Scientific Works. Series C. Veterinary Medicine. Vol. LXIII (2)
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

ANTIMICROBIAL EFFECT OF COMMERCIAL MANUKA HONEY AND CONVENTIONAL LOCAL HONEY AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

Mara GEORGESCU[1*], Mimi DOBREA[1], Vlad Cristian DOBREA[2]

[1]University of Agronomical Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Carol Davila University of Medicine and Pharmacy, Dionisie Lupu no. 37, District 2, 020021 Bucharest, Romania

Corresponding author email: dr_georgescu_mara@yahoo.com

Abstract

Introduction: Due to the escalating antimicrobial resistance of numerous frequent pathogens, research on natural antimicrobial compounds is intensively published. One of the most in trend, yet controversial antibacterial natural products, is the Manuka honey. Manuka honey, produced from the Manuka (Leptospermum scoparium or Leptospermum polygafolium) tree, contains a unique antimicrobial factor (Unique Manuka Factor, UMF), which is absent in other types of honey.
Aims: Commercial Manuka honey was investigated for assessment of antimicrobial effect against different Gram-negative and Gram-positive bacteria.
Materials and Methods: Two types of Manuka honey with different UMF and one local polyfloral honey were assessed for antimicrobial activity against Staphylococcus sp., Streptococcus sp., Listeria sp., E coli, Salmonella sp. Pure broth cultures were pour plated on agar and incubated. Each type of honey was spotted in a marked place on the agar, pending the examination of inhibition areas after another incubation period.
Results: Commercial Manuka honey has antimicrobial activity for Staphylococcus sp., E.coli and Salmonella sp., but not for Streptococcus sp. and Listeria sp. No antimicrobial effect was noticed for regular polyfloral honey.
Conclusion: Using the described method, Manuka honey revealed an antimicrobial effect against Staphylococcus sp., E.coli and Salmonella sp., the intensity of which was directly proportional with UMF.

Key words*: antimicrobial effect, Manuka honey, Unique Manuka Factor .*

INTRODUCTION

Manuka honey, produced from the Manuka (Leptospermum scoparium) tree contains a unique antimicrobial factor (Unique Manuka Factor, UMF), which is absent in other types of honey (Molan and Russel, 1988). The escalating microbial resistance of various pathogens is keeping research concerning natural antimicrobials in the spotlight. The antimicrobial effect of pure New Zealand, Manuka honey was revealed *in vitro* against some pathogenic bacteria (Sherlock *et al.*, 2010; Lin *et al.,* 2010). Nevertheless, in public opinion, the antibacterial properties of commercial Manuka honey are highly controversial (Niko, 2009).
Honey has been known for centuries for its beneficial effects over the human organism, being used as a palliative treatment of various diseases and lesions, such as wounds, mycotic (fungal) infections, eczema, skin infections, ulcers etc. The literature indicates that the antibacterial properties of honey are generally mostly due to hydrogen peroxide content, and to a specific high osmolarity (80% w/v sugar) (Alvarez-Suarez J.M. et al., 2010). Some authors indicate that the antibacterial properties of honey are the result of a complex of synergic factors, such as phenolic compounds, hydrogen peroxide, pH and osmolarity (Alvarez-Suarez J.M. et al., 2014).
Manuka honey has been standing out since the 80's, for its particular, higher antibacterial effect, compared to conventional honey. Dr. Peter Molan from University Waikato of New Zealand was the first who proved by inactivating the peroxidase activity in Manuka honey, that it exhibits an intense antibacterial activity, heat and light resistant, called non

peroxide activity (Molan P.C. et al., 1988). The non peroxide activity was later attributed to certain organic chemical compounds, namely the 1,2-dicarbonyl compounds; the list of 1,2-dicarbonyl compounds was later narrowed down to methylglyoxal (MGO), which was awarded with all the credit for the antibacterial superiority of Manuka honey in comparison with conventional honey (Adam C.J. et al., 2008; Mavric E., et al., 2008). The non peroxide activity is undoubtedly related to the methyglyoxal (MGO) concentration. The MGO comes from the decomposing of dihydroxyacetone, a compound which is found in high concentrations in the nectar of *Leptospermum scoparium* flowers). MGO concentration in Manuka honey is measured in ppm, while the non peroxide activity (NPA) is measured as percent phenolic equivalent, and not by MGO concentration. Molan P. (2008) showed that the antibacterial properties of Manuka honey does not depend directly of its MGO content, revealing the following equation which explains the nature of the MGO-UMF relationship: Antibacterial activity = [0,0275 x MGO (mg/kg)] + 7,826. Therefore, there is a certain synergy in MGO's action when found in honey, proven by comparison with MGO in water solutions. The synergy proves that the pronounced antibacterial activity of Manuka honey is not exclusively due to MGO, but is also related to other factors, such as the nectar quantity of honey.

MATERIALS AND METHODS

We investigated the antimicrobial effect of different types of commercial Manuka honey available in para-pharmaceutical stores in Bucharest, against different Gram-negative and Gram-positive bacteria.
Three types of honey were used in this experiment in order to compare results: Manuka honey UMF 10, Manuka honey UMF 15 and local polyfloral honey. Pure bacterial cultures of *Staphylococcus sp*., *Streptococcus sp*., *Listeria sp*., *E coli*, *Salmonella sp*. were grown in nutrient broth. All 18-24 hours old broth cultures were transferred on nutrient agar, by pour plating procedure. After drying the agar surface, a loop full of each type of honey was spotted in a marked place, pending 24 hours incubation at 37°C (European Committee on Antimicrobial Susceptibility Testing, 2012). Controls were prepared with seeded agar without honey and were incubated in the same conditions. After the incubation interval, all Petri dishes were examined for clear areas of bacterial inhibition.

RESULTS AND DISCUSSIONS

All controls grew noticeable, relatively uniform cultures after the incubation end due time. No clear areas were noticed for the polyfloral honey plates. Following the investigation of the antibacterial properties of various types of commercial Manuka honey towards some Gram negative and Gram positive bacteria, there is noticeable antimicrobial activity of the tested samples of Manuka honey against *Staphylococcus sp*., *E.coli* and *Salmonella sp*. No antimicrobial activity was seen for *Streptococcus sp.* and *Listeria sp.* No antimicrobial effect of the conventional honey used as control, was noticed for the above mentioned bacteria species.
Following the comparative assessment of UMF 10 and UMF 15 Manuka honey (fig. 1, 2 and 3), the inhibition area was greater for UMF 15 than UMF 10 Manuka honey (5 and 2 mm for Staphylococcus -fig. 1, 4 and 3 mm for E.coli - fig.2, 6 and 5 mm for Salmonella sp., respectively- fig.3). (according to the method described by the European Committee on Antimicrobial Susceptibility Testing, 2012).

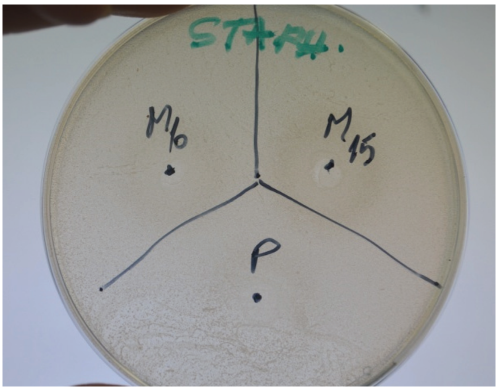

Figure 1. The antimicrobial activity of three types of honey: UMF10 Manuka honey (M_{10}), UMF15 Manuka honey (M_{15}) and polyfloral honey (P), against *Staphylococcus sp.*

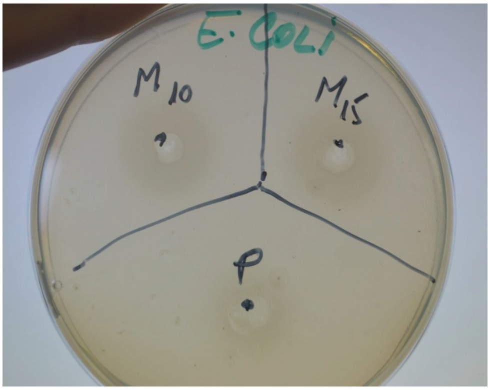

Figure 2. The antimicrobial activity of three types of honey: UMF10 Manuka honey (M_{10}), UMF15 Manuka honey (M_{15}) and polyfloral honey (P), against *Escherichia coli*

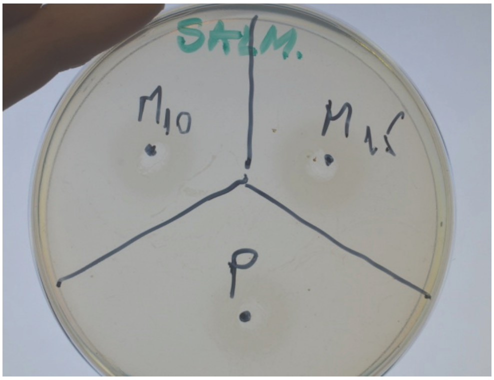

Figure 3. The antimicrobial activity of three types of honey: UMF10 Manuka honey (M_{10}), UMF15 Manuka honey (M_{15}) and polyfloral honey (P), against *Salmonella sp*

Many authors communicated noticeable antimicrobial effect of Manuka honey against various pathogens, but there are insufficient studies comparing different UMF Manuka honeys, amongst each other as well as with other types of honey, in terms of antimicrobial activity. Similar marked differences of antimicrobial activity intensity between Manuka honey and other types of honey were also communicated by other authors, such as Willix (1992), or Sherlock (2010).

Nevertheless, aside this study, there were no comparisons made between samples of Manuka honey with different UMF values and conventional honey. Moreover, most authors used original Manuka honey, tested for its content of antimicrobial compound, while regular commercial Manuka honey, was used in this study.

While the peroxidase activity varies within large limits considering the various honey assortments, and with harvesting time, heat and light exposure, the non peroxide activity is constant and stable in time. This is probably the reason why the non peroxide antibacterial activity of Manuka honey is more intense than the regular peroxide activity in conventional honey.

Nevertheless, there are studies which indicate significant antibacterial activity for certain conventional honey assortments. For example, the minimum inhibitory concentration, MIC (defined as the lowest honey concentration at which there is an inhibition of the visible growth of the organisms on the Petri dish) communicated by Bourabah A. et al. (2014), for conventional Algerian honey, over some bacterial strains of goat mastitis milk, was between 11-14%, which is comparable to Manuka honey MIC values (6-25% v/v) over various bacterial pathogens.

There are insufficient studies indicating a comparative evaluation of the antibacterial properties of Manuka honey and the antibacterial properties of different conventional honey assortments (Tan H.T. et al., 2009; Shahina S. et al., 2013). This is the reason why, the relevance of results concerning this issue may become questionable.

Using conventional honey in research activities that focus on Manuka honey would be extremely valuable, as conventional honey is a control sample group that might help with the relevance of results; in addition, such a control group might be useful in eliminating the suspicions upon the Manuka honey authenticity.

Moreover, such an approach would reduce the consumers' reluctance for Manuka honey (Niko K., 2009), which leads to a higher preference for local, conventional honey, in detriment of exotic Manuka honey, despite the valuable properties of the latter.

Therefore, the cultural aspect of honey consumption would be more easily separated from the medicinal properties and indications of Manuka honey.

CONCLUSIONS

Commercial Manuka honey has antimicrobial activity for *Staphylococcus sp*., *E.coli* and *Salmonella sp*., but not for *Streptococcus sp.* and *Listeria sp.* The intensity of this effect was directly proportional with the UMF. No antimicrobial effect was noticed for regular polyfloral honey.

The differences existing between the synergy of various honey assortments, with different floral sources, remain unexplained, as a current subject of tremendous scientific interest. Therefore, the research aiming the evaluation of the antibacterial properties of Manuka honey should include honey with different UMF values, along with the comparison with conventional honey, as control.

REFERENCES

Adam C.J, Boult C.H, Deadman B.J., Farr J.M., Grainger M.N., Manlez-Harris M., Snow M.J, 2008. Isolation by HPLC and characteriyation of the bioactive fraction of New Zeeland manuka (Leptospermum scoparium) honey. Carbohydr. Res. 343: 651-659.

Alvarez-Suarez, J.M., Tulipani, S., Romandini, S., Bertoli, E., Battino, M., 2010. Contribution of honey in nutrition and human health: A review. Mediterr. J. Nutr. Metab. 2010, 3, 15–23.

Bourabah A., Ayad A., Hammoudi S.M., Boukraa L. and Benbarek H., 2014. Antimicrobial activity of Algerian honey on subclinical mastitis pathogens isolated from goat's milk, Veterinary World 7(4): 248-252.

European Committee on Antimicrobial Susceptibility Testing: Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 1.0. Växjö: EUCAST; 2012.

Lin S.M., Molan P.C., Cursons R.T., 2010. The post-antibiotic effect of manuka honey on gastrointestinal pathogens. Int J Antimicrob Agents 2010, 36(5):467-468.

Mavric E., Wittmann S., Barth G., Henle T., 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka honey from New Zeeland. Mol.Nutr.Food.Res. 52(4): 483-489.

Molan P.C., Russell K.M., 1988. Non-peroxide antibacterial activity in some New Zealand honeys. J Apic Res 1988, 27(1):62-67.

Niko Kloeten, 2009. Consumer confusion allows manuka honey 'rip-offs', article in The National Business Review, www.nbr.co.nz., May 25, 2009.

Shahina S., Krishnan P. (2013). Comparison of the antimicrobial activity of Manuka honey and native honey against methicillin resistant staphylococci from asymptomatic healthcare workers.. International Journal of Phytomedicine, North America, 5, nov. 2013.

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. BMC Complement Altern Med 2010, 10:47.

Tan H.T., Rahman R.A., Gan S.H., Halim A.S., 2009. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complementary and Alternative Medicine: 9:34.

Willix D.J., Molan P.C., Harfoot C.G., 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. J Appl Bacteriol.