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Original Article

EVALUATION OF MICROBIOLOGICAL CONTAMINATION PARAMETERS OF TRADITIONAL MEDICINAL PREPARATIONS CONTAINING RED GINGER

DIAH KARTIKA PRATAMI[1], SHIRLY KUMALA[1*], I GUSTI NYOMAN ARY WIDYASIH[1], NUR MIFTAHURROHMAH[1], SAFIRA NAFISA[1]

[1]Faculty of Pharmacy, Universitas Pancasila, Jakarta, 12640, Indonesia
*Email: fskumala@univpancasila.ac.id

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ABSTRACT

Objective: The purpose of this study was evaluated the presence or absence of pathogenic microbial contamination in traditional medicinal preparations containing red ginger based on specific microbial tests and evaluating the amount of microbial contamination in traditional medicinal preparations containing red ginger based on the Total Plate Number and Mold Yeast values.

Methods: This research was conducted on instant powdered herbal preparations containing red ginger and internal medicinal liquid containing red ginger. This preparation will be tested for safety and quality requirements including Total Plate Number with Tryptic Soy Agar media, Yeast Mold Number with Saboroud Dextrose Agar media, Enterobacteriaceae Numbers with Violet Red Bile Glucose media, *Escherichia coli* with Mac Conkey Agar media, *Clostridia sp* with Reinforced media Medium for Clostridia, *Salmonella sp* with Rappaport Vasiliadis medium Salmonella Enrichment Broth, and *Shigella sp* with Xylose Lysine Deoxycholate Agar.

Results: The results of the study for the Total Plate Count and Mold and Yeast values in red ginger instant powder preparations were<10 CFU/gr, for testing the microbes *Escherichia coli, Salmonella sp., Clostridia, and Shigella sp.* is negative. Then, the results of the research on the medicinal liquid in red ginger for microbial contamination values were<10 CFU/gr, for testing *Escherichia coli, Salmonella sp., Clostridia, and Shigella sp.* is negative/gr.

Conclusion: Based on the results obtained, it is concluded that herbal preparations containing red ginger meet the safety and quality requirements so that they are safe for consumption.

Keywords: Red ginger, Traditional medicine, Microbial contamination, Instant powder

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INTRODUCTION

Over time, the use of ginger as traditional medicine is increasing [1]. Because, it can be used in maintaining and maintaining a healthy body, besides that according to the World Health Organization (WHO), up to 65% of the population of developed countries and 80% of the population of developing countries have used traditional medicine [2]. This increase was driven by the development of science and technology that provides more publications of scientific research data showing the benefits of natural medicine, which is also known as traditional medicine [3]. Moreover, since the Covid-19 pandemic, which has made all parts of the world in an uproar about this virus, research continues to be carried out on drugs that can overcome and cure, including traditional medicine which is the alternative [4]. Because a lot of people are trying to find alternatives to maintain body immunity by consuming efficacious medicinal plants. Thus, the manufacture of traditional medicinal preparations was made in conjunction with an independent project, namely by using the vast vacant land in the Cisadane Valley area, Bogor, which has the potential to be planted with plants that can be used as traditional medicines and have health benefits. Utilization of ginger which is formulated as an instant powder preparation and internal medicinal liquid for body health as well as an ecotourism attraction in the Cisadane Valley can increase added value in the tourist area.

In addition, another reason that makes the use of traditional medicine increasingly trending today is that many people assume that traditional medicine is definitely not dangerous because traditional medicine contains natural ingredients [5]. Some people do not know that traditional medicine is not necessarily safe, if the quality of traditional medicine is not good it can cause various bad effects for its users. The low quality of drugs can be caused by several factors, among which are often found toxic plant species used for traditional medicine, the addition of synthetic materials, inappropriate doses, interactions with conventional drugs, and contamination of traditional medicines by microorganisms which are included in biological contaminants [6].

According to PerKaBPOM RI Number 32 of 2019 concerning the Safety and Quality Requirements of Traditional Medicines, these safety and quality requirements are carried out because they are part of the criteria that must be met to obtain a distribution permit for Traditional Medicines [7]. In this study, two preparations were carried out, namely powder and liquid medicine, one of the safety and quality requirements was the microbial contamination parameter test. Parameter tests for microbial contamination include Total Plate Number, Yeast Mold Number, *Escherichia coli, Salmonella sp.*, and *Shigella sp.*

MATERIALS AND METHODS

Sample and media

Samples of traditional medicinal powders and internal medicine liquids produced in the Cisadane Valley, Ciseeng, Bogor, Wes Java, Indonesia. Red ginger is determined by Herbarium Depokensis (DEB), Departement of Biology, Faculty of Science and Mathematics, Universitas Indonesia (No.701/UN2. F3.11/PDP.02.00/2021). The media Sabouraud Dextrose Agar (SDA), Tryptic Soy Agar (TSA), McConkey Agar (MCA) media, Xylose Lysine Deoxycholate Agar (XLDA) media, media Reinforced Medium for Clostridia, Aquadest, Rubbing alcohol, and Ethanol 70% were purchased from Faculty of Pharmacy, Universitas Pancasila.

Tools

Autoclave, oven, laminar airflow, round loop needle, needle prick, spirit burner, lighter, Erlenmeyer, test tube, gauze, tube rack, petri dish, incubator, vortex.

Herbal medicine production

Samples of traditional medicinal powders and liquid medicinal preparations were obtained from products produced by several students and lecturers of the Faculty of Pharmacy, Pancasila University in the Cisadane Valley, Ciseeng, Bogor, West Java, Indonesia in the Matching Fund program.

Instant Powder Red Ginger: First, prepare materials and tools to be used and then wash all the ingredients. Peel the skin of red ginger. Then, put the red ginger into the chopping machine. Then, enter the red ginger with the addition of water to the squeeze machine until the red ginger is completely extracted. Next, collected red ginger juice and put it into the machine with the addition of cinnamon powder and sugar. Stir at a constant speed over low heat until it becomes a powder. If the powder obtained is not yet fine, do the grinding until a fine powder is obtained. Put 100 grams of powder into a standing pouch with a size of 9x15 cm and then press it using a press machine.

Medicinal Liquid Red Ginger: Heated 200 ml of water then add red ginger, cinnamon, and brown sugar. Then, stir and heat for 15 min. Wait until it cools down and put it in a bottle.

Total plate number

Pipette 1 ml of sample from each dilution and then put into a petri dish containing 15-20 ml of Tryptic Soy Agar (TSA) media at a temperature of 45-50 °C [8]. Next, homogenize by turning the petri dish back and forth or forming a fig. eight. And then, allowed to solidify, then incubated at a temperature of 30-35 °C for 3-5 d. after that it is calculated by the following formula Eq.1:

$$\text{Total Plate Number} = \frac{\sum C}{((1xn1)+(0,1xn2)xd))} \ldots. (1)$$

C = number of colonies from each petri dish

n1 = number of petri dishes from the first calculated dilution

n2 = number of petri dishes from the second dilution

d = first dilution calculated

Yeast mold number

First, put the liquid SDA media that has been cooled at 45 °C as much as 15-20 ml into a petri dish [8]. And then, allowed to solidify, then 1 ml of the diluted sample was put into a petri dish containing solid SDA. Next, the sample is spread with a spreading rod, then incubated at 25 °C for 5-7 d. After that, the number of mold and yeast colonies can be counted.

***Escherichia coli* contamination measurement**

10 ml of the sample was inoculated into Tryptic Soy Broth/TSB, then incubated at 30-35 °C, for 18-24 h [8]. And then, Subculture of 1 ml of suspension in TSB media to 100 ml of McConkey Broth (MCB) media, then incubated at 42-44 °C, for 24-48 h. Next, Subculture suspension from MCB media to McConkey Agar (MCA) media, then incubated at 30-35 °C, for 18-72 h. If there is colony growth, it indicates the presence of *E. coli.*

***Salmonella sp.* contamination measurement**

10 ml of sample was inoculated into Tryptic Soy Broth/TSB, incubated at 30-35 °C, for 18-24 h [8]. Next, Subculture 0.1 ml of suspension in TSB media to 10 ml of Rappaport Vasiliadis Salmonella Enrichment Broth (RVSEB) media, incubated at 30-35 °C, for 18-24 h. And then, the Subculture of the suspension from RSVEB media to Xylose Lysine Deoxycholate Agar (XLD Agar) media, then incubate at a temperature of 30-35 °C, for 18-24 h. The presence of red colonies, with or without a black dot in the center, indicates the presence of characteristics.

***Shigella sp.* contamination measurement**

Weigh the equivalent of 10 g of solid sample or pipette 10 ml of liquid sample into a tube containing 90 ml of Tryptic Soy Broth (TSB) media [8]. Incubate at 30-35 °C for 18-24 h. And then, the sample is declared positive if the TSB is cloudy (there is growth) and if the TSB remains clear (no growth) is declared negative, stop the work step here. Next, if the sample is positive, take a loop from the TSB, scratch on the surface of Xylose Lysine Deoxycholate Agar (XLD) and Mac Conkey Agar (MCA). Minimum 2 two Petri dishes (Duplo). Incubate at 30-35 °C for 18-48 h.

RESULTS AND DISCUSSION

Plant determination

Based on the results of plant identification that has been sent to the Herbarium Depokensis that the plant is actually a red ginger plant (*Zingiber officinale* Roscoe) family Zingiberaceae.

Total plate count number

The total plate count (TPC) test is carried out by the Duplo test. The result of instant powder red ginger and liquid red ginger is shown in table 1 and table 2.

Table 1: TPC test results on instant powder red ginger

No.	Group identification	Dilution	Results
1.	TPC 1	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	
2.	TPC 2	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the total plate count test in duplicate, in the first and second TPC on instant powder red ginger from a dilution of 10^{-1} to 10^{-5} the number of colonies was obtained, the result of the first TPC was<10 CFU/g. This means that in TPC there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the TPC value to meet the requirements, there is <10 CFU/g.

Table 2: TPC test results on medicinal liquid red ginger

No.	Group identification	Dilution	Results
1.	TPC 1	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	
2.	TPC 2	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the total plate count test in duplicate, in the first and second TPC on medicinal liquid red ginger from a dilution of 10^{-1} to 10^{-5} the number of colonies was obtained, the result of the first TPC was<10 CFU/g. This means that in TPC there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the TPC value to meet the requirements, there is<10 CFU/g.

The total plate count (TPC) is one of the safety parameters of traditional medicine that is used as a guide to what level of manufacture of the product in implementing Good Manufacturing Practices of Traditional Medicine (CPOTB) [9]. The result of microbiological quality analysis by TPC of instant powder and liquid medicine of red ginger similar to the ginger product produced by Arifan *et al.* that showed the total microbial value match the BPOM and SNI standard [10]. It can be concluded that this product is safe for consumption.

Yeast mold number

The yeast mold number test is carried out by the Duplo test. The result of yeast mold number on instant powder red ginger and liquid red ginger were shown in Tables 3 and 4.

Table 3: Yeast mold number test results on instant powder red ginger

No.	Group identification	Dilution	Results
1.	YMN 1	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	
2.	YMN 2	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the total plate count test in duplicate, in the first and second YMN on instant powder red ginger from a dilution of 10^{-1} to 10^{-5} the number of colonies was obtained, the result of the first YMN was<10 CFU/g. This means that in YMN there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the YMN value to meet the requirements, there is<10 CFU/g.

The yeast and mold need 5 to 7 d to produce visible growth on solid media. The source of yeast and mold may come in drinking water or raw material. The presence of Yeast and Mold in the medicinal products should be considered a serious threat to consumers. It is necessary measures should be taken to avoid yeast and mold growth as well as bacterial growth in medicinal products to make it safer for public health [11].

Table 4: YMN test results on the medicinal liquid of red ginger

No.	Group identification	Dilution	Results
1.	YMN 1	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	
2.	YMN 2	10^{-1}	
		10^{-2}	<10 CFU/g
		10^{-3}	
		10^{-4}	
		10^{-5}	

Based on the results obtained from the total plate count test in duplicate, in the first and second YMN on liquid medicinal red ginger from a dilution of 10^{-1} to 10^{-5} the number of colonies was obtained, the result of the first YMN was<10 CFU/g. This means that in YMN there are no growing colonies. Then, based on the requirements of BPOM number 32 of 2019 for the YMN value to meet the requirements, there is<10 CFU/g.

Table 5: *Escherichia coli* test results on instant powder red ginger

No.	Sample	TSB media	MCB media	MCA Media
1.	*E. coli* 1	Cloudy	Yellow	Transparent colony
2.	*E. coli* 2	Cloudy	Cloudy violet	Colony not detected

Escherichia coli

The *Escherichia coli* test is carried out by the Duplo test. The result of *E. coli* contamination in instant powder red ginger and liquid red ginger is shown in table 5 and table 6.

E. coli test for instant red ginger powdered herbal medicine in sample 1 was tested with TSB and turbidity was produced, then the next test was carried out on MCB media and obtained a yellow color which means positive, then tested on MCA media and transparent colonies were produced. Then in the second sample carried out on the TSB test, the turbidity was marked positive, then the test was carried out on MCB media and the results were violet and there was turbidity, then continued for testing on MCA media and obtained no colony growth.

Table 6: *Escherichia coli* test results on the medicinal liquid of red ginger

No.	Sample	TSB media	MCB media	MCA Media
1.	*E. coli* 1	Cloudy	Cloudy violet	Colony not detected
2.	*E. coli* 2	Cloudy	Cloudy violet	Colony not detected

E. coli test for medicinal liquid herbal preparations in red ginger on sample 1 and sample 2 tested with TSB media obtained turbidity, then tested on MCB media obtained violet color and turbidity still occurs, then continued with MCA media test obtained no colony growth.

Escherichia coli is the most common pathogen that caused foodborne outbreaks. The pathogenic *E. coli* strains have caused diarrhea, hemolytic uremic syndrome, hemorrhagic colitis, and other indications in human [12, 13]. Red ginger or the other raw material in traditional medicine can be contaminated with *E. coli* at any point from pre-to postharvest.

Salmonella sp.

The *Salmonella sp.* test is carried out by the Duplo test. The result of *Salmonella sp.* contamination in instant powder red ginger and liquid red ginger is shown in table 7 and table 8.

Table 7: *Salmonella sp.* test results on instant powder red ginger

No.	Sample	TSB media	RVSEB media	XLD	Gram stain
1.	*Salmonella sp.* 1	Cloudy	Green cloudy	Yellow colony	Red round
2.	*Salmonella sp.* 2	Cloudy	Blue cloudy	Red colony	Red round

The test for *Salmonella sp*. the preparation of instant red ginger powder herbal medicine for sample 1 was carried out on TSB media and the result was turbidity, then continued the test on RSVEB media and obtained a green color and turbidity occurred after that XLD test was carried out and obtained a yellow color, declared positive then tested with gram staining and red round colonies were obtained. Then, for sample 2 on the TSB media test, turbidity was obtained, followed by the RSVEB media test and blue and cloudy color was obtained, then the test was carried out on XLD media and red colonies were obtained, then continued with the gram staining test which resulted in round red colonies.

Table 8: *Salmonella sp.* test results on medicinal liquid red ginger

No.	Sample	TSB media	RVSEB media	XLD media
1.	*Salmonella sp.* 1	Cloudy	Blue cloudy	Colony not detected
2.	*Salmonella sp.* 2	Cloudy	Blue cloudy	Colony not detected

Test for *Salmonella sp.* in the liquid preparation of red ginger for samples 1 and 2 in the test with TSB media there was turbidity then the test was carried out on RSVEB media, it was obtained blue and turbidity occurred, then continued with XLD media test and obtained no colony growth.

The genus *Salmonella sp.* belongs to the family of Enterobacteriaceae. Its reproduction reaches optimum conditions in temperatures between 35 and 37 °C. *Salmonella sp* is an enteric pathogenic bacterium for humans that can cause septicemia and gastroenteritis [14]. The presence of *Salmonella sp* in traditional medicine is considered as intolerable. The development of a faster and more efficient analytical method to detect those microorganisms in traditional medicine and their derivatives is considered important.

Shigella sp.

The *Shigella sp.* test is carried out by the Duplo test. The result of *Shigella sp.* contamination in instant powder red ginger and liquid red ginger is shown in table 9 and table 10.

Table 9: *Shigella sp.* test results on instant powder red ginger

No.	Sample	TSB media	MCA media	XLD media	Gram stain
1.	*Shigella sp.* 1	Cloudy	Transparent colony	Light yellow-red	round red
2.	*Shigella sp.* 2	Cloudy	Colony not detected	Colony not detected	-

Test *Shigella sp.* In the preparation of instant red ginger powder herbal medicine for sample 1, TSB media was tested for turbidity, then continued to test on MCA media, and transparent colonies were obtained, the results were declared positive, then tested on XLD media, the results were pink yellow colonies, then continued for the Gram stain test and red round colonies were obtained. Then, for sample 2 on the TSB media test, turbidity was obtained and continued with the test on MCA media, it was obtained that there was no colony growth as well as in the XLD media test.

Table 10: *Shigella sp.* test results on liquid medicine red ginger

No.	Sample	TSB media	MCA media	XLD media
1.	*Shigella sp.* 1	Cloudy	Colony not detected	Colony not detected
2.	*Shigella sp.* 2	Cloudy	Colony not detected	Colony not detected

In the test of *Shigella sp*. in medicinal liquid herbal preparations for samples 1 and 2, the test was carried out on TSB media, obtained turbidity, then tested on MCA media and also XLD did not grow colonies.

Shigella sp. is common pathogenic bacteria found in the environment where sanitation is poor, since they are contaminated by faeces of humans and animals [15]. It causes shigellosis due to consumption of pathogenic contaminated water [14]. Children under 5 y old are at highest risk for *Shigella spp.* infection, it may cause symptoms of fever, fatigue, anorexia, and malaise related illness and death [16]. Thus, awareness and provide safe drinking water for traditional medicine production is a must to safeguard people who consume to prevent gastrointestinal diseases.

CONCLUSION

The results of the evaluation of microbiological contamination parameters of traditional medicinal preparations containing red ginger in red ginger instant powder preparations for Total Plate

Count and Mold Yeast Number values were<10 CFU/g, *Escherichia coli, Salmonella sp.,* and *Shigella sp.* was negative/g. Then, in the liquid preparation of red ginger for Total Plate Count and Mold Yeast Number values <10 CFU/g, *Escherichia coli, Salmonella sp*., and *Shigella sp.* was negative/g. Red ginger traditional medicinal preparations, namely instant powder and liquid internal medicine, can be consumed because they have met the safety and quality requirements according to PerBPOM No. 32 of 2019.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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