MELLIFERA RESEARCH ARTICLE

Evaluation of Antimicrobial Activity of Methylglyoxal -The Major Antibacterial Component of Manuka Honey

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Received: 1st December, 2018; accepted: 22nd December, 2018; published: 28th December, 2018

A B S T R A C T

Methylglyoxal (MGO) is the major antibacterial compound in manuka honey, which is formed by a non-enzymatic conversion of nectar-derived dihydroxyacetone. The aim of this study was to investigate the effect of MGO on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Purchased MGO (Sigma-Aldrich) was used for all tests. Disc diffusion test was used for evaluating the antimicrobial activity of MGO. The results showed that each tested bacteria species is susceptible to MGO solutions significantly. Statistical analyses revealed that *S. aureus* is the most and *P. aeruginosa* is the least susceptible bacterial strain to MGO solutions. The results of this study are encouraging, and further studies are necessary to study out MGO's potential.

Keywords: Methylglyoxal,Manuka honey, Disc diffusion test

Introduction

Infectious diseases threat human health and shorten lifetime. Antimicrobial compounds are essential agents that are used for reducing infectious diseases. Resistance formation to current antibiotics, less than a hundred year since the discovery of first antibiotic, complicates the treatment of these infections [1]. In addition, development of new antibiotics has decreased for few decades [2]. Scientists are seeking new natural products, which could substitute antibiotics. Honey has been used as a remedy for several health issues from ancient times. Manuka honey, is derived from Manuka tree *Leptospermum scoporium* in Zealand. It contains high amounts of 1,2-dicarbonyl compound methylglyoxal [3]. Methylglyoxal (MGO), is believed to be the major antibacterial compound in Manuka honey. MGO is formed by a non-enzymatic conversion of nectar-derived dihydroxyacetone. This non-peroxide antibacterial constituent of the Manuka honey gives its unique antibacterial properties since hydrogen peroxide is the main antibacterial factor in other honeys [4-6]. The aim of this study was to investigate the effect of MGO on well known human pathogen bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus. S. aureus* is gram-positive bacteria, which causes nosocomial infections. *Pseudomonas aeruginosa* and *Escherichia coli* are gram-negative bacteria. *E. coli* causes urinary tract infection, diarrhea, septicemia, wound

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infections and *P. aeruginosa* causes wound infection, diabetic foot ulcer, urinary tract infections primarily[7-9].

Materials and Methods

Antibacterial agent

Purchased MGO (Sigma-Aldrich) was used for all tests.

Selection and growth of bacteria

Three common human pathogen bacteria were selected for the evaluation of antibacterial properties of Methylglyoxal. The bacterial strains used in this study were Gram positive bacteria *Staphylococcus aureus* ATCC 29213; Gram negative bacteria *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218. Fresh cultures of bacteria were prepared in Mueller-Hinton Broth at 37 °C and incubated 24 hours. Bacterial cultures' turbidity were adjusted equivalent to McFarland 0.5 standard with nephelometer (Biosan).

Antibacterial susceptibility test

Antimicrobial activity was investigated by the disc diffusion method using Muller Hinton Agar (Merck). After bacterial suspensions were spread on the MHA plate, sterile blank discs were placed on the surface of the plate and impregnated with different concentrations of the MGO (50% and 100%). Ciprofloxacin (5 µg-Bioanalyse®) and sterile distilled water were loaded to blank discs used as positive and negative controls respectively. The inhibition zones were measured after plates were incubated for 24 hour at ±37°C, by measuring the diameter in mm. All trial sets were made in triplicate. General guidelines of National Committee for Clinical Laboratory Standards followed for assessment. (CLSI, 2013)

Statistical analyses

Collected data were compared for antimicrobial activity level of different concentration of methylglyoxal and susceptibility levels of species. One way Anova and Duncan tests were performed in order to determine significant differences. Descriptive analyses were done to determine mean, min., max. and standard deviation. IBM SPSS 23 program was executed for all analyses.

Results and Discussion

Results of the antimicrobial susceptibility test showed up methylglyoxal has significant activity on *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* (Figure 1.). Diameter of inhibition zones and descriptive analyses of collected data were given in Table 1. and Table 2. Mean value of diameter of zones formed in different concentrations of MGO were given as a graph in Figure 2. Statistical analyses revealed that *Staphylococcus aureus* was the most susceptible bacteria and the difference between all bacteria was significant (Figure 3.). (ANOVA df:2; Mean square:737.565; F:14,324 Sig: .000081; P≤0.001). According to Duncan test, *P. aeruginosa*, the least susceptible bacteria, is in group 1 and *E. coli* and *S.*

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aureus in group 2 without taking into account concentration differences (Table3.).

In terms of concentration, difference between 10%, 20% and 40% MGO solutions was significant and the efficacy was increasing compatible with the rate. (ANOVA df:2; Mean square:528.954; F:7.680; Sig: .003; P≤0.005). According to Duncan test, 10% , 20% concentrations are in group 1 and 40% is in group 2. 40% solution of MGO was the most efficient concentration.

Since MGO concentration in Manuka honey depends on to the origin of honey, level of phenolic compounds, age of honey, this study emphasized on MGO' s activity separately [10]. Obtained data showed that methylglyoxal was efficient antibacterial agent accurately.

Due to increased antibiotic resistance among pathogens, it is essential to find ways to manage and prevent resistance. There are few suggestions like tracking the frequency of resistance, isolation of hospitalized individuals and introducing new therapeutic approaches [1]. The most important aspect of the strategy is to find new drugs thorough plenty of unexplored natural products. Manuka honey and its components but majorly methylgloxal, are good representatives of this perspective.

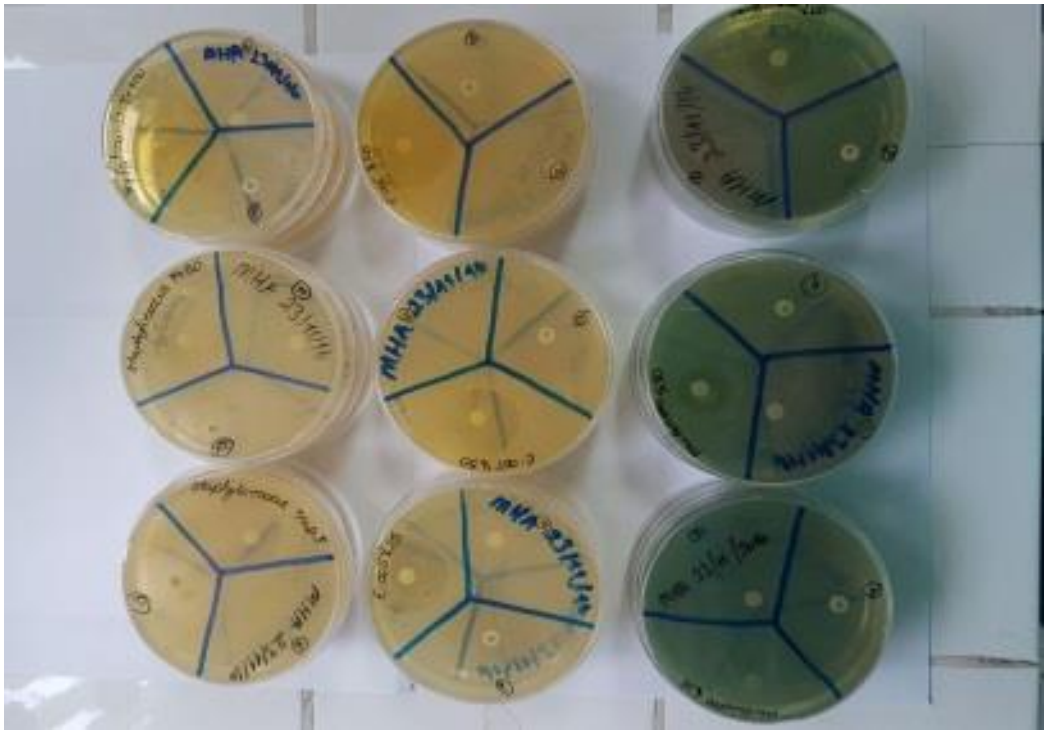

Figure 1. View of inhibition zones in petri dishes inoculated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli.*

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Table 1. Results of disc diffusion tests

	Staphylococcus aureus			*Pseudomonas aeruginosa*			*Escherichia coli*		
	I	II	III	I	II	III	I	II	III
10% MGO	29.00	29.50	28.00	16.00	16.00	16.00	27.00	28.00	28.00
20% MGO	34.00	34.00	34.00	18.00	20.00	20.00	31.00	32.00	30.00
40% MGO	49.00	54.00	50.00	26.00	26.00	25.00	38.00	42.00	40.00
Positive control	30.00	30.00	31.00	26.00	28.00	27.00	35.00	33.00	35.00
Negative Control	0	0	0	0	0	0	0	0	0

Zone diameters are given in mm .The measurements includes the disc diameter (5 mm)

Table 2. Results of descriptive analyses.

Bacteria	Concentration	Min.	Max.	Mean	Std. Deviation
Staphylococcus aureus	10%	28.00	29.50	28.8333	.76376
	20%	34.00	34.00	34.0000	.00000
	40%	49.00	54.00	51.0000	2.64575
	Positive Control	30.00	31.00	30.3333	.57735
Pseudomonas aeruginosa	10%	16.00	16.00	16.0000	.00000
	20%	18.00	20.00	19.3333	1.15470
	40%	25.00	26.00	25.6667	.57735
	Positive Control	26.00	28.00	27.0000	1.00000
Escherichia coli	10%	27.00	28.00	27.3333	.57735
	20%	30.00	32.00	31.0000	1.00000
	40%	38.00	42.00	40.0000	2.00000
	Positive Control	33.00	35.00	34.3333	1.15470

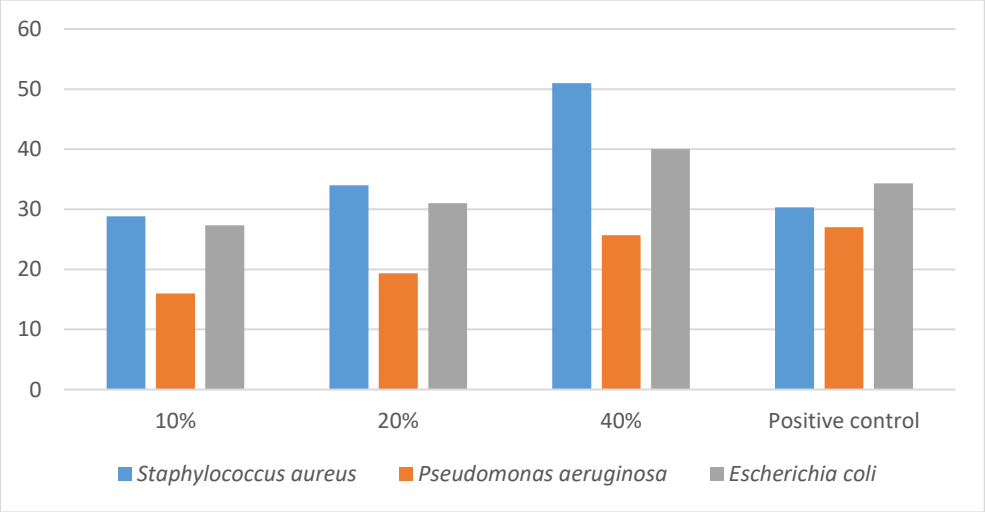

Figure 2. Graph that shows mean value of diameter of zones formed in each trial due to different concentrations of MGO solutions

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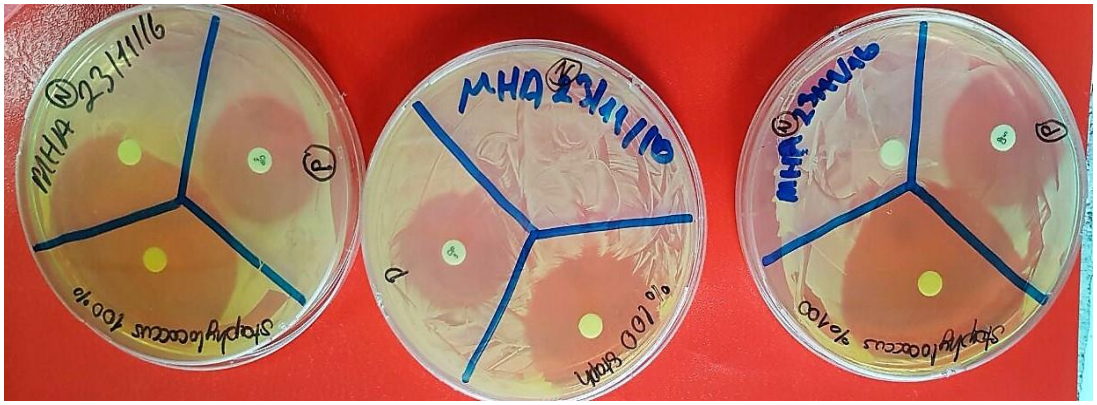

Figure 3. Inhibition zone formation of *Staphylococcus aureus*.

Table 3. Duncan test results for bacteria strains without distinction of concentration

Bacteria	Subset for alpha = 0.05	
	1	2
Pseudomonas aeruginosa	20.3333	
Escherichia coli		32.7778
Staphylococcus aureus		37.9444
Sig.	1.000	.140

Table 4. Duncan test results for concentration without distinction of bacteria strain

Concentration	Subset for alpha = 0.05	
	1	2
10%	24.0556	
20%	28.1111	
40%		38.8889
Sig.	.310	1.000

Conclusion

The biological activity of Manuka honey on various microorganisms has been demonstrated in previous studies [10]. However, this research focused on antimicrobial activity of methylglyoxal as separate chemical. The outcomes of this study were promising, and controlled clinical studies are needed to define the efficacy of methylglioxal in vivo.

Manuka Balının Ana Antibakteriyel Bileşeni Metilglioksal'ın Antimikrobial Aktivitesinin Değerlendirilmesi

Öz: Nektardan elde edilen dihidroksiasetonun, enzimatik olmayan dönüşümü ile oluşan metilglioksal (MGO), manuka balındaki temel antibakteriyel bileşendir. Bu çalışmanın MGO'nun *Escherichia coli*, *Pseudomonas aeruginosa* ve *Staphylococcus aureus* üzerindeki etkisini araştırmaktır. Tüm deneylerde hazır olarak alınan MGO (Sigma-Aldrich) kullanılmıştır. MGO'nun antimikrobiyal aktivitesinin ölçülmesi için agar difüzyon testi kullanılmıştır. Sonuçlar, test edilen

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tüm bakterilerin MGO çözeltilerine karşı anlamlı olarak duyarlı olduğunu göstermektedir. İstatistikler analizler, MGO'ya karşı *S. aureus'un* en hassas, *P. aeruginosa'*nın ise en az hassas olduğunu göstermektedir. Çalışma sonuçları umut vericidir ve MGO'nun potansiyelinin ortaya çıkarılması için ileri çalışmaların yapılası gereklidir.

Anahtar Kelimeler: Metilglioksal, Manuka balı, Disk difüzyon testi.

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