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Otimização do método de extração e efeito sazonal nas atividades biológicas e compostos fenólicos da própolis verde brasileira

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RESUMO

A própolis é considerada uma substância resinosa de complexa composição química que é coletada de várias espécies de plantas por abelhas *Apis mellifera*, e utilizado pelo homem como alternativa para o tratamento de doenças e manutenção de uma boa saúde. A composição química da própolis é dependente da biodiversidade da região visitada pelas abelhas e também da época de coleta. O objetivo deste trabalho foi otimizar a metodologia de produção de extrato etanólico de própolis e avaliar o efeito da sazonalidade na composição química e atividades biológicas deste produto. Para a confecção dos extratos etanólicos de própolis foram utilizadas amostras de própolis coletadas do apiário do IFSULDEMINAS – Campus Muzambinho. Na etapa de otimização foram utilizadas soluções etanólicas em diferentes concentrações e diferentes temperaturas de extração e secagem. Para a análise do efeito sazonal, as próplis foram coletadas no mesmo local em diferentes estações do ano. Os resultados mostraram que a solução etanólica a 80% e a temperatura de extração de 70ºC e secagem a 45ºC foram os que mais se destacaram na otimização do processo de extração da própolis. Já em relação ao efeito da sazonalidade na composição química e atividades biológicas da própolis, foi demonstrado que há diferenças entre as próplis coletadas em diferentes estações do ano.

PALAVRAS-CHAVE: antioxidante, atividade antibacteriana, produtos naturais, fenólicos totais, sazonalidade.

Extraction method optimization and the seasonal effect in the biological activities and phenolic compounds of Brazilian green propolis

ABSTRACT

Propolis has been considered a resinous substance with a complex chemical composition that is collected from several plant species by *Apis mellifera* bees, and used by man as an alternative to the healing of diseases and to keep a good health. The chemical composition of propolis is related to the biodiversity of the region visited by the bees and also to the season of collection. This research purpose was to optimize the production methodology of propolis' ethanol extract production and to evaluate the effect of seasonality in the chemical composition and biological activities of this product. Propolis' samples were collected from the apiary at the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais - Campus Muzambinho and were used to make ethanol extracts. In the optimization stage, different ethanolic solution concentration, extraction method and drying temperatures were used. For the analysis of the seasonal effect, propolis was collected from the same place in different seasons. The results showed that the 80% ethanolic solution, the extraction temperature of 70ºC and drying at 45ºC were the ones that stood out the most. Regarding the effect of seasonality on the chemical composition and biological activities of propolis, it is clear that there are differences between the propolis collected in different seasons.

KEYWORDS: antioxidant, antibacterial activity, natural products, total phenols, seasonality.

1. INTRODUCTION

Propolis is the generic name to the resinous material collected by *Apis mellifera L*. from different parts of plants that is added to wax and pollen (Tiveron et al., 2016). Propolis has a color that varies from yellow, green, red to dark brown, depending on its botanical origin and collection season (Groot, 2013). This material aims to seal small holes, used to reconstruction and to keep the hive temperature, in addition to avoid the entry of unwanted organisms and microorganisms (Pasupuleti, Sammugam, Ramesh, & Gan, 2017).

Currently propolis is one of the most consumed natural product in the world as it has antimicrobial (Gomes et al., 2016; Machado et al., 2012), antiviral (Peter, 2017), anti-inflammatory, immunomodulatory (Franchin et al., 2016), analgesic (Paulino et al., 2006), antiparasitic (Silva, 2015), antioxidant (Batista et al., 2015), antitumor (Fríon-Herrera et al., 2015), antiulcerative and neuroprotective activity (Shimazawa, 2005). Given this range of biological activities, it is used in cosmetics and in popular medicine to treat several diseases (Wagh, 2013).

The methodology optimization for the production of ethanol extract of propolis is important in order to obtain a product that has a high degree of biological activity and that can be used for different purposes. In addition to the variation in extraction methodologies, the biological activities of propolis can vary depending on the collection period, seasonality, temperature and local greenery, elements that can limit the concentration of bioactive compounds in the product (Bankova et al., 1998; Souza, Inoue, Gomes, Funari, & Orsi, 2010). In this context, the present work aimed to optimize the methodology of ethanol extract of propolis production and to evaluate the effect of seasonality on the chemical composition and biological activities of this product.

2. MATERIAL AND METHODS

2.1. Sample collection and preparation

Propolis was collected from the apiary located at the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Muzambinho, and stored at -18 ^{o}C in the Bromatology and Water Laboratory of the same institution.

The propolis was ground for the preparation of ethanolic extracts of propolis (EEPs) by adding liquid nitrogen, homogenized and submitted to extraction, adapting the methodology described by Park, Ikegaki, Abreu and Alcici (1998). The project was divided in three parts: standardization of the ethanolic solution, extraction and drying temperature, seasonal effect in the biological activities and chemical composition of green propolis from Muzambinho/MG.

In order to find the best ethanolic concentration, through different ethanolic solution concentrations, 20 g of crude propolis were solubilized in 200 mL of ethanolic solution with

different concentrations 50%, 60%, 70%, 80%, 90% and 100% (v / v). The extraction was carried out at 70 °C in a thermostatic water bath for 30 minutes with constant shaking.

The samples were left to stand at 8 °C for 24 hours for decanting the wax. Subsequently, they were filtered on filter paper. The extracts were concentrated in a forced air circulation oven at 60°C for about three days, until constant weight, in order to obtain a concentrated extract.

After the analysis of the results from the first part of the project, to standardize the extraction and drying temperature, the ethanolic solution that best stood out in the chemical and microbiological analysis found previously was adopted, and a completely randomized experimental design was used in a factorial scheme containing 2 (baths) x 3 (drying) temperatures (Figure 1). Three samples were submitted to a bath of 45 °C and the other three 70 °C, under constant shaking. The samples were left to stand and were then filtered. After filtration, the extracts were placed in an oven under the temperatures of 45 °C, 55 °C and 65 °C as an alternative to the commonly used method of concentrating the extract in a rotary evaporator (Park et al., 1998).

Figure 1. Experimental design of the standardization step of extraction and drying temperatures of the EEPs. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Water bath temperature	Oven temperature		
45 °C	45 °C	55 °C	65 °C
70 °C	45 °C	55 °C	65 °C

2.2. Seasonal effect

The third part of the project is related to the seasonal effect in the green propolis composition and biological activities. The samples were collected at the apiary of IFSULDEMINAS - Campus Muzambinho at the end of each season, on September 22nd, 2016 (winter), December 13th, 2016 (spring), March 17th, 2017 (summer) and June 9th, 2017 (autumn).

The EEPs extracts were prepared by following the methodology described above, using the optimized conditions obtained in the previous part.

2.3. Chemical and biological analysis

2.3.1. Determination of total phenolic contents

The total phenolic contents were determined by using the Folin-Ciocalteau 1:10 reagent solution. The Folin-Ciocalteau was diluted in 2.0 mL of 4% (w/v) Na_2Co_3 solution and mixed with

2.5 mL of the propolis extract. Following two hours away from light, at room temperature, absorbance was measured spectrophotometrically at 740 nm. Gallic acid was used as standard, and results are expressed as Gallic acid equivalents (Singleton, Othofer, & Lamuela-Raventos, 1999).

2.3.2. Antioxidant activity by scavenging free radicals

The mixture was formed by adding 2.0 mL propolis extract in ethanol and 0.5 mL DPPH at 0.5 mMol. Absorbance was measured at 517 nm following 45 minutes' incubation away from light, at room temperature.

The results were expressed as antioxidant activity (AA), calculated through the DPPH solution's absorbance decline rate after 45 minutes of reaction (stable phase) compared to the reference solution (DPPH in ethanol), by the formula:

% Antioxidant activity = 100 - [(Sample - White) * 100 / Control] where: Sample = absorbance of the DPPH solution (samples); White = absorbance of the sample solution without adding DPPH; Control = absorbance of the DPPH reference solution (ethanol).

The results were expressed as IC_{50} (Brand-Williams, Cuvelier, & Berset, 1995).

2.3.3. Antibacterial activity

According to the protocol M07-A10 developed by the Clinical Laboratories Standardization Institute (CLSI, 2015), with modifications, the antibacterial activity was performed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). For these analyzes, the microorganisms *Staphylococcus aureus* ATCC 0538, *Escherichia coli* ATCC 8739 and *Micrococcus luteus* were grown in liquid Brain Heart Infusion (BHI). After incubation, they were adjusted to 1-2 x 10^8 CFU mL^{-1} in a 0.9% NaCl solution by using the 0.5 Macfarland scale. Then, 52 uL of the bacterial suspensions was inoculated in 52 mL of the liquid BHI medium, in order to obtain a bacterial concentration around 1-2 x 10^5 CFU mL^{-1}. The technique was developed in 96-well microplates, which 180 µL of sterile BHI broth were previously added. Then, 10 µL of the ethanolic propolis extract was added in concentrations ranging from 1000 µg mL^{-1} to 7.8 µg mL^{-1} (serial dilution). For the color control, 180 µL of sterile BHI broth and 10 µL of ethanol extract of propolis were added in the same concentrations already mentioned. The microplates were incubated at 37 °C for 24 h. After incubation, 20 µL of the Resazurin dye (0.01% w / v) was added to verify bacterial growth. Wells which there were no change in the color of the dye compared to the control, the absence of viable bacteria was considered. Any evidence of color change was considered bacterial growth. For the determination of MBC, 10 µL aliquots of the culture medium from the wells considered inhibitory were placed in BHI agar and the plates were incubated at 37 °C for 24h.

2.3.4. Statistical analysis

The statistical evaluation of the results was analyzed using the SISVAR 5.6 software by analysis of variance (ANAVA) and the Scott-Knott test was applied to observe the significant differences between the mean values (p value < 0.05) (Ferreira, 2014).

RESULTS AND DISCUSSION

3.1. Extraction Method Optimization

After preparing the extracts with ethanolic solutions in different concentrations, it was observed that the sample extracted with 50% ethanol showed significant amounts of wax that were noticeable in the final extract, even after the vacuum filtration process. Although we were unable to quantify the wax present in the extracts, the only one that was visibly cloudy, typical of the presence of wax, was the extract obtained with 50% ethanol solution. The wax presented in the extract interferes with the final quality of the product, changing its sensory characteristics and chemical composition (Melo, Matsuda, & Almeida-Muradian, 2012), therefore it is not desirable.

Table 1 shows the values of total phenolic compounds in extracts produced with ethanolic solution in different concentrations. The phenolic content was statistically higher in samples extracted with 50% (v / v) ethanol (121.54 mg EqAG g^{-1} of sample), followed by 80% (v / v) ethanol (115.37 mg EqAG g^{-1} sample). For the Brazilian Law of Propolis Identity and Quality Technical Regulations (Brazil, 2001), the minimum of total phenolic compounds accepted is 5% in propolis. All samples showed values above the minimum required, being 12.15%, 10.57%, 11.21%, 11.53%, 10.32% and 10.49% respectively. Monry et al. (2017), when varying the concentration of ethanol in the production of EEPs, noticed that the extract produced with 80% ethanol presented higher amounts of phenolic compounds when compared to other extracts.

In the present work, the extraction with ethanolic solution at 80% stood out as the second highest presence of phenolic compounds, since the extraction with ethanolic solution at 50%, although obtaining a higher concentration of phenolic compounds, has a high concentration of wax, which is an exclusion factor.

Table 1. Content of phenolic compounds (mg EqAG g^{-1} of sample) and antioxidant potential (IC_{50} µg mL^{-1}) of EEPs samples produced with ethanolic solution in different concentrations. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Sample	Phenolic content	IC_{50}
Ethanol 50%	121.54 ± 2.13[a]	23.45 ± 0.12[b]
Ethanol 60%	105.78 ± 0.6[d]	32.02 ± 1.76[c]
Ethanol 70%	112.11 ± 1.14[c]	32.63 ±0.48[c]
Ethanol 80%	115.37 ± 0.87[b]	33.99 ± 4.92[c]
Ethanol 90%	103.27 ± 3.67[d]	36.48 ± 4.98[c]

Ethanol 100%	104.93 ± 22.03[e]	51.46 ± 2.06[d]
BHT	-	70.11 ± 2.35[e]
Ascorbic acid	-	6.49 ± 0.11[a]

*Average followed by the same letters do not differ in the Scott Knott test ($p<0.05$).

Regarding the antioxidant potential (Table 1), it was observed that to reduce 50% of the DPPH free radicals in the solution (IC_{50}), the ethanol extract obtained with the 50% ethanol solution needed a lower content (23.45 µg mL^{-1}) when compared to the other samples. However, the presence of wax in this extract may have influenced the spectrophotometric reading of the reaction. The samples extracted with ethanol 60%, 70%, 80% and 90% showed no differences regarding antioxidant efficiency. The results support Park et al. (1998) who tested different concentrations of ethanolic solution for the preparation of ethanol extract of propolis and observed that extracts at 70% and 80% showed the highest antioxidant activity, followed by those of 90% and 60%.

Based on the results of antioxidant activity, phenolic compounds can be identified as the main responsible for such activity, which corroborates previous studies by Silva et al. (2006) and Cabral, Oldoni, Alencar, Rosalen and Ikegaki (2012). However, in some cases there is no direct correlation between the amount of phenolic compounds and antioxidant activity, as observed with the samples with ethanol 60%, 70% and 90%, which showed lower amounts of phenolic compounds and antioxidant activity statistically similar to the sample with ethanol 80%. As shown by Toscan (2010), some chemical compounds usually present in propolis samples, such as terpenes, can contribute to the antioxidant activity of this natural product.

The MIC and MBC (Table 2) analysis showed that, at this first part of the project, none of the extract was able to inhibit the growth of *S. aureus* and *E. coli*. However, the extracts obtained with the ethanol solutions at 90% and 100% were able to inhibit the growth of *M. luteus* at 125 µg mL^{-1} and 250 µg mL^{-1} respectively. These results mean that the green propolis collect have bacteriostatic activity but this was not enough to inhibit the growth of *S. aureus* and *E. coli*. The antimicrobial activity of the samples might be related to thermosensitive compounds, and they were probably destroyed during the drying period because the extracts took about three days to dry in a forced air circulation oven at 60 °C. Monroy et al. (2017) showed inhibition of the growth of *S. aureus* with EEP produced with an ethanolic solution of 80%, but they could not inhibit the growth of *E. coli*.

Table 2. Antibacterial activity of the samples against *E. coli*, *S. aureus* and *M. luteus*. The results are expressed as µg mL^{-1} of extract. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Sample	*E. coli*		*S. aureus*		*M. luteus*	
	MIC	MBC	MIC	MBC	MIC	MBC
Ethanol 50%	N.D*	N.D	N.D	N.D	N.D	N.D
Ethanol 60%	N.D	N.D	N.D	N.D	N.D	N.D
Ethanol 70%	N.D	N.D	N.D	N.D	N.D	N.D
Ethanol 80%	N.D	N.D	N.D	N.D	N.D	N.D
Ethanol 90%	N.D	N.D	N.D	N.D	125-250	N.D
Ethanol 100%	N.D	N.D	N.D	N.D	125-250	N.D

*N.D: antibacterial activity not detected.

Because of the results of total phenolic compounds and antioxidant activity and the fact that there was not visible evidence of wax on the sample, the ethanolic solution of 80% was chosen to prepare the extracts of the second part of the project in order to standardize the temperature of extraction and drying of the extracts.

The table 3 shows the values of total phenolic compounds of the EEPs produced varying the temperature of extraction and drying. Based on the results, it was possible to notice that the treatment of the best total phenolic compounds content (109.7 mg EqAG g^{-1}) was the sample submitted to the extraction temperature of 70 ^{o}C and drying of 45 ^{o}C.

The fact that the higher phenolic compounds content was found in the drying temperature of 45 ^{o}C may be related to thermosensitive substances (Georgetti et al., 2018), meaning that higher drying temperatures might destroy these compounds. However, the temperature of 70 ^{o}C, even if it is higher, might not destroy the compounds because the exposition was faster (30 minutes) than the time of drying (about 72 hours) and indirect (water bath).

Table 3. Content of phenolic compounds (mg EqAG g^{-1} of sample) and antioxidant potential (IC_{50} µg mL^{-1}) of EEPs samples produced in different temperatures. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Sample	Phenolic content	IC_{50}
Bath 45/Drying 45	52.6 ± 4.35^{d}	53.93 ± 4.04^{c}
Bath 45/Drying 55	75.88 ± 4.28^{b}	51.24 ± 2.82^{c}
Bath 45/Drying 65	66.15 ± 3.58^{c}	62.82 ± 1.26^{d}
Bath 70/Drying 45	109.7 ± 9.28^{a}	42.74 ± 0.65^{b}
Bath 70/Drying 55	58.87 ± 2.25^{d}	61.81 ± 5.55^{d}

Bath 70/Drying 65	47.33 ± 2.76^{e}	66.03 ± 0.66^{d}
BHT	-	70.11 ± 2.35^{e}
Ascorbic acid	-	6,49 ± 0.11^{a}

*Average followed by the same letters do not differ in the Scott Knott test (p<0.05).

Based on the results of table 3, the sample which had the greater phenolic content value was the same that showed the most efficiency on radical scavenger. Thus, phenolic compound can be the responsible for that antioxidant activity, confirming the previews studies of Silva et al. (2006) and Cabral et al. (2012).

It was possible to observe in the Table 4 that the MIC against *S. aureus* was between 250 and 500 µg mL^{-1}, in all treatments, except for the 70 ºC bath sample submitted to the 45 ºC oven, which presented MIC between 125 and 250 µg mL^{-1}, thus being the most effective. For *M. luteus*, the results showed that the treatments "bath 45 ºC and oven 45 ºC" and "bath 70 ºC and oven 45 ºC" presented MIC between 250 and 500 µg mL^{-1}, being the most effective.

The MBC tests showed that the "bath 70 ºC and oven 55 ºC" showed higher bactericidal efficiency, between 250 and 500 µg mL^{-1} against *S. aureus*. The treatments "bath 45 ºC and oven 45 ºC" and "bath 70 ºC and oven 45 ºC" showed the same result for the same bacteria, with MBC between 500 and 1000 µg mL^{-1}. The remaining treatments did not show bactericidal activity.

Table 4. Antibacterial activity of the EEPs obtained with different bath and drying temperatures against *E. coli*, *S. aureus* and *M. luteus*. The results are expressed as µg mL^{-1} of extract. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Sample	*E. coli*		*S. aureus*		*M. luteus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Bath 45/Drying 45	N.D	N.D	250-500	500-1000	250-500	N.D
Bath 45/ Drying 55	N.D	N.D	250-500	N.D	500-1000	N.D
Bath 45/ Drying 65	N.D	N.D	250-500	N.D	500-1000	500-1000
Bath 70/Drying 45	N.D	N.D	125-250	500-1000	250-500	N.D
Bath 70/Drying 55	N.D	N.D	250-500	250-500	500-1000	N.D
Bath 70/Drying 65	N.D	N.D	250-500	N.D	500-1000	500-1000

*N.D: antibacterial activity not detected.

For *M. luteus*, two treatments showed results between 500 and 1000 µg mL^{-1} (bath 45 ºC/oven 65 ºC and bath 70 ºC/oven 65 ºC), while the other treatments did not show bactericidal action in any concentration.

As for the tests with *E. coli*, no inhibitory or bactericidal action was observed, which is consistent with studies by Kujumgiev et al. (1999) and also by Marcucci (1995). Unlike the work of Silva et al. (2006) who found MIC values above 1000 µg mL^{-1} against *E. coli* for ethanol extracts of green propolis produced using ethanol solution 80% and drying temperature of 50 °C.

The results found in the MIC showed that there was efficiency in inhibiting the growth of *S. aureus,* as well as Cabral et al. (2012) obtained with the same type of propolis, but with values between 40 and 80 µg mL^{-1}. Schmidt et al. (2014) found in their studies with propolis an inhibitory capacity to *M. luteus*, with an average MIC of 400 µg mL^{-1} and Uzel et al. (2005) obtained MIC of 4 µg mL^{-1}.

In addition, the results of the antibacterial activity of the EEPs indicate that there are thermosensitive compounds in propolis related to the activity, which are preserved at lower drying temperatures.

With the results obtained concerning the extraction and drying temperature, to evaluate the effect of seasonality on the chemical composition and biological activities of green propolis, the extraction temperature of 70 °C and drying temperature of 45 °C were used.

3.2. Seasonality Effect

Table 5 shows the results related to the analysis of the content of total phenolic compounds in the propolis extracts collected in different seasons. In the summer season, a higher content of total phenolic compounds was found, followed by spring, autumn and winter, which differ statistically. In studies by Pandolfo (2014) it was also observed that samples from the summer months showed the highest values (80.80 mg Eq AG g^{-1}) for phenolic compounds. Also as claimed by this author, the highest results of total phenolic compounds content were for the months of January, February and March. Souza et al. (2010) also found greater results in the total phenolic content in a sample collected in the summer season. Regueira-Neto et al. (2017) and Nascimento et al. (2019) found different concentrations of the phenolic compounds in red propolis collected in the dry and rainy seasons, thus showing that the concentration of these compounds varies according to the season.

Table 5. Content of phenolic compounds (mg EqAG g^{-1} of sample) and antioxidant potential (IC_{50} µg mL^{-1}) of EEPs samples produced in different seasons. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2018.

Sample	Phenolic content	IC_{50}
Winter	82.92 ± 5.18^{d}	59.05 ± 5.30^{d}
Summer	162.43 ± 4.04^{a}	44.76 ± 0.65^{c}
Spring	149.03 ± 1.27^{b}	29.41 ± 0.43^{b}
Autumn	116.42 ± 1.13^{c}	27.81 ± 1.53^{c}
BHT	-	70.11 ± 2.35^{e}

Ascorbic acid	-	6.49 ± 0.11ª

*Average followed by the same letters do not differ in the Scott Knott test (p<0.05).

Regarding the antioxidant potential (table 5), the extracts obtained from the collections made in the autumn and spring seasons showed a lower IC_{50} (27.81 µg mL^{-1} and 29.41 µg mL^{-1}, respectively) when compared to the other samples. They were, therefore, twice as efficient as BHT and less efficient than ascorbic acid, antioxidants used in processed foods.

As observed in table 5, sometimes there is no direct correlation between the total phenolic compounds and antioxidant activity of the sample. This may happen because of some limitations of the in vitro assay utilized (Rohr, Riggio & Meier, 2000). Also, other chemical compounds present in the samples might have antioxidant activity, such as terpenes (Toscan, 2010).

The results contrasted from those reported by Ferreira (2017), who described a low antioxidant activity in green propolis collected in the southern region of Brazil. This low antioxidant activity was mainly due to the small amount of phenolic compounds in the propolis studied by this author. Salgueiro and Castro (2016) showed more satisfactory results of the antioxidant activity for propolis from the São Paulo region, which it can be attributed to the high content of total phenolic compounds, and these authors also reported results that corroborate this work in the propolis collected in Minas Gerais region. The divergent results can be justified by the different biodiversity of the region and by the time of collection.

Regarding antibacterial activity (Table 6), it was noted that none of the samples showed bactericidal activity at the concentrations tested. It was not possible to test the samples against *S. aureus* because of the viability of the cells. As for the MIC analysis, the autumn and summer samples were the most efficient against the *M. luteus*, both with MIC between 31.25 - 62.5 µg mL^{-1}. Similar results were reported by Cabral et al. (2012) against *S. aureus*.

None of the extracts was able to inhibit the *E. coli*. This result is contradictory to that found by Machado et al. (2016) for green propolis, since the authors observed inhibitory and bactericidal activity against this bacterium, this is perhaps due to the botanical variety present in the propolis collection region of the aforementioned work.

Table 6. Antibacterial activity of the EEPs from different seasons against *E. coli*, *S. aureus* and *M. luteus*. The results are expressed as µg mL^{-1} of extract. IFSULDEMINAS - Campus Muzambinho, Muzambinho / MG, 2017.

Sample	*E. coli*		*M. luteus*	
	MIC	**MBC**	**MIC**	**MBC**
Autumn	N.D	N.D	31.25-62.5	N.D
Winter	N.D	N.D	N.D	N.D
Summer	N.D	N.D	31.25-62.5	N.D

Spring	N.D	N.D	250-500	N.D

*N.D: antibacterial activity not detected.

Castro et al. (2007) demonstrated that the propolis antimicrobial activity may vary depending on the local vegetation and that seasonality may interfere with the MIC and MBC values, probably due to the change in the concentration of bioactive compounds from the plant sources of these propolis. Sforcin, Fernandes, Lopes, Bankova, and Funari (2000) also reported that the local vegetation is directly linked to the antimicrobial activity of propolis, but that seasonality does not interfere.

Finally, according to Nascimento et al. (2019), several factors can directly influence the biosynthesis of plants visited by bees, including temperature range, humidity, solar radiation, rainfall, season, age of plant and interaction with other plants and animals. In the rainy and drought seasons, some modification in plant habitat occur, which is also related to the regulation of the interactions insect-plants. Those factors explain why the phenolic composition and biological activities vary seasonally in the green propolis.

3. CONCLUSION

The 80% ethanolic solution was the one that stood out among the others for being able to extract a greater amount of phenolic compounds and a better antioxidant activity, without the visible interference of wax. Additionally, the extraction temperature of 70 ^{o}C and drying temperature of 45 ^{o}C were able to preserve the sample's thermosensitive compounds, increasing their antioxidant and antibacterial capacity. Thus, it is advisable to use those factor for the preparation of ethanolic extracts of green propolis as an alternative to the methodology commonly used in other laboratories.

Regarding the effect of seasonality on the chemical composition and biological activities of propolis, there are differences between the propolis collected in different seasons. It was not possible to define a season in which the green propolis collected in the apiary of IFSULDEMINAS - Campus Muzambinho had, at the same time, a high content of phenolic and promising biological activities. It can be concluded from this work that the selection of the propolis sample, in relation to seasonality for the preparation of the extract, is directly related to its use, so that it has all its biological potential utilized.

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