International Journal of Natural and Engineering Sciences
E-ISSN: 2146-0086 14 (1): 11-15, 2020

Monitoring of Antimicrobial Effects and Effect Mechanism of Honey Samples in and around Eskişehir, Turkey

Sevil Pilatin[1*]

[1]Department of Biology, Faculty of Science and Art, Eskişehir Osmangazi University, 26040, Meselik, Eskisehir, Turkey.

*Corresponding Author
E-posta: spilatin@ogu.edu.tr

Received: 10 November 2019
Accepted: 28 March 2020

Abstract
Agar well and agar dilution methods were used to determine antimicrobial activity of 41 honey samples collected from different localities of Eskisehir province, Turkey. Totally ten microorganisms, Gram positive and negative bacteria, yeasts and moulds, were used as test microorganisms. And then in order to determine the effect mechanisms of antimicrobial activities of the honey samples which were chosen osmotic effect, catalase test and acidity tests were applied and chemical ingredients analysis was done. In this phase the most sensitive microorganisms to osmotic stress and hydrogen peroxide are *M. luteus* and *C.albicans* and the most resistant bacteria is determined as *P.aeroginosa.* According to this the samples whose activities were determined when treated with catalase it was observed that their antimicrobial activities reduced to a large extent. In this study, it was revealed that hydrogen peroxide is one of the important factors of antimicrobial activity of honey.

Keywords: Eskişehir, Honey, Antimicrobial activity, Effect mechanism

INTRODUCTION

Honey, which is known one of the oldest products, has been used in conventional medicine. Roman doctors indicated that it is a very strong antidote; Hypocrites indicated the equality of honey with air and water; Egyptian, Greek, Arabic doctors indicated that honey was used as syrup or cream to cure variety of eye, psychology and psychiatry problems [6]. Because of the variety of its content honey has a pretty confusing structure. In general, around 80% of honey is different sugars (%35 glucose, %40 fructose, %5 sucrose), 17% is water. The rest 3% is notably enzymes, amino acids, gluconic ascids, phenol compounds, lactone, minerals and variety of vitamins, almost 180 different substances [14]. Among the areas of use respiratory tract, gasrtointestinal system and the treatment of variety of different illnesses are there. Also, it has been known that honey has been used to cure and soothe wounds (even surgery wounds), burns and skin ulcerous [20]. To rehabilitate cancer, to regulate sexual functions, with its antibiotics effect it was found out that honey has a positive effect on infections [24; 10]. It was stated that with the researches done, honey has a potential to be used in different infections caused by different microorganisms [22; 25; 21;16].

There are microbiologic studies about the antibacterial effect of honey and in these studies the effect depends on the other components in honey more than its osmolarity. In the studies where the antibacterial activity level of honey were tested it was stated that even it was added water 10 times or more still it could completely inhibit some types of bacteria to develop [2].

The features of honey such as high osmolarity depending on sugar concentration, low water activity, low pH, and hydrogen peroxide production are effective on its antibacterial activity [7]. The most important antibacterial compound in honey is peroxide, which was the result of oxidation of glucose in honey caused by glucose oxidase enzyme produced in the hipofarengial glands of bees [17]. On the other way, in some samples of honey it was seen that catalase enzym caused by the polens in some herbs inactivates the hydrogen pe.roxide, the antibacterial effect was still seen [18]. It was determined with the results of some researches that *Staphylococcus aureus* (MRSA) bacteria which is known resistant to Meticilin, which is resistant to antibiotics, was inhibited in honey [11]. It was revealed that against 21 types of bacterias especially *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* ve *Pseudomonas aeruginosa* it has an inibitive effect [26]. Apart from pathogenic microorganisms of vegetative forms, honey is also effective for spore forms and it is reported that especially it could exterminate *Clostridium botulinum*'s spore forms [19].

When the literatue was reviewed, it was understood that some honey has specific effects on the test microorganisms whose effectiveness were researched Adebolu [1]. In the in vitro antimicrobial effect study carried out by Aksoy and Dığrak in [2] with agar well method it was found out that honey taken from the in the center of Bingöl and four different regions has antimicrobial effects on different bacteria and yeast [2].

The antimicrobial effect of honey and propolis collected from Muş, Bitlis and around by Alan and friends, was researched and as a result it was found out that honey and propolis extracts have antibacterial activities against gram negative and gram positive bacteria [3].

Our study area is Eskisehir city, province and villages. The reason why Eskisehir was chosen as the study area is its important potential of bee culture, specific climate and vegetation, its having different nectars of herbs and until now there was no such extensive study conducted in this area. In our study, antimicrobial effects of honey collected from Eskisehir and around were researched. Also, the mechanism of antimicrobial activity (osmotic effect, acidity, H_2O_2 formation) tried to be clarified.

MATERIALS AND METHODS

Honey Samples

41 honey samples used in the research were supplied from Eskisehir city center and provinces in July, August, September and October, 2007-2008 (Fig. 1, Table 1).

While collecting the samples the distance of localities, height and if the beehives are settled or not were considered. From each beehive 250 grams of honey were put into the sterile jars and taken to the laboratories. During the research

all honey samples were kept in the room temperature in a dry and dark closet.

Determining Antimicrobial Activity

In vitro antimicrobial susceptibility studies were performed using a panel of pathogenic and nonpathogenic microorganism strains consisted of six bacteria and four fungus strains which are *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIB 196, *Bacillus subtilis* NCIB 3610, *Pseudomonas aeroginosa* NRRL 771, *Proteus vulgaris* NRRL 123, *Escherichia coli* NCIB 9132, *Geotrichum candidum* NRRL Y 552, *Candida albicans* CBS 562, *Aspergillus flavus* NRRL 1957, and *Penicillium crysogenium*.

Overnight grown bacteria and yeast cultures were adjusted to 10^8 and 10^6 cfu/mL that is equivalent to McFarland No 0.5 standard, respectively. Spore suspensions were prepared as 10^6 spore/mL with the using of Thoma slide from a weekly culture of the moulds.

To perform agar well method, a 100 µL cell or spore suspensions was inoculated to surfaces of solidified Mueller-Hinton and malt extract agar plates for bacteria and yeasts, respectively. The 17 mm diameter wells were bored with sterile cork borer. Three hundred microliter honey of each sample was plated into the wells and plates were incubated at 37 and 30°C for 24 and 48 hours for bacteria and yeasts, respectively. On the other hand, the mould were incubated at 30°C for a week. After incubation period, the diameter of the inhibition zones were measured with digital caliper [23].

The minimum inhibitory concentration (MIC) values of the honey samples were determined with the agar dilution method. The appropriate broth media which are include honey samples different final concentrations (10%, 20%, 30%, 40%, and 50%) were prepared and were inoculated with 100 µL cell or spore suspensions. Absorbance values for each test organisms were determined spectrophotometrically on 620 nm after 24 hours [23].

MIC values were calculated with the formula;

$1-[(T_{24} / T_0) \times 100$

where;

T_0: Absorbance on starting time

T_{24}: Absorbance after 24 hours

The selected honey samples for their higher antimicrobial activity were used for subsequent studies.

Determining antimicrobial effect mechanism of honey samples

Osmotic Effect

In this phase of the study, in order to determine if there is a correlation between the antimicrobial feature and sugar concentration an artificial honey solution (40 gr fructose, 30 gr glucose, 8 gr maltose, and 2 gr sucrose, 100 ml distilled water) was prepared [28; 25]. After this artificial honey was sterilized to at least two parallel total three wells were drilled to appropriate mediums. To one of the wells honey sample, which is 80%, to the other in order to evaluate the osmotic pressure effect artificial honey solution was placed. To the last well honey, which was entreated with catalase, was placed in order to evaluate the hydrogen peroxide effect. To each well 300 ml of each sample was added. After the incubation if the artificial honey had an antimicrobial effect against the test microorganisms or not was observed through the inhibition zones.

H_2O_2 Effect

In order to determine if H_2O_2 is responsible for antimicrobial activities 300ml of 0.2% catalase, 14.8 ml (pH 7.0) potassium phosphate tampon and 5ml of honey mixture was added to the wells drilled on the solid media and agar well method was applied. The pH value of honey was also traced.

Acidity

From the honey sample 10 gr was taken and put into the 250 ml Erlenmeyer and 75 ml of distilled water was added and honey was solved. After dropping 4-6 drops of phenolphthalein solution it was titrated with 0.05 N NaOH solution to its turning point. At the turning point, the color red of phenolphthalein must remain at least 15 seconds. The volume of standard NaOH solution spent in the titration was recorded.

RESULTS AND DISCUSSION

As physically and chemically required honey is a dense substance. It is high in rate of sugar and low in rate of water. Because of these features that it has, honey affects the growth of microorganisms inside negatively. Among the test microorganisms studied micro fungus are the ones, which need water the least. This is considered partly the reason of why no antimicrobial effect was seen to any micro fungus during the study. In the literature similar results were obtained when studied with different honey samples and micro fungus.

In a study carried out by Çakır and Tümen in 1990 it was reported that while honey in Balıkesir had an antibacterial effect against *S.aureus B.subtilis, E.coli, Pseudomonas multophica* and *Klebsiella pneumoniae,* it did not have any antimicrobial effect on *Candida albicans* M IV 270, *Aspergillus niger* KUEN 1147 ve *Aspergillus fumigatus* KUEN 1145 [9]. According to Patton the studies carried out by Brady and friends with 'Manuka Honey' and 'Non Manuka Honey', none of these honey had an antifungal effect on the clinically experimented micro fungus. Researchers, similarly, relate these results to the resistance of micro fungus to low water.

In 2006 among the honey samples taken from the Bingöl city center and its four different regions, the sample taken from the Karlıova region was determined the most effective with its 35 mm of inhibition zone [2]. In the scope of project work the experiments done with the honey samples gathered from Eskisehir and around the analysis had the similar findings. It was found out that number 5 and number 11 honey samples were the most effective ones on *E.coli* with its 38 mm inhibition zone (Table 2).

Honey from Bingöl and around the measured inhibition zone of 19-45 mm on *B.subtilis*, on *S.aureus* 16-51 mm, on *P.aeroginosa* 14-21 mm, on *M. luteus* 25-51 mm, according to the inhibition zones antibacterial effect was revealed [2]. Honey from Eskisehir and around number 38 was the most effective on *S.aureus* with its 34 mm of inhibition zone. Honey sample number 20 with its 38 mm inhibition zone was the most effective on *M. luteus.* On *B. subtilis* honey sample number 15 with its 26 mm inhibition zone and on *P. aeroginosa* honey sample number 37 with its 32 mm inhibition zone were determined the most effective samples. (Table 2) The results in both studies where Agar well method was used support each other.

In the study which was conducted by Hazır and Keskin in [12].and the antimicrobial activity was researched, the samples from Eskisehir center and Yakakayık region were analyzed. It was reported that for each three test organisms the samples taken from Eskisehir center and Yakakayık region showed antimicrobial activity in 40-50% concentrations but in lower concentrations no antimicrobial activity was reported. As a result of the studies it was considered hopeful that in low concentrations the honey samples were experimented to show antimicrobial effect.

According to Table 2 the most effective honey on all microorganisms are the ones numbered 3, 20, 22, 26, 28, 35, 38, 40 but number 9 and 18 are the least effective samples. According to the results and findings it was determined that 3, 15, 17, 20, 21, 22, 23, 26, 28, 32, 33, 35, 38 and 40 samples are the most effective on the test microrganisms of Gram (+) bacteria (Table 2). On Gram (-) bacteria the most effective samples are 3, 7, 8, 11, 16, 20, 22, 26, 28, 35, 37, 38, 39 and 40. In this phase to see if there is antimicrobial bacteria or not it was noted that except for numbers 9, 12, 13, 18 and 36 all honey samples have a remarkable antifungal effect on yeast.

Another feature that is expected to be searched in antimicrobial effects is the specific effect that the research material has on the microorganisms. It was determined that some of the honey samples tested showed specific inhibition. Number 9 and number 18 showed antimicrobial effect on just *P. aeroginosa* (Table 2).

According to the results of the scanning considering the inhibition zones among the honey tested (3, 15, 22, 23, 26, 28, 35, 38, 40, 20, 17) the honey samples were chosen because of the antimicrobial effect that they have. In order to determine the minimal antimicrobial activity levels of the samples mentioned Minimal inhibition concentration (MIC) trials were done. And then osmotic and catalase effect tests were applied to determine the effect mechanism of the antimicrobial activity that they have.

MIC tests are reliable tests used to determine the effect value of antibiotics [23]. In this study the main objective is to determine the most effective minimum concentration on test microorganisms in honey samples. According to the findings of the mentioned tests honey sample number 38 was found to be having the most inhibition effect on the test microorganisms in the lowest concentration. Honey sample number 38 in 10% concentration on *P. aeroginosa* 82%, on *P. vulgaris* and *G. candidum* 88%, on *C. albicans* 89% has inhibition. The other samples according to their effectiveness in an order are determined as follows: 20 > 17 > 35 > 22 > 26 > 15 > 23 > 3 > 40> 28

According to Allen [4]. *Actinomyces pyogenes*, *Klebsiella pneumoniae*, *Nocardia asteroides*, *Staphylococcus aureus* (koagülaz positive), *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberi* using test microorganisms in three different honey samples, "Manuka Honey", "Rewarewa Honey' and 'Artitificial Honey' a research done with %0,1, %1, %5 and %10 concentrations while the growth of all microorganisms was minimum 1% in "Manuka Honey" and "Rewarewa Honey" growth was observed in 10% of concentration [4; 27; 29; 15].

The antimicrobial effect sources of honey; depending on the low water osmotic effect, depending on the variety of herb sources of honey hydrogen peroxide that it includes, low acid, low protein content, high sugar concentration, low redox potential, because of its density no touch of oxygen and different phytochemical structure [4; 25].

The honey samples, whose effect values were determined by Broth dillusion MIC tests, were subjected to catalase test and osmotic test in order to determine the possible reasons of antimicrobial effect.

According to the findings osmotic effect is the most remarkable as an antimicrobial activity. It shows that because of the antimicrobial activity of low water potential osmotic effect happens and no antimicrobial activity was observed. Because bacteria and yeast are quiet sensitive to osmotic stress. Their speed of growth reduces in low water activity environments or even stops. Osmotic effect is an important factor to test honey samples. But, antimicrobial activity sources of honey varies.

The pH of honey generally changes between 3.2-4.5 and this low pH shows a repressive feature of most pathogens' improvement. In undiluted honey acidity is an important antimicrobial effect. Because the pH increases in the honey which was diluted by water or tampon solution, its antimicrobial effect reduces on most pathogen [31].

Acidity and the hydrogen peroxide in honey show an important antimicrobial effect. The honey samples used in the experiment in the 80% concentration of test honey pH values chaged 3.30–4.53. This pH range is a limiting factor especially fort he bacteria growing in neutral pH value. Generally the pH value that bacteria can survive is between 6.5 and 7.5 and optimum 7.1-7.4 and bacteria are pretty sensitive to pH changes. Findings show that honey samples take the pH to neutral pH. This condition shows that acidity of honey could be the reason of antimicrobial activity.

The acid pH values that natural honey has refer to the inhibition effect of honey on microorganisms. Because of the low pH values of the honey samples used in the study it is considered that they could have an antibacterial effect. However, as it is known yeasts are more resistant to acid pH than the bacteria. Because of this the antifungal effect of honey used in this study could not be considered as related with the pH value. It is out of possibility that relating the antimicrobial feature of honey to its physical and chemical structures by nature. Thus, not only the pH values were measured, but also the other possible features were searched. According to the literature in the antimicrobial effect of honey another important compound is hydrogen peroxide, which could be found in its content. The source of hydrogen peroxide in honey is the herb nectars [30].

In order to research if the hydrogen peroxide (H_2O_2) effect in honey is effective on antimicrobial activity, honey samples were treated with catalase enzyme and used later on. Here in this phase the change of pH value of honey was traced.

According to the findings it was determined that the last pH values ranged between 6.53 and 6.9. However, honey sample number 3 had an antifungal effect on *C. albicans* with its 30 ml of inhibition zone. While in the condition of 80% honey concentration the pH value was 4.19, after being treated with catalase the pH was measured as 6.73. These results make us think that the antimicrobial especially the antifungal effect that honey has cannot be linked to acidity or H_2O_2 content; besides, different chemical substances of honey could be effective.

In an antimicrobial activity study conducted by Taormina et al. [25] the pH changing between 6.43 and 6.69 before treating the samples with catalase was determined changing between 6.54 and 6.94 after treating with catalase. In the study, it was observed that the antimicrobial effect went down in the samples whose pH values increased [25].

Natural honey contains trace amount of Hydrogen peroxide. Hydrogen peroxide with its strong oxidant feature is a toxic compound for the cell. According to the knowledge gained in the literature in 1979 White determined that a great deal of antimicrobial activity of honey was because of Hydrogen peroxide that it contains [28]. In a study carried out with 'Manuka Honey' it was observed that honey samples, which were not added catalasa enzyme, had an intense antimicrobial activity. In honey samples whose H_2O_2 was removed from its content by adding catalase enzyme low antimicrobial activity was observed [8]. According to the literature knowledge, honey samples, which were not added catalase had an intense antimicrobial activity on test bacteria *Salmonella typhimurium*, *E.coli*, *Shigella sonnei*, *Listeria monocytogenes*, but after treating with catalase they lost their antimicrobial activity feature to a great extent [25].

Within the scope of the project work carried out, in order to research the source of the antimicrobial activity of honey in Eskisehir and around agar well method was used. The

antimicrobial activity of honey which was treated with catalase and which was not added catalase enzyme was measured and compared with each other. In this phase *M. luteus* was determined as the most sensitive to osmotic stress and hydrogen peroxide, *P.aeroginosa* was determined as the most resistant bacteria among yeasts and *C.albicans* was determined as the most resistant microorganism. According to this, it was observed that the antimicrobial activities of the samples, whose antimicrobial activities were determined, when treated with catalase went down. In this study, it was revealed that hydrogen peroxide is one of the important factors of antimicrobial activity of honey.

It is very important that such studies should be widened and conducted in order to use the advantage of the country's natural sources and contribute to the economy. In the country bee culture should be directed with the studies about the serious law regulations to be done, the education and organization of apiarists, and the encouragement about bee culture. Otherwise, the potential of bee culture, which is a very important treasure for our country, will be affected negatively. This study has the quality of completing the studies conducted in our country. We wish the information gained from this study would be beneficial for the world of science and the apiarists.

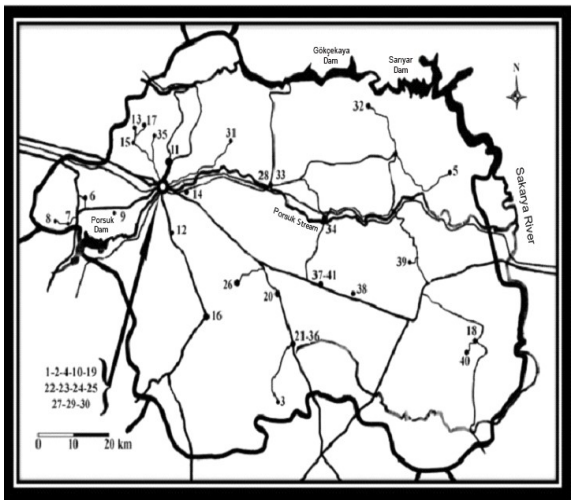

Figure 1. The localities of honey samples

Table 1. Locality names that collected samples of honey

Number	Localities		
001	Merkez Ömür	021	Eskişehir Merkez 2
002	Merkez Tandır	022	Alaköy
003	Çifteler Çatmapınar	023	Merkez Ömür
004	Merkez Karabayır	024	Karaçoban Köyü
005	Mihalıççık Ahurözü	025	Merkez
006	İnönü Dutluca	026	Seyitgazi Doğançayır
007	İnönü Kümbet Yeniköy	027	Merkez Ömür
008	İnönü Kuzfındık	028	Alpu
009	Merkez A. Kartal	029	Yıldırım Çiftliği
010	Merkez Fevzi Çakmak	030	Meşelik ormanı Yenikent
011	Muttalip	031	Alpu Gündüzler
012	Merkez Akpınar	032	Mihalıççık Dinek
013	Merkez Karaçoban	033	Alpu
014	Merkez Karacahöyük	034	Beylikova
015	Eğrioz köyü	035	Sulukaraağaç
016	Seyitgazi	036	Çifteler Karaköprü
017	Buldukpınar Köyü	037	Kaymaz
018	Günyüzü	038	Sivrihisar Paşakadın Köyü
019	Eskişehir Merkez	039	Sivrihisar Dümrek
020	Mahmudiye	040	Günyüzü Atlas Köyü
		041	Sivrihisar Kaymaz

Table 2. The inhibition zones measured at the end of the incubation periods (mm)

HONEY CODE	BACTERIA					YEAST			MOULD	
	Gram (+)			Gram (-)						
	1	2	3	4	5	6	7	8	9	10
Vancomycin	15	25	13	13	-	13	-	-	-	-
1	-	-	-	-	-	-	30	30	-	-
2	-	-	-	20	-	12	-	-	-	-
3	24.5	27	19	21.5	23	19.5	20	28.5	-	-
4	-	-	-	-	-	-	38	20	-	-
5	-	28	-	-	20.5	38	26	30	-	-
6	-	31	-	-	19.5	37.5	28.5	26.5	-	-
7	-	-	-	18	17.75	35	31.5	30.75	-	-
8	-	20	-	21.3	20.25	35	34.25	31.5	-	-
9	-	-	-	16	-	-	-	-	-	-
10	-	-	-	-	-	-	28	30	-	-
11	-	18.5	-	22.3	17.3	38	27	32	-	-
12	12	-	-	16	-	-	-	-	-	-
13	12	-	-	18	-	-	-	-	-	-
14	-	23	19	-	-	34	22.5	25.75	-	-
15	25	25	26	22	-	22.5	22	31	-	-
16	-	22	-	22	19	35.3	31.3	36	-	-
17	27	30	25	-	26	23	24	-	-	-
18	-	-	-	30	-	-	-	-	-	-
19	-	-	-	30	-	-	21	-	-	-
20	25	37.5	24.5	22	26	24	24.5	-	-	-
21	22	23	20	24	21	-	28.5	24	-	-
22	26	29.5	25	19	25.5	24.5	23.5	34.5	-	-
23	21.5	30	19	-	26	-	19	29.5	-	-
24	23	-	-	-	-	-	22	21	-	-
25	22	21	-	-	20	19	30.5	23	-	-
26	27.5	35	25	20.5	26	28	22	34	-	-
27	20	20.5	-	-	19	-	30	20	-	-
28	27	31.5	25.5	21.5	26.5	26	23.5	36	-	-
29	17	-	-	-	22.5	-	22.5	25	-	-
30	17	18.25	-	-	23	-	20	19.5	-	-
31	22	18	-	-	24	18.25	22	25	-	-
32	24.5	21	19	19.5	-	18.25	27	29.5	-	-
33	19	20	19.5	-	23	18.5	27	32.5	-	-
34	22	21.75	-	-	26.5	22.5	28	30.5	-	-
35	26	29	23	19.5	32	22	31.5	29.5	-	-
36	18	-	18	26.5	21	-	-	-	-	-
37	22.5	19	-	31.5	24.5	19.5	25.5	-	-	-
38	34	36	20.5	21	25	21.5	24	36	-	-
39	26.5	21.5	-	31	27.5	21	27	-	-	-
40	22.5	20.5	18.5	30	26.5	22	26	-	-	-
41	-	31	24.5	-	26.5	20.5	22	31	-	-

ACKNOWLEDGEMENT

This study is a part of project "Palynochemical analyses and antimicrobial activity of Honey Samples in and around Eskişehir, Turkey" ESOGÜ Project Number: 200619030, made under the supervision of İsmühan Potoğlu Erkara.

REFERENCES

[1] Adebolu, T. T. 2005."Effect of natural honey on local isolates diarrhea-causing bacteria in southwestern Nigeria," *African Journal of Biotechnology*, *4* (10), 1172-1174.

[2] Aksoy, Z., & Dığrak, M. 2006. "Bingöl yöresinde toplanan bal ve propolisin antimikrobiyal etkisi üzerinde invitro araştırmalar," *Fırat Ünv. Fen ve Müh. Bil. Dergisi*, *18* (4), 471-478.

[3] Alan,Y., Atalan, E., Erbil, N., Bakır, O., Orman, Z., & Kanik, P. 2014. "Antımicrobial activity of honey and propolis collected in Muş and Bitlis region," *Muş Alparslan University Journal of Science*, *2* (1).

[4] Allen, K. L., & Molan, P. C. 1997. "The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey", *New Zealand Journal of Agricultural Research*, *40*, 537-540.

[5] Brady, N. F., Molan P. C., Harfoot, C. G. 1997. "The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey", *Pharm. Sci.*, *2*, 1-3.

[6] Brown, R. 2000. *Honey royden brown's bee hive productbible*. 123-33.

[7] Brudzynski, K., & Kim, L. 2011. "Storage-induced chemical changes in active components of honey de-regulate its antibacterial activity," *Food Chem*., *126*, 1155–1163.

[8] Cooper, R. A., Molan , P. C., & Harding, K. G. 2002. "The efficacy of honey in ınhibiting strains of *Pseudomonas aeroginosa* from infected burns," *Journal of Burn Care & Rehabilitation, 23* (6), 366-370.

[9] Çakır, H., & Tümen, G. 1990. "Balıkesir yöresi ballarının antimicrobial ve antiungal etkileri," X. Ulusal Biyoloji Kongresi (18-20 Temmuz), Erzurum.

[10] Çakmak, İ. 2001. Apiterapi (Polen). *Uludağ Arıcılık Dergisi.* Bursa. *1* (3), 38-39.

[11] Dixon, B. 2003. Bacteria can't resist honey. *The Lancet Infectious Deseases*, *3*, 116.

[12] Hazır, S., & Keskin, N. 2002. "Investigation of

Antimicrobial Effect of Honey Collected from Various Regions of Turkey," *Pakistan Journal of Biological siences 5* (3), 325-328.

[13] Honey Scientific Report Office of Complementary Medicines December 1998.

[14] Kahraman, T., Buyukunal, S. K., Vural, A., & Altunatmaz, S. S. 2010. "Physico-chemical properties in honey from different regions of Turkey," *Food Chem.*, *123*, 41–44.

[15] Lozano- Chiu, M., Nelson, Page W., Paetznick, Victor, L., & Rex, John H. 1999. "Disk diffusion method for determining susceptibilities of *Candida* spp.to MK-0991," *Journal of Clinical Microbiology*, *37*, 1625-1627.

[16] Lusby, P. E., Coombes, A. L., & Wilkinson , J. M. 2005. "Bactericidal activity of different honeys against pathogenic bacteria, Arcives of Medhical Reserch", 36, 464-467.

[17] Mandal, MD. & Mandal, S., 2011. "Honey: its medicinal property and antibacterial activity," *Asian Pac J Tropical Biomed*, *1*, 154-160

[18] Malika, N., Mohamed, F., & Chakib, E. A. 2004. "Antimicrobial activities of natural honey from aromatic and medicinal plants on antibio-resistant strains of bacteria," *Int J Agri Biol*., *6* (2), 289- 293.

[19] Mansour, M. A. 2002. "Epithelial corneal oedema treated with honey. *Clinical and Experimental Opthalmology"*, *30*, 141-142.

[20] Mulu, A., Tessema, B., & Derbie, F. 2004. "Invitro assessment of the antimicrobial potential of honey on common pathogens," *Ethiop, J . Health Dev*., *18* (2), 107-111.

[21] Mundo, M. A., Padilla-Zakour O. I., & Eorobo, R. W. 2004. "Growth inhibition of foodborn pathogens and food spoilage organisms by select raw honeys," *International Journal of Food Microbiology , 97*, 1-8.

[22] Nzeako, B. C., & Hamdi, J. 2000. "Antimicrobial potential of honey on some microbial isolates," *Medical Sciences*, *2*, 75-79.

[23] Patton, T., Barrot, J., Brennan, J. & Moran, N. 2006. "Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey," *Journal Microbial Method*, *64* (1), 84-95.

[24] Sorkun, K. 1987. Arı ürünleri , *Bilim Teknik Dergisi*, *20*, 20-21.

[25] Taormina, T., Niemir, B. A., & Beuchat, L.R. 2001. "Inhibitory Activity of Honey Against Foodborne Pathogens as Influenced by the Presence of Hydrogen Peroxide and Level of Antioxidant Power," Internatiol Journal of Food Microbiology, *69*, 217-225.

[26] Tomoi, S., & Miyata, G. 2000. The Nutraceutical Benefit, Part 3: *Honey Nutritional Pharmaceutical. 16*, 468-469.

[27] Weston, R. J., Brocklebank, L. K., & Lu, Y. 2000. "Identification and guantative levels of antimicrobial components of some New Zealand honey," *Journal of Food Chemistry*, *70*, 427-435.

[28] White, J. W. 1979. Composition of honey. In: Crane, E (ED.) Honey: A compherensive survey. Heinemann, London, 157-158p.

[29] Willix, D. J ., Molan, P. C ., & Harfoot, C. G. 1992. "A comprasion of the sensitivity of waund-infecting species of bacteria to the antibacterial activity of manuka honey and another honey,"The Journal of Applied Bacteriology, 73, 388-394.

[30] Web:http://www.airborne.co.nz/enzymes.html January 2016

[31] Web:www.healinghoney.co.nz/antimicrobial_honey. cfm January 2016