## RELATIONSHIP BETWEEN PHYSICOCHEMICAL PARAMETERS AND ANTIMICROBIAL ACTIVITY OF MOLDAVIAN HONEY

## Nicolae Eremia<sup>1</sup>, Olga Coșeleva<sup>1,2</sup>, Natalia Sucman<sup>2,3</sup>, Greta Balan<sup>4</sup>, Lucian Lupașcu<sup>3</sup>, Tatiana Mardari<sup>1</sup>, Susana Modvala<sup>1</sup>, Fliur Macaev<sup>3,4\*</sup>

 <sup>1</sup> Department of Animal Resources and Food Safety, Faculty of Agricultural, Forestry and Environmental Sciences, Technical University of Moldova, Chisinau, Moldova
<sup>2</sup> Department of Technology of Production and Processing of Agricultural Products, Agro-Technological Faculty, Comrat State University, Comrat, Moldova
<sup>3</sup> Laboratory of Organic Synthesis, Institute of Chemistry, Moldova State University, Chisinau, Moldova
<sup>4</sup> Department of Microbiology, Faculty of General Medicine, "Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Moldova

\*Corresponding author: <u>flmacaev@gmail.com</u>

Abstract: Six samples of Moldavian honey from different regions were analyzed, physical and chemical parameters, the content of macro-, microelements, and aminoacids were determined, as well as antibacterial and antifungal activity. The antibacterial and antifungal properties were determined using the double serial dilution method. It was established that all of the samples of acacia, linden, and sunflower honey possess high antibacterial activity. The bioactivity of the samples of honey was proven to be dependent on the type and origin of honey. Sunflower honey has higher antibacterial potency than linden, but linden honey is more active than acacia. Both Gram-positive and Gram-negative bacterial species proved to be susceptible to Moldavian honey. Acacia, linden, and sunflower honey, possess high antibacterial potency against S. aureus and P. aeruginosa even at a dilution of 1:16 (2.5%). The studied samples showed weak antifungal activity against Candida albicans, with the MIC determined at 1:2 dilution (20%). For linden and sunflower honey, the antifungal activity was higher than for acacia honey. The samples with the best bioactivity (sunflower honey) contain a higher amount of free acids, had lower pH values of the honey solution, and these samples also have the highest content of OMF.

**Keywords:** honey; physicochemical parameters; microelements; macroelements; heavy metals; amino acids; bioactivity; antimicrobial; antibacterial; antifungal.

## **INTRODUCTION**

Honey is a popular food ingredient that is used in a variety of ways, such as in cooking, baking, or as a spread on bread and other foods (Babbar et al. 2022). On the one hand, honey has a long history of use in traditional medicine and has been claimed to have various health benefits, including its antibacterial, antioxidant, antifungal activity, and anti-inflammatory properties (Vorlova et al. 2005; Qamar and Rehman 2020).

The antibacterial activity of honey is due to the combination of several factors, including:

1. Hydrogen peroxide: Honey contains an enzyme called glucose oxidase that produces hydrogen peroxide, a known antiseptic. This gives honey its antibacterial properties (Brudzynski 2006).

2. Acidity: Honey has a low pH, typically around 3.2 to 4.5, which creates an acidic environment that can inhibit the growth of many bacteria (Bogdanov 1997).

3. Osmotic effect: Honey is known to have a high osmotic pressure, which can cause water to be drawn out of bacteria, leading to their death (Molan and Cooper 2000).

4. Methylglyoxal (MGO): Methylglyoxal is a compound that is naturally present in honey and has been shown to have antibacterial properties (Wallace et al. 2010; Israili 2014).

5. Antimicrobial peptides: Some types of honey, such as manuka honey, contain antimicrobial peptides that can directly kill bacteria (Roberts et al. 2015).

Antifungal activity is due to several factors, including the low water content and high sugar content of honey, which create an environment that is inhospitable to many types of fungi Moussa et al 2012).

In addition, honey contains a variety of natural compounds that have been shown to have antifungal properties, including flavonoids, phenolic acids, and certain enzymes. These compounds can help to kill or inhibit the growth of fungi by disrupting their cell membranes or metabolic processes.

On the other hand, recently, the problem of microbial resistance has become very acute. Increasingly, there are cases of insensitivity of microorganisms to the drugs used. This leads to a constant search for new bioactive synthetic compounds. That is why many researchers are currently focusing on the therapeutic properties of natural compounds (Ji and Zhang 2009).

The bioactivity of honey depends on several factors, including its composition and origin. The composition of honey can vary depending on the type of flowers the bees use to make the honey, as well as other environmental factors. Honey from different regions can also have different levels of bioactivity due to differences in the plants and flowers available for the bees to collect nectar from. For example, honey from Manuka flowers in New Zealand is known for its strong antibacterial activity (Wallace et al. 2010), while honey from other regions may have weaker or no antibacterial activity. The concentration of specific compounds in honey, such as hydrogen peroxide and methylglyoxal, also affects its bioactivity and their levels can vary depending on the type of honey and the conditions under which it was produced.

As mentioned earlier, honey is a natural product, the antimicrobial activity of which depends on the origin. Beekeeping is widespread in the world and in the Republic of Moldova too. According to the National Bureau of Statistics of the Republic of Moldova, there are more than 200 289 bee families in January 1, 2023 (Livestock as of January 1 by Districts/Regions, Categories of producers, Species of animals and Years (statistica.md)), with average productivity of 35 kg per bee colony. According to the World Bank data in 2021, Moldova exported natural Honey in quantity 3,594 tones. The leading importers were such European countries as Italy, Romania, Serbia, Slovak Republic, and Poland (Worldbank/MDA/2021/tradeflow).

## REFERENCES

- Babbar N, Bansal P, Aggarwal P, Singh K, Kaur S. Utilisation of honey in processed food products. In: Imran M, Haseeb Ahmad M, Shabir Ahmad R, editors. Honey - composition and properties. IntechOpen; 2023. <u>https://doi.org/10.5772/intechopen.107091</u>
- Berry KA, Verhoef MTA, Leonard AC, Cox G. *Staphylococcus aureus* adhesion to the host. Ann NY Acad Sci., 2022 Jun 15;1515:75–96. <u>https://doi.org/10.1111/nyas.14807</u>
- Jibril FI, Mohd Hilmi AB, Aliyu S. Effect of non-hydrogen peroxide on antibacterial activity of malaysian meliponini honey against *Staphylococcus aureus*. Journal of Pharmacy and Bioallied Sciences. 2020, 12(2): p S831-S835. <u>https://doi.org/10.4103/jpbs.JPBS\_280\_19</u>
- Bogdanov S, Martin P, Lullmann C, Borneck R, Flamini C, Morlot M, Lheritier J, Vorwohl G, Russmann H, Persano L, Sabatini AG, Marcazzan G, Marioleas P, Tsigouri A, Kerkvliet J, Ortiz A, Ivanov T. Harmonised methods of the European Honey Commission. 1997;28:1-59.



- Tsavea E, Vardaka F, Savvidaki E, Kellil A, Kanelis D, Bucekova M, Grigorakis S, Godocikova J, Gotsiou P, Dimou M, Loupassaki S, Remoundou I, Tsadila Ch, Dimitriou T, Majtan J, Tananaki Ch, Alissandrakis E, Mossialos D. Physicochemical characterization and biological properties of pine honey produced across Greece. Foods. 2022, 11(7): 943 <u>https://doi.org/10.3390/foods11070943</u>
- Cetik Yildiz S. *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) Carriage and Infections. In: Bustos-Martínez J, José V-AJ, editors. Staphylococcal infections recent advances and perspectives. IntechOpen; 2023. <u>http://dx.doi.org/10.5772/intechopen.107138</u>
- Chalisova NI, Kontsevaya NE, Linkova NS, Pronyaeva VE, Chervyakova NA, Umnov RS, Benberin VV, Khavinson VH. Biological activity of amino acids in organotypic tissue cultures. Bull Exp Biol Med. 2013 Aug;155:581–585. <u>https://doi.org/10.1007/s10517-013-2200-7</u>
- Council Directive 2001/110/EC relating to honey. Official Journal L 10, 12 January 2002, pp. 47-52. <u>https://www.fao.org/faolex/results/details/en/c/LEX-FAOC037441</u>
- Diggle S, Whiteley M. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. Microbiology. 2020 Oct 10;166(1): 30–33. doi:10.1099/mic.0.000860.
- 10. Garaeva SN, Redkozubova GV, Postolaci GV. Amino acids in a living organism. Chisinau, MD: ASM Press; 2009. 552 p. Russian
- Grynyuk I, Vasyliuk O, Prylutska S, Strutynska N, Livitska O, Slobodyanik M. Influence of nanoscale-modified apatite-type calcium phosphates on the biofilm formation by pathogenic microorganisms. Open Chemistry. 2021 Feb 3;19(1): 39-48. <u>https://doi.org/10.1515/chem-2021-0199</u>
- 12. Hermosin I, Chicon RM, Cabezudo DM. Free amino acid composition and botanical origin of honey. Food Chemistry. 2003 Nov;83(2):263–268. https://doi.org/10.1016/S0308-8146(03)00089-X
- 13. Ji HF, Li XJ, Zhang HY. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? EMBO Rep. 2009 Mar;10(3):194-200. https://doi.org/ 10.1038/embor.2009.12.
- 14. Israili ZH. Antimicrobial properties of honey. American Journal of Therapeutics. 2014;21(4):304–23. https://doi.org/10.1097/MJT.0b013e318293b09b.
- 15. Livestock as of January 1 by Districts/Regions, Categories of producers, Species of animals and Years. -<u>https://statbank.statistica.md/PxWeb/pxweb/en/40%20Statistica%20economica/</u> <u>40%20Statistica%20economica\_16%20AGR\_AGR030/AGR030300reg.px/ta</u> <u>ble/tableViewLayout1/?rxid=b2ff27d7-0b96-43c9-934b-42e1a2a9a774</u>
- 16. McCowan C, Bakhshi A, McConnachie A., Malcolm W, Barry S, Hernandez SV, Leanord, A. *E. coli* bacteraemia and antimicrobial resistance following antimicrobial prescribing for urinary tract infection in the community. BMC Infect Dis. 2022 Oct 28;22:805. <u>https://doi.org/10.1186/s12879-022-07768-7</u>

- Mohamadzade Namin S, Ghosh S, Jung C. Honey Quality Control: Review of Methodologies for Determining Entomological Origin. Molecules. 2023 May 22;28(10):4232. https://doi.org/10.3390/molecules28104232.
- Molan PC, Coo per RA. Honey and sugar as a dressing for wounds and ulcers. Trop Doct. 2000 Oct;30(4):249-50. https://doi.org/10.1177/004947550003000429.
- Moussa A, Noureddine D, Saad A, Abdelmelek M, Abdelkader B. Antifungal activity of four honeys of different types from Algeria against pathogenic yeast: *Candida albicans* and *Rhodotorula sp.* Asian Pacific Journal of Tropical Biomedicine. 2012;2(7): 554–557. <u>https://doi.org/10.1016/s2221-1691(12)60096-3</u>
- 20. Owen ER. Geographical, Entomological and Botanical Origins of Honey. In: Imran M, Haseeb Ahmad M, Shabir Ahmad R, editors [Internet]. Honey -Composition and Properties. IntechOpen; 2023. <u>http://dx.doi.org/10.5772/intechopen.106414</u>
- Qamar W, Rehman MU. Brief history and traditional uses of honey. In: Rehman MU, Majid S, editors. Therapeutic applications of honey and its phytochemicals. Singapore: Springer; 2020. p. 1-10. <u>https://doi.org/10.1007/978-981-15-6799-5\_1</u>
- 22. Roberts A, Brown HL, Jenkins R. On the antibacterial effects of manuka honey: mechanistic insights. Research and Reports in Biology. 2015;6:215-224. https://doi.org/10.2147/RRB.S75754
- 23. Shafiei FK, Sabaa J. Using cluster analysis and principal component analysis to group lines and determine important traits in white bean. Procedia Environm Sciences. 2015;29:38-40. <u>https://doi.org/10.1016/j.proenv.2015.07.145</u>
- 24. Struve C, Krogfelt KA. Pathogenic potential of environmental *Klebsiella pneumoniae* isolates. Environmental Microbiology. 2004 Mar 30;6(6): 584-590. <u>https://doi.org/10.1111/j.1462-2920.2004.00590.x</u>
- 25. Tapalskiy DV, Bilskiy IA. Antimicrobial susceptibility testing by broth microdilution method: widely available modification. Klinicheskaja mikrobiologija i antimikrobnaja terapija. 2018;20(1):62-67. Russian.
- Vorlova L, Karpiskova R, Chabiniokova I, Kalabova K, Brazdova Z. The antimicrobial activity of honeys produced in theCzech Republic. Czech J. Anim. Sci. 2005;50(8):376-384. https://doi.org/10.17221/4180-CJAS.
- 27. Wallace A, Eady S, Miles M, Martin H, McLachlan A, Rodier M, Willis J, Scott R, Sutherland J. Demonstrating the safety of manuka honey UMF® 20+ in a human clinical trial with healthy individuals. Br J Nutr. 2010 Apr;103(7):1023–8. https://doi.org/10.1017/S0007114509992777.
- 28. Worldbank/MDA/2021/tradeflow -<u>https://wits.worldbank.org/trade/comtrade/en/country/MDA/year/2021/tradeflow</u> <u>/Exports/partner/ALL/product/040900</u>
- 29. Zhang G-Z, Tian J, Zhang Y-Z, Li S-S, Zheng H-Q, Hu F-L. Investigation of the Maturity Evaluation Indicator of Honey in Natural Ripening Process: The Case of Rape Honey. Foods. 2021 Nov 22;10(11):2882. <u>https://doi.org/10.3390/foods10112882</u>



30. Zhuk A, Sytnikova I, Fylypchuk T, Bahlei O, Shkrobanets O, Danihlik J, Moskalyk H, Panchuk I, Burkut V, Angelstam P, Fedoriak M. Physicochemical quality indicators of honey: An evaluation in a Ukrainian socioecological gradient. Regulatory Mechanisms in Biosystems. 2022 Nov;13(4):354-361. <u>https://doi.org/10.15421/022246</u>