

Available online on 30.05.2021 at <http://jddtonline.info>

# Journal of Drug Delivery and Therapeutics

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Research Article

## The Effectiveness of Giving Natural Honey as a Bactericide Against the Growth of *Escherichia coli* Bacteria

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### Article Info



#### Article History:

Received 29 March 2021  
 Reviewed 16 May 2021  
 Accepted 21 May 2021  
 Published 30 May 2021

#### Cite this article as:

Djojoputro M, Gultom M, The Effectiveness of Giving Natural Honey as a Bactericide Against the Growth of *Escherichia coli* Bacteria, Journal of Drug Delivery and Therapeutics. 2021; 11(3):183-187

DOI: <http://dx.doi.org/10.22270/jddt.v11i3.4817>

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### Abstract

Honey is one of the natural treatments with antibacterial effects such as hydrogen peroxide, low pH and short water activity that may inhibit bacterial growth. This study aimed to determine the activity of honey as antibacterial to bacteria *Escherichia coli*. Antibacterial activity test was done in vitro through diffusion method by using disk disc in MHA media then measuring the diameter of a clear zone that inhibition zone form growth of *Escherichia coli* bacteria and scaled it millimetre unit. This research was done in the laboratory of the Faculty of Medicine Universitas Kristen Indonesia. The concentrations used are 10%, 30%, 50%, 70%, 100% by using sterile equates as honey diluents. The control used was chloramphenicol, the results obtained at a concentration of 10% with a zone of 7.6 mm inhibition of honey had been able to inhibit the growth of *Escherichia coli* bacteria, and the optimum concentration was 100% with a zone of 24.5 mm. This test showed that natural honey could inhibit bacteria, which conclude that honey might be used as alternative medicine in society.

Keywords: Antibacterial effect, *Escherichia coli*, honey

## INTRODUCTION

Honey is a thick liquid produced by honey bees from flower nectar. Bees suck various kinds of flower and fruit juices, and then they are collected in their bodies and then taken to the hive and formed into honey.<sup>1</sup> Honey is a natural liquid that generally has a sweet taste produced by honey bees from the extract of plant flowers (flora nectar).<sup>2</sup> Honey is an inverted sugar (a mixture of glucose and fructose) dissolved in 14% to 20% water with a small amount of organic acid, along with a mixture of minerals and vitamins.<sup>3</sup>

Honey can be used as an antibiotic. The antibiotic power of honey is because honey contains flavonoids and has an antibacterial mechanism consisting of honey osmotic pressure, acidity, and inhibin compounds for several diseases and infections, one of which is diarrhoea.<sup>2</sup> The bacteria that cause diarrhoea are Shigella, Vibrio cholera, Salmonella (non-typhoid), Campylobacter jejuni, and *Escherichia coli*. *Escherichia coli* is a bacterium commonly found in the human intestine.<sup>4</sup> When consuming unclean food, bacteria that are normal flora in the intestine can turn into active pathogens that infect the digestive tract. It is exacerbated when the immune system decreases. Damage to the digestive tract structure due to infection with *Escherichia coli* bacteria will reduce the function of the digestive tract, causing diarrhoea.

Inhibition of honey from cultivated bees in Padang Pariaman against Staphylococcus aureus and *Escherichia coli* using the disk diffusion method, the results obtained at a

concentration of 30% inhibited the growth of Staphylococcus aureus with an inhibition zone of 10.52 mm on *Escherichia coli* with an inhibition zone of 9.72 mm.<sup>5</sup>

The best concentration that inhibits Staphylococcus aureus bacteria is 60%, with an inhibition zone diameter of 14.9 mm, while the best concentration that inhibits *Escherichia coli* bacteria is 50% with an inhibition zone diameter of 12.55 mm. Based on this research report, the authors are interested in examining the effects of natural honey obtained from Riau Province, Dumai district, as an antibacterial against the growth of *Escherichia coli* bacteria. The problem that will be answered in this research is "How is the effectiveness of giving honey as an antibiotic against the growth of *Escherichia coli* bacteria? Furthermore, at what concentration does honey have the effect of inhibiting the growth of the *Escherichia coli* bacteria? The research aims to determine the effectiveness of honey as an antibacterial against the growth of *Escherichia coli* bacteria and determine the concentration of honey that effectively inhibits the growth of *Escherichia coli* bacteria.

## LITERATURE REVIEW

Honey is a natural substance produced by honey bees from flower extract called nectar, or plant secretions collected by honey bees, converted and stored in the beehive for ripening. Honey has many health benefits, including antibacterial, antioxidant, and many vitamins, including Thiamin, Riboflavin, and Niacin.<sup>6</sup> The properties of honey were introduced by Hippocrates (460 BC-370 BC), who used

honey as an expectorant and cleanser of wounds on the skin and ulcers.<sup>7</sup>

There are several ingredients in honey, such as a) Sugar - Monosaccharides represent about 75% of the sugar found in honey, along with 10-15% disaccharides and small amounts of other sugars. The sugar content in honey is responsible for energy value, viscosity, hygroscopy and granulation; b) Water - Water is the second most significant content of honey. The water content in honey varies from 15-21%. Moisture content is one of the essential characteristics affecting the physical properties of honey such as viscosity, crystallization, colour, aroma, taste, density, solubility and conservation; c) Vitamins: Honey contains small amounts of vitamins, especially B complex vitamins, which are derived from pollen in suspension. The vitamins found in honey include thiamine (B1), riboflavin (B2) and nicotinic acid (B3). These vitamins can survive due to the low pH levels in honey; d) Minerals: The mineral content in honey also differs depending on the type of honey (0.04% in sweet honey and 0.2% in bitter honey). These minerals consist of macro minerals (potassium, calcium and sodium) and micro (iron, manganese, copper); e) Phenolic Compounds: Phenolic compounds are a heterogeneous chemical group, which consists of 10,000 compounds. Phenolics can be divided into non-flavonoids (phenolic acids) and flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidins, isoflavones and chalcones).

These compounds function as antioxidants, eliminate free radicals and inhibit lipid oxidation. The main functional components of honey are flavonoids, which can significantly contribute to the total antioxidant activity and as antibiotics of honey which have beneficial effects on human health; f) pH: pH helps inhibit the growth of micro-organisms in honey. Honey has a pH level between 3.2-4.5 to inhibit the growth of several pathogenic bacteria that have a minimum pH to grow around 7.2-7.4, such as *Escherichia coli*, Salmonella Typhi, Pseudomonas aeruginosa, and Streptococcus pyogenes. The acidity value is low enough to act as an inhibitor for bacteria, and g) Hydrogen Peroxide: Hydrogen peroxide is the primary source of honey as an antibacterial. Hydrogen peroxide is produced from the reaction of the enzyme glucose oxidase (glucosidase) in honey, especially glucose. The glucosidase enzyme will cause a reaction that produces gluconic acid and hydrogen peroxide. This enzyme will work optimally in the presence of water. Increasing the activity of honey as an antibacterial requires less concentrated honey and water levels. The hydrogen peroxide resulting from glucose reaction in honey with water is about 1mmol / litre of honey.

Meanwhile, the medical use of hydrogen peroxide is around 3% by volume. There is no need to worry about

damage to tissues in the body.<sup>8</sup> It has been scientifically proven that bacteria cannot live and thrive in honey. Honey contains elemental potassium (potassium), which is an element that prevents moisture, which is the key to bacterial growth (inhibitory effect). Honey has antibiotics such as interferon, anti-viral, and inhibin which can inhibit bacterial growth.<sup>9</sup>

Flavonoids in honey are derivatives of phenolic compounds that act as antiseptics that are bacteriostatic and bactericidal. Bactericidal properties can cause protein coagulation which releases protein and phenol bonds to enter the skin. Flavonoids interact with bacterial cells through an adsorption process that involves hydrogen bonding and produces an antimicrobial effect.<sup>10</sup>

Honey has sufficient osmolarity to inhibit bacterial growth. The high saturation glucose content in honey can strongly interact with water molecules. Lack of water content can inhibit bacterial growth. Water activity in honey is 0.562-0.620. In general, bacteria will not grow on media that has low water activity.<sup>11</sup> The pH of honey which is acidic in the range of 3.2-4.5, will inhibit the metabolism of Gram-negative bacteria. Inhibition of bacterial metabolism causes bacteria to easily experience lysis so that it can inhibit bacterial growth. In addition, hydrogen peroxide is cytotoxic for bacterial cells. The antimicrobial process of hydrogen peroxide causes oxidation and hydroxyl free radicals, which are more toxic to bacteria, thus facilitating damage to bacterial cells.

*Escherichia coli* - *Escherichia coli* is part of the microflora that generally exists in the digestive tract of humans and warm-blooded animals. *Escherichia coli* belongs to heterotrophic bacteria, which obtain their food in organic substances from their environment because they cannot compile the required organic substances themselves. Organic matter is obtained from the remains of other organisms. These bacteria break down organic substances in food into inorganic substances, including CO<sub>2</sub>, H<sub>2</sub>O, energy and minerals and decomposing bacteria function as decomposers and nutrient providers for plants in the environment. *Escherichia coli* is a short Gram-negative stem (cocobacil) germ, measuring 0.4 - 0.7 µm x 1.4 µm partially positive, and some strains have a capsule. *Escherichia coli* is a type of mesophilic bacteria, namely bacteria whose optimum growth temperature is 15 - 45°C and can live at a pH of 5.5-8. *Escherichia coli* usually colonizes the digestive tract within a few hours of entering the body and establishing a mutualistic relationship. At the same time, non-pathogenic strains of *Escherichia coli* can become pathogenic when there is disturbance indigestion and immunosuppression in the host.<sup>12,13</sup>

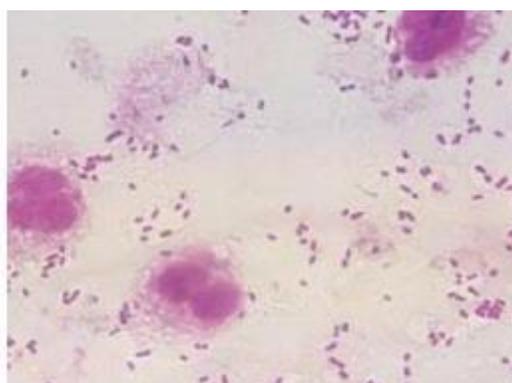
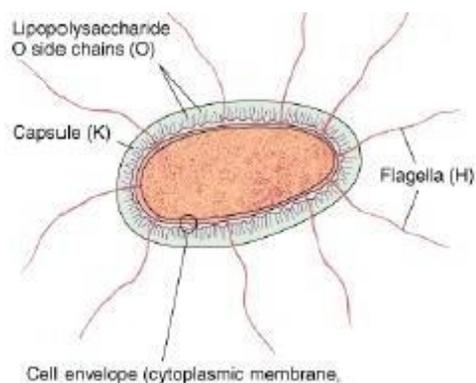


Figure 1: *Escherichia coli*.<sup>14</sup>

Based on nature and virulence characteristics, *Escherichia coli* is classified into five groups, namely: a) Enteroinvasive *Escherichia coli* (EIEC) - These bacteria cause a disease similar to shigellosis by attacking intestinal mucosal epithelial cells; b) Enteroaggregative *Escherichia coli* (EAEC) - These bacteria cause acute and chronic diarrhoea (over 14 days) by adhering to the intestinal mucosa, producing enterotoxins and cytotoxins, resulting in mucosal damage and large mucus secretions; c) Enteropathogenic *Escherichia coli* (EPEC) - This bacterium is a significant cause of diarrhoea in infants, especially in developing countries. These bacteria attach to the small intestine. EPEC infection can result in complicated and chronic watery diarrhoea;<sup>15</sup> d) Enterotoxigenic *Escherichia coli* (ETEC) - These bacteria produce several strains of exotoxins, which are heat-labile (LT) and heat-stable toxins (ST). ETEC infection can cause symptoms of abdominal pain, sometimes accompanied by fever, vomiting, and blood in the stool; and e) Enterohemorrhagic *Escherichia coli* (EHEC) - Serotype E. coli that produces verotoxin, namely EHEC O157: H7. EHEC produces a toxin that has similar characteristics to the Shiga toxin produced by the *Shigella dysenteriae* strain. Verotoxin O157: H7 can destroy the mucosal wall causing bleeding.<sup>16</sup>

**Pathogenicity of *Escherichia coli*** - Pathogenicity is the ability of a pathogenic agent to cause disease. Pathogenicity includes the initiation of the infectious process and the mechanisms that cause disease symptoms. E. coli bacteria can infect the body and are obtained if the number of bacteria that enter the body is less than 100 E. coli bacteria cells. O157: H7 can also be classified according to pathogenic factors in the Enterohaemorrhagic E. coli (EHEC) group because it can cause hemorrhagic colitis through the production of cytotoxins (called Shiga toxin). Based on its pathogenicity, E. coli is classified into two types of pathogens, namely opportunistic and non-pathogenic pathogens. Several factors that influence bacterial virulence are the ability to adhere and invade host cells and tissue toxins, including lipopolysaccharide exotoxins, peptidoglycan and antifagocyte factors. E. coli O157: H7 capable of producing Stx1, Stx2, or both. *Escherichia coli* O157: H7 can produce Shiga toxin, known as Shiga toxin E. coli (STEC). E. coli O157: H7 transmission can be caused by faeces of healthy animals that initially suffered from diarrhoea whose meat was contaminated due to incorrect handling errors starting from the slaughtering process to consumption. Infection with E. coli O157: H7 bacteria can produce hemolytic uremic syndrome (HUS), characterized by hemolytic anaemia, thrombocytopenia, and damage to the kidneys.<sup>17,18</sup>

Table 1: Classification of bacterial growth inhibition responses

Light zone diameter	Growth inhibition response
≥ 20 mm	strong
16 - 20 mm	middle
1 - 15 mm	weak
0 mm	nothing

Based on Table 1, it can be seen that if the diameter of the inhibition zone is greater than 20 mm, the response to growing resistance is strong. At 16-20 mm, growth resistance is moderate, and 1-15 mm growth resistance is weak, while if 0 mm, there is no response to growth restriction.<sup>19</sup>

## RESEARCH METHOD

This study is an experimental study by testing the inhibition power of natural honey on the growth of *Escherichia coli* bacteria. This research was conducted from August 2017 to November 2017 at the Microbiology Laboratory of the Faculty of Medicine, Christian University of Indonesia. The materials tested in this study were natural honey obtained from Riau Province, Dumai district. The samples of this study were 15 *Escherichia coli* bacteria ATCC 25922 obtained from the University of Indonesia. The research instruments used were as follows: Petri dishes, test tubes, test tube racks, filter paper, paper holes, tweezers, bunsen, Erlenmeyer tubes, sterile cotton sticks, and micropipettes. The research method used the Kirby Bauer Diffusion method with the following procedure: a) The bacteria were diluted by mixing 1 ose of the *Escherichia coli* bacterial suspension into a test tube containing Mc Farland's solution; b) Homogenized using a vortex, and the turbidity is standardized with a concentration of 0.5 Mc Farland so that the number of bacteria meets the sensitivity test standard, namely: - / ml; c) Then the standardized bacterial solution was smeared on the Mueller-Hinton Agar growth medium; d) Sterile filter paper discs soaked for 15 minutes in each variable concentration of natural honey, then placed on the surface of Muller-Hinton Agar aseptically; and e) Then the media that we made earlier is incubated into the incubator with a temperature of C for 24 hours, measured the diameter of the resistance zone formed using a calliper.

## RESULT AND DISCUSSION

Based on research conducted by testing the activity of natural honey against *Escherichia coli* ATCC 25922 in vitro using Mueller Hinton Agar media, by looking at the formation or absence of the inhibition zone diameter tested by the Kirby-Bauer method.<sup>20</sup> Obtained results:

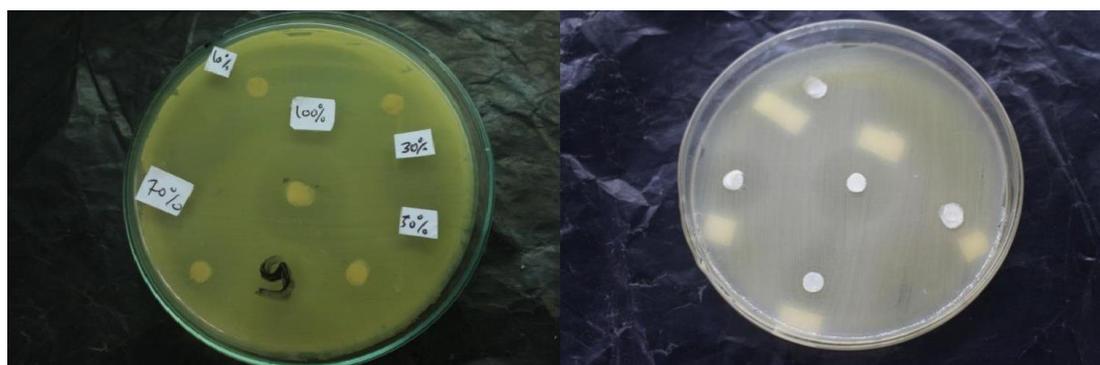


Figure 2. Test results that show an inhibition zone

The tests carried out using filter paper with several different concentrations of honey against *Escherichia coli* bacteria can be seen in Figure 2. An inhibition zone is formed in Mueller Hinton Agar media, given *E. coli* ATCC bacteria with a honey concentration of 10%, 30%, 50 %, 70%, 100%. The concentration of 10% shows a small inhibition zone, and at a concentration of 30%, it begins to enlarge slightly. At a

concentration of 50%, it enlarges the inhibition zone formed around the disc paper, and the concentrations of 70% and 100% are getting bigger. It indicates that the higher the concentration of honey, the higher the inhibition zone is formed, which means that honey as an antibacterial agent increases with the concentration.

Table 2: The results of the honey sensitivity test to *E. coli* bacteria

Number of Samples	10%	30%	50%	70%	100%
1.	8 mm	9 mm	17 mm	19 mm	23 mm
2.	7 mm	9 mm	19 mm	20 mm	23 mm
3.	8 mm	10 mm	15 mm	19 mm	21 mm
4.	9 mm	11 mm	19 mm	20 mm	21 mm
5.	8 mm	9 mm	16 mm	17 mm	28 mm
6.	8 mm	9 mm	13 mm	14 mm	27 mm
7.	7 mm	8 mm	23 mm	26 mm	29 mm
8.	7 mm	8 mm	10 mm	12 mm	24 mm
9.	8 mm	11 mm	19 mm	21 mm	24 mm
10.	7 mm	8 mm	20 mm	22 mm	23 mm
11.	8 mm	9 mm	18 mm	22 mm	23 mm
12.	8 mm	9 mm	16 mm	22 mm	27 mm
13.	7 mm	8 mm	15 mm	19 mm	23 mm
14.	8 mm	9 mm	16 mm	21 mm	28 mm
15.	7 mm	8 mm	15 mm	20 mm	24 mm
Average	<b>7,6 mm</b>	<b>8 mm</b>	<b>16,7 mm</b>	<b>19,6 mm</b>	<b>24,5 mm</b>
Chloramphenicol control			30 mm		

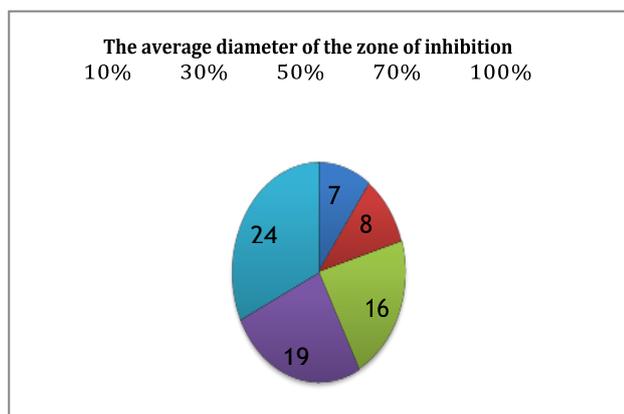


Figure 3: Graph of Average inhibition zone diameter

The observations made after incubation for 24 hours at 37°C obtained variations in the inhibition zone, as shown in table 1 and graph one above. The average diameter of honey with a concentration of 10% was 7.6 mm, a concentration of 30% was 8 mm, a concentration of 50% was 16.7 mm, a concentration of 70% was 19.6 mm, and a concentration of 100% was 24.5 mm.

Based on the research that has been done, it can be seen that using natural honey can suppress the growth of *Escherichia coli* bacteria with a honey concentration of 10%, 30%, 50%, 70%, 100%. In the test with this concentration, the inhibition power of honey against *Escherichia coli* was obtained as follows. The concentration of honey and distilled water was 10%, the inhibition zone diameter was 7.6 mm, 30% inhibition zone diameter was 8 mm concentration, 50% inhibition zone diameter was 16.7 mm concentration, 70% inhibition zone diameter was 19.6 mm, and the concentration was 100% inhibition zone diameter of 24.5 mm. The antibiotic control used was chloramphenicol which

produced an inhibition zone diameter of 30 mm. In the research conducted by Nurul Eliza in 2010, the effect of giving honey on *Staphylococcus aureus* and *Escherichia coli* bacteria in-vitro using the diffusion method using disc discs and the concentrations used were 25%, 50%, 75%, 100%. The zone of inhibition against *Staphylococcus aureus* was obtained in the succession of 8; 9; 10; 11 mm, while the *Escherichia coli* was 7; 9; 10; 11 mm.<sup>21,22</sup> In a study conducted, it was found that the inhibitory power of honey from cultivated bees in Padang Pariaman against *Staphylococcus aureus* and *Escherichia coli*, the study used the diffusion method, obtained results at a concentration of 30% that had inhibited the growth of *Staphylococcus aureus* by 10.52 mm inhibition zone and *Escherichia coli* with 9.72 mm inhibition zone. The best concentration in inhibiting *Staphylococcus aureus* bacteria was 60%, with an inhibition zone diameter of 14.9 mm, while the best concentration in inhibiting *Escherichia coli* bacteria was 50% with an inhibition zone diameter of 12.55 mm.<sup>23,24</sup>

In a study conducted by Wachidah Rizky Nurlailatul et al. in 2016, it was found that the concentration of forest honey (*Apis dorsata*) solution affected the growth inhibition of *Porphyromonas gingivalis* gingivitis dominant bacteria (in vitro study) using the post-test only control group design method, the concentration used 15 %, 30%, 60%, 90%. The mean value of the inhibition zone at a concentration of 15% is 9.75 mm, a concentration of 30% is 11.12 mm, a concentration of 60% is 13.03 mm, and a concentration of 90% is 16.07 mm.<sup>25</sup>

The results in this study compared with the results of research conducted other researchers is that there is a slight difference in the average value of the inhibition zone. It is thought to be caused by the varying quality of the honey itself, depending on the climate, ambient temperature, the type of botanical plant used, and the honey bee species. This study obtained the same data as the two previous studies

(Nurul and Retno) that honey can inhibit the growth of *E. coli* bacteria.

Honey has high osmotic pressure and acidity with a pH range of 3.2 - 4.5 and acid content of 0.17-1.17%, especially gluconic acid, which can reduce the growth of bacteria and organic compounds that are antibacterial such as flavonoids [25]. Flavonoids can damage cell membranes by inhibiting macromolecular synthesis. Flavonoids can also depolarize cell membranes and inhibit the synthesis of DNA, RNA, and proteins that have been observed in *E. coli*. Flavonoids can inhibit nucleic acid synthesis, inhibit cytoplasmic membrane function, and inhibit energy metabolism in bacteria.<sup>26,27</sup>

## CONCLUSION

Based on the research that has been done, it can be concluded: a) The effectiveness test of natural honey at the lowest concentration (10%) can inhibit the growth of *Escherichia coli* bacteria with an inhibition zone diameter of 7.6 mm and the best concentration (100%) with an inhibition zone of 24.5 mm; b) Natural honey without distilled water has a better inhibitory power against *Escherichia coli* bacteria, than the other groups diluted with distilled water; and c) The results of this test indicate that natural honey can inhibit bacteria so that honey can be used for alternative medicine in the community. So it can be suggested that: a) The high inhibition power of honey against *Escherichia coli* bacteria, Indonesian people are encouraged to use more honey as the immune system and at the same time reduce or avoid the use of antibiotic drugs, and further research is needed on the content of honey and its properties and effects. Honey against the growth of other bacteria.

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