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Lemon (*Citrus limon*) Juice Has Antibacterial Potential against Diarrhea-Causing Pathogen

ER Ekawati¹, W Darmanto^{2,*}

¹Doctoral Student, Science and Technology Faculty, Airlangga University, Airlangga Street 4-6, Mulyorejo, Surabaya

²Author Correspondence, Science and Technology Faculty, Airlangga University, Airlangga Street 4-6, Mulyorejo, Surabaya

*windarmanto@fst.unair.ac.id

Abstract. This purpose of this study was to determine the potential of lemon juice in inhibiting the growth of diarrhea-causing bacteria and analyze the optimum dose. The type of this study was a laboratory experiment. The variations of the lemon juice dose used in this study were 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg/ml by using Enterotoxin *Escherichia coli* (ETEC) as the test bacteria. This potential test used a well diffusion method with three repetitions for each dose. The results of this study indicated that lemon juice had the potential to inhibit pathogenic bacteria that cause diarrhea. In doses of 500 mg/ml to 80 mg/ml it inhibited the growth of test bacteria which were characterized by the formation of inhibition zones in a sequence of 10.56, 12.05, 13.02, and 14.58 mm with intermediate categories. Whereas in doses of 900 mg/ml and 1000 mg/ml it inhibited the growth of test bacteria with zone diameter area of 16.04 and 18.70 mm with sensitive categories. Kruskal Wallis test revealed a p-value of $0.000 < \alpha = 0.05$, which means that there was an influence among treatments. This result showed that the optimum dose of lemon juice in inhibiting diarrhea-causing pathogenic bacteria was 900 mg/ml.

Keywords: Lemon (*Citrus limon*); Antibacterial; Pathogen; Diarrhea

1. Introduction

Diarrhea is a liquid defecation more than three times a day, with or without blood and or mucus in the stool. Diarrhea is a disease that can cause pain and death. Diarrhea may attack almost any age and cosmopolitan, but severe diarrheal diseases with high mortality rates are especially found in infants and children [1]. Diarrhea often attacks infants and children. If it is not treated, it may lead to dehydration, which may finally lead to death. The high incidence of infant and childhood death due to diarrhea requires more specific diagnosis, so direct antigen examinations from feces have a high sensitivity ranging from 70%-90% [2].

In Indonesia, diarrheal disease is still one of the main public health problems. As much as 55% of diarrhea cases occur during childhood. The incidence of diarrhea in children under five age is 1.0-1.5 times every year [2]. Many factors, such as bacteria, viruses, malabsorption, allergies, and poisoning, can directly or indirectly trigger diarrhea through intestinal inflammation or, in children, transmission through food and drinks contaminated by those causative pathogens [3][4].

The second most common cause of diarrhea after rotavirus is the infection due to *Escherichia coli* bacteria. *Escherichia coli* is a commensal bacterium, intestinal and extraintestinal pathogens that can cause urinary tract infections, meningitis, and septicemia [5]. The major cause of infectious diarrhea in the developing world is the enterotoxin *Escherichia coli* [6]. These organisms are estimated to be responsible for millions and hundreds of thousands of deaths, particularly of young children. Despite significant declines in death from acute diarrhea [7], enteric infections caused by enterotoxin *Escherichia coli* (ETEC) and other pathogens remain inextricably associated with malnutrition, stunted growth and impaired intellectual development [8]. Inappropriate use of antibiotics can cause resistant bacteria to antibiotics that have been given [9].

The use of natural ingredients in herbal medicines is considered safer than the use of modern chemical drugs, because the side effects of herbal medicines are relatively small if used appropriately [10], [11]. One of the herbal plants often used as medicine is the lemon fruit. Lemon fruit is an herbal



plant that has the main content of alkaloid compounds which have the function as anticancer, antibacterial, antifungal, antiviral and antidiabetic. Alkaloid compounds that responsible for antibacterial is saponin [12]. Lemon (*Citrus limon*) juice contains many bioactive compounds such as flavonoids, carotenoids, limonoid, tannin, and terpenoids. The bioactive compounds contained in lemon (*Citrus limon*) each have an antibacterial [13]. Lemon (*Citrus aurantifolia*) juice besides being used as an antibacterial, it is also useful as an antioxidant. The main content of lemon (*Citrus limon*) juice is vitamin C and citric acid. The content of vitamin C and citric acid makes the degree of acidity (pH) the lemon (*Citrus limon*) juice become acidic [10][14].

This study was aimed to determine the potential of lemon (*Citrus limon*) juice in inhibiting the growth of bacteria causes diarrhea and analyzing the optimum dose.

2. Materials and Methods

2.1. Sample Collection

Lemon (*Citrus limon*) was collected from the traditional market in Larangan Market Sidoarjo, East Java, Indonesia. The sample was placed in a plastic bag and was transported immediately to Bacteriology Laboratory Faculty of Health Science, University of Maarif Hasyim Latif, Sidoarjo, Jawa Timur, Indonesia. The sample was processed immediately in the laboratory.

2.2. Instruments, Reagents, and Medium

The instruments used in this study were a test tube, rack tube, scale pipette, spatula, drop pipette, petri dish, ose, autoclave, bunsen, beaker glass, erlenmeyer, Mc. Farland 0.5 standard, steel pickers, filters, tweezers, measuring cups, beaker glass, micropipette, yellow tip, incubator, stirrer, hot plate, sterile swabs, digital scales, orange squeezer, aluminum foil and sterile gauze.

The material used in this study was lemon (*Citrus limon*) juice, enterotoxigenic *Escherichia coli*, Mac Conkey Agar medium (Merck, Germany), Eosin Methylen Blue medium (Merck, Germany), Nutrient Broth medium (Merck, Germany), Kliger Iron Agar medium (Merck, Germany), biochemical reactions medium (Merck, Germany), sterile physiological zouth, sterile aquadest, MHA medium, ciprofloxacin 500 mg and alcohol 70%.

2.3. Purification of ETEC Suspension

The stock of test bacteria, in this case, was Enterotoxin *Escherichia coli* (ETEC) planted in NB medium and then incubated at 37⁰ C for 24 hours. The bacteria that grew in NB medium were planted on MCA and EMB medium and then incubated at 37⁰C for 24 hours [15]. Colonies grown on EMB medium were planted in KIA medium and then incubated at 37⁰C for 24 hours. Furthermore, it was planted in the biochemical reaction medium and incubated in 37⁰C for 24 hours. The colonies were taken and planted on the NAS medium as the stock of bacteria

2.4. Preparation of ETEC Suspension

The bacterial colonies that have been grown for 24 hours on NAS medium were inserted into 10 mL of sterile physiological zouth and then homogenized. The turbidity level of ETEC suspension was equalized using McFarland 0.5 standard which had a density equivalent to 1.5 x 10⁸ CFU/ml [16].

2.5. Preparation of lemon juice dose

Lemon (*Citrus limon*), that has been washed with sterile aquadest, was cut transversely using a sterile knife and then squeezed using an orange squeezer. The juice was filtered using a sterile gauze, as much as 100 mL was taken and diluted with 100 mL of sterile aquadest to obtain a concentration of 1000 mg/ml dilution. From the dose of 1000 mg/ml, several dilutions were made to obtain doses of 100, 200, 300, 400, 500, 600, 700, 800, and 900 mg/ml.

2.6. Preparation control

Prepared 1 mL of sterile aquadest and taken as much as 100 µl for inclusion in the well for negative control [17]. Prepared of ciprofloxacin 500 mg was dissolved in 100 mL of sterile aquadest and then 100 µl was taken to be put into the well for positive control [17].

2.7. Lemon juice antimicrobial potential test

Prepared two types of tubes and labeled A and B. In tubes labeled A containing 5 ml MHA medium and tube labeled B containing 15 ml MHA medium. Five mL of MHA medium in tubes labeled A was poured into a petri dish and allowed to solidify, then three steel pickers were placed with a spacing between 1.5 cm. One mL of ETEC bacterial suspension was put into 15 mL of MHA medium in tubes labeled B and homogenized, then poured into a petri dish containing a steel tray as a second layer and allowed to solidify. After the medium solidify, steel pickers were aseptically removed from the petri dish so that the wells formed.

Each of the wells was filled with 100 μ l of lemon (*Citrus limon*) juice in various doses and controls, then incubated at 37°C for 24 hours. Observation was done for the formed inhibitory zone [17].

3. Result and Discussion

The results of potential test of lemon (*Citrus limon*) juice on diarrhea-causing bacteria, which in this study used ETEC bacteria, are presented in Figure 1 and Table 1.



Figure 1. Potential test of lemon (*Citrus limon*) juice on MHA medium. The inhibitory zone are formed is shown with red arrow.

Table 1. Potential test results of lemon (*Citrus limon*) juice on ETEC bacterial growth

The concentration of lemon (<i>Citrus limon</i>)	Average resistor zone (mm)	Notes
Positive Control	32,61 mm	S
Negative Control	0 mm	R
100 mg/ml	0 mm	R
200 mg/ml	0 mm	R
300 mg/ml	0 mm	R
400 mg/ml	0 mm	R
500 mg/ml	11 mm	I
600 mg/ml	12 mm	I
700 mg/ml	13 mm	I
800 mg/ml	15 mm	I
900 mg/ml	16 mm	S
1000 mg/ml	19 mm	S

Information: To test the potential and criteria for Enterobacteriaceae inhibitory zone results, except *Salmonella* spp. R: Resistant (≤ 5 mm); S: Sensitive (≥ 16 mm) ; I : Intermediate (10-15 mm) [18]

Based on Table 1 above, at concentrations of 100 mg/ml to 400 mg/ml the lemon (*Citrus limon*) juice showed resistant results, meaning that in the range of the doses it was not able to inhibit ETEC bacterial growth. In concentrations of 500 mg/ml to 800 mg/ml, the lemon (*Citrus limon*) juice showed intermediate results with diameters of ETEC bacteria growth inhibition zones were 11 mm, 12 mm, 13 mm and 15 mm, respectively. In concentrations of 900 mg/ml and 1000 mg/ml, the lemon (*Citrus limon*) juice showed sensitive results with diameters of ETEC bacterial growth inhibition zones of 16 mm and 19 mm.

The data above was tested using the Kruskal Wallis test to determine the differences between lemon (*Citrus limon*) juice doses given to ETEC bacteria growth. The Kruskal Wallis test results obtained p-value $0.000 < \alpha = 0.05$, which means there was difference in the outcome of providing lemon (*Citrus limon*) juice in different doses to ETEC bacterial growth. Based on the data presented in the table and statistical tests, the optimum dose of lemon (*Citrus limon*) juice which can inhibit pathogenic bacteria causing diarrhea, in this case, ETEC bacteria was 900 mg/ml.

This research is almost the same as Henderson, Fachrial and Lister research (2018). The present study shows the antimicrobial activity of the ethanolic extract of the Citrus limon peel of 25%, 50%, 75% and 100% had a strong antimicrobial activity against *Escherichia coli* were 15,03 mm; 16,17 mm; 15,83 mm; 18,77 mm of the average inhibition zone, respectively.

Medicinal plants are one of the natural resources that can be explored by humans. Various sources of secondary metabolites from plants can be used as medicines, agrochemicals, flavors, fragrances, dyes, biopesticides and food additives. Citrus fruits contain nutrients and phytochemicals that are beneficial to health. Citrus juice contains various substances including carbohydrates, fiber, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and various phytochemicals. These substances are needed for the body. Some compounds in citrus fruit can provide additional protection for the body against chronic disease and basic nutrition. Citrus fruits also contain lots of phytochemicals, including essential oils, alkaloids, flavonoids, ouramin, psoralens, and carotenoids. Previous pharmacological studies revealed that citrus fruits have antimicrobial, anthelmintic, insect repellent, antioxidant, anticancer, cardiovascular, anti-inflammatory, analgesic, antidiabetic, reproductive, gastrointestinal, immunological, respiratory and many other pharmacological effects [19].

Flavonoid compounds have properties that are effective in inhibiting the growth of bacteria, fungi, and viruses because flavonoid compounds include groups of phenol compounds that are able to denaturize bacterial cell proteins and damage bacterial cell membranes [20]. Saponin compounds are antibacterial compounds that damage bacterial cell membranes. Besides that, lemon (*Citrus limon*) juice contains vitamin C which is useful as an antioxidant. The main ingredient in lemon (*Citrus limon*) juice is organic acid in the form of citric acid which is contained most in lemon (*Citrus limon*) juice. The citric acid content of citric acid provides a degree of acidity (pH) of the fruit becomes acidic. Acid pH is one of the factors that can inhibit bacterial growth which can cause the internal pH of bacterial cells to decrease and inhibit bacterial cell growth [10].

4. Conclusion

Lemon (*Citrus limon*) juice can inhibit the growth of pathogens that cause diarrhea, in this case, the Enterotoxin *Escherichia coli* (ETEC) bacteria. This results showed that the optimum dose of lemon (*Citrus limon*) juice in inhibiting diarrhea-causing pathogenic bacteria was 900 mg/ml.

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