Research Article

Juice of Red Ginger Water Activity as an Antibacterial against the Growth of *Vibrio Cholera* Bacteria

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Received: 02-Mar-2022, Manuscript No. amhsr-23-91382;
Editor assigned: 06-Mar-2022, Pre QC No. amhsr-23-91382(PQ);
Reviewed: 20-Mar-2022, QC No. amhsr-23-91382;
Revised: 27-Mar-2022, Manuscript No: amhsr-23-91382(R);
Published: 03-Apr-2022, DOI: 10.54608.annalsmedical.2023.89

Abstract

The ginger plant (*Zingiber Officinale* Roscoe) has been widely used by the community as a kitchen spice, as alternative medicine, and for the maintenance of body health. One of the bacteria that damage food and can be harmful is *Vibrio cholera*, which was one of the pathogenic microorganisms that cause harm to humans. The purpose of this study is to analyze the effect of the density of red ginger juice as an antibacterial on the growth of *V. cholera* bacteria. The research method is a quantitative study, which is a laboratory experiment that consists of 5 treatments and 5 replications that accumulate to a total of 25 experimental units were treatments. The details are; 0% (control reference); 40% squeezed water; 60% squeezed water; 80% squeezed water; 100% squeezed water. The results showed the concentration of red ginger juice at 60% was bacteriostatic (inhibited) the growth of *V. cholera*, 80% and 100% were bactericidal (deadly) *V.cholera*.

Keywords: Juice; Red ginger; Antibacterial; *V. cholera*

Introduction

Indonesian people who live in urban and rural areas have known and used traditional medicines for healing their illnesses for a long time. One type of medicinal plant that is often used by people in various regions of Indonesia is ginger (*Zingiber officinale* Roscoe). Ginger is one of the medicinal plants that is widely used by the community both as a spice in the kitchen, as alternative medicine, and as health care. Ginger is also a plant that is used for making traditional and modern medicines. The red ginger that has is a rhizome plant that we often encounter around us which is used by the community [1]. There are three types of ginger variants that are commonly used by the community and have been tested for their antioxidant capacity by, which are Emprit Ginger, Elephant Ginger, and *Zingiber Officinale* var. Rubrum [2].

Red ginger is a type of rhizome plant that can live and grow in lowland and mountainous areas. Besides being used as a cooking spice, ginger is also widely used in various medicinal ingredients, including a herb to treat inflammation, wounds, coughs, and allergies due to insect bites and to increase endurance. Furthermore, it was explained that the research results of red ginger fresh extract at a concentration of 100% had a moderate level of inhibition against S. aureus and weak against *E. coli* [3].

The ginger plant contains compounds in the form of phenols, flavonoids, terpenoids, and essential oils. These compounds can inhibit the growth of pathogenic fungi and bacteria which are detrimental to human life and can cause disease. Further, research by Sari, et al. found that fresh extract of red ginger rhizome had the highest inhibition area against *S.aureus* (15.83 mm) and *E. coli* (15.33 mm) and fresh extract of elephant ginger had highest inhibition against *C. albicans* (10.7 mm). Red ginger plants are more widely used for treatment [4]. While the types of elephant ginger and emprit ginger are more widely used as food and beverage ingredients such as pickles, ginger brew, sweets, and sekoteng [4].

The content of oleoresin in ginger varies depending on the type of ginger in question. Ginger oleoresin can be up to 3%. Among the 3 types of ginger, it is known that red ginger has a high spicy taste. This is because the oleoresin content is higher compared to other types of ginger. Red ginger contains the highest essential oil (2.58%-3.90%), Emprit ginger (1.50%–3.30%), and elephant ginger (0.82%–1.68%), so people often use red ginger as medicine. The active ingredients of essential oils and flavanoids have an antibacterial effect in inhibiting the growth of pathogenic microorganisms [4].

One of the bacteria that damage food and can be detrimental is *V. cholera*. This bacterium is one of the causes of diarrhea, which is also called cholera, where the transmission can be through water or food. The natural reservoir of this bacterium is thought to be in the aquatic environment. Indonesian people are susceptible to infection with this bacterium; because most people work in aquatic environments such as farmers and fishermen. Cholera cases are common in developing countries, due to a lack of clean water sources and poor environmental sanitation [5].

The high incidence rate makes cholera one of the diseases that must be reported to WHO. Incomplete laboratories, low level of care and medical supervision, and inadequate data collection, resulted in reported cases of diarrhea only representing around 5%-10% of the actual number of cases occurring each year worldwide [6].

Food ingredients such as fish and shellfish will not be free from contamination by *V. cholera* bacteria. This bacterium is also resistant to chemical drugs such as Dosycycline and

How to Cite this Article: Majid A, et al. Juice of Red Ginger Water Activity as an Antibacterial against the Growth of *Vibrio Cholera* Bacteria. Ann Med Health Sci Res. 2023;13:516-519

Vibramycin. According to, *V. cholera* bacteria can die within 1 × 24 hours [7]. Types of medicines that cannot be reached by the community, especially people who are in rural areas and have limited access to health facilities. The solution that can be used to inhibit the growth of *V. cholera* bacteria is to utilize red ginger rhizome extract. Ginger is an annual plant that is easy to obtain at a low price. Ginger can be reached by all people, both in urban and rural areas. Ginger is also easy to grow and maintain in the yard. Ginger also contains compounds as antibacterial, namely flavonoids, alkaloids, oleoresin, and essential oils. These compounds have been proven to inhibit the growth of pathogenic microorganisms that can harm humans and animals.

The results of research also show that the fresh extract of red ginger, which has a concentration of 100%, has a moderate category of inhibitory ability on the development of S. aureus bacteria and a weak category on the development of *E. coli* bacteria [3]. On the other hand, additional research studies showed that fresh extract from red ginger rhizome has an inhibitory ability against *S.aureus*, *E. coli*, and *C. albicans* fungi [4]. The purpose of this study was to find the concentration of red ginger juice as an antibacterial that could inhibit growth and kill *V. cholera* bacteria.

Materials and Methods

Research method

Tools and materials: The tools used in this study were: Blender, gauze, funnel, glasses, auto clave, pipette, petri dish, micropipette, test tube and rack, Erlemeyer flask, jar, inoculum needle, aluminum foil, ose needle, knife, bunsen lamps, and analytical scales of the Fijitsu brand and type FSR-A320

The materials used in this study were: red ginger rhizome, cotton, tissue, filter paper, spirit, Nutrient Agar (NA), sterile distilled water, analytical ethanol alcohol, Broth Pepton Water (BPW), and, *V. cholera* ATCC bacteria obtained from Airlangga University Tropical Disease Center Surabaya,

Type of research

This research was a laboratory experimental study consisting of 5 treatments and 5 replications so the number of trials studied was 25 units. The treatments were: A=without red ginger rhizome juice (0% or control reference); B= 40% juice; C=juice 60%; D=80% juice; and E=100% juice.

Research process

The research procedure is as follows:

a. *Making red ginger rhizome squeezed water:* The ginger rhizome plant used was taken from Baumata Village, Taebenu District, Kupang Regency, and then identified at the Biology Laboratory of Nusa Cendana University. The ginger plant taken is the result of maintenance at the Baumata natural tourism park. First of all, the red ginger rhizome is cleaned, cut into small pieces, and then blended with as much as 1 kg. After blending, the ginger rhizome is squeezed and filtered 3 times with gauze (pure ginger rhizome juice) as much as 560 ml. The juice results were stored in an Erlemeyer flask which was tightly closed as a test material.

b. *Rejuvenation of test bacteria:* The *V. cholera* bacteria was first purified and inoculated in a nutrient medium and incubated at 37°C for 1 day (24 hours).

c. *Making concentration of red ginger rhizome:* Making red ginger rhizome juice in this study, namely: control=10 ml distilled water; 40%=4 ml of red ginger extract+6 ml of distilled water; 60%=6 ml of red ginger extract+4 ml of distilled water; 80%=8 ml of red ginger extract+2 ml of distilled water; and 100%=10 ml of red ginger extract without distilled water.

d. *Making culture media:* Weighing was carried out on Broth Pepton Water (BPW) media of 1.25 g. Then mix BPW media with 50 ml of distilled water. The homogeneous results were put into 25 test tubes with a total of 8 ml for each tube.

e. *Preparation of culture (inoculum) of* V. cholera *bacteria:* Prepare 5 test tubes containing 5 ml of distilled water. Take 10 pure culture colonies of *V. cholera* bacteria using a sterile loop needle. Homogenize the results of incubation of *V. cholera* bacteria in a test tube that has been prepared and contains 5 ml of distilled water.

f. *Testing the antibacterial activity of red ginger rhizome juice against* V. cholera *bacteria:* Prepare 25 test tubes and label them. Enter each treatment in the tube as follows: a. 8 ml of BPW medium+1 ml of sterile distilled water+1 ml of *V. cholera* bacterial culture; b. 8 ml of BPW medium+1 ml of ginger rhizome extract 40%+1 ml of *V. cholera* culture; c. 8 ml of BPW medium+1 ml of 60% ginger rhizome extract+1 ml of *V. cholera* culture; d. 8 ml of BPW medium+1 ml of ginger rhizome extract 80%+1 ml of *V. cholera* culture; e. 8 ml of BPW medium+1 ml of 100% ginger rhizome extract + 1 ml of *V. cholera* culture=15,000,000 colonies (1.5×10^6). Each tube with 5 treatments was incubated for 24 hours at 37°C.

Weigh NA Media as much as 10 g. Mix NA media with 400 ml distilled water. Next, heat the media until it boils, then cool it down to 45°C. The NA medium was put into 25 sterile petri dishes as much as 15 ml in an aseptic manner.

The test sample that had been incubated was put as much as 1 ml into the cup using the drop method, then rotated so that the suspension of the test bacteria (*V. cholera*) and the media were homogeneous NA. After the sample was frozen, it was incubated for 24 hours at 37°C. Observation of the growth of *V. cholera* bacteria on NA by counting the number of *V. cholera* bacteria colonies that grew on NA media.

Results and Discussion

Based on Table 1, shows that the number of *V. cholera* bacterial colonies that grew in each treatment of red ginger rhizome juice concentrations were: treatments A and B the number of *V. cholera* bacterial colonies that grew Too Many to Count (TMTC) or more than 300 colonies . Treatment C. The number of colonies of *V. cholera* bacteria that grew could be counted with a mean (average) of 270.5 colonies. In treatments D and E the number of *V. cholera* bacteria colonies that grew 0 (no growth of *V. cholera* bacteria).

The data in the table above were analyzed using the Standard Plate Count (SPC), which is the number of colonies growing

by X dilution [8]. Based on the results of the Standard Plate Count (SPC) test, it was found that the 0% and 40% SPC values ≥ 3.0×10^5 CFU/ml did not inhibit the growth of *V. cholera* bacteria. According to that a large number of bacterial colonies (more than 300 colonies) does not inhibit bacterial growth [8]. A 60% treatment with an average SPC value=2.7×10^5 CFU/ml can be bacteriostatic (inhibits) the growth of *V. cholera* bacteria. Treatments of 80% and 100% were concentrations that were bactericidal (deadly) *V. cholera* bacteria.

Based on the results of research on the concentration of red ginger juice as an antibacterial against the growth of *V. cholera* bacteria, it showed that the concentration of 0% and 40% red ginger juice did not inhibit *V. cholera* bacteria, 60% treatment was bacteriostatic (inhibited) the growth of *V. cholera* bacteria, and 80% and 100% treatment were bactericidal (deadly) *V. cholera* bacteria.

The concentration of red ginger rhizome juice is known to have an active ingredient as an antibacterial. The presence of antibacterial properties of red ginger rhizome juice is inhibitory (bacteriostatic) at a concentration of 60% and lethal (bactericidal) at a concentration of 80%, and 100% means red ginger rhizome juice contains antibacterial compounds. According to the high content of essential oils in red ginger makes red ginger plants widely used in the world of health as medicine [4]. The active ingredients of essential oils and flavonoids have an antibacterial effect in inhibiting the growth of pathogenic microorganisms. Explained that red ginger is suitable for medicinal ingredients because ginger also contains the active substances shogaol and gingerol which function to generate energy as high antioxidants, antimutagenic, antibacterial, antitumor, anti-inflammatory, and anti-carcinogenic [9]. It is explained that the content of red ginger, especially gingerol and shogaol, are compounds that can function as immunomodulatory and red ginger also has anti-inflammatory, antibacterial, and antioxidant effects [10].

The results of the study by Majid, et al. showed that ginger rhizome extract was able to inhibit microbial growth due to the presence of bioactive compounds, namely antimicrobial compounds in the phenol, flavonoid, terpenoid, and essential oil groups which could inhibit microbial growth [11]. It is Stated that important materials from bacteria can be inhibited by the antibacterial activity of bioactive compounds (flavonoids) [12]. This can cause damage, or imperfection of the cell wall in bacteria. In addition, the bacterial cell wall will not be resistant to plasma osmotic pressure so it can be broken/destroyed. Another thing that can happen is the inhibition of protein and nucleic acid synthesis in bacteria which causes total cell damage. Disturbances that can occur are damage to cells and cell membranes and changes in cell permeability.

The effect of red ginger rhizome juice is proven to inhibit the growth of *V. cholera* bacteria at a concentration of 60%, and can kill *V. cholera* bacteria at high concentrations, namely: 80%-100%. Stated that the higher the antibacterial compound, the higher the antibacterial activity [13]. The results of study by Majid, et al. show that the higher the concentration of the extract tested, the higher the levels of antibacterial compounds; so that they can inhibit the growth and kill the tested bacteria [8]. The results of study by Widiastuti, et al. studies showed that ginger rhizome extract was able to inhibit microbial growth due to the presence of bioactive compounds, namely antimicrobial compounds in the phenol, flavonoid, terpenoid and essential oil groups which could inhibit microbial growth [11]. Furthermore, explained that the secondary metabolites (phenols, flavonoids, terpenoids and essential oils) found in ginger rhizomes are a class of bioactive compounds that can inhibit microbial growth [14]. Flavonoids are one of the largest natural phenol group compounds that have many pharmacological benefits, including being antibacterial [15].

Table 1: Number of *V. cholera* bacterial colonies per ml after being treated

Replication	Treatment				
	A (0%)	B (40%)	C (60%)	D (80%)	E (100%)
1	TMTC	TMTC	240	0	0
2	TMTC	TMTC	312	0	0
3	TMTC	TMTC	285	0	0
4	TMTC	TMTC	223	0	0
5	TMTC	TMTC	293	0	0
Mean	TMTC	TMTC	270,5	0	0

0: no bacterial colonies, TMTC: Too Many To Count

Conclusion

Flavonoids are found in plants that produce red, yellow, blue, orange, and purple pigments from fruits, flowers, and leaves. Further research can be carried out regarding the effects of elephant ginger, red ginger, and emprit ginger from East Nusa Tenggara on the development of other types of pathogenic bacteria. For future research, they can carry out further research by testing other types of ginger plants or other medicinal plants, especially medicinal plants typical of East Timor-Nusa Tenggara. Based on the results of the antibacterial test of red ginger juice on the growth of *V. cholera* bacteria, it can be concluded that red ginger juice in treatment C (60%) was bacteriostatic (inhibited) the growth of *V. cholera* bacteria, and in treatment D (80%) and E (100%) is bactericidal (deadly) *V. cholera* bacteria.

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