RELATIONSHIP BETWEEN PHYSICOCHEMICAL PARAMETERS AND ANTIMICROBIAL ACTIVITY OF MOLDAVIAN HONEY

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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).

The purpose of this work was to investigate the antimicrobial properties of honey collected in the environmental conditions of the different zones (South Central) of the Republic of Moldova and to establish a relationship with their physicochemical parameters.

MATERIALS AND METHODS

Material

Samples of honey. Six honey samples were used for this study. Honey were obtained from different beehive keepers of different geographical regions of Moldova, namely: Central zone - Calarasi district with coordinates 47.253305, 28.315715 (acacia honey-the sample **1**, linden honey-the sample **6**), Capriana village, Straseni district (47.117525, 28.503301) (linden honey- the sample **5**), Nisporeni district (47.079566, 28.187005) (sunflower honey -the sample **3**); Southern zone – Comrat district (46.299805, 28.658054) (acacia honey - the sample **2**, sunflower honey - the sample **4**). All samples have been stored at room temperature with lids tightly closed.

Reagens and solvents used were purchased from Sigma-Aldrich.

Microbiological analysis. Antibacterial tests were performed on reference strains of gram-positive and gram-negative bacteria *Staphylococus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603. Antifungal activity of honey was evaluated against the test microorganism- *Candida albicans* ATCC 102331. The used strains were taken from the collection of Microbiological Department of the State University of Medicine and Pharmacy „N. Testemitanu" (Chisinau, Moldova).

Microbiological studies were carried out on six samples of honey (**1-6**) with an initial concentration of 80%.

Methods

Determination of physical and chemical indicators

The analysis of the amino acid content was carried out by ion exchange liquid chromatography on an "AAA T 339M" amino acid analyser using Li-citrate buffer solution with pH 2.90±0.01, 2.95±0.01, 3.20±0.02, 3.80±0.02, 5.00±0.02. The samples were hydrolyzed by 6 M HCl according to the method (Garaeva et al. 2009).

Determination of diastase activity is performed using a photometric method in which an insoluble blue-dyed cross-linked type of starch is used as a substrate which was measured photometrically at 620 nm. Results are expressed as Gothe units (or DN, diastase number) per g of honey (Bogdanov et al. 1997).

Moisture content. Water content was determined by the refractometric method by using Abbe refractometer. Strategy is based on the dependence of refractive index on solid content and following conversion the refraction index in moisture content using the data table (Bogdanov et al. 1997).

Apparent reducing sugars (calculated as **invert sugar**) were defined as those sugars that reduce a Soxlet's modification of Fehling's reagent by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator (Bogdanov et al. 1997).

Sucrose fraction. Apparent sucrose was defined as 0.95 of the difference in apperent reducing sugars before and after the prescribrdhydrolysis procedure (Bogdanov et al. 1997).

 ISSN: 0017-2294

Hydroxymethylfurfural content (HMF)was determibed by high-performance liquid chromatography with UV detection (HPLC-UV) (Shimadzu, Kyoto, Japan), with detection down to 1 mg/kg (Bogdanov et al. 1997). The signal was compared with those from standarts of known concentration. Mobile phase used is water : methanol (90 : 10).
Free acid content, expressed as milliequivalents acid per kg honey, was determined by titration with 0.1M NaOH to pH 8.30 (controlled by pH electrodes) (Bogdanov et al. 1997).
The content of micro- and macroelements and the presence of heavy metals in honey was determined by atomic absorption spectrometry following dry ashingin accordance with SM SR EN 14082:2006 (Determination of trace elements. Determination of lead, cadmium, zinc, copper, iron and chromium by atomic absorption spectrometry (AAS) after dry ashing). Atomic absorption spectrophotometer AAC-1H provided monitoring of absorption spectra in the wavelength range of 190–360 nm with a single exposure time of 5 ms and atomization pulses of 1–2 seconds to determine K, Na, Ca, Mg, Fe, Mn, Cu, and Zn. Cr, Ni, Cd, and Pb were defined by using of Atomic absorption spectrophotometer Shimadzu A-7000 with electrothermal atomizer GFA-7000A.
Antimicrobial susceptibility testing. For testing, the double serial dilution method was used. For this, at the initial stage, 1 mL of peptone broth for test bacteria and Sabouraud broth for test candida was introduced into a series of 10 tubes. Subsequently, 1 mL of the analyzed honey sample was dropped into the first test tube. Then, the obtained mixture was pipetted, after which 1 mL of it was transferred to the next tube, so the procedure was repeated until the tube no. 10 of the series. Thus, the concentration of the initial preparation decreased 2-fold in each subsequent tube. At the same time, 24 hours test-microorganisms cultures were prepared. Initially, suspensions of test microorganisms were prepared with optical densities of 2.0 for tested bacteria and 7.0 for fungus according to the McFarland index. Subsequently, 1 mL of the obtained microbial suspension was dropped in a tube containing 9 mL of sterile distilled water. The content of the tube was mixed, after which 1 mL was transferred to the tube no. 2 of the 5-tube series containing 9 mL of sterile distilled water. From the 5-th tube of the series were taken 0.1 mL of the microbial suspension, which represent the seeded dose and added to each tube with titrated preparation. Subsequently, the tubes with titrated preparation and the seeded doses of the microorganisms were kept in the thermostat at 35°C for 24 hours. On the second day, a preliminary analysis of the results was made. The last tube from the series in which no visible growth of microorganisms has been detected is considered to be the minimal inhibitory concentration (MIC) of the preparation. For the estimation of the minimal bactericidal and fungicidal concentrations (MBC, MFC), the contents of the test tubes with MIC and with higher concentrations were seeded on peptone and Sabouraud agar from Petri dishes with the use of the bacteriological loop. The seeded dishes were kept in the thermostat at 35ºC for 24 hours. The concentration of the tested honey sample that does not allow the growth of any colony of microorganisms is considered to be the minimal bactericidal and fungicidal concentrations of it (Tapalskiy DV and Bilskiy IA 2018).
Cluster analysis.
One of the most successful procedures for classifying objects based on similarities / differences is cluster analysis (Shafiei and Sabaa 2015). By applying the agglomerative-iterational method of constructing the distribution dendrogram of the compounds under

study, in which MIC and MBC are used as cases for the studied bacteria it can be observed an obvious separation of the compounds into different clusters.

RESULTS AND DISCUSSIONS

Acacia, linden, and sunflower honey samples collected from different districts in Moldova were studied in this work. Nisporeni, Capriani, and Calarasi are located in the central region, close to the deciduous forests of Codru. Comrat is located in the south of Moldova, in the Budjak steppe, where there is no continuous forest cover and the climate is much more arid and warmer. As a result, the southern region has lower species diversity of honey plants, which are primarily represented by acacia and sunflower. Acacia honey is nearly colorless, light yellow, and has a fluid-viscous mass with a pronounced sweet taste and an unobtrusive aroma. Sunflower honey is a liquid viscous mass of golden yellow color, homogeneous throughout the product. It has a pleasant smell with an unobtrusive aroma, a pleasant, sweet taste, and no foreign aftertaste.

In the central region, in addition to the aforementioned types of honey, linden honey collection is also popular. The color of linden honey ranges from yellow lemon to orange-yellow, and it has a sweet taste with a pronounced linden aroma, sometimes with a bitter tint. Its consistency is a fluid-viscous or crystallized mass, and crystallization can be initial, partial, or complete, with the crystals being small or large.

Prior to evaluating the antimicrobial properties of the honey samples, different physicochemical parameters were measured to investigate if any correlation exists between them. The analysis results showed that the moisture content of the honey samples ranged from 16.3% to 19.9% (Table 1), which complies with the regulations set by the European Council Directive (Council Directive 2001/110/EC relating to honey. Official Journal L 10, 12 January 2002).

Table 1. Physicochemical parameters of honey

Parameter	Permissible Quantity[a]	Acacia honey		Sunflower honey		Linden honey	
		1	**2**	**3**	**4**	**5**	**6**
Moisture content, %, max.	20.0	16.3	18.2	18.0	17.6	19.9	18.2
Invert sugar fraction, %, min.	60.0	77.3	77.25	76.63	76.5	78.0	77.5
Sucrose fraction, %, max.	5.0	2.3	2.5	1.87	2.75	2.5	2.3
Diastase activity, DN, min.	8.0	7.55	9.0	16.59	11.19	12.81	13.82
Hydroxymethylfurfural content (HMF), mg/kg, max.	40.0	2.21	4.22	3.94	3.94	2.69	1.35
Free acid content, mequivalents per 100 g, max.	5.0	0.98	1.38	2.73	2.08	1.78	1.83

[a] - regulations set by the European Council Directive 2001/110/EC relating to honey. Official Journal L 10, 12 January 2002)

ISSN: 0017-2294

No significant differences were recorded in the mass fraction of invert sugar in honey obtained from the Central and Southern zones, and it exceeded the minimum regulatory requirements by 16.5-18.0%. The sucrose content in acacia and linden honey from these zones was 2.3-2.5%, and in sunflower honey it was 1.87-2.75%. The highest values of DN were observed in sunflower honey **3** (16.59) and linden honey **6** (13.82) from the Central districts. As shown in Table 1, the highest content of hydroxymethylfurfural was found in acacia honey **2** and both sunflower honeys **3** and **4**. It's also worth noting that the latter samples had the highest free acid content as well.

It is known that the amount and composition of minerals in honey depend on their concentration in nectar, which is determined by the botanical origin, soil, and climatic conditions (Mohamadzade Namin et al. 2023; Owen 2022). In this study, honey samples were analyzed for the presence of various macro- and microelements, and the results are presented in Table 2. The total amount of macroelements in the honey ranged from 339.0 mg/kg (acacia honey **2** from the Comrat region) to 1459.4 mg/kg (linden honey **5**). Calcium (Ca^{2+}) content was 14.7-19.5 mg/kg in acacia honey and 65.7-82.5 mg/kg in sunflower honey, while linden honey had a calcium content of 42.4 mg/kg (Capriana) and 68.4 mg/kg (Calaras) for samples **5** and **6**, respectively. A similar trend was observed with magnesium (Mg^{2+}) content, which is higher in the Southern region for acacia honey (**2**) and sunflower honey (**4**) compared to the Central zone. The potassium (K^{+}) content in acacia and sunflower honey from the Central region was higher than that in the Southern zone. Linden honey was found to be the richest in potassium, with a content ranging from 592.7-1128.5 mg/kg. Sodium (Na+) content in the honey samples was found to be quite low, varying from 11.3 to 28.9. Additionally, sunflower honey was found to be the richest in phosphates, with a content of up to 232.0 mg/kg.

Table 2. Content of minerals in honey, mg/kg

Minerals	Acacia honey		Sunflower honey		Linden honey	
	1	**2**	**3**	**4**	**5**	**6**
Calcium (Ca^{2+})	14.7	19.5	65.7	82.5	42.4	68.4
Magnesium (Mg^{2+})	6.91	11.6	21.0	28.8	9.10	18.8
Potassium (K^{+})	171.0	120.7	369.9	319.3	592.7	1128.5
Sodium (Na^{+})	11.3	28.9	23.1	16.0	13.6	15.9
Phosphates (P_2O_5)	198.1	158.3	232.0	225.7	86.9	227.8
Manganese (Mn)	<0.5	<0.5	<0.5	<0.5	<0.5	0.5
Zinc (Zn)	<0.5	0.8	1.65	0.85	0.84	1.24
Copper (Cu)	1.5	1.91	1.49	1.24	1.24	1.63
Iron (Fe)	1.49	1.66	2.48	1.98	1.23	3.0
Chrome (Cr)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Nickel (Ni)	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Lead (Pb)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cadmium (Cd)	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06

It has been found that the total amount of microelements in the honey samples did not exceed 10.37 mg/kg. Among the microelements, the highest amount was detected in sunflower honey from the Nisporen region (as shown in Table 2). Additionally, the total amount of heavy metals in the honey samples was established to be between 2.56-3.70 mg/kg (as shown in Table 2). The value of each of these indicators is below the maximum permissible concentration for food.

It is known that proteins and amino acids in honey are derived from both animal and plant sources, with the major source being pollen. The composition of free amino acids varies in different types of honey and can be used to determine its botanical source (Hermosin et al. 2003). Moreover, many proteinogenic and non-proteinogenic amino acids have self-biological functions and can influence the bioactivity of honey (Chalisova et al. 2013). The qualitative profile of amino acids in the uninvestigated honey samples is presented in Table 3. The total amino acid content in all samples is less than 1%, with proline being the major contributor, accounting for 21-33% of the total amino acids. In addition to proline, there are 18 proteinogenic amino acids in honey, and their relative proportions depend on the type and origin of the honey.

Table 3. Content of amino acids in bee honey, mg/g

Aminoacid	Acacia honey		Sunflower honey		Linden honey	
	1	**2**	**3**	**4**	**5**	**6**
Cysteine acid	0.0401	0.0868	0.0594	0.0793	0.1198	0.1324
Taurine	0.2285	0.2631	0.1652	0.1970	0.2644	0.2666
Aspartic acid	0.1867	0.3265	0.1732	0.2888	0.3331	0.2776
Threonine	0.0460	0.0621	0.0651	0.0760	0.0840	0.0576
Serene	0.0644	0.0890	0.0918	0.1045	0.1378	0.0798
Glutamine acid	0.1327	0.2105	0.2430	0.4560	0.3594	0.1614
Proline	0.4176	0.4347	0.6164	0.5395	0.5711	0.7070
Glycine	0.0432	0.0448	0.0479	0.0726	0.0718	0.0523
Alanine	0.0358	0.0609	0.0627	0.1086	0.1078	0.0501
Valine	0.0190	0.0468	0.0371	0.0757	0.0907	0.0348
Cysteine	0.0116	0.0148	0.0087	0.0200	0.0277	0.0127
Methionine	0.0153	0.0246	0.0085	0.0221	0.0376	0.0251
Isoleucine	0.0311	0.0451	0.0323	0.0449	0.0593	0.0352
Leucine	0.0348	0.0536	0.0406	0.0646	0.0722	0.0455
Tyrosine	0.0065	0.0202	0.0069	0.0115	0.0128	0.0083
Phenylalanine	0.0349	0.0620	0.0674	0.0692	0.0689	0.0896
γ-aminobutyric acid	0.0061	0.0094	0.0087	0.0081	0.0129	0.0085
Lysine	0.0492	0.0738	0.0726	0.0774	0.0974	0.0711
Histidine	0.0236	0.0225	0.0271	0.0333	0.0329	0.0279
Arginine	0.0429	0.0753	0.0269	0.0534	0.0845	0.0320
Ammonia	0.0841	0.1027	0.0789	0.0724	0.1221	0.0568
Σ aminoacids	**1.4762**	**2.0264**	**1.8616**	**2.4028**	**2.6460**	**2.1754**

Proline is unique because this amino acid mainly comes from the honeybee during the conversion of nectar into honey. The amount of proline is one of the indicators of honey

ripeness, with proline accounting for more than 0.2 mg/g (Zhang et al. 2021; Zhuk et al. 2022).
In this study, an assessment was made of the antifungal and antibacterial activity of honey produced on the territory of the Republic of Moldova.
Antibacterial tests were performed on four types of conditionally-pathogenic strains of bacteria: *Staphylococus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. The results are presented in Table 4.
Staphylococcus aureus, is a type of bacteria that can cause a range of infections in humans. Some common infections caused by *S. aureus* include skin infections (such as boils and impetigo), food poisoning, and respiratory infections (such as pneumonia) (Berry et al. 2022). In recent years, a strain of *S. aureus* that is resistant to many antibiotics, known as methicillin-resistant *Staphylococcus aureus* (MRSA), has become a significant public health concern (Cetik 2023). MRSA infections are often more difficult to treat than infections caused by other strains of staphylococcus and can lead to more serious health complications.
The results of this study demonstrate that Moldavian honey is an effective inhibitor of *Staphylococcus aureus* even at rather high dilution. At the same time, sunflower honeys **3**, **4** showed higher activity against the pathogen in comparison with acacia and linden honey. Minimal inhibitory and minimal bactericidal activities were observed even at honey concentrations of 2.5 % (Table 4)
Additionally, the honey samples were found to be highly effective at inhibiting the growth of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which are types of bacteria commonly found in the environment, including soil, water, and plants. These bacteria are opportunistic pathogens, meaning they can cause infections in people with weakened immune systems, and are a common cause of nosocomial infections (Struve and Krogfelt 2004; Diggle and Whiteley 2020). Due to their ability to develop resistance to antibiotics, *P. aeruginosa* and *K. pneumoniae*infections are difficult to treat and often require the use of multiple antibiotics. It's worth noting that *P. aeruginosa*is able to form biofilms, making it even more difficult for drugs to penetrate and traditional antibiotic therapy is often ineffective in treating it.
The analysis carried out showed that sunflower honey samples **3**, **4** inhibited *P. aeruginosa* even at significant dilutions. As in the case of *S. aureus*, inhibition of this bacterium was observed at a 1:16 dilution (2.5%). Linden honey samples **5** and **6** exhibited less bioactivity, with bacterial inhibition observed at a sample dilution of 1:8 (5%). Acacia honey samples **1** and **2** exhibited inhibitory properties at concentrations no higher than 10% of honey. Against *K. pneumoniae*, the honey samples showed moderate activity, with sunflower honey being more antibacterial active, as observed in the previous cases.

Table 4. Antimicrobial activity of Moldavian honey

Sample	***S. aureus***		***E. coli***		***K. pneumoniae***		***P. aeruginosa***		***C. albicans***	
	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMF
1	1:4	1:4	1:2	-	1:2	1:2	1:4	1:4	-	-
2	1:8	1:8	1:4	-	1:2	1:2	1:4	1:4	-	-
3	**1:16**	**1:16**	1:8	-	1:8	1:2	**1:16**	**1:16**	1:2	-

ISSN: 0017-2294

4	**1:16**	**1:16**	1:8	1:4	1:8	1:8	**1:16**	**1:16**	1:2	-
5	1:8	1:8	1:4	1:4	1:4	1:4	1:8	1:8	1:2	-
6	1:8	1:8	1:4	1:4	1:2	1:4	1:8	1:8	1:2	1:2

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration;
MFC –minimal fungicidal concentration
“-“ no antimicrobial activity of the sample

Another gram-negative bacterium against which the antibacterial properties of honey was studied is *Escherichia coli*, a type of bacteria commonly found in the human digestive system and the environment. While most strains of *E. coli* are harmless, some can cause serious illnesses. Traveler's diarrhea is the most common type of *E. coli* infection, which is caused by consuming contaminated food or water. Other types of *E. coli* infections can lead to more serious illnesses (McCowan et al. 2022). Table 4 shows that the honey samples used in these experiments exhibited weak or moderate antibacterial activity against *E. coli*.
In this study, was investigated the ability of honey to inhibit fungal infections, using the *Candida albicans* strain as the fungal pathogen. The results showed that the honey samples tested had weak antifungal activity against this fungus. The minimum inhibitory concentration was observed at a 1:2 dilution. The antifungal activity of linden and sunflower honey was higher than in the case of acacia honey.
After analyzing the relationship between the physicochemical parameters of honey and the biological activity of the samples, it can be seen that sunflower honey contains a higher amount of free acids. As a result, it leads to lower pH values of the honey solution, and these samples also have a high content of OMF. Additionally, the relatively high content of phosphates may have a positive effect on antibacterial properties, which is confirmed by some scientific publications (Grynyuk et al. 2021).
The obtained results are presented in the Figures 1-3.

ISSN: 0017-2294

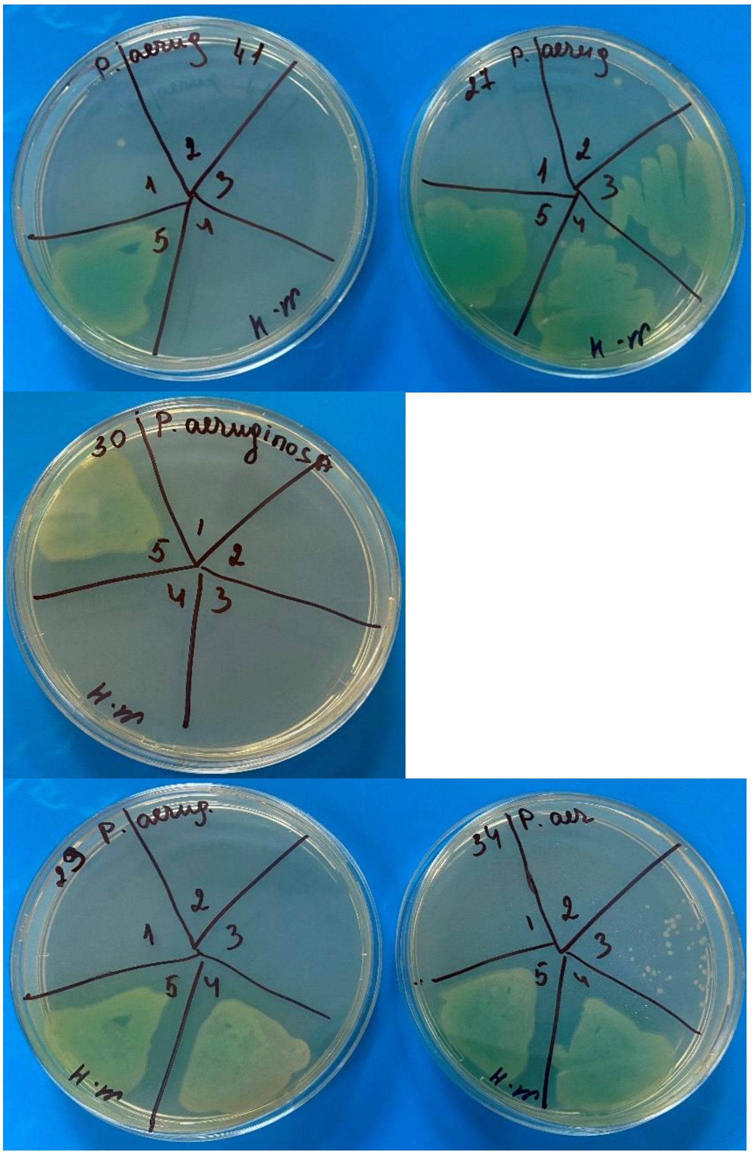


Figure 1. The antibacterial activity of the honey extracts against *Pseudomonas aeruginosa*

1-40% (1:2 dillution); 2-20% (1:4); 3-10% (1:8); 4-5% (1:16); 5-2,5% (1:32)

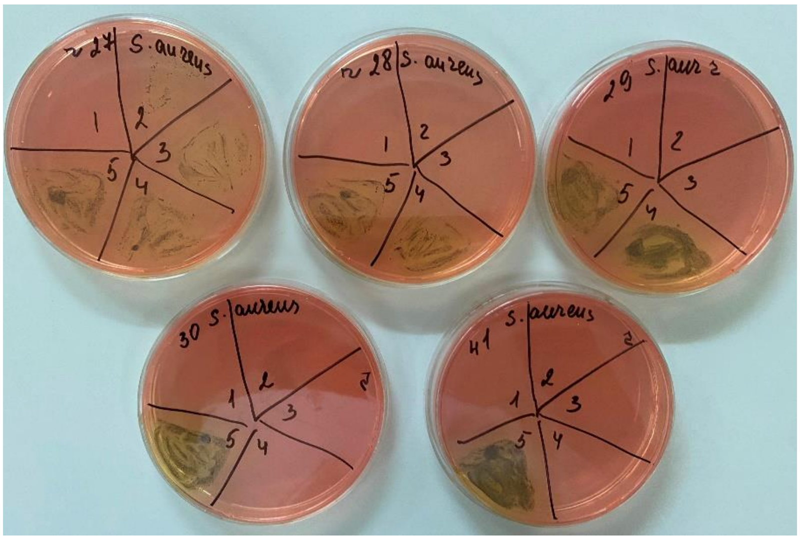


Figure 2. The antibacterial activity of the honey extracts against *Staphylococus aureus*
1-40% (1:2 dillution); 2-20% (1:4); 3-10% (1:8); 4-5% (1:16); 5-2,5% (1:32)

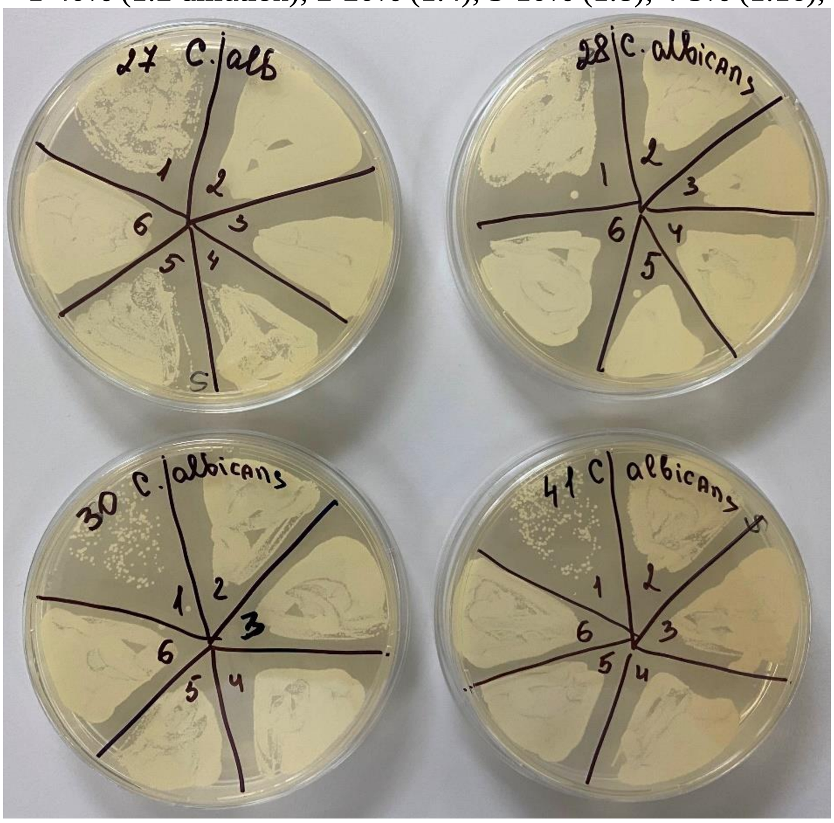
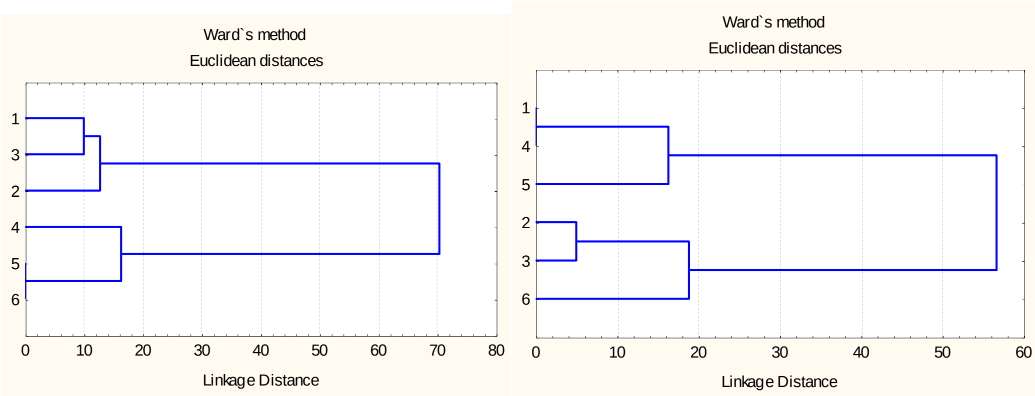


Figure 3. The antifungal activity of the honey extracts against *Candida albicans*
1-40% (1:2 dilution); 2-20% (1:4); 3-10% (1:8); 4-5% (1:16); 5-2,5% (1:32)

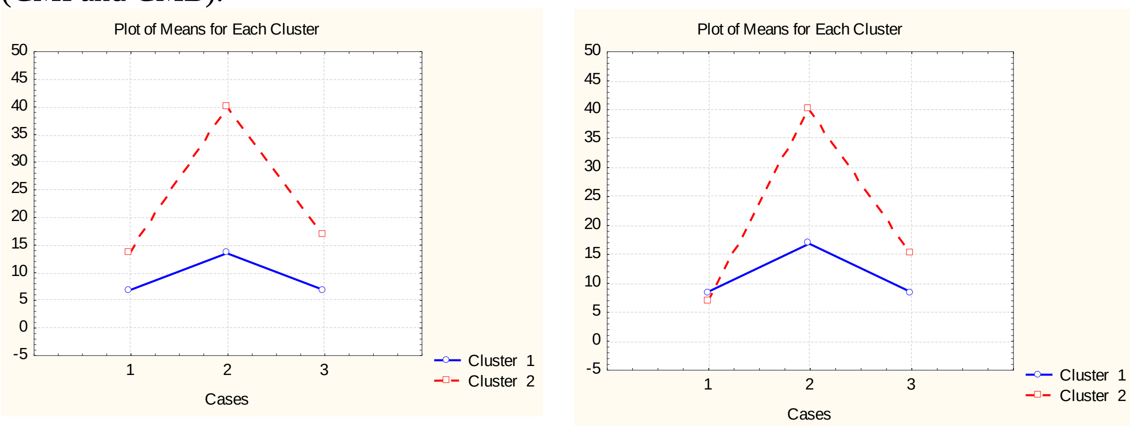
One of the most successful procedures for classifying objects based on similarities / differences is cluster analysis (Shafiei and Sabaa 2015). By applying the agglomerative-iterational method of constructing the distribution dendrogram of the compounds under study, in which MIC and MBC were used as cases for the bacteria *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, an obvious separation of the compounds was registered in 2 clusters, which reveals their pronounced differences based on the mentioned parameters (Figure 4).



MIC MBC

Figure 4. Dendrogram of distribution of honey extracts based on antibacterial activity

Centroid method of classification - k-means (Figure 5) confirmed the data of the method of construction of distribution dendrograms: according to CMI, there is high similarity between compounds **1**, **2**, **3** and **4**, **5**, **6**, and for CMB - high similarity for compounds **1**, **4**, **5** and **2**, **3**, **6**, which were successfully separated into 2 clusters for each parameter (CMI and CMB).



CMI CMB

Figure 5. K-means cluster analysis of the antibacterial activity of some honey extracts

It should be noted that the clusters of compounds in the study showed a pronounced difference in the case of CMI for the bacterium *K. pneumoniae* (2), followed by *P. aeruginosa* (3), then *S. aureus* (1). Regarding CMB for S. aureus, there were no differences between the 2 clusters. The biggest difference was noticed also for the *K. pneumoniae* bacterium, followed by *P. aeruginosa*.

Thus, the compounds from cluster 1 - **1**, **4**, **5** recorded an average of 6.67, 13.33, 6.67% for MIC and an average of 8.33, 16.67, 8.33% for CMB in the case of the bacteria *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, respectively, which denotes the possibility of using of these compounds as preparations with complex antibacterial activity.

CONCLUSION

Six samples of Moldavian honey collected from four districts (three located in the central part, close to the forests, and one located in the south steppe) were analyzed, physical and chemical parameters, the content of macro-, microelements, and aminoacids were determined, as well as antibacterial and antifungal activity. It was demonstrated that the physicochemical parameters of all of the samples fit the regulations set by the European Council Directive 2001/110/EC relating to honey. 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 correlations between MIC and MBC values of the samples of honey were 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 and, as a result, had lower pH values of the honey solution, and these samples also have the highest content of OMF.

ACKNOWLEDGMENTS: The authors are grateful for the funding of this research under the Moldovan State Program (2020–2023), Project Nr. 20.80009.5007.17 “Hybrid materials functionalized with carboxyl groups based on plant metabolites with activity against human and agricultural pathogens”and Project Nr. 20.80009.8007.14 “Complex research for the development of new local anti-infective pharmaceuticals for optimizing the pharmacotherapy of dental, oropharyngeal and auricular diseases”.

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