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by John Jackson Yang, Fri Rahmawati

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Antimicrobial effects of various red ginger (*Zingiber officinale*) extract concentrations on *Escherichia coli* bacteria

John Jackson Yang*, Fri Rahmawati
Department of Biochemistry, Universitas Kristen Indonesia, Jakarta, Indonesia

Abstract
Ginger (Family; *Zingiberaceae*) is one of the well-known herbs, which has been used by the community as a medicinal plant for centuries. The secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of pathogens detrimental to human life, including *Escherichia coli*, *Bacillus subtilis* bacteria, and several other microbes. This study aims to determine the effect of ginger extract on the growth of *Escherichia coli*. The concentration that has the most significant impact on *Escherichia coli* bacteria was determined by inhibition zone. Dried red ginger macerated with 96% ethanol solvent. Variations concentration of red ginger extract used in this study were 10%, 30%, 50%, and 80%, with resin red ginger concentrations of 100% as a positive control. The Kirby Bauer antibiotic test method was performed ten times for each sample. The inhibition zone produced by the ginger extract was measured using a caliper with an accuracy of 0.01mm. The results showed that the best antibacterial activity was 50% of ginger extract, with the largest diameter inhibition zone at 15.03 mm. From this study, we can conclude that red ginger extract has the antibacterial activity to affect the growth of *Escherichia coli* bacteria.

Keywords: antimicrobial, escherichia coli, red ginger

Introduction

Ginger (Family; *Zingiberaceae*) has been known and used by the community as a medicinal plant for centuries. *Zingiber officinale* (ginger) is used as a raw material to manufacture modern and traditional medicines. The red ginger rhizome contains gingerol, which has antioxidant, antibacterial, anti-inflammatory, anti-carcinogenic, anti-mutagenic, and antitumor activities [1]. These secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of pathogens that are detrimental to human life, including *Escherichia coli* and *Bacillus subtilis* bacteria, as well as several other microbes [2].

Ginger plants, perennial herbaceous plants with fibrous roots, belong to the monocot class. Ginger thrives at an altitude of 10-1500 m above sea level, except for the type of elephant ginger at a size of 500-950 m above sea level. The temperature required for optimal ginger growth is 25-30ºC. In the Asian region, ginger plants are spread almost in various wet tropical areas. Currently, ginger is cultivated in different regions in Indonesia, including North Sumatra, Bengkulu, West Java, Central Java, and East Java [3, 4].

The morphology of ginger, in general, consists of roots, stems, leaves, flowers, and fruit. Ginger stems are pseudo stems with a height of 30-100 cm. Ginger root is rhizome-shaped with yellow to reddish rod flesh with a pungent odor. The ginger leaves are pinnate with a length of 15-23 mm and a width of 8-15 mm. Based on the size, color, and shape of the rhizome, there are three types of ginger known, namely: Elephant Ginger (*Zingiber officinale var. Roscoe*), White Ginger, Emprit Ginger (*Zingiber officinale var. Amarum*) or small White Ginger and Red Ginger (*Zingiber officinale var. Rubrum*) or Sunti Ginger [5].

The chemical content in the ginger rhizome determines the aroma and spiciness of ginger. Several factors affect the chemical composition of ginger rhizomes, including the type of ginger, the soil where the ginger is grown, the age of the ginger when it is harvested, the processing of the ginger rhizome, and the ecosystem where the ginger is located [4].

Indonesian people generally know and use ginger daily for various purposes, such as food ingredients, beverages, cosmetics, perfumes, and others [6]. Research in the medical field has shown the ability of ginger extract as an antioxidant, one of the options for anti-cancer, and anti-inflammatory therapy, especially in antimicrobial-bacterial [7, 8, 9].

Ginger contains essential oils consisting of sesquiterpene compounds, zingiberene, zingerone, oleoresin, kamfena, limonene, borneol, cineol, citral, zingiber, felandren, vitamins A, B, and C. Besides, it also contains flavonoid and polyphenolic compounds. Phenotypic substances play a role in flavor formation in which some phenotypic derivatives give an effect called pungence because of the characteristic spicy, sharp, and stinging sensation [10] Essential oils are oils from a mixture of volatile substances with different compositions and boiling points, greenish to yellow in color, and have a characteristic ginger smell [11] Essential oils can be extracted by distillation and hydrodistillation. Essential oils contain the active compound gingerol, which, when it has gone

through the storage and drying process, can turn into shogaol. These chemical compounds actively damage the bacterial cell wall's outer and cytoplasmic membranes [12, 13].

Escherichia coli is one of the main species of gram-negative bacteria. The bacteria discovered by Theodor Escherich can be found in the human colon. *Escherichia coli* is a short rod-shaped bacterium that measures 0.4-0.7 m x 1.4 m [14]. *Escherichia coli* has the interesting characteristic of being both a widespread gut commensal of vertebrates and a versatile pathogen, thought to kill more than 2 million humans per year through both intraintestinal and extraintestinal diseases [15]. The selective pressures in the habitats of commensal strains may coincidentally promote the emergence of virulence factors and antibiotic resistance, rendering commensal *Escherichia coli* strains reservoirs of virulent and resistant strains.

Antimicrobials are chemical substances derived from various kinds of microorganisms in low concentrations but can inhibit the growth of other organisms. Antimicrobials have antibiotic properties, including inhibiting or killing pathogens without damaging the host, being bactericidal, no resistance to bacteria, not allergenic, remaining active in plasma and exudates, soluble in water and stable, and bactericidal level in the body. The body is quickly achieved and persists for a long time [16].

Antimicrobials that have bacteriostatic properties can inhibit bacterial growth and kill bacteria. There are several mechanisms of action of antimicrobials, among others. Folic acid coenzymes are required to synthesize purines, pyrimidines (DNA and RNA precursors), and other compounds necessary for cellular growth and replication. For many microorganisms, p-amino benzoic acid (PABA) is the primary metabolite. Antimicrobials such as sulfonamides are structurally similar to PABA and folic acid and will compete with PABA to form folic acid.

This class of antimicrobials can inhibit peptidoglycan biosynthesis, mucopeptide synthesis, or inhibit cell wall peptide synthesis so that the cell wall becomes weak, and due to turgor pressure from within, the cell wall will rupture or lysis so that bacteria will die [17, 18].

Antimicrobials act directly on the cell membrane, which affects the permeability and causes the release of intracellular compounds of microorganisms so that cells are damaged and even die [19]. Antimicrobials will inhibit the transfer reaction between the donor and the acceptor or inhibit the translocation of peptidyl t-RNA from the acceptor site to the donor site, which causes protein synthesis to stop [20, 21]. It binds to the 30S ribosomal subunit and alters protein synthesis, resulting in cell death. Determination of antibacterial activity in *vitro* can be grouped into two methods, namely the diffusion method, measuring the inhibition of antimicrobial compounds contained in the extract. This method is the most commonly used.

Based on the above background, the formulation of the problem in this study is whether there is an effect of red ginger extract concentration on the growth of *Escherichia coli* bacteria and to know the red ginger extract's concentration that can inhibit *Escherichia coli* bacteria's growth.

Material and Method

This research was conducted at the Physiology Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University, Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, and the Microbiology Laboratory, Faculty of Medicine, the Christian University of Indonesia. The materials needed for the media are Mueller-Hinton Agar, Nutrient Agar, aquades, ethanol 96% ethanol 70%, ginger (*Zingiber officinale* var. Rubrum), and pure culture of *Escherichia coli* ATCC 25922 obtained from the Microbiology section of the University of Indonesia. The red ginger rhizome was obtained from a red ginger seller in Depok, West Java. As much as 1000 grams of red ginger rhizome was cleaned on the surface using 70% ethanol. Red ginger rhizome was cut into small pieces and then dried in the oven. Dried red ginger (100 g) was grounded by a blender, and followed by a maceration process with 96% ethanol for three days. The solution was stirred every 1 hour, and the solvent was separated from the solute every 24 hours. The extraction results were filtered to obtain the dregs and filtrate. The solvent in the filtrate was evaporated at a temperature (50ºC) with pressure below 1 atm until the solvent evaporated to obtained slightly thick ginger resin. The ginger resin was diluted with distilled water to obtain the tested red ginger extract concentrations of 10%, 30%, 50%, 80%, and 100%. Resin red ginger concentrations of 100% as a positive control. The media used was MHA (Mueller-Hinton Agar) media prepared according to the specified composition (Casein hydrolysate 17.5 g; Beef extract 300 g; Starch 1.5 g; Agar 17 g.) The 6mm paper discs was shaped by hole punch and followed by sterilized using an autoclave. Sub-cultured *Escherichia coli* were inoculated in sterile distilled water until a turbidity equivalent to Mc. Farland 0.5 (1% sulfuric acid 9.95 ml and 1% barium chloride 0.05 ml) to meet $1.5x10^8$ CFU/ml. The paper dice with the serial concentration of the ginger extract were placed on the surface of the inoculated growth medium and continued incubated for 18 to 24 hours at 37ºC. Observation and measurement of the diameter of the clear zone formed around the extract using a caliper [22].

Result and Discussion

This research was conducted using the disk diffusion test method using several variations in the concentration of red ginger extract (10%, 30%, 50%, and 80%). Researchers used Mueller-Hinton Agar (MHA) as a test medium and Nutrient Agar as a medium for growing *Escherichia coli* colonies. The result showed that 50% of extract has more inhibition zone compare to other concentration (Picture 1&2).

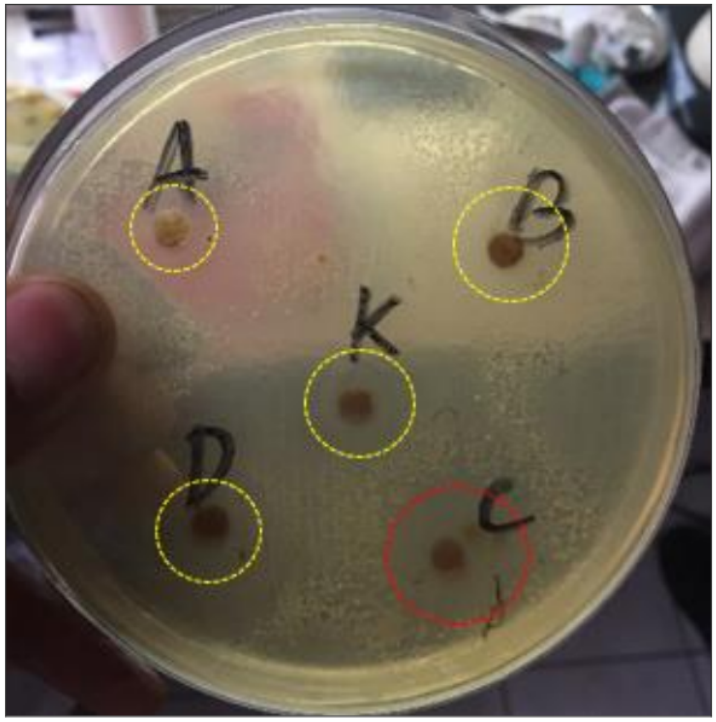

Fig 1: Inhibitory zone diameter in *Escherichia coli*. A: 10%; B: 30%; C: 50% (red circle); D: 80%, K is a positive control (100%).

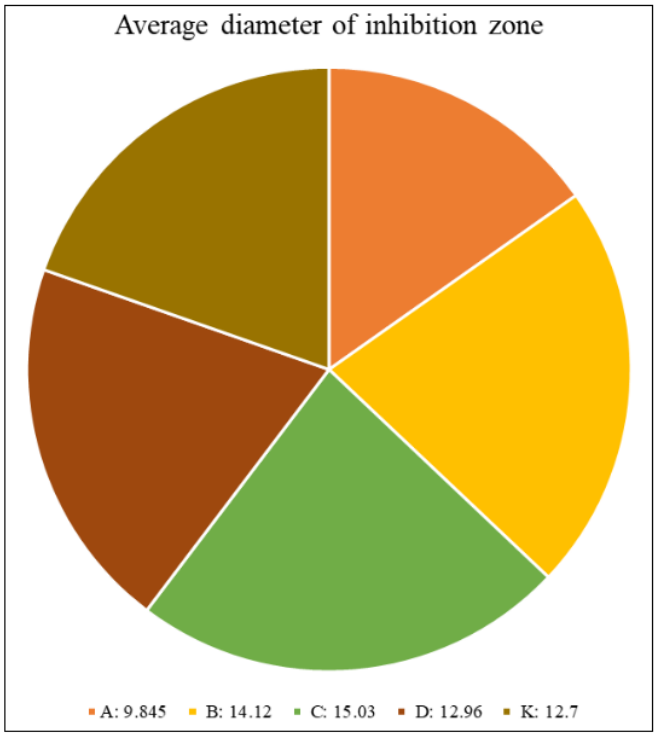

Fig 2: The average diameter of the inhibition zone. Based on the amount of sample concentration: A: 10%; B: 30%; C: 50%; D: 80%, K is a positive control with resin red ginger concentration of 100% (In mm)

The results of observations made after incubation for 24 hours obtained variations in the inhibition zone formed from various samples of the concentration of red ginger extract used. The average diameter of red ginger extract with 10% concentration is 9.845 mm, red ginger extract with 30% concentration is 14.12 mm, red ginger extract with 50% concentration is 15.03 mm, and red ginger extract with 80% concentration is 12.96mm. Red ginger extract with a concentration of 100%, which was used as a positive control, resulted in an inhibition zone diameter of 12.70 mm (Table 1).

Table 1: Inhibition Zone Diameter of Red Ginger Extract to Growth of *E. coli*

Petri dish	Inhibition Zone Diameter (mm)			
	A (10%)	B (30%)	C (50%)	D (80%)
1	12.55	16	19.1	12.7
2	16.3	14.7	10.3	10.2
3	9.1	14.3	11.6	12.6
4	9.8	11.9	10.5	13
5	7.6	8.9	17.2	11.7
6	7.3	9.8	15.9	11.4
7	7.4	14.5	16.6	19.4
8	8.6	19.1	16.1	14.5
9	9.2	21	19.2	15.1
10	10.6	11	13.8	9
Average:	9.845	14.12	15.03	12.96
The large diameter of the control positive inhibition zone				12.7

The results showed antimicrobial properties with weak inhibition at concentrations of 30%, 50%, and 80%. The positive control used had lower inhibitory power than the sample concentration of the trial used, although this did not affect the study's results. The lower inhibitory results may be caused by unbalanced controls and the non-use of antibiotic samples as positive controls whose confidence index has been tested.

Research conducted on the effectiveness of ginger extract (*Zingiber officinale Roscoe*) on the growth of *Streptococcus viridans* bacteria showed that the inhibitory power increased along with the concentration. The results were obtained from weak inhibition at a concentration of 1000mg/ml of elephant ginger extract. The study used a positive control of Penicillin antibiotics with a 95% confidence index [23].

The type of ginger used affects the amount of inhibition. Research conducted to compare the antimicrobial potential of red ginger with elephant ginger showed differences in the inhibitory ability of the types of bacteria tested. Red ginger (*Zingiber officinale var. Rubrum*) had the highest inhibition area against *S. aureus* (15.83 mm), and *Escherichia coli* (15.33 mm), and fresh extract of elephant ginger (*Z. officinale var. Roscoe*) had the highest inhibition area against *C. Albicans*. (10.7 mm) [24].

Essential oils and oleoresins are chemical compounds that can inhibit the growth and kill bacteria by damaging the bacterial plasma membrane, damaging the cell's working system, and causing the lysis of bacterial cells. In addition, the 3-dimensional structure of the protein is disturbed, causing the protein to be denatured. After being denatured, the amino acid sequence in bacteria remains intact but can no longer perform its function [25].

Conclusion

Red ginger rhizome extract with 96% ethanol solvent can provide a weak inhibitory effect on *Escherichia coli* bacteria. The largest average inhibitory effect was found in a red ginger extract with a concentration of 80% with a diameter of 12.9 mm, a concentration of 50% with an inhibition zone diameter of 15.03 mm, and a concentration of 30% with a diameter of 14.12 mm. Based on the results of existing research, it is recommended for further researchers: a) Continue further research on the effect of red ginger extract on *Escherichia coli* using more various concentrations of red ginger; b) Conduct further research on the antibacterial effect of red ginger extract on other bacteria, and c) Use a positive control in the form of antibiotics that are s*ensitive to Escherichia coli*.

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