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Original Article

PHYSICO-CHEMICAL CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF DIFFERENT TYPES OF HONEY TESTED ON STRAINS ISOLATED FROM HOSPITALISED PATIENTS

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Abstract

The first aim of the study was to compare the antibacterial activity of several types of honey of different origins, against some bacterial resistant strains. The strains had been isolated from patients. The second aim was to discover the correlations between the antibacterial character of honey and the physico-chemical properties of the honey. Ten honey samples (polyfloral, linden, acacia, manna, and sunflower) from the centre of Romania were tested to determine their antibacterial properties against the following bacterial species: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enterica* serovar *Typhimurium*, *Bacillus cereus*, *Bacillus subtilis*, and *Listeria monocytogenes*. Bacterial cultures in nutrient broth and the culture medium Mueller-Hinton agar were used. The susceptibility to antibiotics was performed using the disk diffusion method. All honey samples showed antibacterial activity on the isolated bacterial strains, in particular polyfloral (inhibition zone 13-21 mm in diameter) - because it is the source of several plants, and manna (inhibition zone 13-19.5 mm in diameter), and sunflower (inhibition zone 14-18.5 mm in diameter). Pure honey has a significant antibacterial activity against some bacteria which are resistant to antibiotics. Bacterial strains differed in their sensitivity to honeys. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most sensitive. The present study revealed that honey antibacterial activity depends on the origin of the honey. We also found that there was a significant correlation between antibacterial activity of honeys and the colour of the honey but not between acidity and pH. The statistical analysis showed that the honey type influences the antibacterial activity (diameter of the bacterial strains inhibition zones).

Keywords: antibacterial activity, bacteria strains isolated from hospitalised patients, honey, physico-chemical properties, statistical analysis

INTRODUCTION

Honey is a food, sweetener, and medicine for humans. It is produced from different floral sources. The antibacterial activity of honey varies according to its proprieties. Honeys, especially manna honey, have a high antibacterial activity associated with an unidentified phytochemical component (Nzeako & Hamdi, 2000). Throughout the world, research is being carried out over the antibacterial resistance phenomenon. Fundamental research is being done to find out better information about the natural and the acquired resistance mechanisms. Research is also being done on: the development of the epidemiology surveillance networks, the establishment of the antibiotic susceptibility of the isolated bacterial strains, and the conception of new drugs with an increased antibacterial effect, for finding an alternative therapy to an-

tibiotics and for using honey in the treatment of infected wounds, ulcers, and skin grafts (Postmes et al., 1993; Kingsley, 2001; Molan, 2002).

One of the most difficult problems in hospitals is the appearance of an increased number of antibiotic resistant bacteria, such as the Methicillin-Resistant *S. aureus* (MRSA), the Vancomycin-Resistant Enterococci (VRE), *P. aeruginosa*, or other Gram negative bacilli such as *E. Coli* or *S. enterica* (Rosenthal et al., 2008). *Pseudomonas aeruginosa (Rossolini & Mantengoli*, 2005) is one of the leading causes of the nosocomial infections. Severe infections are often difficult to treat, due to the increased antibiotic resistance. Even if some β-lactams, fluoroquinolones, aminoglycosides, and polymyxins can still be used in therapy, *P. aeruginosa* exhibits the remarkable ability to acquire resistance to these agents.

Laboratory studies (Postmes et al., 1993; Nzeako & Hamdi, 2000; Kingsley, 2001; Molan, 2002; Jenkins et al., 2011; Packer et al., 2012) have shown that pure honey has a significant antibacterial activity against some antibiotic resistant bacteria isolated from wounds, ulcers, and skin graft.

Some publications point out the antimicrobial properties of honey but honey's mechanism of action still has not been completely studied (Assadi-Pooya et al., 2003). It is known that strong solutions of sugars inhibit bacterial growth due to their high osmolality. Compared to a solution of artificial honey, natural honey has a higher inhibitory effect against clinically significant Gram-positive cocci isolated from wounds (MRSA and some enterococci). So bacterial inhibition does not rely on osmolality (Cooper et al., 2002). Additional researchers (Cooper et al., 2002) have characterised honey's antibacterial activity, and say that this activity is due to its physical and chemical properties (high content of inverted sugar, high viscosity, high osmotic pressure, low pH, acidity, low water activity, low content of protein), and/or the presence of hydrogen peroxide or of some other chemicals. The nature of these chemical factors and their mechanism of action are not entirely known. It has been shown that some honey types have antibacterial activity and produce bacterial inhibition due to the hydrogen peroxide. Yet, other types of honey had the same antibacterial activity due to some non-peroxide components.

Staphylococcus aureus has been used in various experiments regarding the antibacterial properties of honey. It has been experimentally proven that honey produces large inhibition zones against *Staphylococcus aureus*. Honey produces much larger inhibition zones against *S. aureus*, than the ones given by the sulphacetamidic antibiotics (Tajik & Jalali, 2009).

The antibacterial activity of honeys of different botanical and geographical origins has been the subject of many studies (Hern et al., 2009; Gomes et al., 2010; Brudzynski et al., 2012; Fahim et al., 2014).

A study by Tumin et al. (2005) done on many types of honey, showed that almost all of the honeys inhibited bacterial growth of *E. coli*, *S. aureus*, *S. Sonnei*, and *S. typhi*, and that *S. sonnei* was the most sensitive bacteria to the honey types. Their study also demonstrated that pH or acidity has an important contribution in the honey antibacterial activity. The variable results noticed among the honey types can be estimated when considering factors such as: the different floral sources used by the bees and/or such geographic factors as humidity or temperature, of the areas where the honey was produced.

On the other hand, Bogdanov in a study performed on several types of honey, revealed a significant correlation between the honey antibacterial activity and its acidity, but no correlation between the honey antibacterial activity and its pH (Bogdanov, 1997). It was also proven that small differences between the activities of various types of monofloral honey exist, regarding the inhibition of the bacterial growth. Thus a part of the honey antibacterial activity might depend on the origin of the plants (Bogdanov, 1997).

A major part of the antibacterial activity has been postulated to be of bee origin. However, in two one-floral New Zealand honeys, the main antibacterial substances were shown to have

a flower origin (Russel et al., 1998). According to White and Subers, there are two sorts of antibacterial agents. One of them is heat-and light-sensitive and has its origins in the H_2O_2 produced by the honey glucose oxidase (White & Subers, 1964).

The aims of our study were to test the antibacterial effect of honey, to compare the antibacterial activities of several types of honey, and to specify the most efficient types of honey, for finding an alternative therapy against the circulating resistant bacterial strains to antibiotics.

MATERIAL AND METHODS

A study concerning the honey's capacity to inhibit bacteria-development was performed to see if honey may be used as an alternative therapy for infections rather than using antibiotics to treat infections. Honey samples of different origins (polyflora, linden, acacia, manna, and sunflower) were tested to study the antibacterial properties of the honeys against antibiotic resistant bacterial strains.

Criteria used to choose the specific honey types are in concordance with other studies. These studies revealed marked variations in the antibacterial activity of different types of honey (Maryann, 2000; Tumin et al., 2005).

Honey samples

Table 1 lists the honey samples of known origin (already determinated by physical, chemical, and pollen analysis as well as by using organoleptic methods) that were used. The honey samples were from different areas of Transylvania, Romania: Alba County and surrounding counties (Hunedoara, Cluj, Sibiu) taken in 2011. The samples came from different collecting-packing centres. Each sample was collected in a sterile container and kept in a dark place at 2 - 8°C until tested.

Artificial honey (100g) was prepared by dissolving 40.5 g fructose, 33.5 g glucose, 7.5 g maltose, and 1.5 g sucrose in 17 ml sterile deionised water. This solution represents the proportions of the four predominant sugars in the natural honey samples.

Physico-chemical analysis

Five physic-chemical parameters (SR 784/3, 2009) were analysed: the water content, the acidity, the pH, the water activity, and the colour of the honey.

The moisture content was determined through refractive index at 20°C using an ABBE refractometer. The water content of the samples was set with the help of standard tables according to the refractive indices (SR 784/3, 2009; SR 2213/5, 2009).

Acidity: 10 g of honey were diluted with 50 ml of purified water and titrated with sodium hydroxide solution 0.1 mol/dm^3 in the presence of 2 – 3 drops of *phenolphthalein* (1% alcoholic solution) until a pink colour persisted for 30 seconds (SR 784/3, 2009).

The pH value: an aqueous solution of 10% honey was prepared. The pH was measured with a PH-meter WTW pH 340i (Popescu & Meica, 1997).

The water activity (a_w) was determined with the Aquaspector apparatus AQS-2-TC (AOAC 978.18, 1995).

The colour of honey was based on Pfund scale values obtained using the Hanna Digital Colour Grader (Popescu & Meica, 1997).

Antibacterial assays

Micro-organisms and culture conditions

Before starting the tests, a low-level heat treatment (49.4°C for 15 minutes) was used to reduce potential bacterial contaminations to the honey samples. The microbial purity of the samples was checked.

Eight bacterial species were used for testing the honey antibacterial properties. Some of the eight bacterial species were acknowledged as human pathogens used in other laboratory studies (Kingsley, 2001; Cooper et al., 2002; Tumin et al., 2005). Criteria used for choosing the bacterial species for examination, are in concordance with other studies (Kingsley, 2001; Cooper et al., 2002; Rossolini & Mantengoli, 2005). Each type of honey had been first tested on select bacteria (*B. subtilis*, *L. monocytogenes*, and *S. epidermidis*) to see if they had antibacterial proprieties. Then each type of honey

was tested for each species, on forty clinically isolated strains of *P. aeruginosa, S. aureus*, *E. coli, B. cereus,* and *S. typhimurium*. The results were expressed as the average value obtained.
The strains of *P. aeruginosa* had been isolated from hospitalised patients with urinary tract infections. The *Staphylococcus aureus* strains had been isolated from patients with pyogenic skin infections. Because the research involved human material, it was performed with the approval of the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, with Approval no. 540A/13.02.2012.
The species identification had been achieved by standard procedures, using the API test and by the Vitek 2 system (Biomérieux).

Agar disk diffusion method – determination of the antibiotic resistance of the isolated bacteria

The isolated strains' susceptibility to antibiotics was performed under laboratory conditions. The disk diffusion method (Kirby-Bauer) was used according to the CLSI guidelines (CLSI, 2006) and the Vitek 2 System was used on a panel of antibiotics frequently used in the treatment of the aforementioned infections.

Determination of the antibacterial properties of the honey samples

Bacterial cultures in nutrient broth and Mueller-Hinton agar as culture medium, were used according to the laboratory standard methods (Poiată, 2002). The depth of the agar in these experiments was 4 mm (25 ml in 9-cm-in-diameter Petri dishes). Overnight, broth cultures of each bacterial strain were prepared in nutrient broth. The turbidity was visually compared with the McFarland 0.5 standard, to ensure the uniformity of bacterial inoculum on each plate. McFarland standards are widely used as turbidity standards in the preparation of the suspensions of microorganisms. The McFarland standards are particularly used in the preparation of bacterial inocula for performing antimicrobial susceptibility testing. According to the manufacturer, the McFarland 0.5 standard corresponds approximately to a homogeneous suspension of $1.5x10^8$ colony-forming units/ml. A suspension of the tested microorganism (0.1 ml) was spread on the solid media plates.
The sealed dishes with honey had been submitted to fluidisation in a water bath at 40-45°C until the complete melting of the crystals. Using a sterile device, wells with a diameter of 6.0 mm were excised. The wells were then filled with each of the honey samples from the undiluted honey (the amounts of inoculate being equal, 150 µl). As a negative control, sterile deionised water was used. Artificial honey can also be considered as a negative control. Standard antibiotic discs that had an effect on these bacteria were used as the positive control in the test. The plates were incubated for 18h±2h at 37°C±1°C with their lids up. After the incubation, the diameter on the inhibition zones produced around the wells with the honey samples, were measured. The criterion used to characterise the susceptibility or the resistance of each species to the honey samples was the inhibition zone diameter expressed in mm. The inhibition zone diameter means a quantitative measure of the antibacterial activity of the analysed material. All experiments were performed in triplicate and the zone of inhibition was measured twice for each honey dilution.
When the inhibition zone diameter is greater, it means that the strain is considered more susceptible to the honey activity. If there is no inhibition zone around the wells, the tested strain is considered resistant to the honey sample. If all the forty strains tested are resistant to the action of the honey sample, the bacterial species is considered resistant to that sample.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration zone (MIC) of the different types of honey on the bacterial strains, including clinically isolated strains, was determined throughout the dilutions method. Honey dilutions of 1/1, 1/4, 1/16, 1/32, and 1/64 were used. The minimum inhibitory concentra-

Table 1

The physico-chemical parameters of honey

Sample no.	Honey origin	Electrical conductivity (mS/cm), mean ±SD	HMF (mg/kg), mean ±SD	Glucose (w/w%), mean ±SD	Fructose (w/w%), mean ±SD	Specific pollen grains, with reference to the total number of pollen grains examined (%), mean ±SD	$a_{w,}$ mean ±SD	Colour, Pfund scale (mm)	Colour	Moisture content (%), mean ±SD	pH, mean ±SD	Acidity (meq/kg), mean ±SD
1	Poly-floral honey - Alba County	0.59±0.02	12.2±0.8	27.3±1.0	41.2±0.8	-	0.568±0.041	52	Light amber	15.6±0.8	3.72±04	41.0±6.2
2	Linden honey - Alba County	0.44±0.01	1.0±0.4	36.4±1.8	40.1±3.2	30.0±1.4	0.542±0.038	40	Extra-light amber	17.0±1.3	4.86±01	11.0±4.8
3	Acacia honey - Alba County	0.15±0.02	8.4±0.7	29.1±1.1	36.7±1.9	29.2±1.1	0.608±0.013	8	Extra white	18.0±1.0	3.75±03	12.0±3.0
4	Manna honey - Alba County	1.02±0.03	4.3±0.5	31.0±0.5	38.5±1.3	-	0.579±0.062	94	Amber	16.2±1.5	3.66±01	28.9±7.1
5	Sunflower honey - Alba County	0.50±0.02	22.3±0.9	35.8±1.4	37.7±1.5	40.1±2.2	0.572±0.034	39	Extra-light amber	16.4±1.2	3.67±01	22.6±6.9
6	Poly-floral honey - Cluj County	0.65±0.00	31.8±3.7	29.1±1.5	37.5±1.4	-	0.590±0.025	56	Light amber	16.4±1.3	3.60±04	37.3±5.6
7	Poly-floral honey - Sibiu County	0.70±0.00	4.5± 0.5	28.7±1.8	43.0±1.3	-	0.553±0.030	59	Light amber	15.6±0.6	3.90±05	55.6±10.9
8	Poly-floral honey - Hunedoara County	0.82±0.01	8.5±1.0	29.9±2.1	44.1±1.2	-	0.595±0.047	48	Extra-light amber	16.6±0.8	4.04±01	30.4±9.2
9	Linden honey - Cluj County	0.64±0.01	24.8±3.2	38.2±0.7	42.3±1.5	29.6±1.7	0.528±0.029	36	Extra-light amber	16.4±1.0	4.36±04	20.0±6.7
10	Acacia honey – Sibiu County	0.143±0.03	64.3±7.8	30.2±1.4	39.1±2.3	28.5±0.9	0.557±0.046	10	Extra white	16.4±0.6	3.90±03	14.3±7.5

tion values were determined for all the ten different types of honey. In this method, graded doses (w/v) of different honeys dissolved in sterile deionised water, were used. Overnight, broth cultures of each bacterial strain were prepared in nutrient broth. The turbidity was visually compared with the McFarland 0.5 standard. Then, the above procedure was carried out.

Statistical analysis

All determinations were performed in triplicate and the data were expressed as means ± standard deviations (SD). Pearson's correlation analysis was performed to establish dependence between parameters. A statistical test regarding the one-way analysis of variance (ANOVA) was also performed to test the influence of the diameter of inhibition zones for different bacterial strains (Box et al., 2005).

RESULTS

The composition of honey samples

Table 1 present the results of the analysis for samples of honey collected from different beekeepers from the centre of Romania. The water activity of the honey varied between 0.5 – 0.6. The physico-chemical parameters were within the standards of the regulations, with a slight increase regarding the value of acidity for sample 7.

Our analytical results (moisture content, acidity, pH value, colour) of the honey samples corresponded with profiles describing the data ranges of different unifloral honeys.

The antibiotics' resistance of the isolated bacteria

The most clinically isolated strains are resistant to one or more classes of antibiotics. In particular, the isolated strains of *S. aureus* and

Table 2

The antibiotics resistance in isolated bacteria

Strain	Antibiotic susceptibility
S. aureus isolated from patients with pyogenic skin infections	Methicillin-Sensitive *S. aureus* (MSSA) - 74% MRSA - 8% Macrolide-Lincosamide-Stretogramin B (MLSB) resistant - 12% MRSA + MLSB resistant - 6%
S. aureus isolated from other different types of infections	MRSA - 57.45% (all sensitive to glycopeptides) Erythromycin resistant - 56% MLSB resistant - 38% gentamicin resistant - 72.92% trimethoprim/sulfamethoxazole resistant - 13.97% ciprofloxacin resistant - 9.61%
P. aeruginosa isolated from patients with urinary tract infections	high resistance to some β lactam drugs resistant to Ticarcillin/Clavulanic Acid and to Piperacillin/Tazobactam - 87.5% Cefepime resistant - 91.7% Ceftazidime resistant - 91.7% Carbapenems (Imipenem and Meropenem) resistant - 79.1% Amikacin resistant - 80% Fluoroquinolones resistant: Ciprofloxacin - 79.2% susceptible to Colistin - 62.5% resistant to all antibiotics - 8.33%

Table 3

Diameters of the inhibition zones on the bacterial strains

Strain \ Sample no.	Diameters of inhibition zones (mm)										Artificial honey
	1	2	3	4	5	6	7	8	9	10	
S. epidermidis	18	18	0	18.5	18	17	19	16.5	15.5	17	0
B. subtilis	17.5	17	13	19.5	18.5	15	16	15	16	15	0
L. monocytogenes	16	0	0	16.5	16	14	15	14.5	0	0	0
E. coli (average value – 40 clinically isolated strains)	13	13	0	13	14	13	14	13	0	0	0
S. aureus (average value – 40 clinically isolated strains)	21	16	13	16	18	14	15	16	14	15	0
S. Typhimurium (average value – 40 clinically isolated strains)	14.5	14	0	15	14	13	14	13	13	13	0
B. cereus (average value – 40 clinically isolated strains)	15	0	0	14	14.5	13.5	13.5	0	0	0	0
P. aeruginosa (average value – 40 clinically isolated strains)	17	16	14	18	17	17	16	16	13	15	0

P. aeruginosa presented multiple antibiotic resistances (Tab. 2).

The antibacterial activity of honey samples

The study provided evidence that all the honey samples presented antibacterial activity against the studied strains and that all the honey samples inhibited bacterial growth (Tab. 3). The most sensitive bacteria to the antibacterial activity of the honey samples were *P. aeruginosa, S. aureus*, *S. epidermidis*, and *B. subtilis*. The inhibition zone diameters varied depending on the type of honey and the tested strain, and the diameters reached up to 21 mm.

The *E. coli* strains were shown to be sensitive to seven samples. The diameters of the inhibition zones were 13-14 mm.

The diameters of the inhibition zones against the *S. typhimurium* strains were between 13 and 15 mm. The sensitivity of the *S. typhimurium* strains to the action of honey was higher when compared to the *E. coli* strains.

The *Bacillus cereus* strains were sensitive to 5 of the 10 samples, the diameters of inhibition zones being of about 13.5-14 mm. *Bacillus subtilis* was more sensitive to the honey activity and the inhibition zones were larger; from 15 mm to 19.5 mm, comparable to those obtained in the case of the *Staphylococcus* strains (Tab. 3).

Listeria monocytogenes presented a high sensitivity to the samples of poly-floral, manna, and sunflower honey. The diameters of the inhibition zones were 14 to 16.5 mm.

Pseudomonas aeruginosa recovered from hospitalised patients, was more sensitive to the honey activity. The inhibition zones were larger; from 16 to 17 mm.

The two species of *Staphylococcus* presented a high sensibility to honey, especially *S. epidermidis*. For both species, the diameters of the inhibition areas were large; most over 16 mm. Sample no. 1 presented a higher antibacterial activity against *S. aureus* than all the other samples.

The artificial honey did not inhibit any of the strains.

Table 4

The minimum inhibitory concentration (MIC) of the different types of honey

Sample no.	Honey type	MIC (w/v)
1	Poly-floral	3.12%
2	Linden	25.0%
3	Acacia	25.0%
4	Manna	3.12%
5	Sunflower	3.12%
6	Poly-floral	12.5%
7	Poly-floral	3.12%
8	Poly-floral	6.25%
9	Linden	25.0%
10	Acacia	25.0%

The determination of the minimum inhibitory concentration (MIC)

We determined MIC values for all ten different types of honey using 1/1, 1/4, 1/16, 1/32, and 1/64 dilutions. The honey inhibitory effect in 1/1 and 1/4 dilutions was observed in all samples except for *S. typhimurium* (in samples 9 and 10 in the case of the 1/4 dilution). Lower dilutions (1/16, 1/32) produced partial effects in certain bacterial strains only. The results of the MIC values are presented in Table 4.

Statistical analysis

The Pearson correlative analysis shows a significant correlation between the honey antibacterial activity and its colour ($r=0.79$, $r^2=0.62$ and $p=0.007$). Manna honey was the darkest, followed by the polyfloral, sunflower, linden, and acacia honey (Fig. 1). Also, we can conclude that there is a poor correlation between the number of sensitive strains and honey acidity ($r=0.66$, $r^2=0.43$ and $p=0.039$) and no statistically significant correlation with pH-values ($r=0.03$, $r^2=-0.16$ and $p=0.65$). The result is significant at p-value < 0.05.

The aim of the one-way analysis of variance (ANOVA) was to test the 8 types of isolated strains (independent variable). For this purpose, 10 honey samples were used for each strain. It followed, that the independent variable influenced the diameter of the inhibition zones. If zero means resistant, from Tab. 3 it can be noticed that the average values of the rows are due to the used strain. The computation of variances caused by the independent variable gave the following results: $S_1 = 15\,880$, $S_2 = 13\,879.3$, $S_3 = 12\,903.2$.

For the dispersion analysis and testing the

Table 5

The variance due to the effect of different bacterial strains

Dispersion	Sum of squares	Degree of freedom	Variances	F computed	$F_{0.05}$
Between the diameters of inhibition zones due to strains	$S_2-S_3=976.1$	$m-1=7$	$s_1^2=139.44$	5.02	2.14
Between honey samples	$S_1-S_2=2\,000.7$	$m(n-1)=72$	$s_2^2=27.79$	-	-
TOTAL	2 976.8	79	-	-	-

S_1 – the sum of the square of all the individuals
S_2– the sum of the square of the sums of columns divided by the number of the single observations in the column
S_3– the square of the total sum divided by the total number of individuals
s_1^2, s_2^2 - variances
F - Fisher–Snedecor distribution (F-distribution)

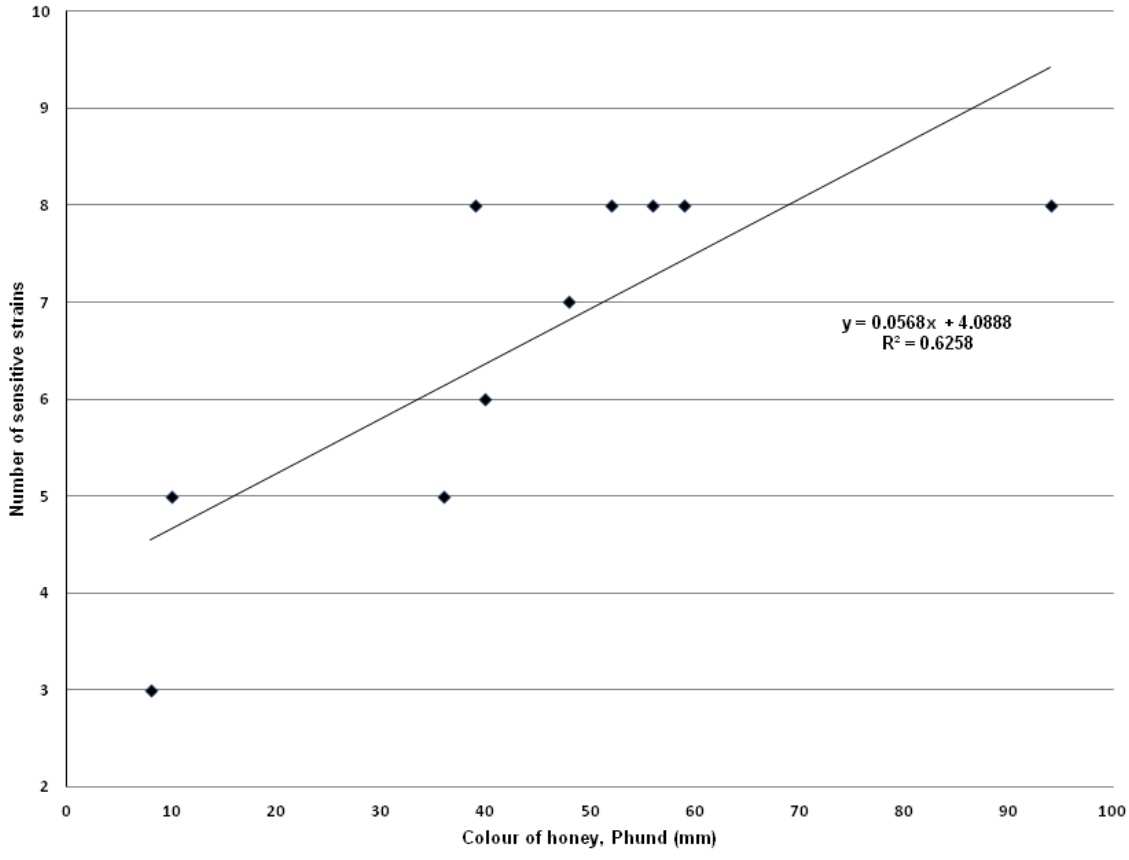

Fig.1 Correlations between the colour of honey samples and their antibacterial activity

equality between the observed value averages, the results are presented in Tab. 5.

Because $F_{computed = 5.02} > F_{0.05} = 2.14$ for $\nu_1 = 7$ and $\nu_2 = 72$ degrees of freedom, the hypothesis that the mean values of the rows are equal was rejected and it was concluded that the type of strain used, influences the inhibition diameter at a chosen threshold significance $\alpha = 0.05$.

DISCUSSION

The water activity of honey varies between 0.5 – 0.6 and is not a proper environment for the majority of bacteria.

The maximum value of moisture content was lower than 20 % (Codex Stan., 12/1981) and the pH was between 3.5 and 4.5 for the studied honey samples. This high degree of acidity prevents the growth of many bacterial forms. The parameter values (pH for sample 2 and acidity in the case of sample 7) were overtaking. This happens only when processing or storage are inadequate, as well as in the case of the fermentation of the honey (Hamid & Saeed, 1991; Sheikh et al., 1995; Popescu & Meica, 1997).

The antibacterial effect of honey was observed in more than forty *Staphylococcus* strains isolated from patients with skin infections (Cooper et al., 2002). In their research, from the total of the isolated strains with different other types of infections, 57.45% were MRSA. MRSA is a strain of *S. aureus* that is resistant to β-lactam antibiotics: methicillin and other more common antibiotics such as penicillin, amoxicillin, and oxacillin. MRSA is also referred as oxacillin-resistant *S. aureus*. The methicillin resistant strains of *S. aureus* were identified shortly after the introduction of methicillin in the clinical practice. In hospitals, the MRSA infections are a particular problem (Cooper et al., 1999; Martínez-Aguilar et al., 2004; Thapaliya et al., 2015; Mamishi et al., 2015). In 1997, approximately 25% of the nosocomial isolates of *S. aureus* were methicillin-resistant (Chini et al., 2006;

Baker et al., 2015). Previous studies showed that in hospitals, the MRSA infection had risen up to 33% (Kloos & Bannerman, 1999). There has been a steady increase in the prevalence of the methicillin-resistant strains of *S. aureus* isolated from hospitals over the years (Chini et al., 2006). The MRSA strains are also becoming a problem in the paediatric settings, including the hospital nurseries. In the UK, the number of deaths attributed to the MRSA strains has been estimated to be in the area of 3,000 per year (Martínez-Aguilar et al., 2004). In 1980, the first Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was reported in the United States (Herold et al., 1998; Martínez-Aguilar et al., 2004). The percentage of the MRSA isolated strains in the study was 57.45%. Other authors (Chini et al., 2006), from different European countries reported similar results.

The resistance of the *P. aeruginosa* strains to third generation cephalosporins, and to third generation Aminoglycosides, and to Quinolones, is very important due to the frequent use of these drugs in hospitals. Such a frequency shows that there is a need to find and introduce alternative therapies based on natural products. Livermore (1995) demonstrated that *P. aeruginosa* has an inducible β-lactamase and is resistant to those β-lactam drugs that induce this enzyme and are hydrolysed by it (e.g., Cephalothin and Ampicillin).

From the results presented in Table 3 , the *P. aeruginosa* and the *S. aureus* strains isolated from patients with hospital acquired infections, are most sensitive to the antibacterial activity of the honey samples.

Regarding the number of strains susceptible to honey (Fig. 2) it was observed that manna

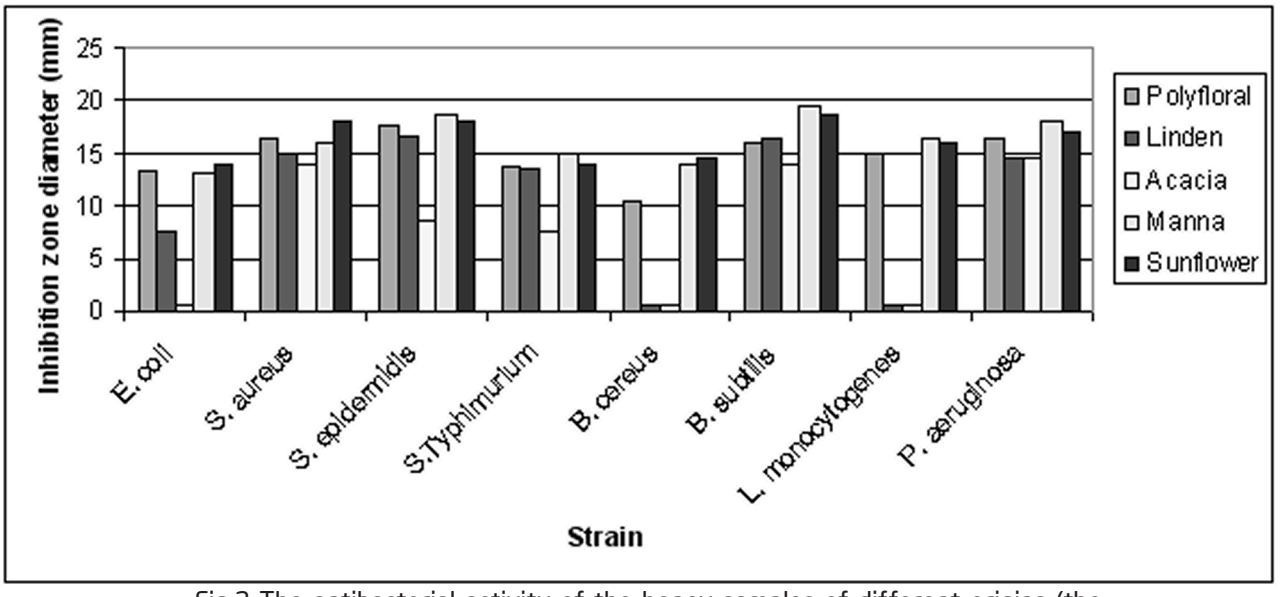

Fig.2 The antibacterial activity of the honey samples of different origins (the diameter on the inhibition zones - mm)

samples and sunflower samples presented a high activity, since they acted against all the bacterial strains. Poly-floral honey samples also had a high antibacterial action and acted against a lot of bacterial strains. None of the strains were inhibited by the artificial honey.

Several authors agree that the sugar content of honey is exclusively responsible for its antibacterial effect (Tovey, 1991; Condon, 1993). But the values obtained in this study demonstrate that natural honeys were significantly more effective in inhibiting bacteria than an artificial honey solution.

A correlation between the origin of honey (the floral source of nectar) and its antibacterial activity was observed in our study. This finding is in agreement with a study that showed correlations between the origin of honey and its antibacterial activity (Russel et al., 1998; Anthimidou et al., 2013). Also, our study emphasised the correlation between honey antibacterial activity and honey colour. In fact, some other studies performed observations on various

honey types and several bacteria, demonstrating that the bacterial inhibition zones depend on the honey type and concentration. Darker honey presented a bacterial-inhibiting activity higher than lighter honey, due to the presence of particular chemical compounds (Taormina et al., 2001; Alvarez-Suarez et al., 2010).
Our results provided evidence that all the honey samples present antibacterial activity against the studied bacterial strains, except artificial honey. The *P. aeruginosa* and the *S. aureus* strains isolated from patients with hospital acquired infections, and the *B. subtilis* strain, were sensitive to the antibacterial activity of all honey samples.
The study showed that between the colour and the antimicrobial character of honey there are appreciable correlations: the darker honey had higher antibacterial activity than the lighter honey. The statistical analysis showed that the honey type influences the diameter of the inhibition zones of the bacterial strains.

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