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Honey has antibacterial properties against *staphylococcus aureus* and *Pseudomonas aeruginosa*, both of which were isolated from an infected wound

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ABSTRACT

The function of antibacterial honey in infected wounds is not simply due to its high osmolality. we used pasture honey and manuka honey to investigate the sensitivity of 58 species of coagulase-positive Staphylococcus aureus isolated from infected wounds. The medicinal value of honey has been described in the world's first medical literature, and it has been noted that there have been antimicrobial properties and healing wounds since ancient times. The healing properties of honey are related to its antimicrobial properties, the ability to retain moisture, and high viscosity, which help to build up the barrier against infection. its immunomodulatory properties are also important in wound healing. Many bees have antimicrobial properties due to the enzymatic generation of hydrogen peroxide. However, even when hydrogen peroxide activity is banned, some types of honey (manuka honey) have the ability to fight germs. How it works can be linked to low honey pH and high sugar (high osmolarity), both of which are important to prevent bacterial growth. In vitro bactericidal activity of honey grade against antibiotic resistant causes various life-threatening diseases in humans. However, due to local and temporary variability in nectar source, some natural bees have a variety of antibacterial action.

INTRODUCTION

Honey is an ancient form of medicine for treating ulcers that have been “recently discovered” by the scientific community [1]. Honey was first mentioned in a Sumerian tablet dated 2100-2000 BC, which cites its use as a medicinal and medicinal plant. Pale honey was described by Aristotle (384-322BC) as "an effective remedy for sore eyes and bruises". Honey was used for both nutritional and medicinal purposes in many ancient cultures. Honey is still considered a nutritious food, a medicine, and an ointment, which is why a branch of alternative medicine has grown there. Honey-based therapies for various diseases, including bacterial infections, have become available in recent years, as well as other bee-related substances [1]. The antibacterial effect of honey is different and is not fully understood. several components of honey have been found to play an important role in the antibacterial activity of honey [2].

METHODS OF MEASUREMENTS OF ANTIBACTERIAL ACTIVITY

Despite the lack of understanding of certain mechanisms, antibacterial properties have been known to work for over a century. In 1892, Van Ketel became the first to describe the antimicrobial properties of honey [3]. The antibacterial component of honey is known as "inhibin", and thionine number "refers to the degree of purification in which a particular type of honey retains its antibacterial activity.These names were coined by doled and Rittenhouse in 1955, and refer to the formation of a scale from 1 to 5 associated with 5% purification of honey, from 25% to 5% (w / v) .Hydrogen peroxide, a common antibacterial ingredient in June, was considered an inhibitor. the molecule in honey inhibin exists, as well as its relationship to honey content [4].

The antibacterial activity of honey has also been tested using a variety of methods. To measure the tendency of bacteria in June, a number of methods can be used, including a (low) purification test, a well / disk diffusion assay, agar purification methods, and a time-killing assay, these procedures are commonly used in microbiological laboratories, according to the guidelines. CLSI. (clinical & laboratories Standards Institute). For example, in agar diffusion experiments, a small amount of honey or honey solution is applied to a 6mm wide diameter that has been carefully cut into a nutritious agar plate that is already incorporated into a microbial culture [3]. As the plates thicken, the honey spreads into the air. Honey is mixed with nutrient agar or nutrient broth where bacterial culture is grown in other ways. Testing for micro- or microdilution broth is the most common test for bacteria. this process involves making twice as much honey as the broth is then distributed to 96 microtiter tubes or plates [3].

Each tube or source is infected and enriched with standard test microorganisms. spectrophotometry is used to measure microbial growth. and is defined as the lowest concentration of night incubation [5] .The spectrophotometric determination of growth or absorption measurement using fluorimetry provides high sensitivity, especially when low honey concentrations are used. Broth microdilution testing, in which inhibition of bacterial growth is assessed spectrophotometrically, is the most appropriate method for its sensitivity. In line with standard plate calculation, this method is often used to determine MIC and MBC values. Additional methods, such as direct microscopic calculations or growth index indicators (e.g., specific lactic acid metabolite), may be used [6]. In general, it is important to remember that the results will be strongly influenced by scientific strategy and perspective,

which should be considered when comparing the results of different approaches [7].

FEATURE OF HONEY RELEVANT TO ITS ANTIMICROBIAL ACTIVITY

Many variables have been linked to honey's antibacterial activity, including's its high viscosity 'which is mostly owing to its high sugar content and low water content, and serves to establish a protective barrier against infection.Furthermore,the moderate acidity and hydrogen peroxide concentration have antibacterial properties [8].

LOW WATER ACTIVITY

The number of water molecules that are not attached to food is measured by water activity; The less water it binds, the harder it is for bacteria to thrive in food. Honey water activity (aw) ranges from 0.562 and 0.62, indicating that it has very little water available to allow the growth of any germs, and is below the threshold where bacterial growth is completely inhibited (aw 0.94-0.99). Either way. , the osmosis process is a major component of antibacterial honey activity, and the degree of inhibition depends on both the concentration of honey and the type of bacteria being investigated [3]. Due to its high sugar content, pure honey has the ability to completely inhibit bacterial growth; high sugar content produces osmotic pressure on bacterial cells, causing water to be transported outside the cells by osmosis. In hypertonic sugar solution, cells become dehydrated and can grow and proliferate. When honey is diluted with body fluids near the diseased area, its antibacterial effect is reduced.

although high concentrations of sugar and low water activity will limit the growth of many bacteria such as staphylococcus aureus, studies have shown that in the presence of "fake" honey, no effective bacterial suppression occurs [3]. In addition, research on the effect of honey on the growth of bacteria such as *S.aureus*, which has a high tolerance for low water performance is due to other factors. To completely prevent *S.aureus*, less than 0.86 aw is required, which is equivalent to 29 percent honey (v / v) [3]. *S.aureus* at a rate of 17 percent. In addition, during the 8-hour incubation period with a concentration of 1.8 (v / v) of Manuka honey completely inhibited *S.aureus* growth. Manuka honey is distinguished from other bees by its high methylglyoxal content, which comes from the nectar of the genus Leptospermum. The function of Manuka honey antibacterial, instead of hydrogen peroxide, is this molecule. All 58 species of *S.aureus* were prevented by 2-3 percent (v / v) Manuka honey and between 3 and 4 percent (v / v) and pasture honey in the same study using honey from the same region in New Zealand. This is obviously much less than the 29 percent required if the effect was specifically based on water activity [9,10]

ACIDITY

Most bacteria grow in a pH range between 6.5 and 7.5, honey acid, between 3.2 and 4.5, is another important factor in its antibacterial activity. Organic acids, especially gluconic acid, found in 0.5% (w / v), contribute to acidity [11,12]. According to White ethel. Gluconic acid is an antibacterial agent created when glucose is released and endogenous glucose is oxidized by endogenous glucose oxidable. In plain june, low pH can be an active antibacterial component, but if diluted in food or human fluids, the pH will not be sufficient to prevent the growth of many bacterial species [4].

HYDROGEN PEROXIDE

Hydrogen peroxide is a powerful oxidizing and sanitizing agent.it is synthesized enzymatically in June and may contribute to its antibacterial properties [13]. Although the enzyme glucose oxidase is naturally present in June. low pH conditions make it inactive in unmixed June. However, when the honey is purified, glucose oxidase is released, allowing it to work on endogenous sugars to form hydrogen peroxide. Indeed, a 30-50% bee purification can produce a maximum amount of hydrogen peroxide between 5 and 100g H2O2 / g honey (corresponding to 0.146-2.93mM) [4]. According to Bang et al. the production of hydrogen peroxide in some honey samples may increase steadily over time, depending on the purification used [14]. Indeed, H2O2 June levels can reach 2.5 mM in 30 minutes, and after prolonged incubation, they may double. The amount of hydrogen peroxide in a significant number of honey samples has been established by researchers [15 - 20]. In this study, the mean H2O2 concentration was 1mM*E.coli*. was killed in 15 minutes using the same concentrate hydrogen peroxide concentration (1mM to 2.5mM) [21,22]. A linear relationship between the level of hydrogen peroxide in June and the antibacterial activity has also been found. levels of hydrogen peroxide mixed juncture [23]. It is noteworthy that the presence and activity of catalase affects the amount of hydrogen peroxide in June. Indeed, Weston found an association between the values of these enzymes and glucose oxidase as well as the values of this enzyme and glucose oxidase and the antibacterial effectiveness of the final product [24]. Weston hypothesized that high glucose oxidase levels are associated with high levels of hydrogen peroxide in the body. Low levels of catalase also indicate high levels of hydrogen peroxide. It was once thought that the antibacterial effect of purified honey was due only to hydrogen peroxide, and that the antibacterial activity of honey could be completely eliminated by adding catalase [25 - 26].

NONPEROXIDE ANTIBACTERIAL COMPOUNDS

Honey has been shown in several studies to have high levels of phenolic compounds, which may have an effect on its antibacterial effect. phenolic acids and flavonoids have been identified as important components of the antimicrobial properties of honey in the early 1990s [27]. The phenolic acid content of honey is influenced by its plant origin and location, as well as the source of nectar. In addition, it is clear that the season contributes to the concentration of the total phenolic (TP) acid content of honey. To illustrate this, Lachman et.al. examined the polyphenol content of honey varieties harvested from May to August 2006 and found that the highest TP acid content was found in the June collection (approximately 170.21 mg / kg) and July (average 170.21 mg / kg) and July. (Average 163.32 mg / kg), while the lowest in the samples collected during the other months (83.60 mg / kg) [28]. The phenolic content of honey is also affected by its type. The values were very low in Lachman et al.'s ranging from 82.5 to 242.5 mg / kg honey, which has flavonoids and phenolic acids as the main phenols [28]. Compared to Manuka honey (424-1575mg / kg) collected at the same time and from the same place, Manuka honey has a concentration of phenolic acid between 430-2706 mg / kg [29]. Viper bugloss and its phenolic acid concentration were found to be very low, ranging from between 132.17 0.05 and 727.77 0.23 mg / kg [30]. Bisaya and Pierzynski noted that all honey samples they tested contained evidence of phenolic chemicals such as echogenic acid, vanillic acid, caffeic acid, syringic acid, myricetin, and apigenin, although in different concentrations [31] .

THE NATURE OF NONPEROXIDE

Snow and Manley-Harris used *S.auerus* as an alkaline honey solution to investigate the effects of antibacterial peroxide in Manuka honey. The effect of 10-fold catalase on an antibacterial assay was investigated, but there was no statistically significant difference in results between normal catalase value and 10-fold, indicating that no antibacterial peroxide activity could be caused by the remaining hydrogen peroxide. [32].

MATERIALS AND METHODS

STUDY AREA

The study area is where the sample isolated from infected wound.

ETHICAL CLEARANCE

Ethical approval is approved by the health service management board.

SAMPLE COLLECTION

The swap sticks containing a total of 52 samples from infected wound were collected from microbiology laboratory for isolation and characterization of *Staphylococcus aureus* and *Pseudomonas* aeruginosa

Isolation and identification of test organism

The samples form swap containing pus form patients were inoculated onto the surface of nutrient agar plate and inoculated for 24 hours at 37□. Each colony was isolated in a pure culture such as colony form, elevation of colony and colony margin were observed. further microbial identification (Gram staining and biochemical characterization including catalase, coagulase and oxidase test characterization including catalase, coagulase and oxidase test) was based on the methods of holt et.al. (13)

Confirmatorytest of natural (raw) honey

A tablespoon of honey was slowly swirled into a tumbler of warm water. this made it made it easier to see if it dissolved in the water. The majority of raw honey clumps together and sinks as a solid lump or remains trapped on the spoon as a lump. Aa candle wick soaked in honey was lit on fire to see if there was any water in the honey that would prevent it from burning [34].

PREPARATION OF HONEY CONCERNTRATION

Honey was used that was both natural (raw) and processed. In sterile distilled water, different concentrations of each honey were created, contributing 25% percent v/v,50 percent v/v,75%v/v, and 100%v/v. This was accomplished by dissolving the appropriate quantities of honey into sterile distilled water [34].

ANTIBACTERIAL ACTIVITY OF HONEY

Honey's antibacterial activity was tested using the agar well diffusion method. The standard inoculums (0.5MacFarland standard) were injected into the skin's surface. For even, a Mueller Hinton agar plate and a sterile glass were used. Distribution through media using a sterile pipette, five wells were created. Cork borer was used, and varying concentration of corn borer were used in each well.25%v/v,50%,75%v/v, and 100v/v of honey the plates were incubated at a temperature of observed for zone of inhibitions at 37□ for 24 hours. This is an in-vitro study. The trial was composed to the usage of Gentamicin 50mg/ml. As a control, (micro lab) was used. The antibacterial activity was measured in micrograms per gram of microorganism. The honey's average inhibitory zone diameter in millimetres [34].

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE EXTRACTS

Using the broth dilution procedure, the honey's MIC was determined .2ml of 100v/v honey was added to a test tube holding 2ml of water to make two-fold serial dilutions of the honey. Nutrient broth, resulting in a solution with 50v/v of the extract. The procedure is repeated in order up to test tubes, resulting in total of five test tubes resulting in concentrations of 50,25,12.5,6.25, and equivalent test standard organism were placed in test tubes and cultured at 37□ for 24 hours. The test tubes were examined after incubation for checking turbidity to see if it's growing [35].

DETERMINATION OF MINIMUM BACTERIAL BACTERICIDAL CONCENTRATIONS (MBC) OF THE EXTRACTS

The test tubes that did not demonstrate apparent growth were used for MBC determination based on the MIC results. A total of 0.1ml was aseptically put over Mueller Hinton agar's surface plates. The plates were incubated for 24 hours at 37□. Malaysian Broadcasting corporation (MBC) the extracts were noted as having the lowest concentration. On Mueller Hinton agar plates, it grew at less than 99% [36].

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