

Antimicrobial activity of honeydew honey that caused larvae mortality (River Disease) in Uruguay

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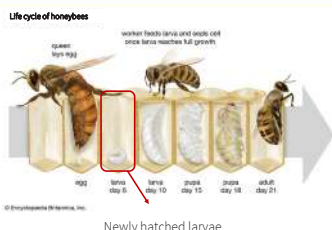
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INTRODUCTION

The "River Disease" (RD) is a disorder that kills newly hatched larvae in honeybee colonies located closed to fresh waterways with abundant riparian vegetation. It usually appears at the end of spring and can lead to massive larval losses in Uruguay [1].



It was observed that bees are highly active on *Sebastiania schottiana* trees (Image 1), in which there are reports of biologically active compounds [2]. It is on these trees that *Epormenis cestr*, a hemiptera that secretes a sugar-rich substance on which the bees feed in the absence of flowering, is often found [1] (Image 2).



Image 1. *Sebastiania schottiana* trees [1].



Image 2. *Epormenis cestr* secretion[1].

The planthopper honeydew collected by the honeybees is then turned into honeydew honey within the colony. Larvae fed with this honeydew die in their first days of life [1]. The affected colonies produce honeydew honey which has no commercial outlet.



RD was first described in 1951 close to Santa Lucia River in Uruguay and appeared sporadically until the 2000s. However, in recent years there has been an increase in the number of cases and, in turn, a spread of the disease along other rivers of Uruguay.

The aim of this work is to study and characterise the biological properties of this type of hive product and to generate strategies to valorize it, which to date not only has no commercial outlet but also represents an economic loss for beekeepers.

METHODOLOGY

To perform the characterization, 10 samples of honeydew honeys were taken from different parts of Uruguay where RD was reported, and the following workflow was carried out (Figure 1)

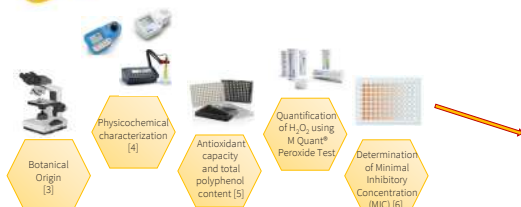


Figure 1. Different methods used to characterise honeydew honeys

A battery of basic physicochemical analyses are performed, including conductivity, moisture, colour, pH, acidity, ash, diastase index and hydroxymethylfurfural (HMF) [3]. Total polyphenol content was studied by Folin & Ciocalteu and their antioxidant capacity by ABTS and ORAC methods as reported by Baez et al [4].

Determination of Minimum Inhibitory Concentration (MIC) was performed as reported by Raffaelli et al [6] with some modifications. 50 %p/v honey solutions were evaluated against *Staphylococcus aureus* ATCC6538P, *Klebsiella pneumoniae* CCMG12716 and *Pseudomonas aeruginosa* ATCC 15442.

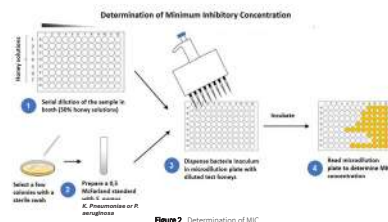


Figure 2. Determination of MIC

MIC analyses were carried out in a microdilution plate with 96 wells containing Mueller-Hinton broth. (Figure 2). The MIC was determined as the lowest honey concentration (antimicrobial agent) that inhibited the visible growth of a microorganism after incubation. Monofloral and polyfloral honeys from Uruguay were also analyzed, as well as honeys recognized worldwide for their nutraceutical properties and biological activity, such as Ulmo honey from Chile and Manuka honey from New Zealand (Manuka MGO > 500).

RESULTS

Physicochemical Analysis:

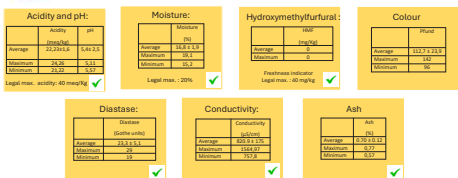


Figure 3. Results of physicochemical analyses

Figure 3 shows the results of the physicochemical analyses. The values of moisture, acidity, ash, HMF and diastase complied with the reference values established for honeydew honey in the National Bromatological Regulations. Conductivity is an indirect measure of ash content and from a nutritional point of view it is an indicator of the mineral composition of the honeys, in this case all the samples presented a conductivity greater than 800 mS/cm, characteristic of Honeydew honeys. Also, the samples showed colors in the darker side of Pfund scale.

Peroxide Content

Peroxide determination indicated that all samples did not contain significant amounts of peroxide. This indicates that the antimicrobial activity observed in the samples is non-peroxide activity, which is not common in honeys and may be due to the presence of other biologically active compounds, such as xanthoxylin.



Nutraceutical Properties:

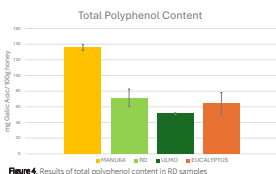


Figure 4. Results of total polyphenol content in RD samples compared to other honeys

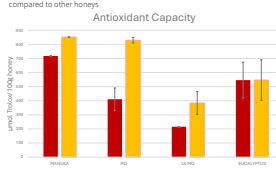


Figure 5. Results of antioxidant capacity (using ABTS and ORAC) in RD samples compared to other honeys

A high total polyphenol content and antioxidant capacity was observed in RD samples, higher than the values observed for honeys internationally known for their polyphenol content, such as Ulmo honey (Figure 4-5).

Antimicrobial Activity

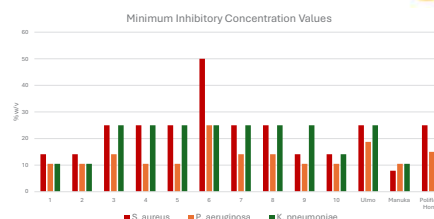


Figure 6. MIC values of RD samples and other honeys against different pathogens of interest

Honeydew honeys from colonies with River Disease showed antimicrobial activity against the three strains studied, mainly against *P. aeruginosa*, at concentrations of 10.5% w/v in most cases. This is comparable to the activity observed for Manuka honeys with more than 500 mg of methylglyoxal/kg honey (MGO>500), widely recognized for its antimicrobial activity. Also, the samples showed activity against *S. aureus* and *K. pneumoniae*, the latter being less commonly reported. The MIC values obtained for *K. pneumoniae* are lower than those observed in polyfloral honeys from Uruguay, indicating high antimicrobial activity (Figure 6). This activity could be due to the presence of biologically active compounds, such as xanthoxylin, which has been reported for its biological activity in some trees.

CONCLUSIONS

Honeydew honey from hives affected by River Disease have remarkable nutraceutical properties and high antimicrobial activity. These results represent advances in the characterization and valorization of a product that tends not to have a commercial use and is associated with economic losses for beekeepers. These results show a way to valorize this product, which is generally considered waste. Future work will focus on evaluating the correlation between the antimicrobial activity and the presence of compounds already reported in these honeys, such as xanthoxylin.

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PP-273 [Beekeeping Technology and Quality]
Antimicrobial activity of honeydew honey that caused larvae mortality (River Disease) in Uruguay

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Honeydew is the sugar-containing excretions of plants-sucking insects and it is very attractive to bees, resulting in the production of honeydew honey. This type of hive product presents difficulties for their commercialization as it has different physico-chemical and organoleptic characteristics to floral honey.

The River Disease (RD) is a honey bee health problem that kills the newly hatched larvae in Uruguay. The causal agent of larval death is the excretions of an aphid, *Epormenis cestri*, when found on *Sebastiania schottiana* trees near the hives. The bees collect these excretions, which end up as food for the larvae. The honey produced from this honeydew affects the larvae in their first days of life. The affected colonies produce honeydew honey which has no commercial outlet, and which contains compounds with potential biological activity and nutraceutical properties like xanthoxylin.

The aim of this work is to study and characterize the biological properties of this type of hive product and to generate strategies to valorize this product, which to date not only has no commercial outlet but also represents a loss for beekeepers.

10 samples of honeydew honey from colonies affected by RD were collected in different departments of Uruguay. Physicochemical characterization and quantification of peroxide content was carried out. Once it was confirmed that they were honeydew honeys, they were screened for antimicrobial activity in a Muller-Hilton diffusion plate assay and Minimal Inhibitory Concentration (MIC) was also determined. 50% w/v honey solutions were evaluated against *Staphylococcus aureus* ATCC6538P, *Klebsiella pneumoniae* CCMG12716 and *Pseudomona aeruginosa* ATCC 15442. MIC analyses were carried out in a microdilution plate with 96 wells containing Mueller–Hinton broth.

The samples presented high antimicrobial activity against all strains with MIC values below 20% w/v honey. No significant peroxide content was found in any of the samples, meaning that the activity is linked to other compounds in the honeys which is not common.

RD honeydew honeys are beehive products with novel antimicrobial potential. This study will continue by working on the correlation of the observed activity with the presence of compounds in this type of honey.