Manuka Honey can Inhibit S. Aureus Biofilm

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Received 2021 October 25**; Revised** 2021 December 20**; Accepted** 2021 December 24

Abstract
Honey has a height nutritional consider and has beneficial effectiveness on human health, so some peoples use it as food and medicine since time immemorial. The current study aimed to estimate the action of Manuka honey (MH) against Gram-positive biofilm.Where the effect of MH on inhibiting *S. aureu* biofilm was compared at different concentrations: 0.025, 0.5, 1, 1.5, and 2 (g). The evaluation of the anti-biofilm effectiveness of this honey was adopted using microtiter plate test (crystal violet) at measuring absorbance (490).The results proved that MH has an inhibitory effect on*S. aureu*biofilm at 1.5, and 2 (g(concentrations.The study concluded that 2g of Manuka honey had the best anti-biofilm activity.When applying this honey in practice for medical purposes, it is recommended to take into account the appropriate concentrations to obtain the best biological and therapeutic effects
Keywords: S. aureus,, Manuka honey, biofilm.

Introduction
Honey is known as a sweetness and tasty naturalistic product, with high nutritional consider. It is made by bees belonging to the genus Apis, which assemble nectar from plants or secreted by aphids [1]. One of the constants is that numerous plant bio-medical qualities can be expressed through honey. It has been proven that mono-flowered honey is more valuable than multi-flowered honey, as it is gained from the nectar of plants for specified sources [2, 3]. Manuka honey (MH) which is black in color, can be expressed as mono-flowering honey that can be acquired from *L. scoparium*or*L.polygalifolium*, descended from Myrtaceae and its growth is either in the form of a shrub or a small tree in some European countries [4,5]. The extensive area of biological activities of this species may be due to its rich content of phenols and antioxidant ability [6]. MH contains high levels of major and minor nutrients such as carbohydrates, proteins, vitamins, in addition to various derivative metabolites. All of these beneficial phytochemicals support their health benefits such as wound healing, anti-oxidant as well as anti-inflammatory activities [7, 8]. As well as,it has a high activity against non-peroxide bacteria which is mainly due to its unique content of methylglyoxal [9].Gram-positive organisms such as members of the genus Staphylococcus are a common major cause of bacterial infection, as these positive organisms colonize the skin and mucous membranes of the host. It is a commensal and pathogenic bacterium in humans with approximately 30% of the population colonized by this bacterium [10, 11].The common presence of host microorganisms increases opportunistic diseases, as it is a frequent source of infection [12]. Because many bacteria have developed resistance to antibiotics, there is a need to find alternatives [13]. Manuka honey has

the ability to reduce and eliminate bacterial activity, so it is one of the candidate alternatives to antibiotics as it is safe and natural.Therefore, the current study is an attempt to estimate the biological effectiveness of HM on the biofilm of positive bacteria.

Material and methods

Bacterial strain

A reference strain of Staphylococcus aureus was taken from clinical patients for the purpose of carrying out this study.Practically, the bacteria were isolated from swabs of wounds of chronic burn patients receiving treatment and health follow-up in an inpatient clinic in Basra city hospitals, Iraq.These bacterial isolates were assured as Staphylococcus aureus by means of Gram stain, oxidase and green pigment product assay.

Honey Treatment

The honey (*Manuka honey*) was purchased from the city of Basra, southern Iraq. To obtain the minimum inhibitory concentrations of MH, a range of concentrations (0.025 g, 0.5 g, 1 g, 1.5 g, 2 g) was generated with whole volume of 50 µl of nutrient broth in a 96-well plate.

Minimum biofilm eliminating concentration

Isolated bacterial strain was (aerobically) cultivated at 37 °C in 5 ml nutrient broth (Oxoid, Cambridge, UK) for 24 h. After the cultured microorganism reached the middle of the exponential phase, it was harvested and the optical density was set at $_{600}$= 0.05. Establish biofilms conform 96-well microtiter dishes in 200 µl NB comprising five diverse honey concentricity (0.025 g, 0.5 g, 1 g, 1.5 g, 2 g), by inoculate every well with 50 µL of balanced cells. Thereafter those plates were incubated at (37°C) for tow days.

Crystal violet assay (Microtiter plate assay)

After 48 h of incubation, plates were used to quantify Staphylococcus aureus, and biofilm growth, unbound planktonic cells were gently aspirated and discarded, and biofilms were steady with 200 µl 99% methanol for fifteen minutes. Using PBS, the biofilm cells were washed twice and with crystal violet all wells were stained with 200 µl for twenty minutes. Next two successive elutions, the cell-bound crystal violet was re-dissolved with 200 µl of 95% ethanol for 30 min, and 100 µl of ethanol was relocated to a fresh plate from each well for absorbance measurement at A_{490}.

Statistical analysis

To reach the minimum concentration that is sufficient to eliminate the bacteria biofilm by examining the microtiter plate, the resulting data were analyzed using SPSS version 25 software. To compare between the different concentration groups of honey with the control, the ANOVA test was used, followed by the Tukey test, taking into account the significant difference at P less than 05.

Results and Discussion

As shown in figure 1, the analysis data for the formation of *S. aureus* biofilms isolated from burn wounds showed that the concentration represents an important source of variance for the effect of the *Manuka honey* used. Considerable differences were observed between biofilms formative in the existence of various concentrations of this honey. Based on the results, *Manuka honey* was highly effective at concentrations of 1.5 and 2 (g) in preventing biofilm formation. In contrast, at concentrations of 1 and 0.05 (g) were less capable to stop biofilm forming than the rest, compared to the untreated control.

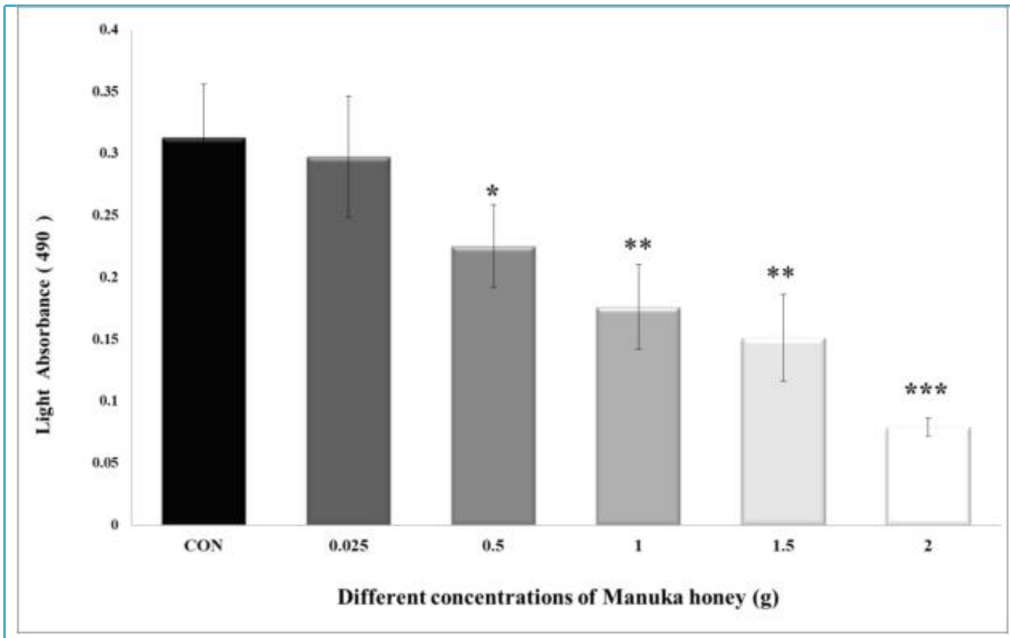

Figure 1: Effects of MH on biofilm formation of *S. aureus*. Data were processed using ANOVA (one way) and followed by Tukey's multiple comparisons, the symbol (*) indicates the significant difference compared to the control.

Natural honey consists of a high percentage of carbohydrates up to 82% in addition to water and the following minor secondary components: proteins, minerals, phytochemicals and anti-oxidants [15]. These minor components bear the responsibility for the bio-medical efficiency of honey in treating inflammations, burns, and others [16,17]. Methylglyoxal (MGO) is one of the main antibacterial components in Manuka honey, which comes from the conversion of another compound in MH known as dihydroxy-acetone, of which there is a high concentration in the nectar of manuka flowers [18,19]. This study proved that Manuka honey has a high performance against *S. aureus*, and this was consistent with what Lu et al., who used types of honey as factors to prevent the production biofilms by S. aureus bacteria [20]. In another recent study by Kot and colleagues investigating the activity of MH on the transcriptional profile of genes necessary for the forming of methicillin-resistant Staphylococcus aureus, they found that MH at a minimum level of MBIC significantly reduced the susceptibility of MRSA cells in biofilms [21].

Manuka honey has a remarkable ability to inhibit the formation of S. aureus biofilms due to the synthesized elements existing in honey such as: MGO, sugar, low pH, H2O2, phenols, flavonoids and others [22, 23]. On the other hand, leptosperin has been identified as a main benefactor to the antibacterial activity of this honey [24]. During the evaluation of the mechanism of manuka honey's activity against S. aureus, marked cellular changes were noted in S. aureus processed with suppressed concentrations [25].It has been proven that MH disrupts the methodical cell division operation of Staphylococcus aureus [26].

Conclusion

The study concluded that MH has anti-biofilm ability of Gram-positive *S.aureus*. It was also confirmed, that the effectiveness of Manuka honey against the growth of S. aureus bacteria was at its highest concentration 2 (g), so in clinical and practical uses, consideration should be given to its use in appropriate concentrations to extract high levels of anti-biofilm activity to increase biological and therapeutic effects

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