better for planktonic cells. Still, the BP assay against *Pseudomonas aeruginosa* (gram-negative) did not show activity.

The VOBP also displayed activity against *Helicobacter pylori*, *Mycobacterium tuberculosis*, and *M. avium* with MIC 3.25, 50, and 62.5 µg/ml, respectively. Tetracycline displayed MIC of 1 µg/ml against *H. pylori* and isoniazid displayed MIC of 1.47 µg/ml against both *Mycobacterium* (Lima et al. 2019). Fernandes et al. (2015) reported the antibacterial evaluation of another VOBP sample with MIC > 1000 µg/ml against five strains of bacteria, including gram-positive and gram-negative bacteria (*Enterococcus faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumonia*). As expected, it was noticed that BP has more significant potential against gram-positive than gram-negative bacteria. Similar results were reported by other authors suggesting that the difference occurs due to more complexity of the cell wall of gram-negative bacteria.

Nevertheless, the variation of the antimicrobial activity found in samples of BP extract and its volatile oil can be explained by the chemical difference among the samples, which leads to different chemical properties. It is known that the chemical composition of propolis depends on its botanical origin, which is directly correlated to the surrounding plant biodiversity (Rodrigues et al. 2020).

Other Activities

The enzymatic analysis of gastric tissues indicated that propolis caused restoration of glutathione and superoxide dismutase activity to normal levels and reduced thiobarbituric acid reactive substances (TBARS) levels. The antioxidant, lipid peroxidation-inhibitory, and anti-ulcer *in vivo* activities of BBP ethanolic extract were also reported (Mohafez

et al. 2010). A dose of 100 mg/kg of BP ethanolic extract, in an oral pretreatment, showed hepatic protection against lipid peroxidation effect of CCl₄, decreasing the malondialdehyde (MDA) level and increasing the glutathione (GSH) concentration. Also, BBP pretreatment reduced hemorrhagic lesions induced by indomethacin in rat stomachs, reducing the gastric ulcer index by approximately 65%, and maintaining the typical histological structure. Even when indomethacin causes ROS-generated inflammation, BP pretreatment attenuates this injury effect, preventing ulcer damage.

BP has been evaluated as a food supplement of lambs in comparison with the traditional dietary additive monensin (Ítavo et al. 2011). The propolis extract was added to the diet of lambs daily with a calculated dry residual/flavonoid proportion of 0.9 g/20.2 mg. The animal group treated with propolis showed good efficiency in weight gain, feed efficiency, and conversion, making BP extract a potential food supplement to replace monensin sodium, which has been indicated as a potential inhibitor of food consumption in sheep.

Production and Economic Aspects

The Brazilian beekeeping activity is a promising national enterprise and has gained traction in the international market since the enormous Brazilian biodiversity favors the diversified bee products. Scientific investigations showing Brazilian propolis's antimicrobial and immunological activities have contributed to increasing propolis's competitiveness and demand nationally and internationally (Berretta et al. 2017).

The range of biological properties of BP has drawn the interest of the pharmaceutical, food, and cosmetic industries, mainly due to its antimicrobial and antioxidant potential with beneficial health effects for consumers. The characteristic organoleptic properties, such as smell, color, and texture of most propolis, are essential for consumer acceptability and commercialization. Its medicinal and food supplement uses are well-established in several parts of the world, especially in Eastern Europe, China, and Japan. Brazil is a big player in the international market, moving millions of dollars annually with bee products. (Hata et al. 2012; Berretta et al. 2017).

CONAP-Brazil (National Beekeeping Cooperative) reported in 2020 that propolis's national and international markets increased by approximately 40 and 50%, respectively, devoid of the COVID-19 outbreak. It is estimated that approximately 90% of CONAP's revenue comes from the exports of bee products, mainly to Asian countries, including Japan, South Korea, and Taiwan, registering an increase of 94% in the exports compared to 2019 (Conap 2020).

Minas Gerais is the largest Brazilian State propolis producer, responsible for 70% of all propolis production in Brazil of the total estimated production of 120 tons per year, with 85% of green propolis and 15% of brown propolis (Guimarães 2020). As the world demand for propolis has grown,

with exportation to Europe and the USA, other regions of Brazil are also increasing their productions, such as the northeast and south regions. However, to consolidate brown propolis in the international market, more scientific studies must be undertaken on propolis from each region, thus adding value to this Brazilian bee product (Nordeste 2021).

Conclusions

BP has a greater chemical variety than other types of Brazilian propolis since several possible botanical sources are listed. Thus, further research using BP to assess its pharmacological activity should consider its chemical variation and its possible botanical sources. Powerful tools like ultra-efficiency liquid chromatography systems coupled with high-resolution mass spectrometry associated with metabolomics studies can be an alternative to propose new propolis classification taking into account their chemical profile. The volatile fractions' composition of different types of propolis has been poorly explored, and it undoubtedly plays an essential role in its biological activities, which have not been fully elucidated. Therefore, different types of Brazilian brown propolis have great potential and should be further investigated to enable its production and commercialization as effective and safe health products.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s43450-023-00374-x>.

Acknowledgements The authors are thankful to Dr. Rogelio Pereda-Miranda, Editor-in-Chief of *Brazilian Journal of Pharmacognosy*, for his suggestions to improve the manuscript. The authors are thankful to Fundação de amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support, Grants # 2017/04138-8 and 2022/00659-1.

Author Contribution VPR contributed to the conception, design, literature search, and writing of the manuscript. Data compilation, and writing and editing of the manuscript were done by JAAM, DMR, GRA, AMFP, and MHT. JKB and SRA supervised and critically revised the manuscript. All the authors have read and approved the manuscript.

Funding The authors received financial support, Grants # 2017/04138-8 and 2022/00659-1, from Fundação de amparo à Pesquisa do Estado de São Paulo (FAPESP).

Declarations

Conflict of Interest The authors declare no competing interests.

References

- Anvisa (2017) RDC 166/2017, Dispõe sobre a validação de métodos analíticos e dá outras providências, Agência Nacional de Vigilância Sanitária, Ministério da Saúde, Brasília, DF
- Araújo C, Mayworm MAS, Yatsuda R, Negri G, Salatino MLF, Salatino A, Timenetsky J, Campos GB (2020) Chemical composition and antimycoplasmal activity of a brown propolis from Southern Brazil. *J Food Sci Technol* 57:4228–4235. <https://doi.org/10.1007/s13197-020-04461-y>
- Arruda C, Ribeiro VP, Almeida MO, Mejía JAA, Casoti R, Bastos JK (2020) Effect of light, oxygen and temperature on the stability of artemillin C and *p*-coumaric acid from Brazilian green propolis. *J Pharm Biomed Anal* 178:112922. <https://doi.org/10.1016/j.jpba.2019.112922>
- Arruda C, Ribeiro VP, Mejía JAA, Almeida MO, Goulart MO, Candido ACBB, dos Santos RA, Magalhães LG, Martins CHG, Bastos JK (2020) Green propolis: cytotoxic and leishmanicidal activities of artemillin C, *p*-coumaric acid, and their degradation products. *Rev Bras Farmacogn* 30:169–176. <https://doi.org/10.1007/s43450-020-00043-3>
- Bankova V, Boudourova-Krasteva G, Sforcin JM, Frete X, Kujumgiev A, Maimoni-Rodella R, Popov S (1999) Phytochemical evidence for the plant origin of Brazilian propolis from São Paulo State. *Z Naturforsch C* 54:401–405. <https://doi.org/10.1515/ZNC-1999-5-616>
- Bankova V, Popova M, Trusheva B (2018) The phytochemistry of the honeybee. *Phytochemistry* 155:1–11. <https://doi.org/10.1016/j.phytochem.2018.07.007>
- Bastos EMAF, Galbiati C, Loureiro EM, Scoaris DO (2011) Indicadores físico-químicos e atividade antibacteriana de própolis marrom frente à *Escherichia coli*. *Arq Bras Med Vet Zootec* 63:1255–1259. <https://doi.org/10.1590/S0102-09352011000500032>
- Bernardes CT, Ribeiro VP, Carvalho TC, Furtado RA, Furtado NAJC, Bastos JK (2022) Disinfectant activities of extracts and metabolites from *Baccharis dracunculifolia* DC. *Lett Appl Microbiol* 75:261–270. <https://doi.org/10.1111/lam.13725>
- Berretta AA, Arruda C, Miguel FG, Baptista N, Nascimento AP, Marquele- Oliveira F, Hori JI, Barud HS, Damaso B, Ramos C, Ferreira R, Bastos JK (2017) Functional properties of Brazilian propolis: from chemical composition until the market. *Superfood and Functional Food - an Overview of Their Processing and Utilization*. <https://doi.org/10.5772/65932>
- Beserra FP, Gushiken LFS, Hussni MF, Ribeiro VP, Bonamin F, Jackson CJ, Pellizzon CH, Bastos JK (2021) Artemillin C as an outstanding phenolic compound of Brazilian green propolis for disease treatment: a review on pharmacological aspects. *Phytother Res* 35:2274–2286. <https://doi.org/10.1002/ptr.6875>
- Brophy JJ, Goldsack RJ, Wu MZ, Fookes CJR, Forster PI (2000) The steam volatile oil of *Wollemia nobilis* and its comparison with other members of the Araucariaceae (*Agathis* and *Araucaria*). *Biochem Syst Ecol* 28:563–578. [https://doi.org/10.1016/S0305-1978\(99\)00090-3](https://doi.org/10.1016/S0305-1978(99)00090-3)
- Burzynski-Chang EA, Ryona I, Reisch BI, Gonda I, Foolad MR, Giovannoni JJ, Sacks GL (2018) HS-SPME-GC-MS analyses of volatiles in plant populations—quantitating compound × individual matrix effects. *Molecules* 23:2436. <https://doi.org/10.3390/molecules23102436>
- Calegari MA, Prasniewski A, Silva C, Sado RY, Maia FMC, Tonial LMS, Oldoni TLC (2017) Propolis from Southwest of Paraná produced by selected bees: influence of seasonality and food supplementation on antioxidant activity and phenolic profile. *An Acad Bras Cienc* 89:45–55. <https://doi.org/10.1590/0001-376520160499>
- Ccana-Capatinta GV, Mejía JAA, Tanimoto MH, Groppo M, Carvalho JCS, Bastos JK (2020) *Dalbergia ecastaphyllum* (L.) Taub and *Symphonia globulifera* L.f.: the botanical sources of isoflavonoids and benzophenones in Brazilian red propolis. *Molecules* 25:2060. <https://doi.org/10.3390/MOLECULES25092060>
- Ceole LF, Cardoso MDG, Soares MJ (2017) Nerolidol, the main constituent of *Piper aduncum* essential oil, has anti-*Leishmania braziliensis* activity. *Parasitology* 144:1179–1190. <https://doi.org/10.1017/S0031182017000452>

- Conap (2020) Exports increase in the pandemic. <https://www.Jornalbelvedere.Com.Br/Index.Php/Estilo/Saude/Item/1653-Conap-Exportacoes-Aumentam-Na-Pandemia>
- Daugusch A, Moraes CS, Fort P, Park YK (2008) Brazilian red propolis - chemical composition and botanical origin. *Evid Based Complementary Altern Med* 5:435–441. <https://doi.org/10.1093/ecam/nem057>
- Deegan KR, Fonseca MS, Oliveira DCP, Santos LM, Fernandez CC, Hanna SA, Machado BAS, Umsza-Guez MA, Meyer R, Portela RW (2019) Susceptibility of *Malassezia pachydermatis* clinical isolates to allopathic antifungals and Brazilian red, green, and brown propolis extracts. *Front Vet Sci* 6:1–12. <https://doi.org/10.3389/fvets.2019.00460>
- Dembogurski DS, Silva TD, Boaretto AG, Rigo GV, Silva RC, Tasca T, Macedo AJ, Carollo CA, Silva DB (2018) Brown propolis-metabolomic innovative approach to determine compounds capable of killing *Staphylococcus aureus* biofilm and *Trichomonas vaginalis*. *Food Res Int* 111:661–673. <https://doi.org/10.1016/j.foodres.2018.05.033>
- dos Santos DC, David JM, David JP (2017) Composição química, atividade citotóxica e antioxidante de um tipo de própolis da Bahia. *Quim Nova* 40:171–175. <https://doi.org/10.21577/0100-4042.20160174>
- Eberhardt TL, Bernards MA, He L, Davin LB, Wooten JB, Lewis NG (1993) Lignification in cell suspension cultures of *Pinus taeda*. *In situ* characterization of a gymnosperm lignin. *J Biol Chem* 268:21088–21096. [https://doi.org/10.1016/S0021-9258\(19\)36897-8](https://doi.org/10.1016/S0021-9258(19)36897-8)
- Elián Guimarães (2020) With therapeutic properties, the search for green propolis increases in the pandemic. *Jornal Estado de Minas*. https://www.em.com.br/app/noticia/economia/2021/06/26/intermas_economia,1280710/com-propriedades-terapeuticas-busca-por-propolis-verde-aumenta-na-pandemia.shtml
- Fabio G, Federica C, Alfredo F, Nicola V (2019) Recent advances in analytical approaches for the standardization and quality of polyphenols of propolis. *J Med Plant Res* 13:487–500. <https://doi.org/10.5897/jmpr2019.6849>
- Fernandes FH, Rosa GZ, Garcez WS, Lopes SM, Corsino J, Garcez FR (2014) Assessment of the (anti)genotoxicity of brown propolis extracts from Brazilian Cerrado biome in a *Drosophila melanogaster* model. *Food Res Int* 62:20–26. <https://doi.org/10.1016/j.foodres.2014.02.029>
- Fernandes FH, Guterres ZDR, Corsino J, Garcez WS, Garcez FR (2019) Assessment of the mutagenicity of propolis compounds from the Brazilian cerrado biome in somatic cells of *Drosophila melanogaster*. *Orbital* 11:307–313. <https://doi.org/10.17807/orbit.al.v11i5.1418>
- Fernandes FH, Guterres ZDR, Violante IMP, Lopes TFS, Garcez WS, Garcez FR (2015) Evaluation of mutagenic and antimicrobial properties of brown propolis essential oil from the Brazilian Cerrado biome. *Toxicol Rep* 2:1482–1488. <https://doi.org/10.1016/j.toxrep.2015.11.007>
- Fonseca YM, Marquele-Oliveira F, Vicentini FTMC, Furtado NAJC, Sousa JPB, Lucisano-Valim YM, Fonseca MJV (2011) Evaluation of the potential of Brazilian propolis against UV-induced oxidative stress. *Evid Based Complement Alternat Med* 2011:863917. <https://doi.org/10.1155/2011/863917>
- Freitas AS, Barth OM, Sales EO, Matsuda AH, Almeida-Muradian LB (2011) A palynological analysis of Brazilian propolis samples. *JAAS* 3:67–74. <https://doi.org/10.3896/IBRA.4.03.2.01>
- Freitas MO, Ponte FAF, Lima MAS, Silveira ER (2008) Flavonoids and triterpenes from the nest of the stingless bee *Trigona spinipes*. *J Braz Chem Soc* 19:532–535. <https://doi.org/10.1590/S0103-50532008000300022>
- Frota VM, Dias FMF, do Nascimento MF, da Silva Mendonça L, dos Santos Moita EC, dos Santos Gomes LC, Leal ALAB, Barreto HM, Silva MFS, dos Santos Fontenelle RO, Gomes GA, do Vale JPC, da Silva Julião MS, Bandeira PN, Pessoa CÓ, dos Santos HS, Zocolo GJ, Rodrigues THS (2021) Inhibitory activity of brown propolis extracts on a norfloxacin-resistant strain of *Staphylococcus aureus*. *Rev Bras Farmacogn* 31:249–255. <https://doi.org/10.1007/s43450-021-00150-9>
- Hata T, Tazawa S, Ohta S, Rhyu MR, Misaka T, Ichihara K (2012) Artepillin C, a major ingredient of Brazilian propolis, induces a pungent taste by activating TRPA1 channels. *PLoS One* 7:e48072. <https://doi.org/10.1371/JOURNAL.PONE.0048072>
- Holmes KK, Bertozzi S, Bloom BR, Jha P (2017) *Disease Control Priorities, Third Edition (Volume 6): Major Infectious Diseases*. World Bank, Washington, DC. <https://doi.org/10.1596/978-1-4648-0524-0>
- Ioannou E, Koutsaviti A, Tzakou O, Roussis V (2014) The genus *Pinus*: a comparative study on the needle essential oil composition of 46 pine species. *Phytochem Rev* 13:741–768. <https://doi.org/10.1007/s11101-014-9338-4>
- Ítavo CCBF, Morais MG, Costa C, Ítavo LCV, Franco GL, Silva JA, Reis FA (2011) Addition of propolis or monensin in the diet: behavior and productivity of lambs in feedlot. *Anim Feed Sci Technol* 165:161–166. <https://doi.org/10.1016/j.anifeedsci.2011.02.020>
- Lima VHM, Almeida KCR, Alves CCF, Rodrigues ML, Crotti AEM, Souza JM, Ribeiro AB, Squarisi IS, Tavares DC, Martins CHG, Miranda MLD (2019) Biological properties of volatile oil from Brazilian brown propolis. *Rev Bras Farmacogn* 29:807–810. <https://doi.org/10.1016/j.bjp.2019.07.004>
- Machado BAS, Silva RPD, Barreto GDA, Costa SS, Silva DF, HNRocha JLCN, Dellagostin OA, Henriques JAP, Umsza-Guez MA, Padilha FF (2016) Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic regions in Brazil. *PLoS One* 11:e0145954. <https://doi.org/10.1371/journal.pone.0145954>
- Machado CS, Finger D, Lima TC, Modesto T, Monteiro MC, Oliveira FCE, Pessoa C, Torres YR (2021) Seeking the regional identity of brown propolis produced in southern Brazil and linked to total levels of bioactive compounds and biological activity. *ACS Food Sci Technol* 1:176–185. <https://doi.org/10.1021/acscfoodscitech.0c00052>
- Machado CS, Mokochinski JB, Lira TO, Oliveira FDCE, Cardoso MV, Ferreira RG, Sawaya ACHF, Ferreira AG, Pessoa C, Cuesta-Rubio O, Monteiro MC, Campos MS, Torres YR (2016) Comparative study of chemical composition and biological activity of yellow, green, brown, and red Brazilian propolis. *Evid-Based Complement Altern Med* 2016:6057650. <https://doi.org/10.1155/2016/6057650>
- Mayworm MAS, Lima CA, Tomba ACB, Fernandes-Silva CC, Salatino MLF, Salatino A (2014) Does propolis contain tannins? *Evid-Based Compl Alt* 2014:613647. <https://doi.org/10.1155/2014/613647>
- Mohafez OMM, Abdel-Raheem IT, Nafady AM (2010) Antioxidant, lipid peroxidation-inhibitory and antiulcer activities of brown propolis. *Bull Pharm Sc* 33:169–177. <https://doi.org/10.21608/bfsa.2010.64741>
- Morrill HJ, Pogue JM, Kaye KS, LaPlante KL (2015) Treatment options for carbapenem-resistant Enterobacteriaceae infections. *Open Forum Infect Dis* 2:ofv050. <https://doi.org/10.1093/ofid/ofv050>
- Nordeste, B (2021) Potential of propolis production in the Northeast. *Publishing Journal Banco Do Nordeste*. https://bnb.gov.br/web/guest/agronegocio/agroinforma/-/asset_publisher/qg5dL6xAGfoP/content/potencial-da-producao-de-propolis-no-nordeste/3760965?inheritRedirect=false&redirect=https%3A%2F%2Fbnb.gov.br%2Fweb%2Fguest%2Fagronegocio%2Fagroinforma%3Fp_id%3D

- Olegário LS, Andrade JKS, Andrade GRS, Denadai M, Cavalcanti RL, Silva MAAP, Narain N (2019) Chemical characterization of four Brazilian brown propolis: an insight in tracking of its geographical location of production and quality control. *Food Res Int* 123:481–502. <https://doi.org/10.1016/j.foodres.2019.04.004>
- Park YK, Alencar SM, Aguiar CL (2002) Botanical origin and chemical composition of Brazilian Propolis. *J Agric Food Chem* 50:2502–2506. <https://doi.org/10.1021/JF011432B>
- Pavlovic R, Borgonovo G, Leoni V, Giupponi L, Ceciliani G, Sala S, Bassoli A, Giorgi A (2020) Effectiveness of different analytical methods for the characterization of propolis: a case of study in Northern Italy. *Molecules* 25:504. <https://doi.org/10.3390/molecules25030504>
- Pimenta HC, Violante IMP, Musis CR, Borges ÁH, Aranha AMF (2015) *In vitro* effectiveness of Brazilian brown propolis against *Enterococcus faecalis*. *Braz Oral Res* 29:1–6. <https://doi.org/10.1590/1807-3107BOR-2015.vol29.0058>
- Ribeiro VP, Arruda C, Abd El-Salam M, Bastos JK (2018) Brazilian medicinal plants with corroborated anti-inflammatory activities: a review. *Pharm Biol* 56:253–268. <https://doi.org/10.1080/13880209.2018.1454480>
- Ribeiro VP, Arruda C, Mejia JAA, Bastos JK, Tripathi SK, Khan SI, Khan IA, Ali Z (2021) Phytochemical, antiplasmodial, cytotoxic and antimicrobial evaluation of a southeast Brazilian brown propolis produced by *Apis mellifera* bees. *Chem Biodivers* 18:e2100288. <https://doi.org/10.1002/cbdv.202100288>
- Ribeiro VP, Arruda C, Mejia JAA, Candido ACBB, Santos RA, Magalhães LG, Bastos JK (2021b) Brazilian southeast brown propolis: gas chromatography method development for its volatile oil analysis, its antimicrobial and leishmanicidal activities evaluation. *Phytochem Anal* 32:404–411. <https://doi.org/10.1002/pca.2988>
- Ribeiro VP, Ccana-Ccapatinta GV, Mejia JAA, Berretta AA, Moraes LA, Bastos JK (2022) Chemical characterization of Brazilian propolis using automated direct thermal desorption–gas chromatography–mass spectrometry. *J Sci Food Agric* 102:4345–4354. <https://doi.org/10.1002/jsfa.11788>
- Ribeiro VP, Símaro GV, Mejia JAA, Arruda C, Bastos JK (2021) Anti-inflammatory and antinociceptive activities of the hydroalcoholic extract and the volatile fraction of southeastern Brazilian brown propolis. *Rev Bras Farmacogn* 31:59–66. <https://doi.org/10.1007/s43450-020-00122-5>
- Rivera-Yañez N, Rivera-Yañez CR, Pozo-Molina G, Méndez-Catalá CF, Méndez-Cruz AR, Nieto-Yañez O (2020) Biomedical properties of propolis on diverse chronic diseases and its potential applications and health benefits. *Nutrients* 13:78. <https://doi.org/10.3390/NU13010078>
- Rodrigues CRF, Plentz LC, Marcucci MC, Dihl RR, Lehmann M (2016) *In vivo* evaluation of mutagenic and recombinogenic activities of Brazilian propolis. *Food Chem Toxicol* 96:117–121. <https://doi.org/10.1016/j.fct.2016.07.037>
- Rodrigues DM, de Souza MC, Arruda C, Pereira RAS, Bastos JK (2020) The role of *Baccharis dracunculifolia* and its chemical profile on green propolis production by *Apis mellifera*. *J Chem Ecol* 46:150–162. <https://doi.org/10.1007/s10886-019-01141-w>
- Ruhnke M (2006) Epidemiology of *Candida albicans* infections and role of non-*Candida albicans* yeasts. *Curr Drug Targets* 7:495–504. <https://doi.org/10.2174/138945006776359421>
- Salas AL, Alberto MR, Zampini IC, Cuello AS, Maldonado L, Ríos JL, Schmeda-Hirschmann G, Isla MI (2016) Biological activities of polyphenols-enriched propolis from Argentina arid regions. *Phytomedicine* 23:27–31. <https://doi.org/10.1016/J.PHYMED.2015.11.007>
- Salomão K, Pereira PRS, Campos LC, Borba CM, Cabello PH, Marcucci MC, Castro SL (2008) Brazilian propolis: correlation between chemical composition and antimicrobial activity. *Evid-Based Complement Altern Med* 5:317–324. <https://doi.org/10.1093/ecam/nem058>
- Santana LCLR, Carneiro SMP, Caland-Neto LB, Arcanjo DDR, Moita-Neto JM, Citó AMGL, Carvalho FAA (2014) Brazilian brown propolis elicits antileishmanial effect against promastigote and amastigote forms of *Leishmania amazonensis*. *Nat Prod Res* 28:340–343. <https://doi.org/10.1080/14786419.2013.856904>
- Santos MFC, Oliveira LC, Ribeiro VP, Soares MG, Moraes GOI, Sartori AGO, Rosalen PL, Bastos JK, Alencar SM, Veneziani RCS, Ambrósio SR (2021) Isolation of diterpenes from *Araucaria* sp. Brazilian brown propolis and development of a validated HPLC method for its analysis. *J Sep Sci* 44:3089–3097. <https://doi.org/10.1002/jssc.202100374>
- Sartori G, Pesarico AP, Pinton S, Dobrachinski F, Roman SS, Puletto F, Rodrigues LC, Prigol M (2012) Protective effect of brown Brazilian propolis against acute vaginal lesions caused by herpes simplex virus type 2 in mice: involvement of antioxidant and anti-inflammatory mechanisms. *Cell Biochem Funct* 30:1–10. <https://doi.org/10.1002/cbf.1810>
- Sartori GO, Spada FP, Ribeiro VP, Rosalen PL, Ikegaki M, Bastos JK, Alencar SM (2021) An insight into the botanical origins of propolis from permanent preservation and reforestation areas of southern Brazil. *Sci Rep* 11:22043. <https://doi.org/10.1038/s41598-021-01709-1>
- Sawaya ACHF, Cunha BS, Marcucci MC (2011) Analytical methods applied to diverse types of Brazilian propolis. *Chem Cent J* 5:27. <https://doi.org/10.1186/1752-153X-5-27>
- Serafim MS, Rodrigues DM, Ribeiro VP, Ccana-Ccapatinta GV, Groppo M, Martins CHG, Ambrósio SR, Bastos JK (2022) *Eucalyptus botryoides* resin and its new 2-*O*-galloyl-1,6-*O*-di-*trans-p*-coumaroyl- β -D-glycopyranoside compound display good antimicrobial activity. *Nat Prod Res* 5:1–10. <https://doi.org/10.1080/14786419.2022.2065486>
- Silva RPD, Machado BAS, Barreto GA, Costa SS, Andrade LN, Amaral RG, Carvalho AA, Padilha FF, Barbosa JDV, Umszaguez MA (2017) Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. *PLoS One* 12:e0172585. <https://doi.org/10.1371/journal.pone.0172585>
- Simões-Ambrosio LMC, Gregório LE, Sousa JPB, Figueiredo-Rinhel ASG, Azzolini AECS, Bastos JK, Lucisano-Valim YM (2010) The role of seasonality on the inhibitory effect of Brazilian green propolis on the oxidative metabolism of neutrophils. *Fitoterapia* 81:1102–1108. <https://doi.org/10.1016/J.FITOTE.2010.07.008>
- Simone-Finstrom M, Borba RS, Wilson M, Spivak M (2017) Propolis counteracts some threats to honeybee health. *Insects* 8:46. <https://doi.org/10.3390/INSECTS8020046>
- Tazawa S, Arai Y, Hotta S, Mitsui T, Nozaki H, Ichihara K (2016) Discovery of a novel diterpene in brown propolis from the state of Parana, Brazil. *Nat Prod Commun* 11:201–205. <https://doi.org/10.1177/1934578X1601100218>
- Waller SB, Peter CM, Hoffmann JF, Picoli T, Osório LG, Chaves F, Zani JL, Faria RO, Mello JRB, Meireles MCA (2017) Chemical and cytotoxic analyses of brown Brazilian propolis (*Apis mellifera*) and its *in vitro* activity against itraconazole-resistant *Sporothrix brasiliensis*. *Microb Pathog* 105:117–121. <https://doi.org/10.1016/j.micpath.2017.02.022>
- WHO (2019) World Health Organization. Control of Neglected Tropical Diseases. <https://www.who.int/teams/control-of-neglected-tropical-diseases/overview>

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.