Assessment of the antibacterial activity of goat milk kefir on *Escherichia coli* ATCC 8739 and *Salmonella enteric* subsp. *enterica serovar typhimurium* ATCC 14028 using a well diffusion method

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Abstract. Kefir is a fermented dairy product that uses kefir grains, which contain lactic acid bacteria and yeast, as a starter. Kefir has various health-promoting properties as a prebiotic food product. The purpose of this research was to measure the antibacterial activity of goat milk kefir against the pathogenic bacteria *Escherichia coli* ATCC 8739 and *Salmonella enteric* subsp. *enterica serovar typhimurium* ATCC 14028 using a well diffusion method. A total of five treatments were tested: kefir grain, kefir curd, kefir whey, distilled water as a negative control, and tetracycline antibiotic as a positive control. The research used a Completely Randomized Design (CRD), and the data were analyzed by ANOVA if it showed significant effects by Least Significant Difference Test (LSD). The highest antibacterial activity against *E. coli* ATCC 8739 was seen with kefir curd, 2.98±0.65 mm, and the lowest with kefir grains, 2.82±0.83 mm. Similarly, antibacterial activity against *S. typhimurium* ATCC 14028 was lowest with kefir grains, 2.22±1.05 mm, and the highest with kefir curd, 2.34±1.65 mm. The results showed that kefir goat milk, both curds, whey and kefir grains have potential as antibacterial against pathogenic *E. coli* ATCC 8739 and *S. enteric* subsp. *enterica serovar typhimurium* ATCC 14028.

1. Introduction
Kefir is a fermented milk product that uses kefir grains as the starter culture. Kefir is characterized by distinctive smell and flavor, where smell and flavor of kefir like of yeast, and the effervescent effect in the mouth [1,2,3]. The major component contribution product of kefir such as lactic acid, ethanol and CO2, which desired its viscosity, sour taste and a low ethanol content, the minor components contributing to the flavor composition fermented products, such as diacetyl, acetaldehyde, ethyl and amino acids [2,3]. Kefir also contains live microflora derived from the kefir grains and mixed cultures contain of lactic acid bacteria (*Lactobacilli, Lactococci*, and *Leuconostoc* spp.), acetic acid
bacteria (*Acetobacteraceti*), and yeasts (*Saccharomyces cerevisiae*, *Candida kefir*, and *Kluyveromyces marxianus*) [4].

Various fermented products are known and recommended for consumption as probiotics and prebiotics. According to [5], probiotics are beneficial if consumed by humans and animals in amounts above 6 log cfu g\(^{-1}\) and recommended around 8–9 log cfu g\(^{-1}\). [6,7,8,9], there are kefir products in the world contain differences microbial. But, several research have showed the health benefits of this beverage, and the result of isolated from kefir grains have potential probiotic [10], [11,12].

The purpose of this research was to observe and measure the antibacterial activity of goat milk kefir against the pathogenic bacteria *Escherichia coli* ATCC 8739 and *Salmonella enterica* subsp. enterica serovar typhimurium ATCC 14028 using a well diffusion method.

### 2. Research methods

#### 2.1. Research material

The materials used in this research were cultures of *E. coli* ATCC 8739, *S. enterica* subsp. *Enterica serovar typhimurium* ATCC 14028, McFarland 0.5, goat’s milk, water one [one lab], MRS agar [Cat No. 1043.00, Conda Pronadisa], Salmonella Shigella Agar [CM0099, Oxoid], Lactobacillus MRS broth [M369-1006, Himedia], EMB Levine agar [Liofilchem], Distilled water pro injection, Mac Conkey agar [CM0007, Oxoid], Mueller-Hinton Agar CM0337 [Oxoid], 1 ml eppendorf tubes, tetracycline 500 mg [Novapharin].

Tools used were: autoclave [Webecco], incubator [Memmert], centrifuge [k-centrifuge series], stirrer, oven, Vernier Caliper 0.05 [Cricle Brand], reaction tubes [Pyrex], Petri dishes [Herma], and a digital scale [Camry].

#### 2.2. The process of making kefir goat milk

The method of [13] was used to make kefir goat milk. Goat milk is obtained from people's farms in Majene, West Sulawesi and Kefir grain are obtained from commercial grain Kefir. Briefly, goat milk was pasteurized at 105\(^\circ\)C for 5 minutes, then cooled to room temperature (37\(^\circ\)C); 2% kefir grain were added, and the milk incubated for 12 hours at 37\(^\circ\)C. The physiochemical characteristics of the goat milk kefir produced can be seen in table 1. The types of lactic acid bacteria and yeast found in the kefir grain used identified as the lactic acid bacteria *Lactococcus cremoris*, *Streptococcus cremoris*, *Lactobacillus plantarium* and the yeast *Saccharomyces cerevisiae* [14].

#### 2.3. Growth and Purity test of E.coli ATCC 8739 and S.enterica subsp. enterica serovar typhimurium ATCC 14028.

The *E. coli* and *S. enterica* test bacteria were inoculated on nutrient agar (NA) media and incubated at room temperature for 24 hours. The purity of the *E. coli* cultures was tested via macroscopic observation and microscopic observation.

#### 2.4. Testing of antibacterial substances using the well diffusion method

The activity of the antibacterial substances was tested using one of the two test *E. coli* or *S. enterica* suspensions, with a bacterial density of 1.5\times10^6 cells per ml, inoculated on a 10 ml media Mueller
Hinton agar in a petri dish. The inoculated cultures were left at 4°C for 1–1.5 hours, then a total of 5 wells were made, with a diameter of 9 mm in each plate. The first well contained sterile distilled water, the second well contained kefir curd, the third well kefir whey, the fourth well kefir grain and fifth well tetracycline at a 10% concentration. The plates were incubated at 37°C for 48 hours. The ability to inhibit fermented milk against pathogenic bacteria was shown by the presence of clear zones around the well, which were measured as the clear zone diameter minus the well diameter in mm [15].

2.5. Data analysis
The data obtained were analyzed by analysis of variance (ANOVA) and when there was a significant treatment effect, the differences between treatments were tested with the smallest real difference test (BNT) [16]. The data were processed using the software SPSS 19.0 for Windows and MS Office Excel 2007.

3. Result and discussions
3.1. Growth and test of purity of E. coli and S. enterica
The purity test was carried out to ensure the E. coli and S. enterica used in this study were pure and not contaminated by other bacteria or did not undergo mutations. E. coli bacteria were inoculated in Mac Conkey agar media and Levine-Agar EMB selective media; S. enterica were inoculated in salmonella shigella agar selective media and their growth and colony morphology assessed (fig. 1).

Figure 1. Observation of E. coli ATCC 8739 bacterial colonies and S. enterica in Salmonella Shigella Agar (SS-Agar) Media (Description: a. Growth of E. coli in Mac Conkey agar media, b. Growth of E. coli in Levine-agar EMB media, and c. Growth of S. enterica in SS-agar media)

Colonies of E. coli ATCC 8739 turned red grown in Mac Conkey medium and malachite green in EMB-Levine media as seen in fig. 1a and 1b. Fig. 1c shows that colonies of S. enterica ATCC 14028 grown on SS-agar media had a round shape with black and clear colored edges.
3.2. Antibacterial test of kefir curd, kefir whey and kefir grain against E. coli and S. enterica
Antibacterial activity of the treatments against E. coli is shown in fig. 2, and against S. enterica is
shown in fig. 3. The areas of clear zones caused by the kefir curd, kefir whey, kefir grain and controls
can be seen in table 2.

Table 2. The area of clear zones caused by Kefir curd, kefir whey and kefir grain (mm) against E. coli and S. enterica.

| Observation type          | Area of Inhibitory Zone (mm) * |
|---------------------------|---------------------------------|
|                           | E. coli                         | S. enterica                    |
| Kefir curd                | 2.98±0.65a                     | 2.34±1.65a                     |
| Kefir whey                | 2.86±0.63b                     | 2.32±0.90b                     |
| Kefir grain               | 2.82±0.83b                     | 2.22±1.05b                     |
| Negative control (dd H2O) | 0.00±0.00b                     | 0.00±0.00b                     |
| Positive control (tetracycline) | 33.64±1.58c                   | 33.40±1.43c                   |

*The diameter of the well is 10 mm
Description: Different superscript letters show significant differences (P <0.05).

Table 2 shows that the highest inhibitory zone area of pathogenic E. coli is from kefir curd, 2.98 ± 0.65 mm, and the lowest inhibition zone area is from kefir grain, 2.82 ± 0.83 mm, while the highest inhibitory zone for S. enterica is from kefir curd, 2.34 ± 1.65 mm, and the lowest activity from kefir grain, 2.22 ± 1.05 mm. These results showed that kefir curd had the highest antimicrobial activity compared to the other kefir treatments, but the BNT test results showed no significant differences (P> 0.05).

According to [17], the inhibition category of pathogenic bacteria consists of several criteria, namely, low inhibition if it has a barrier zone area <3 mm, moderate inhibition at 3–6 mm, and high inhibition, >6 mm. According to [18], antimicrobial activity has several criteria: moderate activity (6–9 mm), strong (10–14 mm) and very strong (15–18 mm).

4. Conclusions
The highest antibacterial activity against E. coli ATCC 8739 was 2.98 ± 0.65 mm, with kefir curd and the lowest with kefir grain, 2.82 ± 0.83 mm. Similarly, antibacterial activity against S. enterica ATCC 14028 was lowest on the kefir grain, 2.22 ± 1.05 mm, and highest on the kefir grain, 2.34 ± 1.65 mm. Overall, the results show that kefir goat milk curd, whey, and kefir grain have potential as antibacterial agents against pathogenic E. coli and S. enterica.
Figure 2. Antibacterial test against *E. coli* ATCC 8739 ATCC 14028 (Description: 1. Negative control, 2. Kefir curd, 3. Kefir whey, 4. Kefir grain, 5. Positive control (tetracycline).
Figure 3. Antibacterial test for *S. enterica* ATCC 14028 (Description: 1. Negative control, 2. Kefir curd, 3. Kefir whey, 4. Kefir grain, 5. Positive control (tetracycline).
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