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Short communication

Antibacterial Activity of Polyfloral and Honeydew Organic Honey

Galina Satchanska*, Galia Ivanova, Anna Doycheva
BioLab, New Bulgarian University, Sofia, Bulgaria

Abstract

Organic honey is produced by non-genetically modified bees, does not contain colorants, sweeteners, odorants, heavy metals, pesticides or other organic pollutants. The aim of the investigation was to study the antibacterial properties of polyflower and honeydew organic honey. Four organic honey samples originated from different regions in Bulgaria: two samples of Sofia vicinity (Lozen and Negushevo), one from the fields near the town of Kubrat, North-Eastern Bulgaria, and the fourth one from Belogradchik, North-Western Bulgaria. The honey samples did not contain any food additives and were free of pollutants. Antibacterial activity was examined on nutrient agar with paper discs swollen with concentrated honey. The test microorganisms were Gram-negative *Escherichia coli* NBMCC No8751 and Gram-positive *Bacillus subtilis* NBMCC No8752. Our results demonstrated that both polyfloral and honeydew samples possess antibacterial activity towards the examined test bacteria. The strongest inhibition inhibitory effect was exerted by the Kubrat polyfloral honey sample (M3), followed by the polyfloral sample of Belogradchik (M4)and the honeydew honey sample of Negushevo (M2). Almost no antibacterial activity was shown by the polyfloral honey sample delivered from Lozen Mountain (M1). Out of all four, three organic honey samples exhibited antibacterial properties. The polyfloral honey samples were more effective than the honeydew sample.
Keywords: food microbiology, organic honey, polyfloral, honeydew

Резюме

Органичният пчелен мед се произвежда от пчели, които не са генно-модифицирани и не съдържа оцветители, подсладители, аромати, тежки метали, пестициди или други органични замърсители. Целта на настоящото изследване беше да се изучи антибактериалната активност на многоцветен и манов органичен мед. Бяха изследвани четири проби, които бяха събрани от различни области на България – две проби от района на София - Лозен и Негушево, една – от полята край град Кубрат, Североизточна България и една проба от района на Белоградчик, Северозападна България. Пробите не съдържаха добавки и замърсители. Антибактериалната активност беше изследвана върху месопептонен агар с посети тестови щамове бактерии, върху който бяха поставени хартиени дискове, напоени с пробите от пчелен мед. Като тестови микроорганизми бяха използвани Грам-отрицателният *Escherichia coli* NBMCC No8751 и Грам-положителният *Bacillus subtilis* NBMCC No8752. Нашите резултати показаха, че и многоцветният и мановият мед проявяват антибактериална активност спрямо посочените бактерии. Най-силен антибактериален ефект упражняваше пробата от многоцветен мед от Кубрат (M3), следвана от пробата от Белоградчик (M4) и на трето място - от пробата от манов мед от района на с. Негушево, София (M2). Съгласно получените резултати, единствено пробата от многоцветен мед от района на с. Лозен край София не показа антибактериална активност. В заключение, от изследваните четири проби три показаха антибактериален ефект, като пробите от многоцветен мед имаха по-висока активност в сравнение с пробата от манов мед,а актвността на пробите беше по-висока към Грам-положителните бактерии, отколкото към Грам-отрицателните.

*Corresponding author: E-mail: gsatchanska@nbu.bg

Introduction

For centuries honey has attracted the attention of peoples and scientists, and has been intensively studied for its content and health benefits (Mavric *et al.*, 2008; Jaganathan and Mandal, 2009; Pandey and Rizvi, 2009; Khalil and Sulaiman, 2010; Petrova *et al*., 2010; Trusheva *et al*., 2010; Matzen *et al*., 2018). The oldest document about its use is a painting in a Valencian cave, Spain, dated 8 000 years ago. It has been accepted both as food and medicine by all generations and civilizations. In 2016, the worldwide production was approximately 1.8 million tons, the largest honey producers being China, Turkey and the USA. Three honey types are known worldwide: blended, polyfloral and monofloral. Most of the commercially available honey is blended i.e. a mixture of a few different types of honey of different source, color and geographic origin. Polyfloral honey is produced from the nectar collected by honeybees from several different flowers while monofloral honey derives from the nectar of only one flower. A special variety of honey is the honeydew – produced when the honeybee collects not nectar but the honeydew excreted by some insects (aphids) while they feed on the plant sap. Trees of the *Pinaceae* family are the most common site visited by those insects, but also broad-leaved trees (Somova *et al*., 2012).The honeydew liquid contains high sugar concentration and, besides insects, it can be produced also by some fungi such as ergot.

Clinical studies on the antimicrobial properties of honey cover a long list of pathogens as bacteria, viruses, fungi and mycobacteria. The antimicrobial effect is believed to be due to the honey acidity, osmotic effect, high sugar concentration and abundance of bacteriostatic and bactericidal substances as hydrogen peroxide, antioxidants, lysosime, polyphenols, phenolic acids, flavonoids, methylglyoxal and bee peptides (Al-Waili *et al.,* 2011, Israili, 2014, Matzen *et al*., 2018). Despite antibacterial effect, the honey possess proven antiviral activity against influenza virus and herpes virus (Serkedjieva, 2006). Although the antibacterial activity of honey has been extensively studied, data on the antibacterial effect of organic honey are scarce.

The aim of our study was to investigate the antibacterial activity of polyfloral and honeydew organic honey samples, collected from different geographical regions in Bulgaria.

Materials and Methods

Sampling

The organic honey samples were collected as follows: M1 – polyfloral honey from Lozen Mountain, near the town of Sofia, Central-West Bulgaria; M2 is the single sample of honeydew type which was collected in the fields of the village Negushevo at the foot of the Balkan Mountains, near the town of Sofia; M3 – polyfloral honey from the meadows of the village of Zavet, near the town of Koubrat, North-East Bulgaria; M4 - polyfloral honey from the area of Belogradchik, North-West Bulgaria (Fig. 1). The honey samples did not contain any food additives and were free of pollutants. In Fig.1 ,the darker color of the honeydew sample (M2) is clearly visible.

Test bacteria

Antibacterial activity was examined on nutrient agar with paper discs swollen with concentrated honey. Disc diameter according to the manufacturer (National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria) was 0.6 cm. The test microorganisms applied were Gram (-) *Escherichia coli* NBMCC No8751 and Gram (+) *Bacillus subtilis* NBMCC No8752 (Bulgarian National Collection for Microorganisms and Cell Cultures, Sofia, Bulgaria).

Assessment of antibacterial activity

The antimicrobial activity of the honey samples was evaluated using the standard agar diffusion method. Bacteria were inoculated on plates at a concentration of $1x10^9$ CFU/ml and cultivated for 48 ^{o}h at 37^{o}C.

Results and Discussion

Organic honey is produced by non-genetically modified bees, does not contain colorants, sweeteners, odorants, heavy metals, pesticides or other organic pollutants. Our investigation was conduct-

Fig. 1. Studied honey samples

ed with four honey samples of organic origin, both polyfloral and honeydew honey, originating from different geographical regions across Bulgaria.

Table 1 presents the antibacterial activity of the honey samples towards *E. coli* and *B. subtilis*. As shown in the table, both polyflower and honeydew honey samples display higher antibacterial effect against Gram (+) bacteria - *B. subtilis* and a lower one - to the Gram (-) *E. coli.*

Of the four samples studied, the polyflower honey M3 collected in North-East Bulgaria was the most active and showed consistent antibacterial effect against both *B. subtilis* and *E. coli,* forming 1.4 and 1.0 cm inhibition zones, respectively. The second most active sample was the M4 organic honey sample from Belogradchik, which was powerful against both Gram (+) and Gram (-) bacteria, but to a lower extent compared to the M3 sample. The M4 sample inhibition zones were smaller than those of M3 polyfloral honey – 1.1 against *B. subtilis* and 0.9 – towards *E. coli.* Similarly to the M3 sample, it preferentially affected the Gram (+) bacteria. M2 (honeydew sample) activity towards *B. subtilis* was equal to that of M3 (1.1 cm zone). Only the M1 polyfloral sample from Lozen Mountain showed almost no antibacterial activity, as seen from Table 1.

Some reports (Matzen *et al*., 2018), comparing the antibacterial effect of commercial and organic honey, described that all organic honey samples showed antibacterial effect in contrast to the commercial ones. Among the test bacteria in these studies were *E. coli* and *P. aeruginosa* as well as *S. aureus* and *S. epidermidis*. Najdenski (2006) reported as well an activity of honey against *S. aureus*. Several research groups as those of Mavric *et al.* (2008) and Israili (2014), reported the substance methylglyoxal (Fig. 2) as the dominant antibacterial constituent in honey along with hydrogen peroxide, lysosime, polyphenols, phenolic acids, flavonoids, and some bee peptides.

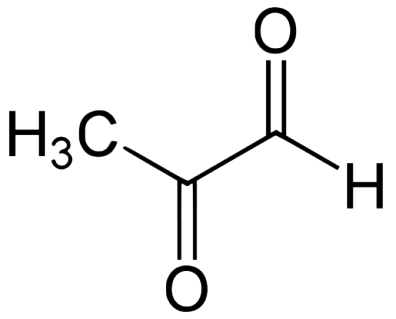

Fig. 2. Structural formula of methylglyoxal

In recent years, another honey substance - 5-Hydroxymethylfurfural (HMF) was reported by Shapla et al. (2018) as a new suitable indicator of honey quality (Fig. 3).

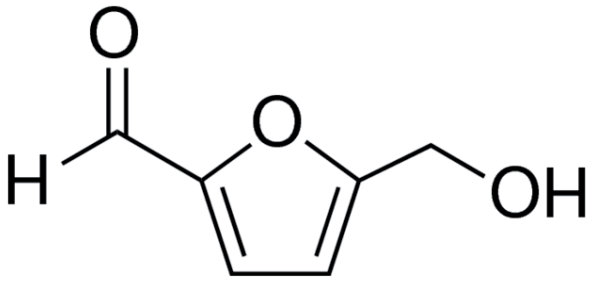

Fig. 3. Structural formula of 5-Hydroxymethylfurfural

HMF is beneficial to human health by providing anti-oxidative, anti-allergic, anti-inflammatory, anti-hypoxic, and anti-hyperuricemic effect. It is produced by reducing sugars in honey under acidic conditions. HMF is converted to 5-sulfoxymethylfurfural and then can exert a genotoxic effect and can become non-excretable. Further investigations on HMF in the organic honey samples analyzed in this study will be performed.

Table 1. Antibacterial activity of organic honey samples

	Organic honey sample	Inhibition of *B. subtilis* NBMCC No8752 (d of zones in cm)	Inhibition of *E. coli* NBMCC No8751 (d of zones in cm)
1.	Polyflower honey M1	0.6	0.7
2.	Honeydew honey M2	1.1	0.7
3.	Polyflower honey M3	1.4	1.0
4.	Polyflower honey M4	1.1	0.9

Conclusions

Three out of four organic honey samples have shown antibacterial properties against both Gram-positive and Gram-negative bacteria. The polyfloral honey samples were more effective than the honeydew ones. Investigated samples were more powerful against Gram (+) bacteria compared to the Gram (-) ones.

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