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Original Article

EVALUATION OF MICROBIOLOGICAL CONTAMINATION PARAMETERS OF HERBAL DRINK AND INSTANT POWDER

DIAH KARTIKA PRATAMI[1], TIKA MALIKHAH[2], DESI NADYA AULENA[1], NOVI YANTIH[2], SHIRLY KUMALA[3]*

[1]Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Pancasila University, Jakarta, 12640, Indonesia, [2]Faculty of Pharmacy, Pancasila University, Jakarta, 12640, Indonesia, [3]Laboratory of Microbiology, Faculty of Pharmacy, Pancasila University, Jakarta, 12640, Indonesia

***Email: fskumala@univpancasila.ac.id**

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ABSTRACT

Objective: The aim of this study was to determine whether the microbial contamination contained in herbal medicine in the form of herbal drink (liquid) and instant powder fulfil(s) the requirements of BPOM No. 32 of 2019.

Methods: Determination of microbial contamination was carried out by testing the total plate count using TSA (Tryptic Soy Agar) media, Yeast and Mold count plates using SDA (Sabouraud Dextrose Agar) media, *Escherichia coli* using MCB selective media (MacConkey Broth) and MCA (MacConkey Agar), Salmonella using RVSEB (Rappaport Vassiliadis Salmonella Enrichment Broth) and XLD (Xylose Lysine Deoxycholate) media, and Shigella using XLD (Xylose Lysine) media Deoxycholate) and also MCA (MacConkey Agar).

Results: The result of the herbal drink the total plate count is 1.2 x 10^2 CFU/g, yeast and mold count plates was<10 CFU/g, *Escherichia coli* was negative/g, *Salmonella enterica* Serovar Thypimurium was negative/g, and *Shigella sonnei* which was negative/g. while in instant powder samples test showed that the total plate count was<10 CFU/g, the yeast and mold count plates was<10 CFU/g, *Escherichia coli* was negative/g, *Salmonella enterica* Serovar Thypimurium was negative/g, and *Shigella sonnei* was negative/g.

Conclusion: Based on the results of data analysis from the two samples, the herbal medicine produced as herbal medicine has fulfilled the applicable requirements

Keywords: Herbal medicine, Total plate count, *Escherichia coli*, *Salmonella enterica* serovar Thypimurium, and *Shigella sonnei*

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INTRODUCTION

Consumption of herbal medicine is one of the alternative choices for Indonesian people in an effort to increase body immunity. Jamu is one of the traditional Indonesian medicines that has been practiced for centuries in the community, although there are many modern medicines, herbal medicine is still very popular among the people. The increasing demand for herbal medicine has encouraged small/home industries to manufacture herbal and traditional medicines to produce instant herbal medicine as well as herbal drinks that can increase endurance.

In general, Indonesian people consume herbal medicine in the form of liquid and instant powder. Herbal medicine in liquid form has a relatively short shelf life and is less practical than herbal medicine in powder form. The process of making herbal medicine, starting from the selection of raw materials to processing using tools and methods that are still very simple, does not rule out the possibility that the herbs are contaminated by microorganisms [1].

Based on a survey and interview in 2020 conducted on home industry herbal medicine business players in the North Semarang area which were sampled with the aim of knowing sanitation and hygiene in the herbal medicine processing. BPOM provisions for not applying good hygiene and sanitation, and other samples are eligible for implementing hygiene and sanitation [2].

Routine testing conducted by the Banjarmasin POM Center in 2018 in the context of checking production facilities and distribution of traditional medicines, still found home industries that did not meet the applicable hygiene and sanitation standards because they did not apply Good Traditional Medicine Manufacturing Methods (CPOTB) [3].

The herbs to be tested are concoctions or mixtures of *Curcuma zanthorrhiza* Roxb simplicia, *Zingiberaceae officinale* Roscoe and *Centella asiatica* made with the addition of sugar. Medicinal products in circulation must meet safety and quality standards. As a result of the increasing circulation of illegal herbal medicine, the government carries out surveillance and prevention. Based on a regulation through the Ministry of Health of the Republic of Indonesia in the Decree of the Minister of Health of the Republic of Indonesia No: 661/MenKes/SK/II/1994, it is stated that it is necessary to prevent the circulation of traditional medicines that do not meet the requirements of safety, benefit and quality.

Based on this, the researchers were conducted microbial contamination tests on liquid and powdered herbs including quantitative and qualitative tests. Quantitative microbial contamination tests are Total Plate Number and Yeast Mold Number While the qualitative microbial contamination test is the identification of *Escherichia coli, Salmonella enterica* serovar thypimurium*, Shigella sonnei*.

MATERIALS AND METHODS

Material

Herbal medicine in liquid form and instant powder made by students of the Faculty of Pharmacy, Pancasila University, Aqua destillata, Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), Sabouraud Dextrose Agar (SDA), MacConkey Agar (MCA). MacConkey Broth (MCB), Xylose Lysine Deoxycholate (XLD), Rappaport Vassiliadis Salmonella Enrichment Broth (RVSEB), NaCl 0.9%, Cristal Violet, Alcohol 96%, Immersion oil, Lugol, safranin, Reagent Kit GN A+B.

Tool

Equipment needed in this study were incubator, Autoclave (121 °C, 20 min), Vortex Stirer, Micropipette, Petri dish, Beaker Glass, Test Tube Racks, Test Tubes, Object Glass, Erlenmeyer, inoculating loops, Microscope, spirit lamp, and analytical balance.

Source of plant origin

Raw materials were obtained from the land of the Lembah Cisadane, Kec. Ciseeng, Bogor Regency, West Java, Indonesia.

Plant determination

Based on the results of the determination test from Herbarium Depokensis (DEB), Biology Departement, University of Indonesia stated that the raw material was a species of *Curcuma zanthorrhiza* Roxb and *Zingiberaceae officinale* Roscoe of the Zingiberaceae family.

The preparation of traditional medicine

The raw material used was simplicia which was dried in an oven. Herbal medicine was made in liquid and powder dosage forms by students Faculty of Pharmacy, Pancasila University. Liquid herbal medicine was made using the decoction process. Simplicia *Curcuma xanthorrhiza* Roxb, *Zingiberaceae officinale* Roscoe, and *Centella asiatica* were extracted with water as a solvent at 90 °C for approximately 30 min calculated after boiling, then add brown sugar to taste. The extract was filtered and allowed to cool and packed in plastic bottles. The step of making herbal powder begins with making extracts through the decoction process, namely extracting ginger and ginger simplicia with water as a solvent at 90 °C for approximately 30 min calculated after boiling then added granulated sugar followed by heating until crystallization occurs to form dry granules or solids, pulverized to form a fine powder and sieved to obtain a powder with uniform size.

Total Plate Count (TPC) test

A total of 10 ml or 10 g of sample was put into an erlenmeyer containing 90 ml of Tryptic Soy Broth (TSB) media. Pipette 1 ml each into 5 tubes containing 9 ml of Tryptic Soy Broth (TSB) to obtain a dilution of 10^{-1} to 10^{-6}. The results of the dilution were pipetted as much as 1 ml into each Petri dish (done in duplicate), as much as 15-20 ml of Tryptic Soy Agar (TSA) media at a temperature of±45 °C was poured into each dish, homogenized and allowed to solidify. The plates were incubated upside down at 30-35 °C for 3-5 d. then counted the number of colonies in each plate with the highest number of colonies less than 250 colonies, stating the results in Colony Forming Units (cfu/g or cfu/ml) [4].

Yeast and mold count plate test

A total of 10 ml or 10 g of sample was put into an Erlenmeyer containing 90 ml of Tryptic Soy Broth (TSB) media. Pipette 1 ml each into 5 tubes containing 9 ml of Tryptic Soy Broth (TSB) to obtain a dilution of 10^{-1} to 10^{-6}. The results of the dilution were pipetted as much as 1 ml into each petri dish (done in duplicate), as much as 15-20 ml of Sabouraud Dextrose Agar (SDA) media at a temperature of±45 °C was poured into each dish, homogenized and allowed to solidify. The plates were incubated in an inverted position at 20-25 °C for 5-7 d. then count the number of colonies in each plate with the highest number of colonies less than 50 colonies, state the results in Colony Forming Units (cfu/g or cfu/ml) [4]

Identification of *Escherichia coli*

A total of 10 ml or 10 g of sample was put into an Erlenmeyer containing 90 ml of Tryptic Soy Broth (TSB) media. Incubated at 30 °C for 18-24 h. The sample was declared positive if there was turbidity in the TSB media. as much as 1 ml of the sample that was tested positive was inoculated into 100 ml of MacConkey Broth (MCB), incubated at 42-44 °C for 24-48 h. the sample that is declared positive if the MCB media changes color from purple to yellow but if the MCB media does not change color then it is declared negative and the work step is stopped. A total of one loop of positive MCB media was etched on the MacConkey Agar (MCA) surface. At least it was done in duplicate and then the petri dishes were incubated at 30-35 °C for 18-72 h and observations were made [4].

Identification of *Salmonella enterica* serovar thypimurium

A total of 10 ml or 10 g of sample was put into an Erlenmeyer containing 90 ml of Tryptic Soy Broth (TSB) media. Incubated at 30 °C for 18-24 h. The sample was declared positive if there was turbidity in the TSB media. A total of 0.1 ml of positive TSB was inoculated into 10 ml of Rappaport vassiliadis *Salmonella* Enrichment (RVSEB). incubated at 30-35 C for 18-24 h. The sample was declared positive if the RVSEB medium was turbid. if the RVSEB media does not occur turbidity is declared negative and the work step is stopped. One loop of positive RVSEB media was etched on the surface of Xylose Lysine Deoxycholate (XLD). At least it was done in duplicate and then the petri dishes were incubated at 30-35 C for 18-48 h and observations were made [4].

Identification of *Shigella sonnei*

A total of 10 ml or 10 g of sample was put into an Erlenmeyer containing 90 ml of Tryptic Soy Broth (TSB) media. Incubated at 30 °C for 18-24 h. The sample was declared positive if there was turbidity in the TSB media. One swab of TSB which was tested positive was etched on the surface of Xylose Lysine Deoxycholate (XLD) and MacConkey Agar (MCA). At least it was done in duplicate and then the petri dishes were incubated at 30-35 °C for 18-48 h and observations were made [4].

Gram stain confirmatory test

Prior to gram staining, the object glass was cleaned and fixed first. One drop of NaCl was given. Colonies were taken from the culture media and then placed on the object glass. Re-fixed to dry. Followed by giving crystal violet (set aside for 1 minute). Drops of lugol (leaved for 1 minute). The object glass was washed with running water and dripped with 96% alcohol until the purple color disappeared. The object glass was washed again with running water, then safranin dye was added (it was left for 30 seconds). Clean with running water. The object glass is air-dried. Added immersion oil and observed object glass under a microscope.

Biochemical reaction confirmation test

Bacterial cultures were taken with a needle and placed on an oxidase test strip to be tested for oxidase activity. If the oxidase test shows a positive result (the oxidase test strip changes color to purplish blue), then the bacteria are tested with the A+B GN kit. if the oxidase test shows a negative result (the oxidase test strip does not change color), then the bacteria are tested with GN A or GN A+B kits. Single colonies were taken aseptically using a needle loop, the colonies were suspended in 3 ml sterile 0.9% NaCl solution (GN A) or 5 ml sterile 0.9% NaCl solution (GN A+B). 3-4 drops (100 L) of the colony suspension were added to each strip hole. Coat the well with suitable mineral oil. Incubated at 35-37 °C for 18-24 h. Then the next day the reagent was added.

RESULTS AND DISCUSSION

Herbal product results

Herbal medicine made by applying hygiene and sanitation standards to produce quality and safe herbal drink and instan powder for consumption. The herbs tested were the best formulas from the hedonic test results. Organoleptic tests were carried out on both dosage forms. The herbal drink and instan powder showed a sweet taste, brownish orange color and aromatic aroma. While the herbal medicine powder showed a sweet taste, yellowish color and aromatic aroma. In this study, a 'hedonic test was carried out, it was necessary to find out the most preferred formula. The research using the hedonic test was in line with the food research conducted by negara JK *et al.* [5].

Total plate count

Results of Total Plate Count (TPC) in liquid and powdered herbal medicine samples was shown in table 1 and fig. 1. TPC is one of the indicators of the hygiene and sanitation process that is used as a basis for suspicion of a product based on its microbiological quality. The Total Plate Count must be kept as small as possible even though these microbes are not harmful to health, but sometimes due to the influence of something they can become harmful microbes. The calculation of the Total Plate Count is carried out to find out the level of contamination by bacteria in herbal products.

Table 1: Results of Total Plate Count (TPC) in liquid and powdered herbal medicine samples

Type	Sample	Dilution	Cup		Yield (CFU/ml)
			I	II	
Liquid	ALT 1	10^{-1}	12	12	1.2 x 10^2
		10^{-2}	1	1	
		10^{-3}	0	0	
		10^{-4}	0	0	
		10^{-5}	0	0	
Powder	ALT 2	10^{-1}	0	0	<10
		10^{-2}	0	0	
		10^{-3}	0	0	
		10^{-4}	0	0	
		10^{-5}	0	0	

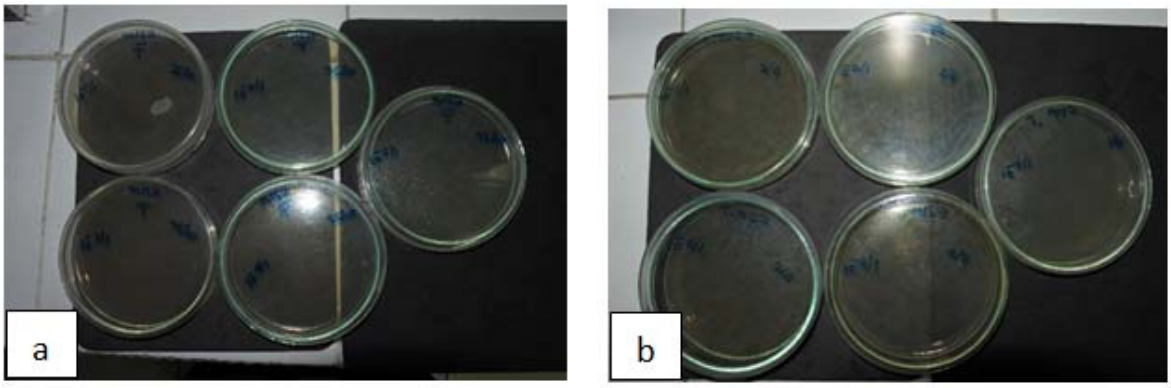

Fig. 1: Results of observation of microbial growth in herbal drink and instant powder. (a) The results of the observation of the herbal drink; (b) The results of the observation of the instan powder

The total plate count was selected from the dish with the highest colony of less than 250 colonies. Dilution of bacterial suspension using Tryptic Soy Broth (TSB) Media as a place for the growth of microorganisms. Serial dilutions are intended to reduce the density or number of microbes in the sample. Then from each dilution put into Trytic Soy Agar (TSA) media and incubated at 30-35 °C for 3-5 d. The results of the analysis of the Total Plate Count (TPC)) in the herbal drink sample showed that there was bacterial growth in the 10-1 dilution of 12 colonies/g and the $10^{-2,}$ 10^{-3}, 10^{-4}, 10^{-5} dilution showed no bacterial colony growth, total calculation result is obtained 1.2 x 10^2 CFU/ml. Whereas the results of the analysis of Total Plate Count (TPC) on instant powder samples did not show bacterial growth in all dilutions indicated by the absence of bacterial colonies in the media which was expressed as<10 CFU/ml

According to BPOM RI Regulation Number 32 of 2019, the Total Plate Count (TPC) contamination limit is10^5 colonies/g so that the herbal drink and instan powder were declared to meet the safety and quality requirements [6].

Yeast and mold count plate

Results of Yeast and Mold Count Plate in liquid and powdered herbal medicine samples was shown in table 2 and fig. 2. Yeast and Mold Count Plate is a parameter that shows the number of yeast molds that contaminate a sample. Mold is a group of filamentous multicellular fungi. Filament is a distinctive morphological part of molds that shows fibrous colonies like cotton so that they can distinguish them from yeast [7]. Yeast is a group of unicellular fungi that grow well in watery or moist places, including plant sap. Yeasts reproduce by way of cell division from the parent cell or called asexual.

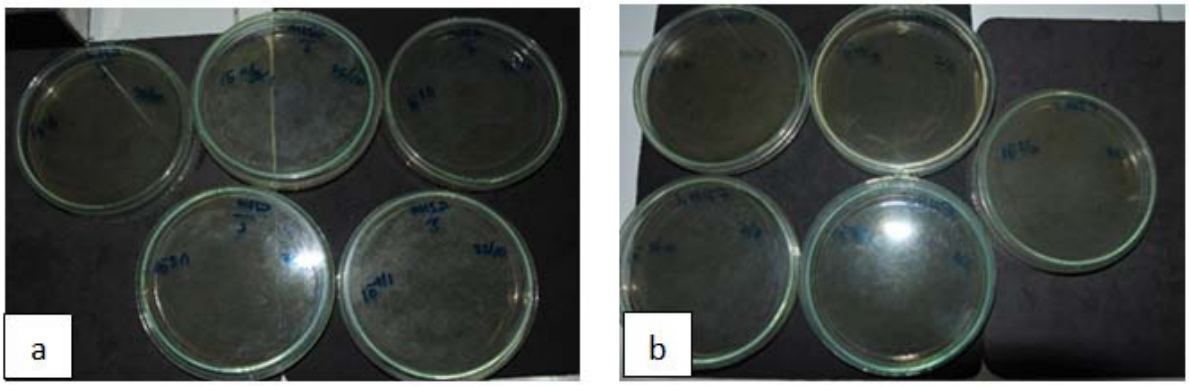

Fig. 2: Results of observation of mold and yeast growth in herbal drink and instant powder. (a) The results of the observation of the herbal drink; (b). The results of the observation of the instant powder

Table 2: Yeast and mold count plate results on herbal drink and intant powder

Type	Sample	Dilution	Cup		Yield (CFU/ml)
			I	II	
Liquid	AKK 1	10^{-1}	0	0	<10
		10^{-2}	0	0	
		10^{-3}	0	0	
		10^{-4}	0	0	
		10^{-5}	0	0	
Powder	AKK 2	10^{-1}	0	0	<10
		10^{-2}	0	0	
		10^{-3}	0	0	
		10^{-4}	0	0	
		10^{-5}	0	0	

Molds/yeasts can contaminate herbs through the raw materials used, such as rhizomes which generally grow in the soil. The raw materials that grow in the soil have environmental conditions that can support the growth of molds and yeasts, such as moist or wet soil conditions and the water content contained in traditional medicinal raw materials. Therefore, it must be ensured that the raw materials used must be clean. Determination of Yeast ande Mold Count Plate was carried out by making serial solutions or dilutions using Tryptic Soy Broth (TSB) media, then the bacterial suspension was inoculated into the incubated Sabouraud Dextrose Agar (SDA) temperature 20-25 °C for 5-7 d. The results of the analysis of Yeast and Mold are based on Count Plate. The sample of herbal drink and instant powder showed results <10 CFU/mL or in other words the sample did not contain mold and yeast. Based on BPOM RI Regulation Number 32 of 2019, the contamination limit of Yeast ande Mold Count Plate is 10^3 colonies/g so that the herbal drink and instant powder were declared to meet the safety and quality requirements. One of the factors that support the quality of herbal products is drying. Drying aims to remove or reduce to a minimum the water content to prevent the growth of microbes including molds and yeasts [8].

Identification of *Escherichia coli*

The results of the identification of Escherichia coli in herbal drink and instant powder was shown in table 3 and fig. 3. *E. coli* is a negative-Gram bacteria, short rod-shaped, has a flagellum, measuring 0.4-0.7 □mx 1.4 □m and have hoops. These bacteria can grow in almost all media, can ferment lactose and have microaerophilic [9]. *E. coli* is the most commonly used microbe as an indicator of fecal contamination in water, food and beverage ingredients, including herbal medicine. The habitat of *E. coli* is in the digestive tract and non-digestive tract such as soil and water. Microbes of this type are always present in human feces.

Table 3: The results of the identification of *Escherichia coli* in herbal drink and instant powder

Type	Sample/Control	Media		
		TSB	MCB	MCA
Liquid	K+	cloudy	cloudy violet	Brick red colony
	K-	Clear	cloudy violet	Do not grow colonies
	I	cloudy	cloudy violet	Do not grow colonies
	II	cloudy	cloudy violet	Do not grow colonies
Powder	K+	cloudy	cloudy violet	Brick red colony
	K-	Clear	cloudy violet	Do not grow colonies
	I	cloudy	clear violet	Do not grow colonies
	II	cloudy	clear violet	Do not grow colonies

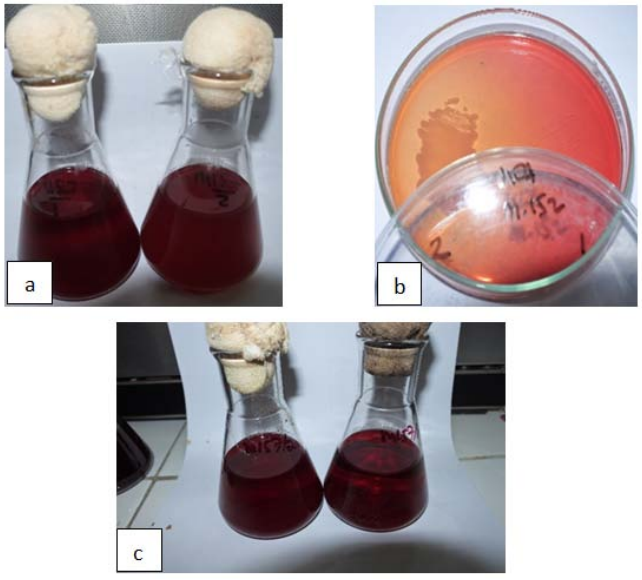

Fig. 3: Observations on the identification of *Escherichia coli* in herbal drink and instant powder on MCB and MCA media, (a). The results of observations of samples of herbal drink medicine on MCB media; (b). The results of observations of samples of herbal drink on MCA media; (c). Observation results of instant powder on MCB media

The research was conducted by making positive and negative controls as a comparison. The initial stage is done by growing bacteria using TSB media. At this stage, homogenization of the sample was carried out. Bacterial growth was indicated by the presence of turbidity. Next positive TSB media were tested using MCB media to enrich bacteria by accelerating their growth. MCB contains 2 elements, namely lactose and salt where lactose is a source of energy and salt that can inhibit the growth of microorganisms other than *E. coli*. The MCB medium was originally reddish-purple but if there was bacterial growth, it was marked by the occurrence of turbidity. Further tests were carried out by scratching the isolates on MCA media. MCA contains bile salts and crystal violet so that it can inhibit the growth of gram-positive bacteria. This shows that not all bacteria can grow well because this medium is used specifically to identify the presence of gram-negative growth. MCA media was used to differentiate colonies based on their ability to ferment lactose. The growth of *E. coli* on MCA media produced red to brick red colonies. Observations on the TSB media of the two samples, namely liquid and powdered herbal medicine, indicated the suspicion of bacterial growth which was indicated by the occurrence of turbidity. Furthermore, the samples of herbal drink and instan powder were tested using MCB media. Samples of herbal drink showed positive results, this was indicated by the occurrence of turbidity in the MCB media while the instant powder samples showed negative results which were indicated by the absence of turbidity so that no further test with MCA media was needed. Samples of positive herbal drink on MCB media were tested with MCA selective media. The results of the observation of the herbal drink samples showed negative results. This means that all samples have met the safety and quality requirements as stated in BPOM RI Regulation No. 32 of 2019.

Identification of *Salmonella enterica* serovar thypimurium

Identification of *Salmonella enterica* Serovar thypimurium in liquid and powdered herbal medicine samples was shown in table 4 and fig. 4. *Salmonella enterica* Serovar thypimurium is a negative-Gram bacterium of the Enterobacteriaceae family. One type of pathogenic bacteria that can cause disease in humans, rod-shaped with a size of 1-3,5 m x 0.5-0.8 m. *Salmonella enterica* Serovar thypimurium grows fast on ordinary media but does not ferment lactose or sucrose. These bacteria produce acid and some gases from glucose and mannose, but are more likely to produce hydrogen sulfide. These germs can live in frozen water for a long time. *Salmonella* is resistant to certain chemicals, such as diamond green, sodium tetrathionate and sodium dioxycholate. These compounds inhibit coliform bacteria and are therefore useful for the isolation of *Salmonella* from feces [10].

Table 4: Identification of *Salmonella enterica* serovar thypimurium in liquid and powdered herbal medicine samples

Type	Sample/Control	Media		
		TSB	RVSEB	XLD
Liquid	K+	cloudy	cloudy blue	Black
	K-	Clear	clear blue	chocolate
	I	cloudy	cloudy blue	Do not grow colonies
	II	cloudy	cloudy blue	Do not grow colonies
Powder	K+	cloudy	cloudy blue	Black
	K-	Clear	clear blue	chocolate
	I	cloudy	clear blue	Do not grow colonies
	II	cloudy	clear blue	Do not grow colonies

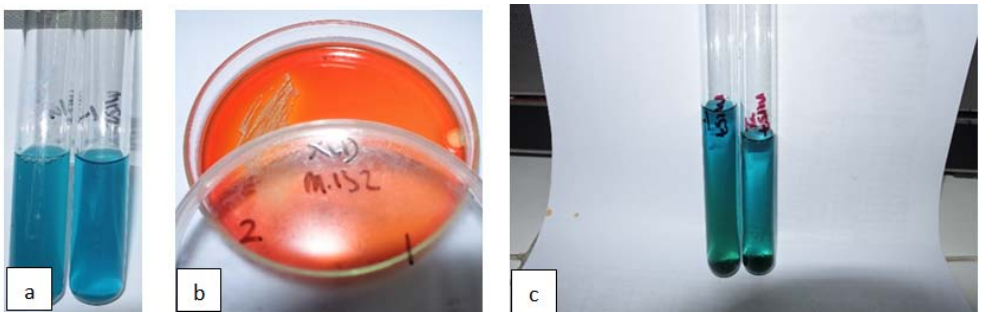

Fig. 4: Observations on the identification *Salmonella enterica* serovar thypimurium of in herbal drink and instant powder on RVSEB and XLD, (a). The results of observations of samples of herbal drink on RVSEB media; (b). The results of observations of samples of herbal drink on XLD media; (c). Observation results of instant powder on RVSEB media

Early stage research was conducted using TSB media. All samples showed bacterial growth which was indicated by a cloudy color and then both samples were tested with RVSEB media. RVSEB is one of the blue enrichment media used for the isolation of Salmonella bacteria. At this stage the optimization of Salmonella growth occurs and inhibition of other bacteria that can interfere with the growth of Salmonella. Bacterial growth is indicated by a cloudy blue color. The third stage was carried out by isolating the positive sample results on RVSEB media into XLD media using a ose needle by scratching. *Salmonella enterica* Serovar Typhimurium will use the components of xylose, lactose and sucrose to become acidic substances that can cause phenol red to turn yellowish. *Salmonella enterica* Serovar Thypimurium also produces hydrogen sulfide which causes black colonies. The results of the research on samples of herbal drink and instant powder on TSB media showed positive results because there was turbidity in TSB media. Then the positive results were tested with RVSEB media. The sample of herbal drink gave positive results, it was indicated by the presence of turbidity in the RVSEB media and continued with the test using XLD media. While the instan powder sample gave a negative result or in other words, there was no turbidity so there was no need for further testing with XLD media. Samples of herbal drink on XLD media showed negative results because no black colonies were found in the media.

Identification of *Shigella sonnei*

Identification of *Shigella sonnei* on herbal drink and instant powder was shown in table 5 and fig. 5. *Shigella sonnei* is a negative Gram, rod-shaped bacterium, single, has'nt flagella, is aerobic or facultatively anaerobic and does not form spores, and its habitat is in the digestive tract with infection through the oral phase [11]. The results of identification observations showed not growth was founds *higella* in both samples.

Table 5: Identification of *Shigella sonnei* on herbal drink and instant powder

Type	Sample/Control	Media		
		TSB	XLD	MCA
Liquid	K+	cloudy	Colony Pink edging yellow	Transparent Colony
	K-	Clear	Do not grow colonies	Do not grow colonies
	I	cloudy	Do not grow colonies	Do not grow colonies
	II	cloudy	yellow colony	Transparent colony
Powder	K+	cloudy	Colony Pink edging yellow	Transparent Colony
	K-	Clear	Do not grow colonies	Do not grow colonies
	I	cloudy	Do not grow colonies	Do not grow colonies
	II	cloudy	Do not grow colonies	Do not grow colonies

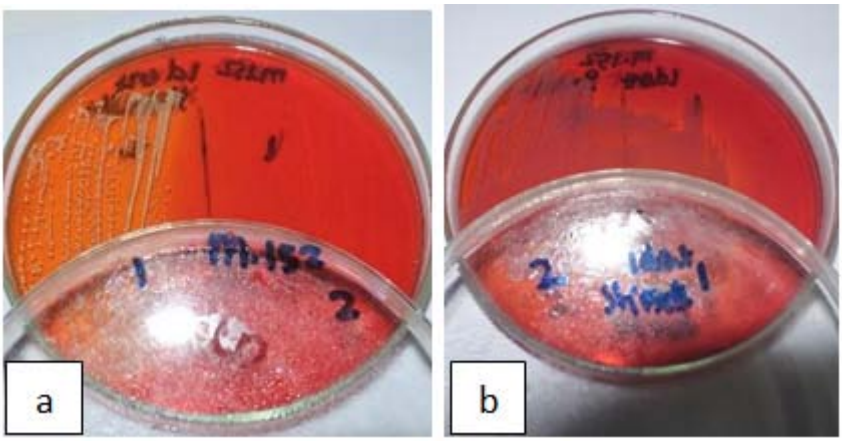

Fig. 5: Observations on the identification of *Shigella sonnei* in herbal drink on XLD and MCA media, (a) the results of Shigella's observations on herbal drink on XLD media; (b). results of Shigella's observations on herbal drink on MCA media media

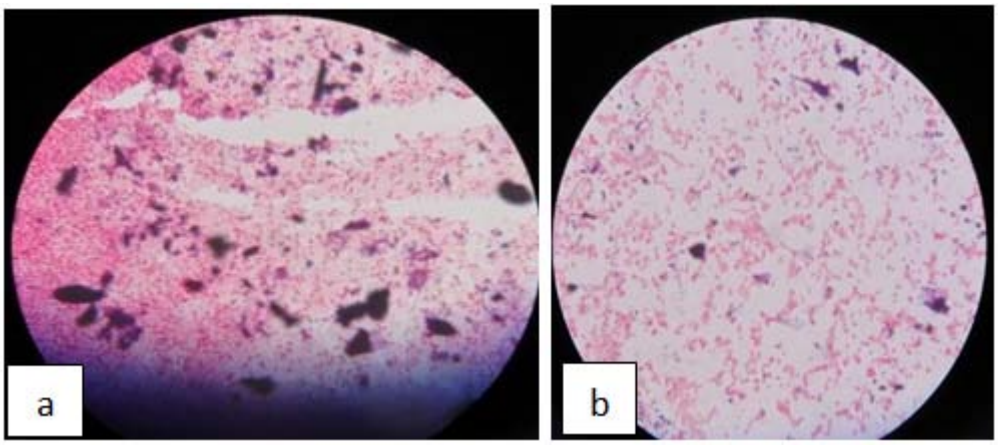

Fig. 6: Observation results of gram staining of shigella sonnei identification on herbal drink. a. the result of gram staining of herbal drink medicine in XLD media b. the result of gram staining of herbal drink in XLD media

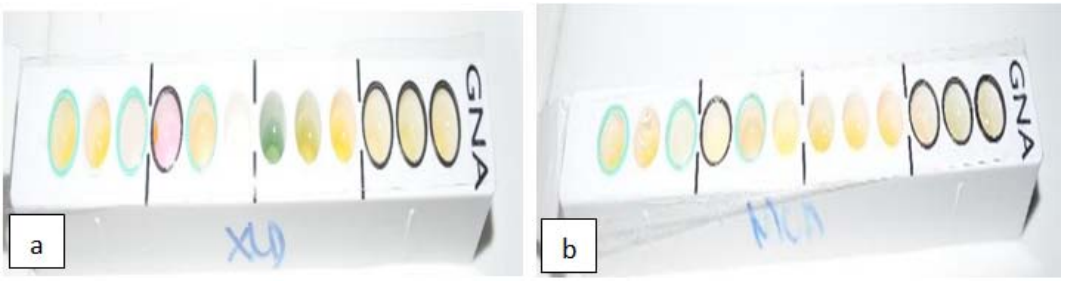

Fig: 7: Observation results of biochemical tests with the GN A kit for *Shigella sonnei* identification on herbal drink, a. the results of the biochemical test of herbal drink medicine in XLD media b. the results of the biochemical test of herbal drink medicine in MCA media

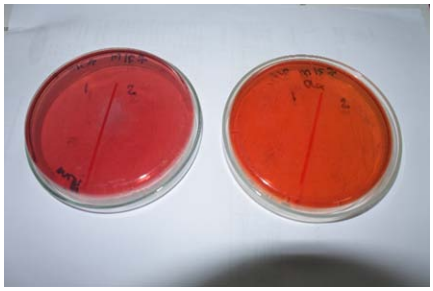

Fig. 8: Observation results of *Shigella sonnei* identification in instant powder on XLD and MCA media

The study was conducted using a bacterial growth medium, namely TSB media. The sample obtained a positive result of turbidity. Both samples were tested using selective media, namely XLD and MCA media. Shigella bacteria cannot ferment lactose and do not produce H2S or thiosulfate reductase enzymes so that the colonies formed are white or colorless (transparent). The results showed that the sample of herbal drink had colony growth, while the results of instant powder were negative or met the requirements. In the sample of herbal drink, a confirmation test was carried out with gram staining, showing a red color, which means the bacteria are negative-Gram and have a rod shape.

CONCLUSION

Based on the results of the calculation of the Total Plate Number and Yeast Mold Number, the samples of herbal drink and instant powder made by students of the Pharmacy Faculty of Pancasila University showed that the samples met the microbial limit standards. The test results for specific microbial contamination of *Escherichia coli*, *Salmonella enterica* Serovar Typhimurium*, and Shigella sonnei* did not show any microbial growth. This proves that the sample has met the safety and quality requirements according to BPOM Regulation No. 32 of 2019.

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AUTHORS CONTRIBUTIONS

All authors make equal contributions.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Susanti E, Apiliyani R. Uji cemaran mikroba pada jamu keliling yang dijual di kelurahan simpang baru pan am pekanbaru dengan metode MPN (Most Probable Number). J Penelitian Farmasi Indones. 2017;6:56-60.
2. Priamsari MR, Susanti MM. Analisis cemaran mikroba pada jamu gendong kunir asem yang beredar di wilayah semarang utara. J Akad Farmasi Prayoga. 2020;10(5):29.
3. Banjarmasin BalaiB POM. Pengawasan Mutu, Keamanan, dan Kemanfaatan Obat Tradisioanl. Banjarmasin; 2018.
4. Departemen kesehatan. Farmakope Indonesia. V. Jakarta: Departemen Kesehatan; 2014. p. 1348-52.
5. Negara JK, Sio AK, Rifkhan R, Arifin M, Oktaviana AY, Wihansah RRS, Yusuf M. Aspek mikrobiologis, serta sensori (Rasa, Warna, Tekstur, Aroma) Pada Dua Bentuk Penyajian Keju yang Berbeda. JIPTHP. 2016;4(2):286-90. doi: 10.29244/jipthp.4.2.286-290.
6. BPOM. Peraturan Badan Pengawas Obat dan Makanan Tentang Persyaratan Keamanan dan Mutu Obat Tradisional; 2019.
7. Mikrobiologi Pangan FS. Jakarta: Gramedia; 1992.
8. Guine R. The drying of Fpods and its effect on the physical-chemical, sensorial, and nutritional properties. Int J Food Eng. 2018;2:93-100.
9. Radji. Buku Ajar Mikrobiologi Panduan Mahasiswa Farmasi dan Kedokteran. Jakarta: Penerbit Buku Kedokteran EGC; 2011.
10. Jawetz, Melnick. Adelberg. Mikrobiologi Kedokteran. 20th ed. Jakarta: EGC; 1996.
11. Aini F. Isolasi identifikasi shigella sp penyebab diare pada balita bio-site; 2008.