Anti-microbial activity of the red beet extract (*Beta vulgaris* L.) with solvent ethanol and acid addition variation

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**Abstract.** The aim of this study was to identify the effectiveness of antimicrobial extract of red beets against *E. coli* and *S. aureus*. Extraction was carried out by maceration using ethanol solvents which added by acid variation then stored in an airtight, and light-resistant container for 24 hours at room temperature. The extraction results were then filtered using filter paper and the filtrate obtained. This filtrate was concentrated using a rotary evaporator and stored in a sealed dark bottle in the refrigerator. The research method was experimental with a total of 9 treatments and replicated (r) 2 times, data analysis was performed using descriptive methods. The treatment carried out was antimicrobial activity of red beet extract with ethanol solvent with variations of acid (citric, ascorbate and tartaric) and variations in concentration (10, 30, 50%) towards *E. coli* and *S. aureus*. The results showed that the higher concentration of the acid added increases the antimicrobial activity. Meanwhile, the use of acid variations showed tartaric acid gave the highest antimicrobial activity compared to citric acid and ascorbic acid.

1. Introduction
Red beet (*Beta vulgaris* L.), or often known as beet root is shaped plant similar root tubers, including from family Amaranthaceae. Physical characteristics of Beet red among others shaped grass with stalks that are very short and almost invisible has a taproot that grows into a tuber, tuber is round like a potato and red to dark purple that when the fruit is cut will look white stripes with colors pink.

During this time the red bits are usually used as natural dyes and can also be used as an anti-cancer because it contains high antioxidant derived from vitamin C and betacyanin contained in red Bit (8). In addition to the group betacyanin red-violet, red Beet also contains betaxantin yellow. The concentration ratio of the betacyanin and betaxantin Bit vary depending on the variety. Differences ratio of the two pigments that cause a red color variation on the bit [1].

Both groups are under the parent pigment pigments betalain alkaloids which are suspected of having antimicrobial activity. Alkaloids have anti-microbial activity by interfering constituent of peptidoglycan in the cell wall lining selmikroba thus not fully formed and cause the death of microbial cells [5].

In addition, red beet extract is also known to contain flavonoids, sterols, triterpenes, saponins, and fetus [11]. Flavonoid compounds are phenolic compounds that can lead to the inhibition of cell wall synthesis (6). Flavonoid compounds contained in red beets can function as an antimicrobial by inhibiting the synthesis of microbial cell membranes so that microbes can not grow and proliferate.

Bit Antimikrobapada red can be obtained by extraction. One method that is widely used is the extraction using solvent extraction. The solvents used in the extraction process has a different polarity.
Ethanol is the safest solvents for extraction which will be applied to the food because ethanol is not toxic [9].

Moreover, the addition of acid to the solvent that aims to provide optimal conditions for betalain which is an antimicrobial compound at the same pigment in red beets with a pH range of 4-6 [10]. Research [1] reported a 2% addition of citric acid in ethanol can maintain stability Bit betalain contained in red. The combination of ethanol with other acids (citric, tartaric) will provide different levels of polarity so that the solubility betacyanin and antimicrobial effectiveness can occur in various ways.

The antimicrobial effectiveness of red beet extract can be tested with various concentrations of activity against pathogenic bacteria and food destroyer. Pathogenic bacteria are bacteria that have the ability to cause disease in humans. Testing the antimicrobial activity of red beet extract was carried out against *E. coli* and *S. aureus* because these two bacterial pathogens often attack humans and are very dangerous if the contaminate food and be consumed by humans. Therefore, the aim of this research to identify the antimicrobial activity of extracts of red Bit with ethanol and the addition of acid.

2. Materials and methods

Raw materials used in the study were classified into two parts, namely: (1) the material to extract: pieces of red beet (*Beta vulgaris* L) obtained from one of the farmers in Bogor age of 1 month, (2) material for analysis: culture *E. coli* and *S. aureus*, 2% citric Acid, Ascorbic Acid 0,05M, tartaric acid 3%.

Extraction was done by maceration using acidified ethanol for 24 hours at room temperature, airtight and watertight light. Comparison of materials with a solvent was 1:10 (w/v). Extraction result was then filtered using filter paper and the filtrate obtained was separated from the pulp. The filtrate was concentrated using a rotary evaporator and stored in a dark bottle with a lid in the refrigerator [7].

The method used was experimental with a total of 9 treatments and replicates (r) 2 times, after which the data analysis using descriptive methods. The treatment was done in this test is testing the antimicrobial activity against *E. coli* and *S. aureus*:

A = red beet extract with ethanol + citric acid concentration of 10%
B = red beet extract with ethanol + citric acid concentration of 30%
C = red beet extract with ethanol + citric acid concentration of 50%
D = red beet extract with ethanol + ascorbic acid concentration of 10%
E = red beet extract with ethanol + ascorbic acid concentration of 30%
F = red beet extract with ethanol + ascorbic acid concentration of 50%
G = red beet extract with ethanol + tartaric acid concentration of 10%
H = red beet extract with ethanol + tartaric acid concentration of 30%
I = red beet extract with ethanol + tartaric acid concentration of 50%

The goal was to determine the antimicrobial activity of red beet extract with ethanol added tartaric acid with a concentration of 5%, 7.5% and 10% of *E. coli* and *S. aureus* by measuring the diameter of the clear zone produced. Here is a diagram of the process of testing the antimicrobial activity of extracts of red beet.
3. Results and discussions

Extraction of red beet (*Beta vulgaris* L.) was made by maceration using ethanol solvent added with citric acid, ascorbic acid, tartaric acid by 10%, 30% and 50% in all three treatments, the test results showed different results from the three. The test showed that there was an effect of inhibition on the extraction results from each concentration, where the antimicrobial activity in the figure 2 showed that 10% of the concentration at the level of the scale of 10 to 15 ml was categorized as a strong inhibition zone.

For the next 10% of ascorbic acid and tartaric acid at a strong and very strong inhibitory level, indicated by the inhibition zone scale above 15 mm to past 20 mm, it is assumed that this occurs between the equilibrium of the extraction results with the added acid which is strongly influenced by the solution concentration. The next inhibition zone at a concentration of 30% shows the results. The addition of ascorbic acid and tartaric acid is at a strong level with a range of 10 mm to 20 mm. Then at 50% concentration shows that the inhibitory power is on the same scale with the addition of 10%, in general, the acid which is very concentrated will affect the tissue and will even break the connective chain in the tissue, the inhibitory power produced by acid into the solvent optimal for betalain which is a pigment and antimicrobial compound in red beets with a pH range of 4-6 [9].
Figure 2. Antimicrobial activity of the red beet extract with ethanol solvent and acid addition variation against \textit{E. coli}.

Figure 3. Antimicrobial activity of the red beet extract with ethanol solvent and acid addition variation towards \textit{S. aureus}.

Good acidity to maintain the stability of red beet extract is in line with the increasing concentrations of acid variation added by the test results showing that this test in accordance with the opinion [2] who reported the addition of 2\% citric acid to ethanol solvents can maintain the stability of betalain contained on red beets. The combination of ethanol solvents with other acids will give different levels of polarity so that the solubility of betasianins and the effectiveness of antimicrobials can occur in various ways.

The more polar it is, the better the effectiveness of extraction will be towards the power of the obstacle, the more concentrated it will increase its effectiveness. This indicates the power to inhibit the \textit{E. coli} bacteria, the effectiveness of inhibition in all three of them with the most effective inhibitory power is 10\%, 50\% then 30\% based on test results data.

The next test results with ethanol variations in the addition of different acids (10\%, 30\% and 50\%) to \textit{S. aureus} is a concentration of 10\% at a strong inhibitory level against \textit{S. aureus}, this is also shown in the remaining 30\% concentration in the inhibitory range of 10 to 20\%, in contrast to the 50\%
concentration which shows that variations in citric acid and ascorbate are at a strong temporary inhibitory level in tartaric acid at the level of a strong level based on test data the results of this test [3], the criteria for antibacterial strength are the inhibition zone diameter of 5 mm or less categorized as weak, 5-10 mm inhibition zone categorized as medium, inhibition zone 10-20 mm categorized as strong and a 20 mm inhibition zone or more is categorized as very strong. In general, the acid variation added is the highest effectiveness of tartaric acid which tends to be higher than the two other added acids at the three concentrations tested. Inhibiting the activity of E. coli bacteria was more effective compared to S. aureus in red beets because of porin protein contained in the outer membrane of E. coli bacterial cell walls has the ability to transport antimicrobial compounds, so that the antimicrobial compounds contained in the sample extract will easily enter and damage the activity of cell enzymes that cause damage to E. coli cells [11].

4. Conclusion
The process of inhibiting the rate of bacterial activity using ethanol added with 3 types of acids including citric acid, ascorbic acid, and tartaric acid had different inhibitory power. The strongest and most effective inhibition was found in ethanol with a mixture of tartaric acid. Each substance produces an inhibitory effect on bacterial activity which gradually increases with increasing concentration of the acid used. The three variations were considered effective in inhibiting the activity of E. coli bacteria compared to S. aureus in red beets.

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