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Original Article

Antifungal potential of Brazilian green and brown propolis against clinically relevant yeasts

Potencial antifúngico das própolis verde e marrom brasileiras contra leveduras clinicamente relevantes

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Abstract

Brazilian biodiversity is rich in compounds with biological potential. Propolis is a natural product formed from bee saliva and plant exudates. Brazilian brown and green propolis exhibit antioxidant, antibacterial, antifungal, antiviral, and antiparasitic activities. These properties arise from their rich phenolic content; molecules such as artepellin-C, quercetin, and kaempferol are important phenolic biomolecules described in propolis. The objective of this study was to determine the antifungal activity and chemical parameters of brown and green propolis samples commercialized in Santa Rita do Sapucaí, southern Minas Gerais, Brazil. The total phenolic compound content was assessed using the Folin-Ciocalteu colorimetric method. Total flavonoid concentration was determined by the aluminum chloride complexation method. The pH and density were also determined. The antifungal activity of the extracts was evaluated using the disk diffusion technique against strains from the American Type Culture Collection (ATCC), following the Kirby-Bauer methodology. Both extracts showed similar densities; the green propolis extract presented a pH of 4.37, while the brown propolis extract showed a pH of 3.61. The ethanolic extract of brown propolis (706.79 mg GAE/g) exhibited a higher content of phenolic compounds compared to green propolis (697.65 mg GAE/g), whereas the green propolis extract (415.75 mg QE/g) presented a higher flavonoid content than brown propolis (378.75 mg QE/g). Clinically relevant strains, *Candida albicans* (EPM: 12.43 mm; EPV: 12.78 mm), *Candida parapsilosis* (EPM: 14.88 mm; EPV: 13.96 mm), and *Cutaneotrichosporon dermatis* (EPM: 11.95 mm; EPV: 12.28 mm), were the most susceptible to both extracts. It can be inferred that the extracts possess excellent antifungal activity, possibly associated with their rich phenolic and flavonoid content.

Keywords: antifungal activity, *Candida* spp. and yeast pathogens, ethanolic extract, flavonoids, propolis.

Resumo

Potencial antifúngico das própolis verde e marrom brasileiras contra leveduras clinicamente relevantes. A própolis é um produto natural formado a partir da saliva de abelhas e exsudatos vegetais. A biodiversidade brasileira é rica em compostos com potencial biológico. A própolis marrom e verde brasileira apresentam atividade antioxidante, antibacteriana, antifúngica, antiviral e antiparasitária. Essas propriedades são oriundas de seu rico conteúdo fenólico, moléculas como o artepellin-C, a quercetina e o kaempferol são importantes biomoléculas fenólicas descritas para a própolis. O objetivo deste trabalho foi determinar a atividade antifúngica e parâmetros químicos de amostras de própolis marrom e verde comercializados em Santa Rita do Sapucaí, sul de Minas Gerais, Brasil. O teor de compostos fenólicos totais foi avaliado utilizando o método colorimétrico de *Folin-Ciocalteau*. A concentração de flavonoides totais foi determinada pelo método de complexação com cloreto de alumínio. O pH e a densidade também foram determinados. A atividade antifúngica dos extratos foi avaliada através da técnica de disco-difusão frente a cepas da *American Type Culture Collection* (ATCC) seguindo a metodologia de *Kirby-Bauer*. Ambos os extratos apresentaram densidades similares, o extrato de própolis verde apresentou pH referente a 4.37, enquanto o extrato de própolis marrom apresentou pH referente a 3.61. O extrato etanólico de própolis marrom (706,79 mg EAG/g) apresentou maior conteúdo de compostos fenólicos se comparado ao de própolis verde (697,65 mg EAG/g), enquanto o extrato de própolis verde (415,75 mg EQ/g) apresentou maior conteúdo de flavonoides se comparado a própolis marrom (378,75 mg EQ/g). As cepas de relevância clínica, *Candida albicans* (EPM: 12,43 mm; EPV: 12,78 mm), *Candida parapsilosis* (EPM: 14,88 mm; EPV: 13,96 mm) e *Cutaneotrichosporon dermatis* (EPM: 11,95; EPV: 12,28 mm), foram as mais susceptíveis frente ambos os extratos. Pode-se inferir que os extratos possuem excelente atividade antifúngica, possivelmente associada ao rico conteúdo fenólico e de flavonoides.

Palavras-chave: atividade antifúngica, *Candida* spp. e leveduras patogênicas, extrato etanólico, flavonoides, própolis.

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1. Introduction

Brazilian biodiversity harbors a wide range of bioactive molecules with antioxidant (Reis et al., 2024a), antibacterial (Paiva et al., 2024), antifungal (Reis et al., 2022, 2025a), antiviral (Silveira et al., 2021), antiparasitic (Pontin et al., 2008; Ferreira et al., 2014; Rebouças-Silva et al., 2023), and wound-healing activities (Costa et al., 2024), among others. Within this context, propolis stands out as a resinous substance produced by bees from plant resins collected from tree bark and buds. Bees use it to safeguard their hives by sealing cracks, filling gaps, and lining the inner walls. In addition, propolis helps protect the colony against invaders and pathogens, including bacteria, fungi, and other insects (Marcucci, 1995).

The chemical composition of propolis is shaped by factors such as geographic origin, surrounding flora, seasonality, and the bee species involved in its production. Despite this variability, certain classes of compounds are consistently present and are linked to a broad spectrum of biological activities. Propolis typically contains flavonoids, phenolic acids, esters, essential oils, and other bioactive molecules. Owing to this complex composition and diverse biological potential, it has been traditionally used in different cultures worldwide (Ribeiro et al., 2023; Marcucci, 1995).

Phenolic compounds are the predominant class in propolis. Among them, flavonoids—a widely distributed group of plant polyphenols—are particularly abundant and account for many of its biological properties, including antioxidant, anti-inflammatory, and antimicrobial activities. The most common flavonoids identified in propolis include quercetin, apigenin, and galangin. Propolis also contains phenolic acids such as caffeic, ferulic, and p-coumaric acids (Marcucci, 1995). In some types, small amounts of essential oils are present, which may contribute additional antimicrobial and anti-inflammatory effects. Beyond these classes, propolis can also harbor diverse components such as terpenes, alkaloids, and fatty acids (Bankova et al., 2000).

The biological properties of propolis are broad and well established (Reis et al., 2021). It displays antimicrobial activity against bacteria, fungi, and viruses, as well as antioxidant effects that help reduce oxidative stress and protect cells from free radical damage. Owing to these properties, propolis holds considerable potential for medicinal and health-related applications and is widely incorporated into pharmaceutical formulations, cosmetics, and dietary supplements (Veiga et al., 2017; Bankova et al., 1995).

Given the biological potential of propolis, this study aimed to evaluate the in vitro antifungal activity and determine the total phenolic and flavonoid content of ethanolic extracts from two samples of green and brown propolis marketed in Santa Rita do Sapucaí, Minas Gerais, Brazil.

2. Material and Methods

2.1. Procurement of the extracts

Ethanolic extracts of brown and green propolis samples were obtained from local commerce in Santa Rita do Sapucaí (22°17′8.082″S, 45°48′18.174″W), Minas Gerais, Brazil. To determine the total soluble solids, aliquots of the extracts were weighed and dried in an oven at 50 °C for 2 hours. Subsequently, after cooling, the aliquots were weighed, and the soluble solids content was determined according to Reis et al. (2021). The resulting dry extracts were then stored under refrigeration and protected from light for subsequent assays.

2.2. Density determination

Density determination was performed according to Reis et al. (2024b). Samples of the dried extract were placed into graduated cylinders with previously recorded masses. The cylinders were then sealed and subjected to the manual compaction method for solids. The apparent density was determined using the following Formula 1:

$$D = (mf - mi) / v \quad (1)$$

Where: D = apparent density (g/cm³); mi = mass of the empty cylinder (g); mf = mass of the cylinder with solids (g); v = volume occupied by the solids in the cylinder (cm³).

2.3. pH determination

For pH determination, 1 g of the dried extract was solubilized in a volumetric flask containing 10 mL of absolute ethanol. After complete dissolution, 90 mL of distilled water was added. The solution was left to rest for 10 minutes, and the pH was measured using a calibrated Kasvi® pH meter (Reis et al., 2024b).

2.4. Quantification of total phenolic compounds

The total phenolic compound content was evaluated using the Folin-Ciocalteu colorimetric method, as described by Reis et al. (2024a), with some modifications. For this assay, the dried extract was solubilized to obtain a concentration of 100 µg/mL. First, a standard curve was prepared using gallic acid at concentrations ranging from 1 µg/mL to 7 µg/mL, diluted in volumetric flasks. Then, after the addition of gallic acid, each flask received 8 mL of Folin-Ciocalteu reagent and 12 mL of 20% sodium carbonate solution, and the mixtures were kept at rest, protected from light, for 2 hours. After this period, the volume was adjusted to 100 mL with distilled water. The extracts were evaluated using a colorimetric solution prepared in the same manner as the standard curve, replacing gallic acid with 100 µL of the propolis extract, followed by the addition of Folin-Ciocalteu reagent and sodium carbonate, and subsequent resting protected from light for 2 hours. All analyses were performed in triplicate. Readings were carried out using a spectrophotometer (model UV-M51, BEL®) at an absorbance of 760 nm. The standard curve equation was determined using the least squares method. Results were expressed as micrograms of gallic acid equivalents per gram of extract (µg GAE/g).

2.5. Quantification of total flavonoids

The total flavonoid content determination followed the method described by Reis et al. (2024a), with modifications. For this assay, the dried extract was solubilized to obtain a concentration of 100 µg/mL. Initially, a methanolic

quercetin standard curve was prepared at concentrations of 12 µg/mL, 6 µg/mL, 3 µg/mL, and 1.5 µg/mL. Samples of 4 mL from each concentration were transferred to test tubes, to which 4 mL of 2% methanolic aluminum chloride ($AlCl_3$) solution was added. For the test samples, 4 mL of extract was added to the test tubes, followed by 4 mL of aluminum chloride solution. After 30 minutes of rest, the samples were analyzed spectrophotometrically at a wavelength of 425 nm. All readings were performed in triplicate. The standard curve equation was determined using the least squares method. Results were expressed as micrograms of quercetin equivalents per gram of extract (µg QE/g).

2.6. Evaluation of antifungal activity

The antifungal activity of the extracts was evaluated using the disk diffusion technique against strains from the American Type Culture Collection (ATCC), following the Kirby-Bauer methodology based on protocols CLSI (2003) and CLSI (2009), with some modifications. Reference strains employed included *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida glabrata* ATCC MYA 2950, *Issatchenkia orientalis* ATCC 6258, *Candida parapsilosis* ATCC 22019, *Cryptococcus neoformans* var. *grubii* ATCC 90113, and *Cutaneotrichosporon dermatis* ATCC 204094. Small fragments of colonies grown for 48 hours were inoculated into 0.9% saline solution. The resulting suspension was homogenized using a vortex mixer, and the optical density was adjusted to 0.5 McFarland standard using a spectrophotometer at an absorbance of 625 nm. After inoculum standardization, 200 µL of the microbial suspensions were spread across the surface of Mueller-Hinton agar supplemented with 2% glucose (MHAG). Subsequently, sterile Whatman filter paper disks, 6 mm in diameter, impregnated with 10 µL of extract at a concentration of 100 µg/mL, were placed on the agar surface. Ethanol-impregnated disks were used as solvent controls. It is important to emphasize that the discs corresponding to the test groups and the control groups were placed on the solid medium only after complete evaporation of the solvent. Experiments were performed in sextuplicate. Plates were incubated at 35 °C for 48 hours for the yeasts. After incubation, inhibition zones, when present in the group test, were measured, and the mean diameters were compared.

2.7. Statistical analysis

After verifying the assumptions of normality using the Shapiro-Wilk test and homogeneity of variances with Bartlett's test, the data were subjected to analysis of variance (ANOVA). A one-way ANOVA was employed to evaluate the influence of fungal species on susceptibility to propolis extracts, while a two-way ANOVA was used to examine the interaction between propolis type and fungal species. Means were compared using Tukey's test, considering a significance level of 5%. Additionally, a direct comparison between the effects of green and brown propolis extracts was performed using Student's t-test. All analyses were conducted using R software (version 2.5.1).

3. Results and Discussion

3.1. Density, total phenolics, and total flavonoids

The results regarding density, pH, and the quantification of total phenolic compounds and total flavonoids for the green (GPE) and brown (BPE) propolis extracts are presented in Table 1.

Density is an important parameter as it aims to describe the chemical content present in the extracts. Interestingly, the density was similar in both extracts evaluated. Regarding another important physicochemical parameter, the pH was slightly more acidic for the brown propolis. As is well known, the pH of propolis is influenced by the content of organic and inorganic acids, and the higher phenolic compound content in brown propolis may be associated with this pH reduction, since many compounds of this class are phenolic acids (Abduh et al., 2023). As observed, the ethanolic extract of brown propolis presented a higher content of total phenolic compounds, whereas the green propolis extract exhibited a higher flavonoid content. Flavonoids are known to constitute an important class of phenolic molecules associated with various biological activities, such as antimicrobial and antioxidant effects (Agati et al., 2020).

In the study by Machado et al. (2016), different ethanolic extracts of brown propolis exhibited phenolic compound concentrations ranging from 110.92 to 117.03 mg GAE/g, whereas ethanolic extracts of green propolis ranged from 160.98 to 181.71 mg GAE/g. These results highlight the high phenolic content and potential of green propolis. Interestingly, in the work of Devequi-Nunes et al. (2018), phenolic compounds were quantified at 249.28 mg GAE/g for the brown propolis extract and 374.10 mg GAE/g for the green propolis extract. In the same study, flavonoids were measured at 29.67 mg QE/g and 131.69 mg QE/g, respectively. Although these values were lower than those reported in the present study, it is noteworthy that the flavonoid content in both studies—along with that of Machado et al. (2016)—was consistently higher in the ethanolic extract of green propolis.

Furthermore, Machado et al. (2016) also reported a high antioxidant capacity in green propolis, in addition

Table 1. Chemical profile of brown and green propolis.

Assays	Green	Brown
Density (g/mL)	0.75 ± 0.01	0.73 ± 0.01
pH	4.37 ± 0.22	3.61 ± 0.17
Total Phenols (mg AGE/g)	697.65 ± 8.10	706.79 ± 2.91
Total Flavonoids (mg QE/g)	415.75 ± 1.14	378.75 ± 1.11

to its flavonoid content. This enhanced antioxidant potential, when compared to brown propolis, may be associated with its primary botanical source: *Baccharis dracunculifolia* (family Asteraceae). This plant is known to be the origin of phenolic acids (such as caffeic acid) and prenylated derivatives, particularly artepillin C, a chemical marker of green propolis (Chang et al., 2008). The rich phenolic content of the samples, especially when considering the elevated levels of flavonoids (compounds directly associated with biological activities) found in the present study compared to those reported in the literature, suggests their potential application in areas such as dietary supplementation and even the development of cosmetic formulations, owing to their antioxidant capacity (Zheng et al., 2025). Historically, polyphenols such as flavonoids have been associated with a wide range of biological activities (Daglia, 2012; Peixoto et al., 2019), including antifungal activity, which will be discussed in the following section.

3.3. Antifungal activity

The potential of Brazilian propolis, particularly the brown and green types, has been extensively explored by several research groups against a wide range of relevant etiological agents, such as anaerobic dental bacteria (Assis et al., 2022), *Staphylococcus aureus*, and the protozoan *Trypanosoma cruzi* (Salomão et al., 2008). However, as previously noted, the present study reports the antifungal activity of ethanolic extracts of brown and green propolis against different etiological yeast strains.

The green propolis exhibited inhibitory effects against all tested strains, with mean inhibition zone diameters ranging from 10.56 mm to 14.46 mm (Table 2). Statistical analysis revealed significant differences among the microorganisms ($p < 0.001$), highlighting *C. parapsilosis* as the most sensitive, followed by *C. albicans* and *C. glabrata*. In contrast, *C. utilis* and *C. neoformans* showed lower susceptibility to the extract.

Interestingly, in the study by Aldana-Mejía et al. (2024), the crude extract of green propolis showed activity against *C. neoformans*, with some of its fractions proving even more promising—this contrasts with the lower susceptibility of this strain observed in the present study. Furthermore, in the study by Aldana-Mejía et al. (2024), the IC_{50} values were reported as 192.50 µg/mL for *C. neoformans*. In line with the findings reported here, Sokolonski et al. (2021) demonstrated that the ethanolic extract of green propolis was effective in inhibiting various clinical oral isolates of *C. albicans*. Furthermore, supporting the results presented herein, the study by Reis et al. (2021) identified C. parapsilosis as the most susceptible strain among those tested against the green propolis extract, which exhibited an inhibition zone of 15 mm.

The brown propolis extract exhibited antifungal activity against all tested strains, with mean inhibition zones ranging from 10.45 mm to 15.38 mm (Table 3). Statistical analysis ($p < 0.001$) indicated significant differences among the microorganisms, with *C. parapsilosis* being the most sensitive to the extract, followed by *C. neoformans* and *C. albicans*. The lowest mean inhibition values were

Table 2. Antifungal activity of green propolis.

Microorganisms	Mean*	Statistical group
C. parapsilosis ATCC22019	14.46	**a**
C. albicans ATCC10231	13.35	**ab**
C. glabrata ATCCMYA-2950	13.08	**abc**
C. dermatis ATCC204094	12.71	**bcd**
I. orientalis ATCC6258	11.45	**cde**
C. utilis ATCC9950	11.03	**de**
C. neoformans ATCC90113	10.56	**e**

*Diameter in mm. Means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($p<0.05$).

Table 3. Antifungal activity of brown propolis.

Microorganisms	Mean*	Statistical group
C. parapsilosis ATCC22019	15.38	**a**
C. neoformans ATCC90113	13.61	**ab**
C. albicans ATCC10231	13.00	**bc**
C. dermatis ATCC204094	12.38	**bcd**
C. glabrata ATCCMYA-2950	12.1	**bcd**
C. utilis ATCC9950	11.33	**cd**
I. orientalis ATCC6258	10.45	**d**

*Diameter in mm. Means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($p<0.05$).

Table 4. General effect of propolis extracts against different fungal species.

Microorganisms	Mean*	Statistical group
C. parapsilosis ATCC22019	14.92	**a**
C. albicans ATCC10231	13.18	**b**
C. glabrata ATCCMYA-2950	12.59	**b**
C. dermatis ATCC204094	12.55	**b**
C. neoformans ATCC90113	12.09	**bc**
C. utilis ATCC9950	11.18	**c**
I. orientalis ATCC6258	10.95	**c**

*Diameter in mm. Means followed by the same letter in the column do not differ significantly from each other according to Tukey's test (p<0.05).

Table 5. Influence of propolis type on the antifungal susceptibility of the tested fungal species.

Propolis	Microorganisms	Mean*	Statistical group
Brown	*C. parapsilosis* ATCC22019	15.38	**a**
Green	*C. parapsilosis* ATCC22019	14.47	**ab**
Brown	*C. neoformans* ATCC90113	13.62	**abc**
Green	*C. albicans* ATCC10231	13.35	**abcd**
Green	*C. glabrata* ATCCMYA-2950	13.08	**bcde**
Brown	*C. albicans* ATCC10231	13.00	**bcde**
Green	*C. dermatis* ATCC204094	12.72	**bcde**
Brown	*C. dermatis* ATCC204094	12.38	**cdef**
Brown	*C. glabrata* ATCCMYA-2950	12.10	**cdef**
Green	*I. orientalis* ATCC6258	11.45	**def**
Brown	*C. utilis* ATCC9950	11.33	**def**
Green	*C. utilis* ATCC9950	11.03	**ef**
Green	*C. neoformans* ATCC90113	10.57	**f**
Brown	*I. orientalis* ATCC6258	10.45	**f**

*Diameter in mm. Means followed by the same letter in the column do not differ significantly from each other according to Tukey's test (p<0.05).

observed for *C. utilis* and *I. orientalis*, suggesting lower susceptibility of these species to the brown propolis extract.

In the study by Freires et al. (2016), among the strains evaluated, *C. albicans* and *C. parapsilosis* were susceptible to the ethanolic extract of red propolis. These findings suggest that although different types of propolis accumulate distinct compounds, the antifungal activity observed against various strains may not be attributed solely to their chemical composition, but also to specific biological features of each fungal species. Furthermore, the study by Reis et al. (2021) supports this notion by revealing the higher susceptibility of *I. orientalis* and *C. parapsilosis* to red propolis extract, with inhibition zones of 21.5 mm and 19 mm, respectively.

When comparing the inhibition zone results between the green and brown propolis extracts using Student's t-test, no statistically significant difference was observed between the two products ($p = 0.5404$). The mean inhibition zones were 12.61 mm for brown propolis and 12.38 mm for green propolis, with a 95% confidence interval. These findings indicate that, when considering all fungal species collectively, both types of propolis exhibit similar efficacy in inhibiting fungal growth.

Analysis of variance (ANOVA) showed that the inhibition zones varied significantly among the tested fungal species ($p < 0.001$), indicating that antifungal susceptibility depends on the microorganism. Tukey's test confirmed this variation, revealing that *C. parapsilosis* was the most susceptible species to the propolis extracts, as discussed above (Reis et al., 2021; Freires et al., 2016), while *I. orientalis* exhibited the lowest inhibition ($p < 0.05$; Table 4). Additionally, the interaction between the type of propolis and fungal species was significant ($p < 0.001$), suggesting that the antimicrobial effect of the extracts varies depending on the fungal species analyzed.

The analysis of the interaction between propolis type and fungal species revealed statistically significant differences in the inhibition of the tested microorganisms ($p < 0.001$; Table 5). Tukey's test indicated that *C. parapsilosis* exhibited the largest inhibition zones for both brown propolis extract (15.38 mm) and green propolis extract (14.47 mm), forming

statistical group "a". In contrast, *I. orientalis* treated with brown propolis (10.45 mm) and *C. neoformans* treated with green propolis (10.57 mm) showed the smallest values, being grouped into category "f". These findings demonstrate that the antifungal response of propolis extracts varies according to the fungal species analyzed, suggesting that species-specific metabolic factors influence susceptibility to the bioactive compounds present in the extracts (Daglia, 2012).

4. Conclusion

As expected, both extracts exhibited high levels of phenolic compounds, particularly flavonoids, which represent an important subclass of phenolics due to their notable biological activity. Furthermore, the present study demonstrated that green and brown propolis extracts display significant antifungal activity against all tested strains, although susceptibility varied among different fungal species. Among the susceptible strains, *C. parapsilosis* was the most sensitive, whereas *I. orientalis* and *C. neoformans* showed lower levels of inhibition. Moreover, the efficacy of the extracts was found to depend not solely on the type of propolis but also on the fungal species, suggesting that intrinsic biological factors of the microorganisms influence their response to the bioactive compounds (Reis et al., 2025b). A direct comparison between green and brown propolis extracts revealed no statistically significant differences, indicating that both possess similar antifungal potential when considered overall.

These findings reinforce the potential use of propolis extracts as natural antifungal agents, which may represent a promising alternative for the control of fungal infections. Future studies are recommended to characterize the bioactive compounds responsible for the antifungal activity—such as through high-performance liquid chromatography and mass spectrometry—as well as to assess in vitro cytotoxicity and in vivo toxicity. Additionally, future research should explore the clinical application of these extracts, incorporating them or their isolated chemical constituents into ointments or creams, for example, for the treatment of fungal infections caused by the important etiological agents evaluated in this study.

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Data Availability Statement

The research data analyzed in this study are not publicly available by any means.

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