

## IN VITRO SCREENING OF ANTIBACTERIAL ACTIVITY OF HONEY SAMPLES COLLECTED FROM KOSOVO

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### ABSTRACT

Since the ancient times, it is well known that honey has a therapeutic effects on human health. However, the effectiveness of antibiotics is diminished as resistant pathogens develop and spread. So in this case, we need alternative antimicrobial agents and it is important to use medicinal important materials such as plants, plant based products including honey to struggle this situation. There are too many studies conducted on antimicrobial activity and researchers have been reported both bacteriostatic and bactericidal effects of honey. The aim of this research is screening the antimicrobial effects of six different honey samples from Kosovo against some gram-positive (*Enterobacter faecalis* ATCC 29212, *Staphylococcus aureus*, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25923, *Bacillus cereus*) and gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 51812) bacteria by using the agar well diffusion method on Mueller Hinton Agar. According to our results all the honey samples have effectively high antibacterial activity against especially *Staphylococcus* strains and *Salmonella typhimurium* ATCC 51812 when comparing the reference antibiotics used in the study.

**Keywords:** Antibacterial activity, antimicrobial activity, honey, agar well diffusion method, Kosovo

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### 1. INTRODUCTION

Honey is a very important and special product which is produced from flower nectars (floral honey), combined with an enzyme secreted by honey-bees. Bees gather these sugary substances, enrich them with their own substances and store them in the honeycombs. Honey is a valuable nutritive food used for its antibacterial activity and widely in the food industry, which provides energy to the organism due its high percentage of carbohydrates, which are easily assimilated (Akçiçek and Yücel, 2015; Cenet et.al., 2017). The use of honey has become popular again today due to its strong antibacterial activity against to resistant bacteria in vitro and the usage of as an antibacterial agent in chronic wound infections that unresponsive to antibiotic treatment.

Also, due to phenolic compounds and other valuable compounds in honey, it has many medicinal properties such as antioxidant, anti-inflammatory, antimutagenic, antitumor

and antimicrobial activity (Suarez et al., 2013). In addition, recent studies reported that honey can exert anti-proliferative effects against cancer cells (Suarez et al., 2014). The antibacterial activity of honey have been related to well known antibacterial factors in honey like the high sugar concentration, osmolarity, hydrogen peroxide, the low pH-acidity and more recently methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey (Kwakman et al., 2010; Kwakman et al., 2011; Kwakman and Zaat, 2012). However, another kind of honey, called non-peroxide honey (manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (Deb Mandal and Mandal, 2011). The floral source of honey plays an important role on its biological properties (Molan, 2002). The rich multiflora of honey increases not only its nutritional quality as well as antimicrobial potential on various clinically important microorganisms (Cenet et al., 2017). According to Eteraf-Oskouei and Najafi (2013) researchers have been reported both bacteriostatic and bactericidal effects of honey and they are especially effective on pathogenic strains like *Klebsiella pneumonia*, *S. aureus*, *Salmonella typhimurium* etc. Honey has been used as a topical antibacterial agent for the treatment of surface infections such as ulcers and bed sores and those resulting from burns, injuries and surgical wounds.

Honey is composed of about 181 components and is basically a solution supersaturated in sugars, of which fructose (38%) and glucose (31%) are the most important (Nagai et al., 2006). In addition, there is a great variety of minor components, including phenolic acids, flavonoids, the enzymes glucose oxidase and catalase, ascorbic acid, carotenoids, organic acids, amino acids, proteins and alfa-tocopherol (Carina et al., 2014). However, the composition of honey varies depending on many factors such as the floral source, climate, environmental conditions and the processing it undergoes as pasteurization or storage (Gheldof et al., 2002).

The aim of the research is to determine the in-vitro antimicrobial effects of six different honey samples from Kosovo against some gram-positive and gram-negative bacteria by using agar well diffusion method and then to analyse the zones of inhibition around the wells as qualitative and finally to compare the results of inhibition zones with standard antibiotics as used in the research.

## 2. MATERIALS AND METHODS

### 2.1. Preparation honey samples for antibacterial activity

6 different honey samples from Kosovo were obtained from the bee-keepers on January-February 2016, transferred to Palynology Laboratory in Çanakkale Onsekiz Mart University, Biology Department, in glass jars and stored at room temperature in the dark. They were described as mountain honey, floral honey, meadow flowers honey and pinus honey by the bee-keepers. The localities are given in **Table 1**. A map of Kosovo indicating the location of the honey samples is shown in **Figure 1**.

**Table 1.** Localities of honey samples and honey types from Kosovo

Sample Number	Country	District	Honey types determined by bee keepers
B1	Kosovo	Srecka-Prizren	Mountain
B2	Kosovo	Giakove	Floral
B3	Kosovo	Prizren	Meadow flowers
B4	Kosovo	Rahovec	Pinus
B5	Kosovo	Mitrovica	Floral
B6	Kosovo	Prishtine	Floral

## 2.2. Test microorganisms

Some gram-positive (*Enterobacter faecalis* ATCC 29212, *Staphylococcus aureus*, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25923, *Bacillus cereus*) and gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 51812) bacteria were used to evaluate the antibacterial activity of honey samples. Bacterial strains obtained from first researcher's personal culture collection from Basic and Industrial Microbiology Research Laboratory in Çanakkale Onsekiz Mart University, Biology Department.

## 2.3. Screening of antibacterial activity

Agar well diffusion method was used to screen antibacterial activity of honey samples (Moussa et al., 2012; Balouri et al., 2016; Cenet et al., 2017). They were dissolved in distilled water (1:1) and then kept in +4°C until the experiment. All the bacterial strains were incubated for over-night at 37°C after their inoculation into Tryptic Soy Broth (TSB-Merck). Bacterial inoculum was set up to 0.5 Mac Farland ( $10^6$  bacterial cells/mL) before transferred to petri dishes containing Mueller Hinton Agar (MHA-Merck) and 100 µL inoculum was spread on MHA. Wells on MHA were made after the bacterial inoculation on MHA and honey samples were filled into wells approx. 50-60 µL. Inhibition zones formed around the wells on agar plates were measured by inhibition zone ruler (Bioanalyse) in mm to determine the activity and they were analysed as qualitatively. While Mueller Hinton Agar was used as medium, penicillin (P10), streptomycin (S10) and ampicillin (AM10) were used as standard reference antibiotics for positive control. Studies were performed in triplicate.



**Figure 1.** Research area (Kosovo) and stations of honey samples that were obtained (<https://www.ezilon.com/maps/europe/kosovo-physical-maps.html>)

### 3. RESULTS AND DISCUSSION

In this study totally six different types of honey samples collected from different localities from Kosovo were evaluated for their antibacterial activity against some gram-positive (*Enterobacter faecalis* ATCC 29212, *Staphylococcus aureus*, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *S. aureus* ATCC 25923, *Bacillus cereus*) and gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 51812) bacteria. As a result, antibacterial activity was determined in different values against different microorganisms in all the honey samples and the inhibition zones were varied from 10 mm to 40 mm in diameters. But none of the honey samples were effective against two bacteria *Enterobacter faecalis* and *E. coli* ATCC 25922. However, the honey samples used in our study showed

more antibacterial activity than the antibiotics used in some species, even higher than the strains of *S. aureus* and *S. tyhimurium*. So, it can be said *Enterobacter faecalis* and *E. coli* ATCC 25922 are more resistant to honey samples than other microorganisms used in the study.

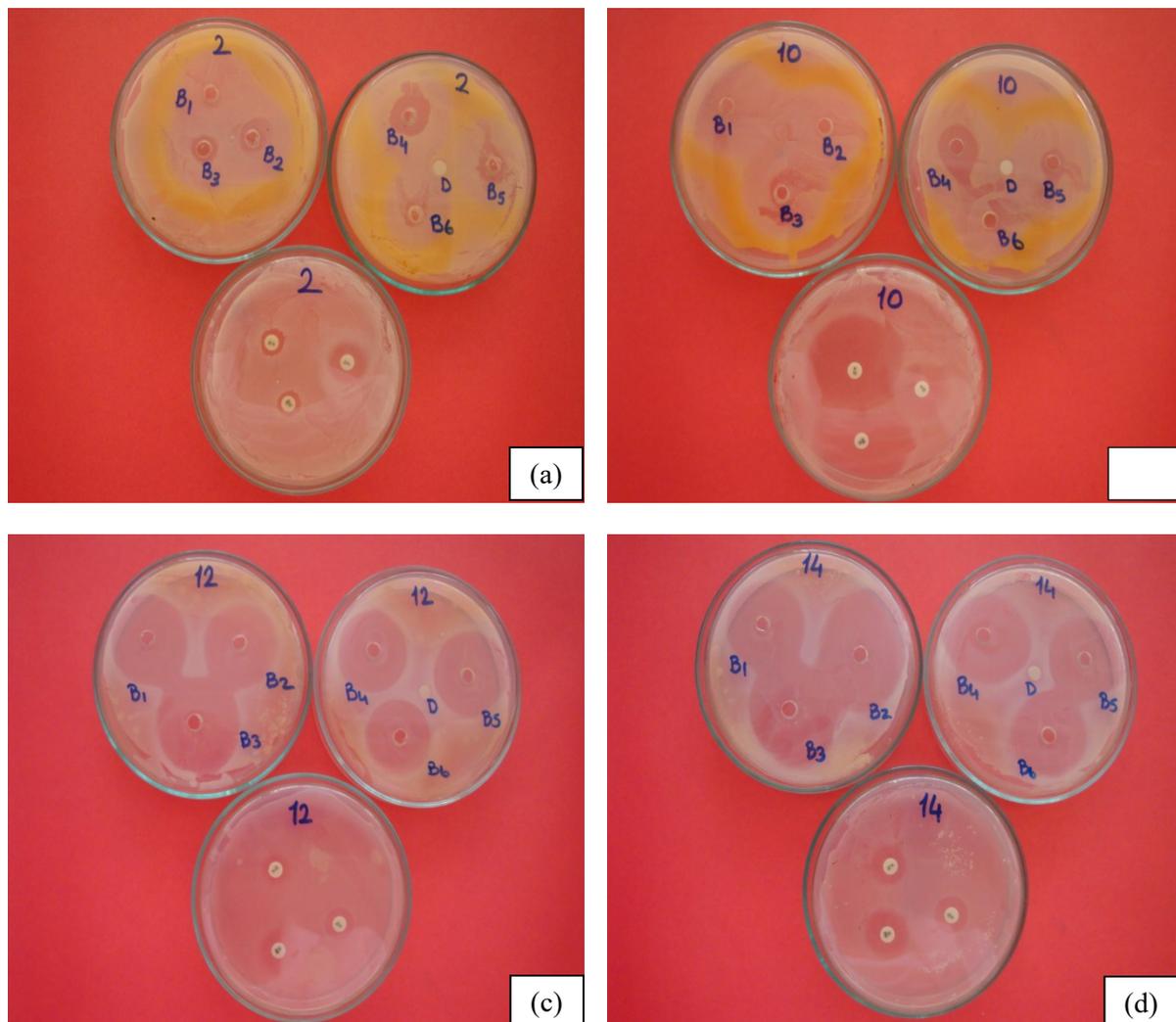
Among the honey samples floral honey from Giakove-Kosovo (sample no:B2) forms the maximum inhibition zone 40 mm in diameters against *Salmonella tyhimurium* ATCC 51812. The results of antimicrobial activity were given in **Table 2** according to the inhibition zone diameters formed around the wells. In generally, two honey samples (B4 and B5) have antibacterial activity against six bacteria and they are more effective than the others. The least effective honey sample is B1.

**Table 2.** Antibacterial activity of honey samples from Kosovo

Microorganisms	Inhibition zones (mm)*								
	B1	B2	B3	B4	B5	B6	P(10)	S(10)	AM(10)
<i>E. faecalis</i>	-	-	-	-	-	-	-	-	-
<i>S.aureus</i> ATCC 29213	-	10	12	18	14	12	12	16	12
<i>S.aureus</i> ATCC 6538 p	-	-	12	14	14	10	42	22	40
<i>S.aureus</i>	-	-	10	18	10	-	42	26	40
<i>S.aureus</i> ATCC 25923	36	34	38	34	34	32	12	14	14
<i>E.coli</i> ATCC 25922	-	-	-	-	-	-	8	20	12
<i>B.cereus</i>	-	-	-	12	10	-	-	26	8
<i>Salmonella typhimurium</i> ATCC 51812	36	40	36	36	36	36	16	16	20

(-): no inhibition P (10): Penicillin G (10 U), S (10): Streptomycin (10 µg), AM (10): Ampicillin (10 µg) \*: inhibition zones includes 6 mm disk diameter, data are average of three measurements

The high activity of honey samples and reference antibiotics tested in-vitro are shown in **Figure 2** as inhibition zones formed around the wells.



**Figure 2:** In-vitro tested honey samples (a) *Staphylococcus aureus* ATCC29213 (b) *S. aureus* (c) *S.aureus* ATCC25923 (d) *Salmonella typhimurium* ATCC51812 bakterilerine karşı inhibisyon etkileri. (B1): Srecka (B2) Giakove (B3): Prizren (B4): Rahovec (B5): Mitrovica (B6): Prishtine P (10): Penicillin G (10 U), S (10): Streptomycin (10 µg), AM (10): Ampicillin (10 µg) \*: inhibition zones includes 6 mm disk diameter.

Mahendran and Kumarasamy (2015) in their study, a total of twelve honey samples from different origins were evaluated for their antibacterial activity against the Gram positive species such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and the Gram negative species such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Among the twelve honey samples studied S1(summer honey) and W1(winter honey) honey samples show maximum antibacterial activity especially against *Staphylococcus aureus*.

According to Rani et al., (2017) both Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) isolates were found sensitive to honey. But MRSA were resistant to all antimicrobials tested except linezolid whereas MSSA were sensitive to all except penicillin.

Cenet et al. (2017), investigated honey specimens from Southeastern Anatolia and found that they were effective on five bacterial species like *S. aureus* 29213, *S. aureus* BAA-977, *E. faecalis* 29212, *E. coli* 25922, *E. hormaechei* 700323.

The activity against *S. aureus*, *E. coli* and *Salmonella sp.* has been expressed despite honey originating from different floral sources and countries. The inhibition of growth in those bacteria is principally due to the peroxide effect, which is very common in honey worldwide, and as it is a derivative compound from bees, it is expected that it is present in all honeys (Molan, 1992; Carina et al., 2014; Deb Mandal and Mandal, 2011). As seen in our study, nearly all types of honey from Kosovo have high activity against *S. aureus* strains and *Salmonella typhimurium* and the findings of our study together with three of our previous studies show similarities (Bican Süerdem et al., 2013; Bican Süerdem et al. 2014; Bican Süerdem et al., 2016). It can be one of the reasons for the high antibacterial activity which exceptionally rich in plant and tree species considering Kosovo's relatively small area ([http://pdf.usait.gov/pdf\\_docs/Pnact349.pdf](http://pdf.usait.gov/pdf_docs/Pnact349.pdf)).

Finally, the activity of all honey samples against *S. aureus* ATCC 25923 and *Salmonella typhimurium* ATCC 51812 are nearly three times higher than that of standard antibiotics discussed as reference in our study (Table 2.). It is therefore very likely that honey can be used as a potential alternative antibiotic to treat bacterial infections caused by these species especially of the skin and soft tissue.

#### 4. CONCLUSION

Because of many negative situations such as; increasing bacterial resistance to antibiotics day by day, side effects after antibiotic usage, high cost of production and long process, alternative treatment methods like apiteraphy (treatment with bee products like honey, pollen, propolis etc.) should be re-activated again. It is advisable to use honey as an alternative natural product, generally in children and older people and should be used in medicine and pharmaceutical industry. In all the researches about antibacterial activity of honeys, the common point is that the potency of the antibacterial activity can vary very markedly. The number of variable factors involved makes it impossible to predict with any certainty that a particular honey will have a high antibacterial activity. Because of this, honeys preferred for therapeutic use should be tested for their antibacterial activity against the pathogens to be get sure. In addition to many benefits of honey, there are some critical points while consuming honey. Firstly, it is necessary to guarantee its quality, secondly to consider the lesion type and the patient state (risk of allergy, diabetes, etc.).

Such preliminary researches should be carried out further on the subject of identifying the antimicrobial properties of the substances in honey. The determination of botanical origins of different types of honeys makes these studies even more meaningful.

#### Acknowledgement

We wish to thank to Andra Kanlinuro for her help in obtaining the honey samples from Kosovo and also to bee-keepers. This research was presented in ISEEP-2017 VIII. International Symposium on Ecology and Environmental Problems as an oral presentation.

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