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Antioxidant and Anti-*Helicobacter pylori* Activities of Apis Mellifera Honey from Central Brazil

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ABSTRACT

The aim of the study was to evaluate the antioxidant and anti-Helicobacter pylori activities, and the botanical origin of Apis mellifera honey from different regions of Mato Grosso, Central Brazil. Eight honey samples were analyzed for the total phenolic and flavonoids content, antioxidant activity, antibacterial activity and pollen analysis. The total phenolic content varied from 5.6 to 12.78 mg/GAE/100 g and flavonoids varied from 3.52 to 9.36 mg/QE/100 g. The honey samples showed antioxidant activity for nitric oxide and reduction of ferric ion, but not for DPPH. The honey 1 showed 93.84% inhibition of Helicobacter pylori growth, indicating promising antibacterial activity. Sixteen pollen types were identified in the samples with Myracrodruon dominance. The study of anti-H. Pylori in honey were unprecedented in Brazil and expand the knowledge about therapeutic activities of honey produced in the State of Mato Grosso and its bioactive compounds. The honey contributes benefit to human health.

Keywords: Honey, *Helicobacter pylori*, Biological activity, Functional properties, Nitric oxide.

INTRODUCTION

Helicobacter pylori is a spiral, gram-negative, microaerophilic bacterium that colonizes the gastric mucosa, partially neutralizing the acid pH with the continuous production of ammonia and causes disorders that can progress to gastritis, ulcers, dyspepsia, gastric adenocarcinoma and lymphomas. The world estimated is that more than half of the world population carries this bacterium; however, only 10% of this population develops clinical problems related to *H. pylori*. The prevalence of *H. pylori* is significantly higher in developing countries, affecting 70% to 90% of the population. In Brazil, the prevalence in adults is around 82%. The bacteria are frequently acquired in childhood and may persist throughout life; however, the majority of the population is unaware of its presence [1].

The most commonly used treatment for *H. pylori* infection is the conventional triple scheme, which consists of a therapy that combine two antibiotics (clarithromycin and amoxicillin or metronidazole) and a proton pump inhibitor. The increased prevalence of antibiotic resistant strains, the noncompliance of patients with the therapeutic regimen due

to side effects and the high cost of the therapy make it difficult to eradicate the bacteria, especially for people with low income [2].

The resistance of *H. pylori* strains to the antibiotics of the triple therapy scheme encourages the development of new forms of treatment and products, including natural based products on plants and honey. The anti-*Helicobacter pylori* activity has been confirmed in honey extract and in natura from different regions of the world such as Germany, Cameroon, Switzerland, Iran and South Africa, with promising bacteriostatic action [3].

The antibacterial activity of honey is just one of its biological activities. This natural product is a complex nutritional sweetener, composed mainly of carbohydrates, water and other compounds such as organic acids, amino acids, minerals, enzymes, proteins, vitamins, volatile compounds, Maillard reaction products and numerous bioactive compounds (phenolic and flavonoid compounds).

One of these biological activities that honey stands out is the antimicrobial in inhibiting the growth of a wide spectrum of bacteria, fungi and viruses. The inhibitory activity of gram-negative bacteria was found in honeys from India, Egypt and Portugal and Brazil in the South region and the Midwest region [4].

Regular honey consumption has significantly reduced the risk of developing *H. pylori* infection and reinfection, associated with its potential to prevent gastric ulcers caused by this bacteria, as demonstrated in clinical studies by and honey also presents antioxidant activity, probably due to the phenolic compounds present in its composition in addition to other bioactive compounds capable of capturing free radicals, which have been identified in honey. The antioxidant activity was found in Brazilian honey from different geographic regions, such as in Rio Grande do Sul, Santa Catarina, Paraná and Sao Paulo. However, research on *Apis mellifera* honey from the Midwest region of Brazil (Mato Grosso) is still scarce, indicating the need to expand scientific production in this region of high potential for bee production.

Therefore, the present study aimed to evaluate the antioxidant and anti-*Helicobacter pylori* activities of *Apis mellifera* honey from the Mato Grosso state, Central Brazil, as well as its botanical origin [5].

MATERIALS AND METHODS

Study Areas and Honey Samples

The study area covers the state of Mato Grosso located in the Central region of Brazil, which has a territory of 903,357 km^2, 53.6% composed of the Amazon biome, 39.6% by the Cerrado and 6.79% by the Pantanal (IBGE, 2019). Four municipalities were selected within these different biomes: Alta Floresta and Marcelândia (Amazon Biome), Nossa Senhora do Livramento (Cerrado Biome) and Poconé (Pantanal Biome). The municipalities of Nossa Senhora do Livramento and Pocone are in a transition zone and are occupied by two biomes, thus, they were grouped within the biome with the largest area of territorial occupation. The honey from these four municipalities was selected based on previous studies that showed the presence of phenolic and flavonoid contents that confer antioxidant and antibacterial potential [6].

The studied honey samples have their origins distributed as follows: Nossa Senhora do Livramento (3 samples-honey 1, 2 and 3), Pocone (3 samples-honey 4, 5 and 6), Marcelandia (1 sample- honey 7) and Alta Floresta (1 sample-honey 8) and were acquired directly from beekeepers. The honeys came from the 2015/2016 harvest, collected between October 2015 and November 2016. The samples were stored at 5°C and diluted in aqueous, ethanol and methanol solution according to the specifications of each experiment [7].

Chemical Substances and Reagents

All chemicals were of the highest analytical grade. Folin-Ciocalteau, DPPH (2,2-diphenyl-1-pycrilhidrazila), ascorbic acid, Griess reagent, Fetal Bovine Serum (FBS), Skim-Milk, skirrow, amoxicillin, amphotericin, triphenyltetrazoliun chloride and resazurin were supplied by Sigma-Aldrich® (Saint Louis, Missouri, USA). Gallic acid, quercetin, sodium nitroprusside, chloroform, methanol and formic acid supplied by Dinâmica® (Quimica Contemporânea Ltda, Diadema, Sao Paulo, Brazil). Hydrated aluminum chloride, BHI broth and BHI agar supplied by Acumedia®/Neogen® (Lansing, Missouri, USA) and Kasvi® (Sao Jose dos Pinhais, Parana, Brazil) respectively [8].

Quantitative Analysis of Secondary Metabolic Classes

Phenolic content: The total phenolic content was determined by the Folin-Ciocalteau method described by Singleton and Rossi Jr. Briefly, a 100 μL aliquot of the aqueous honey solution (1:1) was mixed with 10% Folin-Ciocalteau and 2.5 mL of sodium carbonate, with the final volume of 10 mL integrated with distilled water. After 2 hours of rest in the dark, the absorbance was measured at 540 nm using spectrophotometer (Femto-600 s). Total phenolic content was determined by interpolation of the absorbance values with a calibration curve constructed with different concentrations of galic acid standard. The test was performed in independent triplicates, presenting the average expressed in mg of Gallic Acid Equivalents (GAE)/100 g of honey [9].

Total flavonoid content: The total flavonoid content was determined according to the method described by Arvouet-Grand, Vennat, Pourrat and Legret, with adaptations. Briefly, a 50 μL aliquot of the honey solution in methanol and water (1:1) was mixed with methanol solution of hydrated aluminum chloride (2%) and the final volume of the mixture was solubilized with 50% methanol (methanol: water) up to 5 ml. After resting for 30 minutes, the absorbance reading was taken at 415 nm. The total flavonoid contents were determined by extrapolating the absorbance of the samples against a calibration curve constructed with different concentrations of the quercetin standard. The test was performed in independent triplicates, showing the average expressed in mg of Quercetin Equivalents (QE)/100 g of honey [10].

Evaluation of Antioxidant Activity

The antioxidant activity was evaluated by the 2,2-Diphenyl-1-Pycrylhidrazila free radical (DPPH) sequestration tests, the capture of the Nitric Oxide (NO) free radical and the reduction of the ferric ion (FRAP). The results were expressed in terms of $IC_{50\%}$ (minimum concentration necessary for the antioxidant to reduce in 50% the initial concentration of the radical), through the average obtained in the equations that related the percentage of activity with the concentration of the substance in test, that is, the lower the value of IC, the greater is the antioxidant capacity of the substance. The ascorbic acid (1.56-200 μg/ mL) was used as positive standard for the three tests [11].

Sequestration of the free radical 2,2-Diphenyl-1-Pycrilhidrazil (DPPH): The antioxidant activity was evaluated by the sequestration of the DPPH radical, according to Mensor with adaptation. The method is based on DPPH's ability to react with hydrogen donors. In the presence of antioxidant substances, DPPH receives H+ being reduced. The honey samples were diluted in methanol at concentrations of 0.15-20 mg/mL (serial dilution) and mixed with a methanol solution of the DPPH radical (0.15-40 μg/mL) in 96-well microplates. The readings were taken after 30 minutes of incubation in the dark at 515 nm, in spectrophotometer (Elisa). The percentage of remaining DPPH was calculated from the DPPH calibration curve according to the equation:

$$\%DPPHREM=(DPPHT=t/DPPHT=0) \times 100,$$

Where,

DPPHT=t is the concentration of DPPH in the medium after the reaction with the extract,

DPPHT=0 is the initial concentration of DPPH. The samples were tested in independent triplicates.

Nitric Oxide (NO) free radical capture: The capture of the free radical was done according to Sreejayan and Rao with adaptations. Sodium Nitroprusside (NPS) degrades spontaneously in the presence of light at room temperature (24 ± 1°C) forming nitrite radical, which reacts with oxygen, resulting in nitric oxide. The honey samples were diluted in water at concentrations of 50-6400 μg/ mL (serial dilution), mixed with sodium nitroprusside (10 mM) in PBS in 96 well microplates. After incubation at room temperature and in the presence of light for 150 minutes, the Griess reagent was added and read at 540 nm in spectrophotometer (Elisa). The experiment was done in triplicate [12].

Determination of Ferric ion Reducing Power (FRAP): In this method originally developed in 1999, the ferric-tripyridiltriazine complex (Fe^{3+}) is reduced to the ferrous complex (Fe^{2+}), in the presence of an antioxidant and under acidic conditions, forming a complex of intense green coloration. The honey samples were solubilized in 50% methanol, tested in independent triplicates and mixed with sodium phosphate buffer (0.2 M, pH 7.2) and potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 minutes. After that, distilled water, and ferric chloride (0.1%) were added to the trichloroacetic acid solution, followed by a second incubation in the dark for 30 minutes and read at 630 nm in a spectrophotometer (Elisa).

Evaluation of Antibacterial Activity

Microorganism: *Helicobacter pylori* cag A and vac A positive (ATCC 43504, Maryland, USA) provided by Fiocruz (Rio de Janeiro, Brazil) was used. The microorganism was maintained at - 80°C in Skim Milk agar and reactivated from the cultures stock in BHI broth enriched with 5% SBF and Skirrow® (1:500), later cultivated in BHI agar enriched with 5% sheep blood, for 3 to 5 days at 37°C, under microaerophilic conditions in a candle jar [13].

Determination of Minimum Inhibitory Concentration (MIC): The antibacterial activity was evaluated by the method of microdilution in BHI broth, in 96 well microplates, added 100 µl of enriched BHI broth (SBF), 100 µl of honey solution in final concentrations of 12.5-1000 µg/mL (serial dilution) and 100 µl of bacterial suspension in 0.85% sterile NaCl solution McFarland Scale 2 (6×10^8 CFU/mL). The result was expressed in terms of growth inhibition percentage. The positive control used was amoxicillin at concentrations 0.19-100 µg/mL. After incubation for 3 to 5 days at 37°C, the spectrophotometer (plate reader) was read at 450 nm. Subsequently, microbial growth was confirmed with resazurin dye (0.01% m/v), after 6 hours of incubation, in independent triplicates [14].

Minimum Bactericide Concentration (MBC): MBC was determined by taking an aliquot of 6 µL from samples of the microplate wells in which no microbial growth was observed and was added to petri dishes with enriched BHI agar. The plates were incubated at 37°C for 3 to 5 days and microbial growth was observed. MBC was considered the lowest solution concentration that resulted in total inhibition of the growth of microorganisms visible in the agar plates.

Antibacterial activity by thin-layer chromatography: The metabolic classes were identified by thin layer chromatography on 60-GF254 silica gel chromate plates, according to Christy E Manyi-Loh with adaptations. The chromatoplates received 8 µL of the honey samples in the minimum inhibitory concentration ($MIC_{50\%}$) and the amoxicillin positive control (100 µg/mL). The mobile phase used was chloroform/methanol/water/formic acid (30:18:1, v/v), the dots were visualized under UV light at wavelengths of 254 and 365 nm. The chromatography plates were made in duplicate, and a set was used as reference chromatogram [15].

The bioautography technique used was according to Holetz with adaptations performed 24 h after elution. The chromatoplates were covered with 20 mL of 10%? SBF enriched agar containing 500 µL of the microbial suspension adjusted in a 0.85% sterile NaCl solution McFarland scale 2 (6×10^8 CFU/mL) and 500 µL of the revealing substance (Triphenyltetrazoliun 1% chloride). After homogenization, this mixture was poured on eluted chromate plates, placed on Petri dishes and incubated at 37°C for 24 h. Inhibition was evidenced by colorless halos in the plates where no ostensive growth of microorganisms occurred and the results were expressed by the Retention factor (R_f) observed on the chromate plate after disclosure [16].

Botanical Origin of Honey

The botanical origin of honey was determined by pollen analysis, with preparation of the sample slides according to Can and Louveaux, Maurizio and Vorwohl. The pollen profile of the samples was determined by qualitative and quantitative analysis of pollen types by microscopy. The qualitative analysis identifies the pollen types, organized by botanical family, genus and when possible, species. For this purpose, the pollen types were compared by morphology with the pollens of the reference collection of pollen grains of the CetApis Laboratory (Centro de Estudo em Apicultura) -UNEMAT-Caceres Campus. Specific literature was used to assist in the identification of pollen types.

The quantitative analysis was performed by counting 500 pollen grains per slide and classifying the frequency of each pollen type comparing to the total of quantified pollens. The pollen frequency was grouped in three classes: Dominant Pollen (DP) with more than 45% of the total grains, Accessory Pollen (AP) between 15 and 45% of pollens and isolated pollen (IP) less than 15%.

Data Analysis

For total phenolic, flavonoid and antioxidant activity content, the results were analyzed by means of descriptive statistics, with mean and standard deviation. The antioxidant activity (y) was correlated with the total phenolic (x) through Spearman's test due to non-normal error distribution. The same statistical model was used for the flavonoids.

The minimum inhibitory concentration and minimum bactericide concentration were expressed as percentage of inhibition per honey sample. The honey samples were compared with amoxicillin through the *Chi-square test*. The bioactive compounds were expressed by measuring the Retention factor (R*f*) absolute value. The botanical origin of honey was determined by the dominant or accessory pollen species, using the average of two honey slides per sample. All statistical analyses were performed in the R software (R version 3.31, 2016). The Shapiro-Wilk test was used to check the adjustment of the Normal error distribution.

RESULTS

Phenolic and Total Flavonoid Contents

In the samples evaluated, the phenolic content ranged from 5.6 to 12.78 mg GA/100 g of honey and the total flavonoid content ranged from 3.52 to 9.38 mg QE/100 g of honey. Mato Grosso samples from honey 1, 2, 6 and 7 have high flavonoid content. The calibration curve constructed with different concentrations of the galic acid and quercetin standard gives the equation (y=0.093 × -0.0222, with R^2=0.9974) and (y=0.0067 × -0.0126, with R^2=0.9947), respectively [16-18].

Antioxidant Activity

The samples of honey 1, 2, 5 and 6 presented antioxidant activity by the capture of Nitric Oxide (NO) with $CI_{50\%}$ from 3.02 to 3.64 mg/mL. On the other hand, the remaining samples presented $CI_{50\%}$ greater than 6.4 mg/mL. The honey samples showed antioxidant activity by reducing the ferric ion (FRAP) with $CI_{50\%}$ from 10.66 to 19.24 mg/mL. Antioxidant activity by the sequestration of the free radical DPPH was greater than 20 mg/mL for all evaluated honey from Mato Grosso. All the results are shown in Table 1.

Table 1: Content of phenolic and total flavonoids and antioxidant activity of *Apis mellifera* honey by free radical sequestration (DPPH), capture of the free nitric oxide radical (ON) and iron reduction (FRAP), Central Brazil, Mato Grosso. 2018.

Samples	Phenolic[1]	Flavonoids[2]	ON[3]	FRAP[3]	DPPH[3]
Honey 1	12.7 ± 0.0	9.3 ± 0.1	3.4 ± 0.91	10.6 ± 1.8	> 20
Honey 2	10.9 ± 0.0	7.3 ± 0.0	3.6 ± 1.13	17.4 ± 0.1	> 20
Honey 3	7.0 ± 0.0	4.3 ± 0.0	>6.4	19.2 ± 0.2	> 20
Honey 4	8.6 ± 0.0	6.2 ± 0.0	>6.4	18.9 ± 0.1	> 20
Honey 5	5.6 ± 0.0	3.5 ± 0.0	3.1 ± 3.39	18.1 ± 0.4	> 20
Honey 6	7.9 ± 0.0	6.9 ± 0.0	3.0 ± 1.14	19.0 ± 0.1	> 20
Honey 7	7.7 ± 0.0	7.4 ± 0.00	>6.4	11.0 ± 1.8	> 20
Honey 8	7.9 ± 0.0	4.1 ± 0.00	>6.4	10.9 ± 1.0	> 20
Ascorbic Acid	--	--	25.8 ± 11.0	14.9 ± 2.1	1.6 ± 0.2
Footnote: [1] (mg galic acid equivalent/100 g honey ± SD); [2] (mg quercetin equivalent/100 g honey ± SD); [3] (CI50% honey=mg/mL ± SD and IC50% ascorbic acid=µg/ mL ± SD).					

Anti-*Helicobacter pylori* Activity

Minimum Inhibitory Concentration (MIC): Up to the tested concentration of 1000 µg/mL, honey samples did not present 100% inhibition on *H. pylori* growth, however, at this concentration, honey 1 showed 93.84% of inhibition. On the other hand, honeys 5, 6, 7 and 8, at this concentration, presented inhibitions between 52% and 60%. For the other honeys, the MIC_{90} is greater than 1000 µg/ mL Table 2.

Table 2: Anti-*Helicobacter pylori* activity of *Apis mellifera* honey from Mato Grosso evaluated by Minimum Inhibitory Concentration (MIC), bacterial growth inhibition percentage and $MIC_{90\%}$, Central Brazil, Mato Grosso, 2018.

Samples	Atividade Antibacteriana		
	MIC (µg/ mL)	Inhibition (%)[1]	MIC_{90}[2]
Honey 1	1000	93.84 ± 4.65	971.39 ± 31.76
Honey 2	1000	22.00 ± 28.91	x
Honey 3	1000	34.60 ± 28.10	x
Honey 4	1000	41.70 ± 33.74	x
Honey 5	1000	52.90 ± 7.54	x
Honey 6	1000	60.79 ± 25.81	x
Honey 7	1000	60.08 ± 25.01	x
Honey 8	1000	59.46 ± 16.37	x
Amoxicillin	100	94.59 ± 5.48	81.53 ± 2.86
Footnote: [1] ug/ ml ± SD; [2] inhibition percentage ± SD.			

Minimum Bactericide Concentration (MBC): Aliquots of honeys 1, 5, 6, 7 and 8 were chosen due to the inhibition of the growth of *H. pylori* in the concentration of 1000 µg/mL. After 72 h of incubation, was observed an increase in the colonies of *H. pylori* leading to conclude that the honeys have bacteriostatic action [19-20].

Anti-bacterial activity by thin layer chromatography: Fractions of the honey samples 1, 5, 6, 7 and 8 showed halos of inhibition in the thin layer chromatography, which indicate the presence of substances capable of inhibiting *H. pylori* growth, as well as its antioxidant capacity.

Honey 1 showed nine bioactive compounds (R*f* 0.04-0.96), seven with *H. pylori* inhibition halos (R*f* 0.04-0.89), near the starting point of the chromate plate, of which five bioactive compounds with R*f*s 0.04 to 0.28, the bioactive compound 6 had R*f*=0.34 and the bioactive compound 7 R*f*=0.89 linked to the phenolic content.

The honey 5 exhibited nine bioactive compounds, three with inhibition halos and two at the starting point and one near half of the chromate plate (R*f* 0.03-0.09 and 0.24). Honey 6 exhibited nine bioactive compounds, three with inhibition halos (R*f* 0.78- 0.88) at the end of the race. The honey 7 showed six compounds, four with inhibition halos (R*f* 0.04 - 0.19) and honey 8 showed seven bioactive compounds, four with inhibition halos, all at the starting point of the chromate plate Table 3.

Table 3: Anti-*Helicobacter pylori* activity of *Apis mellifera* honey from Mato Grosso by the thin layer chromatography method, Central Brazil, Mato Grosso, 2018.

Sample	Total number of compounds and R*f* Values	Number of compounds and R*f* Resistant	Color λ 365 nm
Honey 1	09 (0.04 - 0.96)	07 (0.04 - 0.39)	Lilac
Honey 5	08 (0.03 - 0.94)	03 (0.03 - 0.24)	
Honey 6	09 (0.01 - 0.88)	03 (0.78 - 0.88)	
Honey 7	06 (0.04 - 0.95)	04 (0.04 - 0.19)	
Honey 8	07 (0.04 - 0.96)	04 (0.04 - 0.20)	

Botanical Origin of *Apis Mellifera* Honey

Pollen analysis identified 16 types of pollen in honey samples, comprised in 12 botanical families. The pollen of *Myracrodruon urundeuva* Allemão was dominant in six samples of honey (1 to 6). The honey 7 showed *Mimosa pudica* L. pollen as dominant. Honey 8 was not analyzed because it contained less than 200 pollens on the slides.

The isolated pollens common to honey samples were *Astronium fraxinifolium* Schott, *Brachiaria* sp., *Mimosa pudica*, *Protium heptaphyllum* (Aubl.) Marchand and *Cecropia pachystachya* Trécul. As uncommon characteristics, honey 1, 2 and 5 presented as exclusive pollen those of *Vernonia* sp., *Miconia* sp. and *Hyptis suaveolens* Poit., respectively.

DISCUSSION

It is widely known that honey, besides its nutritional value, presents several biological activities that derive from the presence of secondary metabolites in its constitution. These metabolites vary a lot in the composition of the honeys due to several factors, such as bee genetics, the period of harvest, and especially the type of the flora visited for the collection. Ultimately, we can say that each honey has its own chemical identity and pharmacological property.

Among the secondary metabolites contained in honey are polyphenols, a large chemical family that can be divided into flavonoids (flavones, flavonols, flavanols, flavanones, isoflavones, anthocyanidin and chalcones, for example, quercetin, kaempferol, genistein, apigenin, among others) and non-flavonoids, the phenolic acids (cafeic acid, gallic acid, vallinic acid, chlorogenic acid, among others). Their presences can be used as a tool for the honey classification and authentication.

It is widely known that the good antioxidant activity of honey is due to the presence of the polyphenols and their free radical scavenger property. Polyphenols exert this effect by releasing hydrogen from one of their hydroxyl groups, and the degree of antioxidant activity of honey is related to the number of hydroxyl groups of phenolic present in its composition. Thus, knowing the identity and content of secondary metabolites present in the composition of a honey can provide clues to its antioxidant capacity.

The honey considered with a high content of total flavonoids, as it presents values of 1.61 mg QE/ 100 g, 1.79 mg QE/ 100 g and 3.10 mg QE/100 g, respectively. However, Dor and Mahomoodally was more rigorous when considering high flavonoid content in honey, higher values, between 8.75 and 11.80 mg QE/100 g. The honeys evaluated in this study showed concentrations between 3.52 and 9.38 mg QE/100 g of honey, consistent with all the mentioned parameters, so it is possible to consider that the honeys from Mato Grosso present high content of total flavonoids.

Studies show that the total phenolic contents in different types of honey can present a broad spectrum of values. In the honey samples evaluated in this study, the amount of phenolic was relatively low (5.6 to 12.78 mg GA/1 00 g) when compared with some literature works, such as honeys from Turkey (16.02 to 120.04 mg GA/100 g), Tunisia (32.17 and

119.42 mg GAE/100 g) and even southern Brazil (11.37 to 54.01 mg GA/100 g). However, other studies found similar values, such as monofloral honey from southern Brazil and different regions of Italy (4.88 to 12.14 mg GA/100g and 4.7 to 11.4 mg GA/ 100g, respectively. Among the honeys studied, honey 1 had the highest content of both flavonoids and total phenolic. The identification of honey components is fundamental for the development of a pharmacological product with known activity, and flavonoids and polyphenolics are important compounds in the chemical profile of the honey.

Free radicals are reactive chemical species of oxygen or nitrogen (ROS and RNS, respectively), which have highly reactive unpaired electrons in the external orbital. Superoxide (O_2•), hydroxyl (OH•), peroxyl (RO_2•), hydroperoxyl (HO_2•) radicals can be generated from cellular metabolism and can damage DNA and oxidize lipids and proteins. Other oxidizing agents that are not radicals, such as Hydrogen peroxide (H_2O_2), Hypochlorous acid (HOCl) and ozone (O_3), can be easily and quickly converted into radicals and cause the same damage to DNA. These oxidants act on the molecular mechanisms that trigger various pathological processes such as neurodegenerative, renal, gastrointestinal, pulmonary, cardiovascular diseases, autoimmune, cancer, diabetes, among others.

Antioxidant compounds (endogenous or not) promote defense of the organism from ROS and RNS. The oxidative stress occurs when these reactive molecules overload the body's natural antioxidant defenses, resulting in the oxidation of lipids, proteins or DNA and therefore, to several pathologies. Thus, compounds with antioxidant activity play an important role in preventing damage generated by ROS and RNS. Several authors reported the antioxidant action of honey, but no study had been done on this aspect with the honey samples from Mato Grosso used in this study.

It is known that antioxidant tests have limitations and do not allow a precise measurement of the antioxidant action that occurs *in vivo*. Antioxidants can exert their effect throughout several mechanisms, such as scavenger effect, ion sequestration of transition metals, hydrogen peroxide or hydro peroxides in decomposition, reduction of active pro-oxidants and repair of biological damage. For this reason, there is a wide range of tests to evaluate the antioxidant action and each one evaluates one of the aspects of this complex process, making it desirable to combine several tests to assess the overall antioxidant capacity, which includes reactivity to aqueous and lipid radicals directly *via* radical reduction mechanisms and indirectly *via* metal. In this study DPPH, FRAP and NO tests was used.

DPPH is a stable free radical with scavenger properties and the test can evaluate the transfer of electrons and hydrogen atoms. The method is widely used in the evaluation of the antioxidant action, even despite its limitations. It is based on a kinetic evaluation that can be influenced by several factors, among which, the stoichiometry of the solvent used and pH. These factors are especially relevant for complex substances, where each component can react in its own way. In this test, all honey samples from Mato Grosso had CI_{50} >20 mg/mL, showing no antioxidant activity for DPPH, when compared with honey from other Brazilian regions with $CI_{50\%}$ from 0.27 to 1.60 and 17.21 mg/mL. Honey from other countries presented IC_{50} lower than 20 mg/mL like Turkey (CI_{50} 0.35 to 2.56 mg/mL), Spain (CI_{50} 13.86 mg/mL) and Morocco (CI_{50} 15.0 to 23.5 mg/mL). Honey from these countries has, in its composition, a predominance of phenolic acids; this suggests that the antioxidant activity for DPPH could be related to the presence of phenolic acids. Thus, as the samples evaluated in this study showed relatively low amounts of phenolic acids; low antioxidant activity for DPPH was expected.

The FRAP method is based on electron transfer and can detect compounds capable of reducing Fe (III) in Fe (II). Like any non-enzymatic antioxidant test, FRAP has its limitations. The test is performed at acid pH, which prevents the complete oxidation of phenolic compounds as occurs in plasma at physiological pH, not allowed for extrapolate the antioxidant action to *l* conditions. Still, it is a simple, practical and robust test, widely used, and can offer a putative index of the antioxidant capacity. The honey samples evaluated presented CI_{50} values between 10 and 20 mg/mL, higher than multifloral honey samples from Taiwan, India and Morocco with CI_{50} 0.05-7.0 mg/mL, 1.87-4.40 mg/mL and 1.5 to 6 mg/mL, respectively. This result can be attributed to the phenolic content of the samples that could provide stronger eliminating radicals and consequently greater reduction activity.

Nitric Oxide (NO) is an important chemical mediator involved in various biological functions, including neurotransmission, vascular homeostasis, antimicrobial and antitumor activity. On the other hand, it contributes to oxidative damage since the Nitric Oxide radical (•NO), the free radical and weak oxidant, can react with the radical O_2• generating RNS as nitrite and peroxynitrite anions, oxidizing potentials. Excess NO is implicated in the cytotoxic effects observed in disorders such as HIV, cancer, Alzheimer and arthritis. The Griess test, used in this study to

evaluate antioxidant activity of honey on NO, is a well-known colorimetric method for the analysis of biological samples such as saliva, urine, serum, cerebrospinal fluid and culture medium, indirectly. Sodium nitroprusside, in aqueous solution and physiological pH, generates nitric oxide spontaneously, which, combined with O_2, produces nitrite ions, which reacts with the Griess reagent generating pink coloring.

Dor and Mahomoodally evaluated monofloral ginger and eucalyptus honeys from Mauritius (Africa) by the Griess method, which showed CI_{50} of 2.77 and 6.76 mg/mL, respectively. Compared to these, the honeys 1, 2, 5 and 6 from Mato Grosso evaluated in this study, which showed CI_{50} between 3.0 and 3.4 mg/mL, can be considered of pharmacological interest.

The antioxidant activity for the capture of nitric oxide and reduction of ferric ions had significant positive correlation with the content of flavonoids in the honeys (r^2=0.60), suggesting that the antioxidant capacity in the trials is linked to the content of flavonoids present in honey, corroborating several findings of other authors. The flavonoids present in the honey samples helped to reduce the formation of nitric oxide and ferric ion and respond, at least in part, to the antioxidant activity of the honey samples. Although some samples present antioxidant activity even with low flavonoid content, as the honey sample 5 for example, suggests that this activity may be due to the presence of other secondary compounds, besides the flavonoids.

The lower $CI_{50,}$ the greater the sample efficiency in neutralization the reactive radical. Extracts of plants and pure substances have values of CI_{50} in the greatness of µg, with greater efficiency compared to honey, that the values of CI_{50} are of the order of mg. However, it is worth mentioning that honey is a food product, the amount consumed is naturally higher. In addition, when comparing with other samples of honey, fractionated or not, we can say that the honey samples of this study showed good antioxidant activity.

Helicobacter pylori is a gram-negative, microaerophilic, flagellated bacteria, considered the most prevalent human pathogen, linked with the chronic active gastritis, peptic and duodenal ulcer disease and cancer. *H. pylori* infection promotes an inflammatory response in the gastric mucosa that leads to the production of free radicals. It also affects the level of antioxidants measured in gastric juice. Studies also show that some free radical scavengers, such as vitamin C, have anti-*H pylori* activity. Honey is a product with recognized antioxidant activity and reported anti-*H pylori* activity, but no study had yet been conducted with honeys from Mato Grosso under this perspective.

The mechanisms of antibacterial action of honey are different from antibiotics, which destroy the bacterial cell wall and inhibit intracellular metabolic pathways. Its composition can extract moisture from the environment, and thus dehydrate bacteria and due to its high sugar content, low pH, Maillard reaction products, volatile organic acids, beeswax, nectar, pollen and propolis, which are important bacterial components.

The honeys evaluated in this work, regarding its anti-*H. pylori* activity, honey 1 stands out for presenting MIC_{90} of 0.97 mg/ mL and an inhibition of 93.84% of bacterial growth at a concentration of 1 mg/ mL. reported 95% growth inhibition at a concentration approximately 5 times higher (5 mg/mL) for a honey from South Africa, while showed that Australia's "mountain honey" inhibited the growth of three isolates of *H. pylori* at a concentration of 0.938 mg/mL, equivalent to honey 1. The other honeys evaluated showed less expressive anti-*H. pylori* activity than honey 1.

The anti-*H pylori* activity for medicinal plants was categorized by Wang into four distinct classes according to MIC: strong activity (MIC<10 µg/mL), strong-moderate activity (MIC 10-100 µg/mL), weak-moderate activity (MIC 100-1000 µg/mL) and weak activity (MIC>1000 µg/mL). Wang's classification is the most rigorous, Holetz consider endowed with strong activity those compounds that have MIC<100 µg/mL and Aligiannis, Kalpoutzakis, Mitaku and Chinou MIC<500 µg/mL. Fabry considered plant extracts with MIC<8000 µg/mL as having usable antibacterial activity. If we were to evaluate the result of honey 1 by these parameters, it would be considered bacteriostatic and with weak anti-*H. pylori* activity. However, honey is a food product, whose consumption reaches quantities much higher than the MIC found. For example, one teaspoon, which contains approximately 5 g of honey, can be consumed daily. Thus, the insertion of a small daily amount of honey in the diet can represent a benefit in the anti-*H. pylori* treatment.

The pharmacological activity of a complex product such as honey, can be the result of the synergistic action of several constituent compounds, or can be carried out by only one of them, even if they are minority, such as rutin or gallic acid

for antioxidant activity. Honey 1 from Mato Grosso did not present high phenolic content compared to other honeys, but still presented antioxidant and anti *H. pylori* activity, possibly due to some component between flavonoids and polyphenols or even by the synergistic action of some of its compounds.

The phenolic compounds and flavonoids present in honey, in addition to exerting antioxidant action, are also linked to antibacterial activity in front of a wide spectrum of gram-negative and gram-positive bacteria. There is increasing evidence that flavonoids interfere with various bacterial virulence factors, including enzymes and toxins. In the case of honey, the antibacterial activity can occur by synergy of several of its components. Although the components of honey 1 have not been identified, it is certain that the presence of its flavonoids may have contributed to this activity. For Cushnie and Lamb antibacterial activity of the flavonoids can be attributed to three mechanisms of action: damage to the cytoplasmic membrane, inhibition of nucleic acid synthesis and inhibition of energy metabolism.

The thin layer chromatography showed seven bioactive compounds in the inhibition of *H. pylori* growth in honey 1. If we take as basis the R*f* values and the solvent system used and compare to data found in literature, we can point out some possibilities of identity of these compounds. The bioactive compound 6 presented results compatible with the gallic acid found by De Souza, with R*f* values between 0.39 and 0.42. Galic acid is a major phenolic acid in honey from *Apis mellifera*. The bioactive compound 7 of honey 1 had R*f* values compatible with that of caffeic acid found by Wagner and Bladt, another phenolic acid widely present in the composition of honey. However, studies must be conducted to certify the identity of these bioactive compounds.

The botanical origin of honey 1 showed that the dominant pollen comes from the species *Myracrodruon urundeuva*, a tree popularly known as aroeira, native to Brazil with wide geographical distribution. There are records of pollen of this species in honey from other regions of Brazil, such as Minas Gerais, Paraíba and Mato Grosso do Sul. *Myracrdruon urundeuva*, rich in flavonoids, has known antimicrobial, anti-inflammatory and antiulcerogenic activity is used as an antiseptic and in the treatment of stomatitis. The bactericidal and bacteriostatic activities of the aroeira on *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus sobrinus*, *Streptococcus sanguis*, *Lactobacillus casei* and antifungal action on *Candida albicans*, *Candida tropicalis* and *Candida krusei* are also reported.

All honey samples evaluated in this work presented *M. urundeuva* as dominant botanical origin and five other types of accessory pollens in common and they showed a variation as to flavonoid content, and still. This suggests that the marker of the botanical origin of Mato Grosso honey with high flavonoid content should be associated with isolated pollen present in honey.

CONCLUSION

Honey from Mato Grosso showed good antioxidant and anti-*Helicobater pylori* activity in *in vitro* experiments. Among the samples evaluated, honey 1 from Nossa Senhora do Livramento- MT, Cerrado biome, showed the best results, possibly duea to its good content of flavonoids and 7 bioactive compounds in its constitution, which still requires identification in future studies. The other samples also showed antioxidant and bacteriostatic action, although less promising than honey 1.

The antioxidant activity of honey samples involves the reduction of ferric ion and the capture of nitric oxide, which may be related to the content of flavonoids and none of them showed scavenger action. The botanical origin of honey from Mato Grosso has *Myracrodruon urundeuva* as a dominant pollen species in all analyzed samples.

The study of anti-*H pylori* of honey was unprecedented in Brazil, expanding the knowledge about honey produced in state of Mato Grosso, Central Brazil and suggesting that it can bring prophylactic benefits or as a complementary therapy in *Helicobacter pylori* infection, mainly honey 1.

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