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BIOTECHNOLOGY

Antibacterial activity of Aroeira honeys produced in Minas-Gerais against bacteria of clinical importance

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ABSTRACT. The biological activity of honey has been the subject of great scientific investigation. Bee products are widely used in folk medicine to treat human diseases due to their antibacterial and inhibitory potential action on different human pathogens. Ten honey samples produced by *Apis mellifera* in the northern of Minas Gerais state were tested against pathogenic microorganisms *Staphylococcus aureus* TSST (clinical isolated) and enterohaemorrhagic *Escherichia coli* ATCC 43895 for determination of their minimum inhibitory concentrations. The microdilution technique in broth Mueller-Hinton broth (MHB) was used in four concentrations (25, 12.5, 6.25 and 3.125%). There was a reduction of bacterial growth for the two target species at all concentrations tested. The optimal concentration for inhibition of *S. aureus* and *E. coli* were 3.125% (w/v). Therefore, the antibacterial activity of the tested samples evidences the potential of Aroeira honey produced in the north of Minas Gerais for therapeutic use, thus contributing to the aggregation value and commercialization of this type of honey.

Keywords: bee products; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*.

Atividade antibacteriana de méis de Aroeira produzidos em Minas-Gerais frente a bactérias de importância clínica

RESUMO. A atividade biológica do mel tem sido objeto de relevante investigação científica. Produtos apícolas são amplamente utilizados na medicina popular para tratar doenças humanas por causa de sua potencial ação antibacteriana e inibitória a diferentes patógenos humanos. Dez amostras de mel produzidas por *Apis mellifera*, no norte do Estado de Minas Gerais, foram testadas contra os micro-organismos patogênicos *Staphylococcus aureus* TSST (isolado clínico) e *Escherichia coli* enterohemorrágica ATCC 43895 para determinação de suas concentrações inibitórias mínimas. A técnica de microdiluição em caldo Mueller-Hinton (CMH) foi utilizada em quatro concentrações (25, 12,5, 6,25 e 3,125%). Houve uma redução do crescimento bacteriano para as duas espécies alvo em todas as concentrações testadas. A concentração ótima para a inibição de *S. aureus* e *E. coli* foi de 3,125% (p/v). Portanto, a atividade antibacteriana das amostras testadas evidencia o potencial do mel de Aroeira produzido no norte de Minas Gerais para uso terapêutico, contribuindo para a agregação de valor e comercialização deste tipo de mel.

Palavras-chave: produtos de abelhas; atividade antibacteriana; *Staphylococcus aureus*; *Escherichia coli*.

Introduction

Honeys are complex substances produced by bees and may contain different compounds with bioactive properties, which vary according to the region where they are produced and collected (Pita-Calvo & Vásquez, 2017). The interest in using a natural product with therapeutic properties has increased in recent years. Honey has antibacterial activity, which has been widely recognized, against a broad spectrum of Gram-positive and Gram-negative bacteria (Jantakee & Tragoolpua, 2015; Fyfe, Okoro, Paterson, Coyle, & McDougall, 2017; Poovelikunnel et al., 2018). The antibacterial properties of honey are attributed to its chemical composition such as osmolarity, the presence of hydrogen peroxide, low pH and the role of phytochemical compounds such as polyphenols, antioxidants, antimicrobial peptides as well as Maillard reaction products (Anthimidou & Mossialos, 2013).

The Aroeira honey is produced by *Apis mellifera* bees in the Brazilian Dry Forest. It is characterized by predominance of *Myracrodruon urundeuva* and in this biome, the scarcity of floral resources, associated with high temperatures and low humidity, induce bees to seek these massive food sources to ensure their food supply. This leads to the productions of a honey which has peculiar characteristics that

differentiate it from other types of honey from other Brazilian regions: predominance of pollen grains of *M. urundeuva*, dark amber color, less sweet taste and does not crystallize at room temperature. This honey also has physical-chemical characteristics such as high electrical conductivity, high levels of ash content, less acid pH and acidity, high levels of invertase and the presence of melezitose and erlose (Bastos, Calaça, Simeão, and Cunha, 2016). It is a little-known honey and because of its organoleptic characteristics is less attractive for commercialization.

The use of natural products and the research for new applications have increased significantly in several areas such as agriculture and food industry and mainly in the pharmaceutical industry in search of products with therapeutic properties to assist in the treatment of diseases related to pathogenic and resistant microorganisms (Costa & Júnior, 2017).

S. aureus is one of the pathogens most involved in nosocomial infections and represents a serious public health problem because it is easily capable of acquiring resistance to routinely used antibiotics (Souza, et al., 2016). The toxic shock syndrome triggered by *S. aureus* is an acute and multisystemic toxin-mediated disease (TSST - Toxic Shock Syndrome Toxin). Symptoms involve fever, skin infections (redness/rush), hypotension and diarrhea, and may be potentially fatal (Suga, Shiraishi, Takushima, & Harii, 2016).

E. coli is associated with infectious diseases involving all human tissues and organ systems. This species is one of the microorganisms commonly involved in Gram-negative septicemia and endotoxin-induced shock. Enterohemorrhagic *E. coli* (EHEC) is a pathogenic *E. coli* line capable of causing disease in humans and is responsible for a large number of food-borne diseases associated with outbreaks of bloody diarrhea, which may be complicated by the development of hemolytic uremic syndrome (HUS) (Jost, Bidet, Carrère, Kurkdjian, & Bonacorsi, 2016). Antimicrobial therapy is not recommended during EHEC infection because of the risk of developing HUS through the induction and/or production of Shiga toxin, the main virulence factor of EHEC (Licznerska, et al., 2016).

The objective of this work was to determine the minimum inhibitory concentration of honeys produced by *Apis mellifera* bees in the north of Minas Gerais against pathogenic microorganisms of clinical importance *S. aureus* TSST and *E. coli* (EHEC) cause of hemorrhagic diarrhea.

Material and methods

Honey samples

Ten honey samples were collected in apiaries located at "Fazenda Coqueiros" (43º 18' 31" W and 15º 48' 09" S). Honeys were identified and stored in plastic bottles sheltered from light and colling temperature (5 +/- 2°C). For the antimicrobial assays, the honey samples were diluted in sterile Mueller-Hinton broth in a ratio of 1: 1 and filtered on polyethersulfone membrane Millipore (0.22 µM). This solution was immediately used.

Target microorganisms

The following microorganisms were used for antimicrobial assays: *S. aureus* TSST (clinical isolate - Department of Food Microbiology and Toxicology, University of Wisconsin–Madison, Madison, Wisconsin) and *E. coli* (ATCC 43895). The microorganisms were grown in Petri dishes containing Mueller-Hinton agar (MHA) and incubated at 37°C for 18 to 20 hours before to antimicrobial inhibition assays for purity and viability observation.

Spectrophotometric assay for MIC determination

The MIC of the honeys was determined by the plate microdilution technique in CLSI (former NCCLS) guidelines (National Committee for Clinical Laboratory Standards [NCCLS] 2003), with slight adaptations. The plates were incubated at 37°C for 16 to 20 hours. The triphenyltetrazolium chloride dye 2, 3, 5 was used as an indicator of cell viability. The inhibition of microbial growth was measured on a microplate reader at 620 nm. MIC was defined as the lowest concentration of the sample capable of reducing 70% of microbial growth in natural products (Hughes, Lima, Lucchese, Góes Neto, & Uetanabaro, 2013). The assays were done in triplicate.

Results and discussion

Table 1 shows that honey samples inhibited the microbial growth of pathogenic bacteria of clinical importance.

Table 1. Minimal inhibitory concentration (MIC) of Aroeira honeys against *S. aureus* TSST and *E. coli* (EHEC) in different concentrations.

Samples	*E. coli* EHEC	*S. aureus* TSST
1	12.5	3.125
2	3.125	12.5
3	3.125	12.5
4	3.125	3.125
5	12.5	3.125
6	6.25	3.125
7	3.125	6.25
8	3.125	6.25
9	12.5	3.125
10	6.25	3.125

Percentage (MIC) considered: ≥ 70%.

For the two target species tested was observed at all concentrations tested the reduction of bacterial growth. Inhibition values were distinct among the samples analyzed for the inhibition established percentage (≥ 70%). The antimicrobial activity of dark honeys was also observed for *E. coli* (Tenore et al., 2012) and for *S. aureus* (Anthimidou & Mossialos, 2013). The presence of different components of honey are responsible for the antimicrobial activity of honey, related to its acidity, high osmolarity and hydrogen peroxide. Non-peroxide factors such as lysozyme, phenolic acids and flavonoids also contribute for antibacterial properties (Anthimidou & Mossialos, 2013). The Aroeira honey shows a high level of phenolic compounds produced and provided by flowers to bees for honey production (Bastos et al., 2016).

The results of the microbial growth inhibition frequency for each target microorganisms are shown in Figure 1.

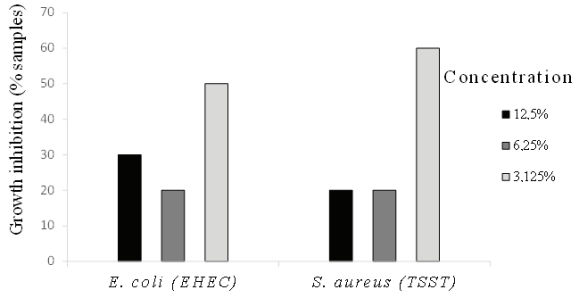

Figure 1. Frequency (%) of inhibition of Aroeira honey against *S. aureus* TSST and *E. coli* (EHEC) in different concentrations.

The MIC for *S. aureus* was 6.25 and 12.5% (w/v) respectively in 20% of the samples, followed by MIC from 3.12 to 60% of the honeys tested. In contrast to *E. coli*, the highest inhibition potential was found in 50% of honey samples analyzed with MIC (3.125%) (w/v). For the other samples, the MIC of 6.25% (w/v) was observed in 20% of the analyzed honeys followed by 12.5 in 30% of the samples. The antimicrobial potential will be directly related to the botanical source and geographical location of each honey (Sherlock et al., 2010). In our study Aroeira honey showed high antimicrobial activity. The strong presence of the phenolic compounds detected in the idioblasts canals and other parts of Aroeira inflorescence may be related to the defense of the plant against the attack of herbivores and the growth of fungi, as well as in protection against water loss and temperature increase (Bruneton, 1999). These phenolic compounds are also present in different percentages according to the kind of honey and its origins (Tenore et al., 2012; Pita-Calvo & Vázquez, 2017). Therefore, it is one of the main components which grant antimicrobial activity in Aroeira honey. This observation is in agreement with previous studies with Manuka honey against *E. coli* and *S. aureus* (Inoue, Muryama, Seshimo, Takeba, & Yoshimur, 2005; Kwakman & Zaat, 2012) and Tualang honey (Boateng & Diunase, 2015), both dark honeys with strong antimicrobial potential.

Conclusion

Aroeira honey has few studies and its activity against bacteria, especially Gram-negative bacteria such as *E. coli*, is little known. Considering the results, the ideal concentration for inhibition of *S. aureus* and *E. coli* was 3.125% (w/v). The use of honeys as antimicrobials has shown to be advantageous for reducing the microbial load, since it was able to inhibit the growth of pathogenic bacteria. Moreover, the antibacterial activity of the honeys observed in this work reinforces the potential for therapeutic use of Aroeira honey produced in the north of Minas Gerais, thus contributing to the aggregation value of this honey.

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References

Anthimidou, E., & Mossialos, D. (2013). Antibacterial activity of Greek and Cypriot honeys against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in comparison to manuka honey. *Journal of Medicinal Food*, *16*(1), 42-47. doi: 10.1089/jmf.2012.0042

Bastos, E. M. A. F., Calaça, P. S. S. T., Simeão, C. M. G., & Cunha, M. R. R. (2016). Characterization of the honey from *Myracrodruon urundeuva* (Anacardiceae - Aroeira) in the Dry Forest of northern of Minas Gerais/Brazil. *Advances and Agricultural Science*, *4*(4), 64-71.

Boateng, J., & Diunase, K. N. (2015). Comparing the antibacterial and functional properties of Cameroonian and Manuka honeys for potential wound healing - Have we come full cycle in dealing with antibiotic resistance? *Molecules*, *20*(9), 16068-16084. doi: 10.3390/molecules200916068

Bruneton, J. (1999). *Pharmacognosy, phytochemistry, medicinal plants*. Londres: UK: Intercept.

Costa, A. L. P., & Júnior, A. C. S. S. (2017). Resistencia bacteriana aos antibióticos e Saúde Pública: uma breve revisão de literatura. *Estação Científica (UNIFAP)*, 7(2). 45-47. doi: 10.18468/estcien

Fyfe, L., Okoro, P., Paterson, E., Coyle, S., & McDougall, G. J. (2017). Compositional analysis of Scottish honeys with antimicrobial activity against antibiotic-resistant bacteria reveals novel antimicrobial components. *LWT - Food Science and Technology*, *79* 52-59. doi: 10.1016/j.lwt.2017.01.023

Hughes, A. F. S., Lima, F. G., Lucchese, A. M., Góes Neto, A., & Uetanabaro, A. P. T. (2013). Antimicrobial activity of *Syagrus coronata* (Martius) Beccari. *Brazilian Archives of Biology and Technology*, *56*(2), 269-274. doi: 10.1590/S1516-89132013000200012

Inoue, K., Muryama, S., Seshimo, F., Takeba, K., & Yoshimur, H. (2005). Identification of phenolic compound in Manuka honey as specific superoxide anion radical scavenger using eléctron spin resonance (ESR) and liquid chromatography with coulometric array detection. *Journal of the Science of Food and Agriculture*, *85*(5), 872-878. doi: 10.1002/jsfa.1952

Jantakee, K., & Tragoolpua, Y. (2015). Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. *Biological Research*, *48*(4), 1-11. doi: 10.1186/0717-6287-48-4

Jost, C., Bidet, P., Carrère, T., Kurkdjian, P. M., & Boncarosi, S. (2016). Susceptibility of enterohaemorrhagic *Escherichia coli* to azithromycin in France and analysis of resistance mechanisms. *Journal of Antimicrobial Chemotherapy*, *71*(5), 1183-1187. doi: 10.1093/jac/dkv477

Kwakman, P. H. S., & Zaat, S. A. J. (2012). Critical Review. Antibacterial components of honey. *Life*, *64*(1), 48-55. doi: 10.1002/iub.578

Licznerska, k., Nejman-FaleNczyk, B., Bloch, S., Dydecka, A., Topka, G., Gdsior, T., … Wwgrzyn, G. (2016). Oxidative stress in shiga toxin production by enterohemorrhagic *Escherichia coli*. Review Article. *Oxidative Medicine and Cellular Longevity*, *2016*(6), 1-8. doi: 10.1155/2016/3578368

National Committee for Clinical Laboratory Standards [NCCLS]. (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard-Sixth Edition. *NCCLS document M7-A6*, *23*(2), 14-18.

Pita-Calvo, C., & Vásquez, M. (2017). Differences between honeydew and blossom honeys: A review. *Trends in Food Science & Technology, 59*, 79-87. doi: 10.1016/j.tifs.2016.11.015

Poovelikunnel, T. T., Gethin, G., Solanki, D., McFadden, E., Codd, M., & Humphreys, H. (2018). Randomized controlled trial of honey versus mupirocin to decolonize patients with nasal colonization of meticillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection*, *98*(2), 141-148. doi: 10.1016/j.jhin.2017.10.016

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S., & Humphreys, H. (2010). Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BioMed Central Complementary and Alternative Medicine*, *10*(47), 1-5. doi: 10.1186/1472-6882-10-47

Souza, C. S. M., Fortaleza, C. M. C. B., Witzel, C. L., Silveira, M., Bonesso, M. F., Marques, S. A., & Cunha, M. L. R. S. (2016). Toxigenic profile of methicillin-sensitive and resistant *Staphylococcus aureus* isolated from special groups. *Annals of Clinical Microbiology and Antimicrobials*, *15*(9). doi: 10.1186/s12941-016-0125-5

Suga, H., Shiraishi, T., Takushima, A., & Harii, K. (2016). Toxic shock syndrome caused by methicillin-resistant *Staphylococcus aureus* (MRSA) after expander-based breast reconstruction. *EPlasty*, *16*(2), 7-12. doi: 10.1016/j.asj.2009.01.010

Tenore, G. C., Ritieni, A., Pietro Campiglia, P., & Novellino, E. (2012). Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (*Apis mellifera* ssp. *sicula*). *Food and Chemical Toxicology*, *50*(6), 1955-1961. doi: 10.1016/j.fct.2012.03.067

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