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STUDIES REGARDING THE ANTIBACTERIAL ACTIVITY, SOME PHYSICO-CHEMICAL AND BIOCHEMICAL PARAMETERS IN A FEW VARIETIES OF HONEY FROM BIHOR COUNTY

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Abstract

According to EU, Honey is the natural sweet substance, produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature. It plays an important part in our nutrition and it is well-known for its positive effects on health.

The aim of the study was to determine the antibacterial activity, physico-chemical parameters and bioactive compounds of some selelcted honey from Bihor County, such as Honeydew honey, Meadow honey, Acacia and Linden honey.

The antibacterial activity of honey was tested against Echerichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Streptococcus pneumoniae (ATCC 49619), Staphylococcus aureus (ATCC 25923). Among the studied honey, honeydew and meadow honey had the highest antibacterial activity.

Key words: honey, antibacterial activity, physico-chemical parameters, bioactive compounds.

INTRODUCTION

Honey is an excellent food with great nutritional, biological and energetycal value, easily assimilated with real bactericidal properties, due to its content in antibiotics, enzymes and vitamins. Honey is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honeycombs" (Codex Alimentarius Commission, 2002). It is a concentrated aqueous solution of invert sugar, that contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex (Ajlouni and Sujirapinyokul, 2010; Manzanares et al., 2011; Rashed and Soltan, 2004).

Honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments. Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broadspectrum antibacterial activity when tested against pathogenic (Mandal M.D, Mandal S., 2011, Lusby et al, 2005, Mundo et al., 2004).

The antimicrobial activity may be different depending on the types of honey, its geographical, seasonal and botanical sources, as well as the conditions of harvesting, processing and storage (Sherlock et al, 2010). The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components (Taormina et al, 2001). Honey contains antioxidants and flavonoid that may function as antibacterial agents (Bosio et al., 2000).

Phenolic compounds contribute significantly to honey color, taste and flavor and have beneficial health effects (Estevinho et al., 2008). The composition of honey, including its phenolic compounds, is variable, depending mainly on the floral source and also other external factors, including seasonal and environmental factors as well as processing (Arreaz-Roman et al., 2006). Honey inhibits the growth of dangerous bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella, Shigella,* and *Vibrio cholera* and is superior to several well-known antibiotics (Zumla and Lulat, 1989, Rahman et al 2010). The aim of this study on the one hand was to evaluate the antibacterial potential of four varieties of honey at a concentration of 75% w/V, against bacterial strains of *Echerichia coli, Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, and on the other hand to determine physico-chemical and biochemical parameters, in a few varieties of honey (acacia, linden, honeydew and meadow), obtained in the year 2020 and 2021 in Bihor County.

MATERIAL AND METHOD

A number of 4 honey samples (acacia-AH, linden LH, honeydew-HD and meadow honey-MH) were analyzed. All samples were obtained directly from beekeepers of Bihor county. Acaccia and linden honey were obtained in 2021, while meadow and honeydew honey in 2020.

Antibacterial activity

The sensitivity of bacterial strains to different types of honey was determined by using the disk diffusion (Kirby-Bauer test).75% aqueous honey solution was used for Kirby Bauer method.

The following bacterial strains provided by Sanimed International Impex were used: *Echerichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923).

Kirby Bauer method

An inoculum of each clinical isolate was prepared from subculture of bacterial suspension. Briefly, it was prepared as follows: 4–5 colonies of the isolates were emulsified in sterile distilled water and the turbidity adjusted to 1.5 × 108 CFU/mL, corresponding to 0.5 McFarland standards (Koneman et al, 1992 cited by Ndip et al 2007). A sterile cotton swab was dipped into the standardized bacterial suspension and used to uniformly inoculate sterile Petri dishes (Ø90mm) with Nutrient Broth (code: 41185). The plates were allowed to dry for 3–5 minutes. Thereafter, 8 pieces of 6mm discs (Blank discs - Oxoid Ltd) were placed on each plate and pressed gently to ensure complete contact with agar. Disks were impregnated with 10µl of all prepared honey samples. A gentamicin disk 10µg (Liofilchem SRL was used as the positive control.

The plates were incubated at 37°C for 2-5 days and examined by measuring the diameter of the inhibition zones. The experiment was repeated 3x for each strain.

Physical-chemical parameters

Physical-chemical parameters: water, pH, acidity, HMF, were analyzed according to the Romanian Standard Analysis Methods (National Standard, 2009) and Harmonized methods of the IHC (Bogdanov, 2009), or with specific methods.

Water and total sugar - of the tested samples were determined with digital refractometer KRUSS model AR 2008.

pH – HACH Sension 378 multiparameter meter was used to measure the pH of a honey solution prepared from 10 g of honey in 75 ml of distilled water.

Total acidity - by volumetric method (Bogdanov, 2009).

HMF content was determined by spectrophotometric method (White, 1979). Each of the honey samples was divided into 2 clarified aliquots; water was added to one of the aliquots and absorption was read at λ=284 and 336 nm. This was compared to a second solution in which this absorption was eliminated by the addition of sodium bisulfate. Results were expressed in milligrams of HMF per kilogram of honey.

Biochemical parameters

Extraction of antioxidant components - Antioxidant components from honey were extracted with water (10% solution).

Total polyphenols (TP) content was determined by using the Folin-Ciocâlteu (1927) colorimetric method developed by Singleton and Rossi (1965). A diluted extract (0.5 ml) or phenolic standard was mixed with 2.5 ml Folin-Ciocâlteau reagent and after 5 minutes 2.0 mL sodium carbonate (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. For the preparation of calibration curve 0.5 ml aliquot of 0.2, 0.4, 0.8 and 1.2 μM/ml aqueous gallic acid solution was used as the standard and expressed as mg of gallic acid equivalent (GAE).

Antioxidant activity – FRAP assay (Benzie and Stain,1996) using the calibration curves for ascorbic acid (5 to 100 mg/L). The result is expressed as the corresponding activity in ascorbic acid equivalent of a 10% honey solution.

RESULTS AND DISSCUSIONS

Antibacterial activity

Diameter of inhibition zones are registered in Table 1 and the graphical representation of the inhibition percentages of the honey samples taken in the study, are shown in Fig. 1. The reference value is the area of inhibition of the positive control gentamicin, considered 100%. Except *Escherichia coli* strain ATCC 25922b, which is resistant to all the studied honey samples, and *Pseudomonas aeruginosa* strain ATCC 27853 which is resistant to acacia and linden honey, all other pathogenic strains tested show different sensitivity to the concentration used, depending on the types of honey used in the experiment. Honeydew honey had the strongest antibacterial effect on the *Staphylococcus aureus* strain ATCC 25923, the diameter of the inhibition zone formed being 12.2 mm followed by Meadow honey forming a diameter of inhibition zone of 11.7 mm, being significantly and distinctly significantly smaller than the inhibition zone diameter measured in the case of gentamicin control, 13mm (Table 1). The areas of inhibition in the case of *Pseudomonas aeruginosa* strain ATCC 27853 were 6.2 mm for meadow honey and 6.8 mm for honeydew honey, the diameter of inhibition zone being very significantly lower than in the case of the control represented by gentamicin=12mm (Table 1).

Table 1.

Estimated mean values for the diameter of the inhibition zone in mm, in the studied honey solutions, compared to the same parameter in the control group (gentamicin)

Sample/CTR Microorganism	MH	HH	AH	LH	Gentamicina
Streptococcus pneumoniae ATCC 49619	7.4±0.3 **	7.8±0.3 **	6.6±0.1 ***	6.4±0.1 ***	8.8±0.2
Pseudomonas aeruginosa ATCC 27853	6.2±0.2 ***	6.8±0.2 ***	0	0	12±0.1
Staphylococcus aureus ATCC 25923	11.7±0.2 **	12.2±0.3 *	6.5±0.1 ***	6.2±0.1 ***	13±0.2
Escherichia coli ATCC 25922	0	0	0	0	6.6±0.1

0=rezistent

p>0.05= non-significant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot.

In the case of *Streptococcus pneumoniae* strains ATCC 49619, honeydew honey and meadow honey generate an inhibition zone with a diameter of 7.8 mm and 7.4 mm, respectively, which represents a distinctly significant decrease in comparison to the diameter of inhibition zone of the control (gentamicin), and acacia and linden honey have a reduced antibacterial effect, generating inhibition zones diameters between 6.6 mm and 6.4 mm respectively - representing very significant decreases compared to the control represented by gentamicin-8.8mm (Table 1).

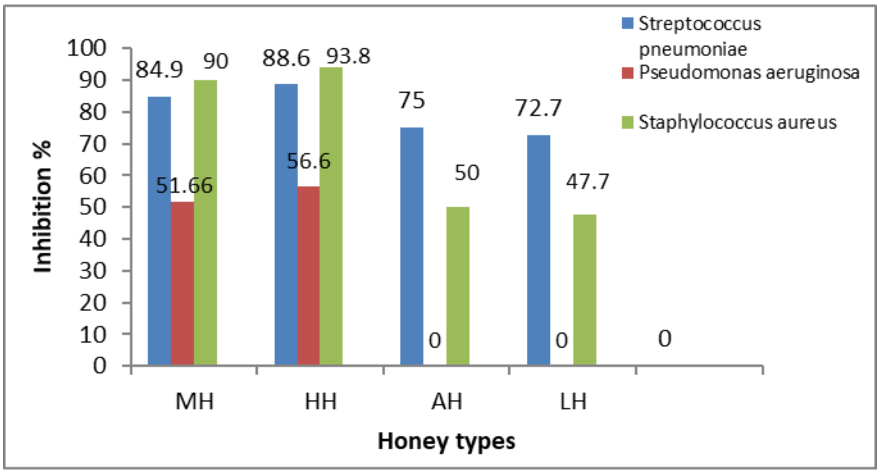

Fig. 1 – Graphical representation of the inhibition percentages of the honey samples taken in the study. The reference value is the area of inhibition of the positive control gentamicin, considered 100%

All the samples present lower antibacterial activity than the gentamicin.

Honeydew and meadow honey present the highest inhibition in case of *Staphylococcus aureus* (93.8 and 90%) and *Streptococcus pneumoniae* (88.6 and 84.9%), followed by linden and acacia honey.

The development of *Pseudomonas aeruginosa* was inhibited only by honeydew and meadow honey and only 56.6 and 51.6%.

Escherichia coli strain ATCC 25922b, was resistant to all the studied honey samples.

The results obtained in this study are consistent with the results published by several authors (Molan 1992; Manisha Deb Mandal and Shyamapada Mandal 2011; Wilkinson and Cavanagh, 2005; Sherlock et al.2010; Vică et al, 2021) regarding the effect of honey on some strains of pathogenic bacteria.

Physical-chemical parameters

The content of water, total sugar, pH, total acidity and HMF (Table 2) respect the limits established for honey in the Romanian and the International Regulatory Standards. Table 2, also contains the values for the analyzed bioactive compounds.

Table 2.

Mean values for physical-chemical parameters determination and the limits in the Romanian Regulatory Standards

Sample	pH	Free Acidity Meq/kg	Total sugar %	Water %	HMF mg/kg	Total polyphenols mg GAE/100g honey	FRAP value for a 10% honey solution (Ascorbic acid $Equiv_4$)
AH	4.01	21.9	81.9	16.2	1.65	23.97±0.787	4.087±0.005
LH	3.97	26.1	80.0	17.6	20.66	60.83±0.337	12.42±0.001
MH	3.14	30.2	81.7	16.6	9.92	79.82±0.449	16.34±0.004
HH	3.08	33.4	80.7	17.8	2.94	159.82±1.348	52.38±0.002
STAS* 784/ 3-89/	-	Max 40	Max 83	Max 20	Max 40		

In general, honey is characteristically acidic with pH between 3.2 and 4.5, which is low enough to be inhibitory to several bacterial pathogens (Haniyeh et al, 2010).

pH values in our study ranged from 3.08 and 4.01 and total sugar between 80 and 81.9%, and as described by Sweda, 2017, high concentration of sugars and low pH are universal antibacterial factors of all honeys.

The total phenolic content of the tested honey samples, were between 23.97 (acacia) and 159.82 GAE/kg (honeydew). Similar results were obtained by Mărghitaş et al., 2009, who determined the total phenolic content in Romanian acacia honey to be in the range from 2.0 to 39.0 mg of GAE/100g of honey and by Bobiş et al, 2008 in Romanian honeydew honey (93,5 – 144,94 mgGAE/100 g honey).

Antioxidant activity has the same trend as polyphenolic compounds, growing from acacia to meadow and honeydew honey.

A very strong correlation was observed between the polyphenol content, the antioxidant activity of studied honey, and their antimicrobial activity against *Streptococcus pneumoniae* (ATCC 49619), *Staphylococcus aureus* (ATCC 25923) for all the studied honey samples, $|r| > 0,75$.

The same strong correlation was registered *Pseudomonas aeruginosa* ATCC 27853 between the polyphenol content, the antioxidant activity of honeydew and meadow honey, and their antimicrobial activity. Our are in accordance with those obtained by Alzahrani et al., 2012, Sousa et al. 2016, etc.

CONCLUSIONS

Among the studied honey, honeydew and meadow honey had the highest antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49619), *Pseudomonas aeruginosa* (ATCC 27853). *Escherichia coli* strain ATCC 25922b, which is resistant to all the studied honey samples.

This potency is attributed to its physicochemical and some biochemical characteristics. Hight phenolic compounds, high antioxidant capacity and low pH, play a major role in the antimicrobial activity of honey.

In the future we intend to test the antibacterial activity of other types of honey, as well as against other strains of bacteria.

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