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Ethnomedicine Study: Mitigating *Escherichia coli* Contamination in Jamu Gendong

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KEYWORDS Gram Staining. Herbal Medicine Recipe. Indole Test. Isolation. Oxidase

ABSTRACT The purpose of the study was to document and scientifically analyse the content of various Jamu Gendong recipes so that it can be ensured that they are safe when consumed. The study used a combination of traditional (qualitative) and modern (quantitative/microbiology) knowledge. The microbiological method used is by isolating selective media followed by indole test, MR test, TSIA test, oxidase, and Gram staining. The results showed that there were five results of the study of Jamu Gendong herb recipes, namely *jamu kunyit asem*, *jamu pahitan*, *jamu beras kencur*, *jamu kunyit sirih*, and *jamu gula jahe*. Of the 40 samples tested in the laboratory, 10 percent were identified as containing *E. coli*. Overall, the quality of Jamu Gendong sold in the Padang City area, West Sumatra is quite safe and hygienic for consumption by the community, but monitoring of hygiene factors must always be carried out.

INTRODUCTION

One of the main sources of health services in social life in the community is traditional and complementary medicine systems. Based on WHO data more than 88 percent (170) WHO member states prevent and manage various diseases in their communities, and they recognize and tend to use traditional and complementary medicine systems (World Health Organization 2019). UNESCO even officially at the 18th Intangible Cultural Heritage Convention Committee Session in Kasane, Botswana designated Jamu Wellness Culture from Indonesia as an Intangible Cultural Heritage (TropBRC 2024). At the beginning, the purpose of traditional medicine tended to emphasise the desire to find a cure or solution to the disease suffered by the community, so that the relationship between health maintenance is the main factor that must receive attention. This makes people's perceptions of traditional medicine known as Jamu, or native herbs and decoctions, be placed in their position as medicine. Thus, there is an element of attachment between traditional medicine and medical, clinical, pharmacology, pharmacy and toxicology.

Based on this understanding, the role of the discipline of ethnomedicine has a very special significance as it contributes to helping preserve and understand traditional healing practices, which have played an important role in healthcare for many communities around the world. It also provides insight into the cultural, social and healing aspects of health. Researchers in the field of ethnomedicine study these traditional healing practices, their effectiveness, and their cultural significance to bridge the gap between traditional and modern medicine.

Thus, it can be emphasised that the ethnomedicine study to mitigate *Escherichia coli* contamination in Jamu Gendong is a research that combines two fields of knowledge, that is, ethnomedicine and microbiology. The main focus of the study is to understand and address public health-related disorders that arise due to indications of *Escherichia coli* contamination in Jamu Gendong, a traditional Indonesian herbal concoction.

Selling Jamu Gendong is a form of home-based business carried out by individuals, in the form of traditional medicinal ingredients, and in the form of fresh liquids that are directly sold to buyers, so legally its marketing does not require a distribution permit and certification from the Food and Drug Administration (Wulandari and Azrianingsih 2014; Artha 2017; Rostiana et al. 2021). In general, people consume Jamu Gendong because there is a tendency not to cure diseases but rather just to

manage and increase their body stamina to stay healthy and energetic (Artha 2017; Fatimah et al. 2017; Sitoresmi et al. 2019). The concoction technique in the processing of Jamu Gendong is knowledge, experience, and expertise passed down from generation to generation from the ancestors of the Indonesian people, so it is an integral part of medicine, traditional culture, and local wisdom that needs to be preserved (Wulandari and Azrianingsih 2014; Prasanti 2018; Harefa 2020; Isnawati and Sumarno 2021; Yolanda et al. 2021). Based on archaeological evidence in the form of inscriptions, palm leaves, and temple reliefs, it is known that the history of the use of traditional herbal medicine by people in the archipelago has existed since the time of the Majapahit Kingdom in the 14th century AD (Wijayakusuma 2002; Isnawati and Sumarno 2021).

The term Jamu Gendong comes from the Javanese language which means, Jamu in the form of fresh liquid, efficacious as traditional medicine packaged in glass bottles (plastic bottles), consisting of various types of herbs, which all glass bottles are arranged and collected neatly into one basket (*bakul* or *wadah* in Javanese language). The basket containing the various types of Jamu is carried behind the backs of women using a Jarit shawl, while walking to sell to buyers (Riswan and Roemantyo 2002; Primiani et al. 2021; Rostiana et al. 2021). The various types of herbal medicine packaged in glass bottles inside the basket include, *kunyit asam*, *pahitan*, *beras kencur*, *kunyit sirih*, and *gula jahe* (Isnawati and Sumarno 2021; Rostiana et al. 2021).

In general, the raw materials used in making Jamu Gendong concoctions come from plants that grow in various land forms (Isnawati and Sumarno 2021). Some of the criteria that become the requirements for the selection of fresh raw materials in Jamu Gendong include that the selection of the name of the plant species used must be correct and precise between the intended regional name and its scientific name, the utilisation of plant organ parts must be appropriate, they must free from the influence of contaminating materials that have been related such as soil, sand and grass, the plants used have sufficient age when harvested, and the plants used are free from pests (Rostiana et al. 2021; Irwan et al. 2021). Plant organs that are often used as raw materials for Jamu Gendong concoctions include rhizomes/tuber, stem bark, roots, leaves, seeds, fruit, and the entire plant body (Isnawati and Sumarno 2021; Prasetyo 2021; Rostiana et al. 2021). As for the technique of making traditional medicine, it is generally done in five ways, namely boiling, burning, chewing, squeezing, and pounding (Prasetyo 2021), but in Jamu Gendong the processing is only done by boiling (Rostiana et al. 2021). Basically, the processing of Jamu Gendong concoctions is relatively very simple and it is quite easy to obtain the raw materials. The processing process is very close to the use of water and raw materials embedded in the soil. In fact, it is known that these two elements are triggers for pathogenic bacterial contamination in the Jamu Gendong herb. This is in accordance with Horman (2011) that pathogenic bacteria in the soil have the ability to attach to plant organs. It is also emphasised that some research results mention that in fresh beverage products can be found pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Bacillus* sp., and *Shigella* sp. (Esimone et al. 2003; Oyetayo 2008; Abba et al. 2009).

Microbiologically, *Escherichia coli* is included in one of the members of the Enterobacteriaceae family, grouped as Gram-negative bacteria that are rod-shaped, non-capsular, flagellated, 2-6 μm long, 1.2-15 μm wide, growing at temperatures of 10-45 ^{o}C with an optimum temperature of 37 ^{o}C (Faridz et al. 2007). In general, it is found in the lower digestive tract of warm-blooded animals (homoiterm), one example is mammals and humans. Its presence in the human intestine serves to suppress the growth of harmful microbes, accelerate decay in the colon, and synthesise vitamin K in blood clotting, so it is not harmful to health (Sutiknowati 2016; Rahayu et al. 2018).

The growth and development of *E. coli* in the environment is the result of the disposal of faeces and wastewater (Artha 2017), therefore, if the environmental sanitation conditions, materials, and equipment related to the food and beverage processing process are not clean and sterile, then *E. coli* can be said to be the main pathogenic bacteria that trigger contamination of food and beverages (Utami 2018; Yolanda et al. 2021). This condition can occur because initially this normal microflora is non-pathogenic, then obtains additional virulence genes from other microorganisms through the mechanism of gene transfer (transformation), plasmid transfer (conjugation), or gene transfer

through bacteriophage (transduction), thus turning into pathogenic bacteria (Rahayu et al. 2018). Several serious human diseases can be caused by contamination of pathogenic *E. coli* strains in beverages and food. Clinical symptoms caused by *E. coli* pathotypes are threefold. First, gastrointestinal or enteric tract disorders that usually cause diarrhoea (Villalobos et al. 2008; Ochoa et al. 2009; Arini and Wulandari 2017). Second, urinary tract infections, and third, sepsis-meningitis. The types of *E. coli* that cause gastrointestinal disorders come from six variants, namely *Enterophatogenic E. coli* (EPEC), *Enterohaemorrhagic E. coli* (EHEC), *Enterotoxigenic E. coli* (ETEC), *Enteroaggregative E. coli* (EAEC), *Enteroinvasive E. coli* (EIEC), and *Diffusely Adherent E. coli* (DAEC) (Kaper et al. 2004). The *E. coli* causing urinary tract infection is of the *Urophatogenic E. coli* (UPEC) type (Rahayu et al. 2018), and the *E. coli* responsible for sepsis and meningitis is the *Sepsis-Meningitis-Associated E. coli* (EMNEC) type (Kaper et al. 2004).

The unfavourable view of a small part of the community towards the cleanliness of processed Jamu Gendong (ethnomedicine) products, triggered by the appearance attributes of the seller who seems to look less clean, and the water and napkins used to wash and dry drinking glasses also seem less clean, encourages the need for microbiological testing of the feasibility of processed Jamu Gendong, whether there are indications of *E. coli* contamination. This is so that the traditional herbal medicine of Indonesia's heritage is safe and hygienic in terms of health when consumed.

Objectives of the Study

The purpose of the study was to document and scientifically analyse the content of various Jamu Gendong recipes so that it can be ensured that they are safe when consumed.

MATERIAL AND METHODS

Time, Location and Research Methodology

The research was conducted for three months at the Microbiology Laboratory of the Centre for Drug and Food Control, The Indonesian Food and Drug Authority Padang, West Sumatra Province. The research used a combined method between traditional (qualitative) and modern (quantitative/microbiology) knowledge. The microbiological method used is by isolating selective media followed by indole test, MR test, TSIA test, oxidase, and Gram staining. This method refers to the procedure described in Quality Control methods for Herbal Materials (World Health Organisation 2011).

This research was conducted because the results of the same study conducted by Hanifah (2016) using the Most Likely Number of Coliforms (MPN) test gave invalid results. This method cannot confirm the presence of *E.coli* contamination in processed Jamu Gendong. The results showed 86.4 percent positive coliforms in 19 out of 22 samples, strongly suggesting that the coliform bacteria belonged to four genera, namely, *Citrobacter, Enterobacter*, *Escherichia* and *Klebsiella* (Hanifah 2016). Another cause is the inaccurate results of the study by Tivani at al. (2019) on *E. coli* testing in four samples of sour turmeric herbal medicine wherein all were positive for *E. coli* (100%). Testing is based on colony characteristics and Gram staining on certain media. In this case, biochemical tests should be carried out, especially the indole test, to ensure that the suspected colonies on certain media are actually *E. coli.*

Tools, Materials and Samples

Some of the equipment and materials used in this research include 35°-37 °C incubator, 42°-44.5 °C incubator, microscope, hot plate, erlenmeyer, test tube, petri dish, pipette. The materials used are solvent media *Lactose Broth* (LB), *MacConkey Broth* (MCB), *MacConkey Agar* (MCA), *Trypton Broth*, MRVP medium, *Triple Sugar Iron Agar* (TSIA), *Tryptic Soy Agar* (TSA), Kovac's reagent, Oxidase reagent, and Gram staining set.

The sample consisted of 10 Jamu Gendong sellers in the Adabiah area, Padang City. These Jamu sellers travel around almost all areas of Padang City. From each Jamu Gendong seller (Fig. 1), samples were collected consisting of four types of Jamu, namely, *kunyit asem*, *beras kencur*, *pahitan*, and *jahe*, resulting in a total of 40 samples. Samples were collected using sterile containers to avoid contamination from pathogenic microbes.

Data Analysis

The data analysis of the samples was conducted as follows.

1) Sample testing is done by shaking so that it becomes homogeneous. Homogeneous liquid was taken using a pipette as much as 10 ml and put into a sterile erlenmeyer and added 90 ml of *Lactose Broth*. The mixture was then incubated at 30°-37 °C for 2-5 hours. After 2-5 hours the mixture was aspirated using a pipette and then put into 100 ml of *MacConkey Broth* for enrichment and incubated at 43°-45 °C for 18-24 hours. The enrichment results were then inoculated onto the surface of a specific *MacConkey Agar* media plate using an ose. Furthermore, observations were made to determine the specific colonies on the surface of the agar.
2) Colonies suspected to be *E. coli* were picked from 1-5 colonies and inoculated on a tilted *Tryptic Soy Agar* (TSA) surface and incubated at 35°-37 °C for 18-24 hours. Cultures grown on this tilted TSA were used to perform biochemical tests and Gram staining.
3) Biochemical tests for confirmation of *E. coli* bacteria are indole test, methyl red (MR) test, acid formation, gas and thiosulfate reduction tests using TSIA slant media and oxidase test.
4) The indole test is carried out by inoculating one ose of culture from tilted TSA into 10 ml of *Trypthone Broth* and incubated at 43°-45 °C for 18-24 hours. The culture in *Trypthone Broth* is then dripped with 2-3 drops of Kovac's reagent. Observe the formation of a red ring on the surface of the culture. In the methyl red (MR) test, cultures from TSA were inoculated on 10 ml of MRVP media, incubated at 35°-37 °C for 18-24 hours. The culture was then dripped with 2-3 drops of methyl red reagent. Observe the colour change in the culture. In the test with *Triple Sugar Iron Agar* (TSIA), cultures from TSA tilted were inoculated on TSIA tilted agar by poking and scratching, then incubated at 35°-37 °C for 18-24 hours. Observations on TSIA include colour changes on the surface of the media (*slant*), the bottom of the tube (*butt*), and gas formation including H_2S. In the oxidase test, cultures from TSA are inoculated on oxidase test strips. Discoloration of the strip to purple indicates positive oxidase. Also perform Gram staining using cultures from TSA.
5) For comparison, the same test procedure was also performed on the positive control *E. coli* ATCC 25922.
6) Interpretation of results, the sample is said to be positive for *E. coli* if on selective media specific colonies are found with the characteristics of a round red, non-mucoid shape, sometimes there is a reddish zone of bile precipitates, colony size 2-3 mm (World Health Organisation 2011; Akter et al. 2018) or pink to red colonies with bile precipitates. While in the positive indole test, a red ring forms on the surface of the culture. MR test is positive, the culture turns red. With TSIA on the surface of the media is yellow, at the base is yellow and gas is formed, which is characterised by the presence of air bubbles at the bottom of the tube or media cracking and lifting, negative H_2S gas. In the oxidase test no purple colour is formed (negative oxidase). Gram stain: Gram negative bacilli.

RESULTS

Potion Recipes for the Types of Herbs Found in the Jamu Gendong Basket

Based on the results of the study of herbal concoction recipes found in the Jamu Gendong baskets, five types of recipes were obtained consisting of *jamu kunyit asem*, *jamu pahitan*, *jamu beras kencur*, *jamu kunyit sirih*, and *jamu gula jahe* (Ikhtiariana 2011; Sukini 2018; Rostiana et al. 2021). The five Jamu recipes consist of various ingredients derived from plants, which are then mixed in certain ways until boiled to produce a ready-to-eat liquid Jamu Gendong concoction.

Detection of *Escherichia coli* Contamination in Jamu Gendong Preparations

The results of testing 40 Jamu Gendong samples at the homogenisation stage with Lactose Broth media, showed that the culture became slightly cloudy and there was sediment. At the enrichment stage with MacConkey Broth (MCB) media, the herbal ingredients appear cloudy with low to moderate turbidity intensity, except for the *jamu*

pahitan code number 7 P, the intensity is very cloudy, and the MCB media turns yellowish accompanied by the formation of gas bubbles.

Table 1 shows the observation of test results on MacConkey Agar selective media at the isolation stage, all samples indicated to contain *E. coli*

Table 1: Observation of test results on *MacConkey Agar* selective media

Positive control/ type of herbal medicine	*Sample code*	*Observation*	*Annotation*
E. coli control		Pink colony with pink sediment on the media around the colony	Specific
Beras Kencur	1 BK	No growth	
	2 BK	Small transparent, pale pink colonies	Not specific
	3 BK	No growth	
	4 BK	No growth	
	5 BK	No growth	
	6 BK	- Small transparent, pale pink colonies	Not specific
		- Pink colony	Suspected *E. coli*
	7 BK	- Small transparent, pale pink colonies	Not specific
		- Pink colony	Suspected *E. coli*
	8 BK	Small transparent, pale pink colonies	Not specific
	9 BK	Pale pink colonies with a dark pink spot in the center of the colony	Not specific
	10 BK	- Small transparent, pale pink colonies	Not specific
		- Pink colony	Suspected *E. coli*
Kunyit Asam	1 KA	No growth	
	2 KA	No growth	
	3 KA	No growth	
	4 KA	No growth	
	5 KA	No growth	
	6 KA	No growth	
	7 KA	No growth	
	8 KA	No growth	
	9 KA	No growth	
	10 KA	Transparent colonies	Not specific
Pahitan	1 P	No growth	
	2 P	Small transparent, pale pink colonies	Not specific
	3 P	No growth	
	4 P	No growth	
	5 P	No growth	
	6 P	No growth	
	7 P	- Small light yellow colonies	Not specific
		- Transparent light pink colony, pink colony edge	Not specific
		- Pink colony with pink sediment on the media around the colony	Suspected *E. coli*
	8 P	No growth	
	9 P	No growth	
	10 P	No growth	
Jahe	1 J	No growth	
	2 J	- Small transparent, pale pink colonies	Not specific
		- Pink colony	Suspected *E. coli*
		- Transparent light pink colony, pink colony edge	Not specific
	3 J	No growth	
	4 J	No growth	
	5 J	No growth	
	6 J	No growth	
	7 J	No growth	
	8 J	No growth	
	9 J	Transparent pale pink colonies	Not specific
	10 J	No growth	

at this isolation stage are still temporary results and more stages need to be carried out through biochemical tests to confirm the *E. coli* content in the sample. As in the sample codes 6 BK, 7 BK, 10 BK, 7 P, and 2 J there are pink colonies, which are predicted to be positive for *E. coli*, but if the colonies are pale pink, small transparent, light yellow, and the edge of the colony is pink, it means not yet clear whether *E. coli* is present or not (not specific). Meanwhile, other sample codes showed no growth of microorganisms.

Table 2 is a confirmation test of the observation of several samples suspected of containing *E. coli* on MCA media. If there is *E. coli* in the sample, it is marked with the following indicators. The Indol and MR tests show positive, the Oxidase test shows negative, while the Gram stain test shows pink rods/bacilli (Gram negative). The TSIA test on the surface (slant) and bottom (butt) is yellow, forms gas but H_2S is negative. All of these indicator conditions are found in the 7 P samples, especially the 1st, 2nd, 4th and 5th colonies. On the other hand, if the Indol and MR tests show negative and the TSIA test does not produce gas, then it is certain that there is no *E. coli*.

DISCUSSION

Potion Recipes for the Types of Herbs Found in the Jamu Gendong Basket

There are usually 4-5 different types of Jamu that are sold on foot or by bicycle/motorbike to buyers. The various types of Jamu are sold in the form of fresh liquid with the condition still warm because it has just finished cooking. The following presents the results of a study of the types of Jamu recipes found in the Jamu Gendong baskets, which consist of *jamu kunyit asem*, *jamu pahitan*, *jamu beras kencur*, *jamu kunyit sirih*, and *jamu gula jahe* (Ikhtiariana 2011; Sukini 2018; Rostiana et al. 2021) as follows.

Jamu kunyit asem, is a traditional herbal drink that is widely consumed in Indonesia because it has positive benefits for health, maintaining and increasing body stamina to always be energetic and strong. *Jamu kunyit asem* is made from a combination of ingredients including 1 kg of fresh turmeric rhizome (*Curcuma longa*), ¼ kg of tamarind fruit (*Tamarindus indica*), 2 litres of water, ¼ kg of beet sugar, ¼ kg of palm sugar, and 10 grams of iodised salt. The process of processing *jamu kunyit asem* includes fresh turmeric rhizomes are washed thoroughly, then turmeric rhizomes are sliced until the size of the thickness becomes thin, followed by pounding until smooth and adding 250 ml of fresh water that has not been cooked, then squeezed on a sieve until the turmeric liquid is free from residue. The next step is peeling the tamarind fruit skin to take the pulp. Next, a mixture is made consisting of filtered turmeric liquid with tamarind fruit pulp and 250 ml of fresh water and continued by boiling until boiling, also adding brown sugar, granulated sugar and salt to taste. The last step of the herbal mixture solution is filtered to be put into a glass bottle, and when it is full, it is tightly closed, labelled.

Table 2: Observation of biochemical test results of suspected *E. coli* samples and positive control *E. coli* ATCC 25922

Sample code	*Indol*	*MR*	*TSIA (slant/butt, gas, H_2S)*	*Oksidase*	*Gram staining*	*Conclusion*
E. coli ATCC 25922	(+)	(+)	A/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (+)
7P 1st colony	(+)	(+)	A/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (+)
7P 2nd colony	(+)	(+)	A/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (+)
7P 3rd colony	(-)	(-)	A/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
7P 4th colony	(+)	(+)	A/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (+)
7P 5th colony	(+)	(+)	A/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (+)
2J	(-)	(-)	A/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
6BK	(-)	(+)	A/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
10BK 1st colony	(-)	(-)	K/A, G (+), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
10BK 2nd colony	(-)	(+)	A/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
10BK 3rd colony	(-)	(-)	K/K, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
10BK 4th colony	(-)	(-)	A/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)
7BK	(-)	(-)	K/A, G (-), H_2S (-)	negatif	Bacillus Gram (-)	*E. coli* (-)

Annotation: A= yellow on the slanted surface, K= red on the bottom, G= cracking or lifting of the agar from the bottom of the tube (gas formation)

Jamu pahitan is a type of traditional herbal drink that has a distinctive bitter taste and is widely consumed in Indonesia for its potential health benefits. This bitter taste comes from a combination of bitter ingredients that are believed to have various medicinal properties, including 2 pieces of dried brotowali (*Tinospora crispa*) stems with a length of 6-8 cm, 2 ounces of dried sambiloto herb (*Andrographis paniculata*), ½ ounce of dried secang wood shavings (*Biancaea sappan*), 1 ounce of dried lempuyang emprit (*Zingiber amaricans*), 1 tablespoon dried fennel fruit (*Foeniculum vulgare*), 1 tablespoon ceplik sari fruit (*Eucalyptus alba*), 1 ounce dried temulawak (*Curcuma xanthorrhiza*), 5-10 grams palm sugar, 5-10 grams of iodised salt, and 2 litres of water. Processing of *jamu pahitan* includes all ingredients used are washed thoroughly, add 2 litres of fresh water then boiled until boiling and leave the amount of water until it becomes 1 litre. The whole solution is filtered and put in a glass bottle, then the bottle is closed and labelled. The bitter taste is often associated with its potential to stimulate various bodily functions and improve overall comfort.

Jamu beras kencur is a traditional Indonesian herbal drink that contains rice and kencur, which gives rise to a distinctive and unique odour among its ingredients. This unique combination is believed to offer various health benefits and is a popular choice among traditional herbal medicine lovers. The ingredients used include ½ kg of kencur rhizome (*Kaempferia galanga*), 1 ounce of white rice (*Oryza sativa*), ½ ounce of cinnamon bark (*Cinnamomum zeylanicum*), 1 stem of masoyi bark (*Cryptocarya massoy*) 7 cm long, 1 fresh ginger rhizome (*Zingiber officinale*), 1 star anise (*Illicium verum*), 5 kedawung seeds (*Parkia roxburghii*), 5 betel leaves (*Piper betle*), 10 cardamom pods (*Amomum compactum*), 10 fresh kaffir lime leaves (*Citrus hystrix*), 10 fresh pandan wangi leaves (*Pandanus amaryllifolius*), ¼ kg of beet sugar, ¼ kg of palm sugar, 5 grams of iodised salt, and 2 litres of fresh, uncooked water. The process of processing *jamu beras kencur* comprises white rice washed thoroughly then soaked in fresh water for 15 minutes, then drained until free from soaking water, then fried roasted (without cooking oil) on a frying pan until the rice is browned, followed by pounding until smooth or blended. In the next step, kedawung seeds are roasted until cooked and then ground until smooth. Fresh ginger and kencur rhizomes are sliced to a thin thickness, then finely ground and add 250 ml of fresh water, then squeezed on a sieve so that the resulting solution is free from residue. Add 1.75 litres of fresh water to the solution then boil and mix all other ingredients except rice, brown sugar, granulated sugar, and salt until the solution boils. The next step is to add brown sugar, granulated sugar, and salt while stirring. When it is felt that all the ingredients have been mixed then it is filtered until the herb is free of pulp. Then the mixture is still stirred and 7 tablespoons of white rice are added that have been roasted and finely ground, then filtered again and the results are put into a glass bottle and labelled. *Jamu beras kencur* is prized for its potential in providing comfort to the digestive system and alleviating various stomach-related problems.

Jamu kunyit sirih is often enjoyed for its potential to invigorate the body and promote an overall sense of comfort. The combination of turmeric and betel leaves results in an aromatic drink. Some of the ingredients used include 30 fresh betel leaves (*Piper betle*), 1 kg fresh turmeric rhizome (*Curcuma longa*), 3 areca nut seeds (*Areca catechu*), ¼ kg tamarind fruit (*Tamarindus indica*), ¼ kg fresh temu kunci rhizome (*Boesenbergia rotunda*), ¼ kg beet sugar, ¼ kg of palm sugar, 5-7 grams of iodised salt, and 2.5 litres of freshwater. The process of processing *jamu kunyit sirih* involves using fresh temu kunci and turmeric rhizomes sliced into thin slices and then finely ground and added with 250 ml of fresh water. The results are squeezed by hand on a sieve until the mixture is clean from the pulp. The next step is to peel the tamarind fruit to get the pulp, followed by splitting the areca nut and taking the seeds to be pulverised by beating/pounding. Another step is to slice fresh betel leaves into small pieces. Mix the results of the temu kunci and turmeric rhizome filtering process and add tamarind pulp, crushed areca nut seeds, betel leaves that have been cut into small pieces, and 2 litres of freshwater, all boiled until boiling. In order to make the mixture more complete, sugar, brown sugar and salt are also added until the herb tastes good, the last step is filtered and put into a glass bottle and labelled.

Jamu gula jahe is a traditional Indonesian herbal drink that combines the sweetness of brown sugar and the warmth of ginger rhizome. In terms of health benefits, this mixture creates a distinctive aromatic beverage and is a popular choice for peo-

ple especially during winter because it can evoke a sense of warmth in the body. The ingredients used include 1 ounce of fresh emprit ginger (*Zingiber officinale* var. amarum) 1 cinnamon bark (*Cinnamomum zeylanicum*) 8 cm long, 5 fresh pandan wangi leaves (*Pandanus amaryllifolius*), 10 fresh kaffir lime leaves (*Citrus hystrix*), ¼ kg beet sugar, ¼ palm sugar, 5 grams of iodised salt, and 2.5 litres freshwater. Processing of ginger sugar herbal medicine involves fresh ginger rhizomes that are washed, sliced into thin slices, ground until smooth and added 250 ml of freshwater and then squeezed by hand on a sieve until the result is clean from the pulp. In the next stage, the ginger water from the filter is added with cinnamon, pandan leaves, lime leaves, and 2¼ litres of water and brought to a boil. Finally, sugar, brown sugar and salt are added to the stew and stirred, leaving it for a while and if it is deemed sufficient both in taste and ripeness, wait until the potion becomes warm, then put it in a glass bottle and labelled.

Detection of *Escherichia coli* Contamination in Jamu Gendong Preparations

The detection of *E. coli* contamination in processed Jamu Gendong is of particular concern because it has the potential to cause serious impacts on public health conditions. There is often a negative perception among the public towards the safety and hygiene of processed Jamu Gendong products. This is triggered by the seller's appearance style, which does not look clean and sterile, because the connotation of clean is synonymous with white or bright colours, and sterile is synonymous with wearing hand shirts when serving buyers. In addition, the water used to wash the glass and the napkins to dry the glass also seem less clean. This makes it necessary to conduct microbiological testing on the feasibility of processed Jamu Gendong so that it is safe for health when consumed.

In the results of testing 40 Jamu Gendong samples obtained from 10 Jamu Gendong traders in Padang City, it was found that at the homogenisation stage with *Lactose Broth* media, which was incubated at 30°-37 °C for 2-5 hours, the culture became slightly cloudy and contained sediment. Turbidity and sediment in *Lactose Broth* media are mainly caused by the increase in lactic acid from the fermentation of lactose by bacteria, so that the lactose component clumps and the culture becomes cloudy and contains sediment (Kamaliah 2017).

It is known that *MacConkey Agar* and *Broth* have been recommended to be used in microbiological examination of food and beverages. One of the ingredients used in making Jamu Gendong concoctions is beet sugar and palm sugar. Both ingredients contribute strongly to the enrichment results. At the enrichment stage with *MacConkey Broth* (MCB) media, the herbal concoction will appear cloudy with low to moderate turbidity intensity, except for the *jamu pahitan* code number 7 P, the intensity is very cloudy and the MCB media turns yellowish accompanied by the formation of gas bubbles. This situation is in accordance with what Akter et al. (2018), Putri and Kurnia (2018) and Panjaitan et al. (2020) that, the carbohydrate fermentation test is characterised by a change in purple colour to yellow colour and the formation of gas bubbles in the Durham tube. The occurrence of colour changes in MCB media from purple to yellow due to the formation of acid from the fermentation of lactose by *E. coli* microbes. This condition is in accordance with the opinion of Putri and Kurnia (2018) and Panjaitan et al. (2020) who stated that the colour change from purple to yellow indicates that these bacteria form acids from the fermentation of glucose, sucrose, and lactose. The formation of gas bubbles can occur due to the lactose fermentation reaction (Wulansari et al. 2017; Romesberg and Henderson 2018; Putri and Kurnia 2018). To ensure the formation of acid, Bromocresol Purple (BCP) is used as a pH indicator, which has a pH range of 5.2-7.5 and the result is yellow, indicating an acidic atmosphere.

Table 1 shows the observation of the test results on *MacConkey Agar* selective media at the isolation stage, from four types of herbal medicine that were observed based on the colour of the colonies formed and the presence or absence of bacterial growth, it was found that there were three code numbers in the kencur rice herbal medicine sample that were predicted to contain *E. coli*, namely 6BK, 7BK, and 10BK. In all samples of sour turmeric herbal medicine no *E. coli* was found. In the bitter herbal medicine sample, which was strongly suspected of containing *E. coli* was code number 7P, while in the ginger herbal medicine sample code number 2J was suspected of containing *E. coli*. All samples indicated to contain *E. coli* at this isolation stage are still provisional results and more

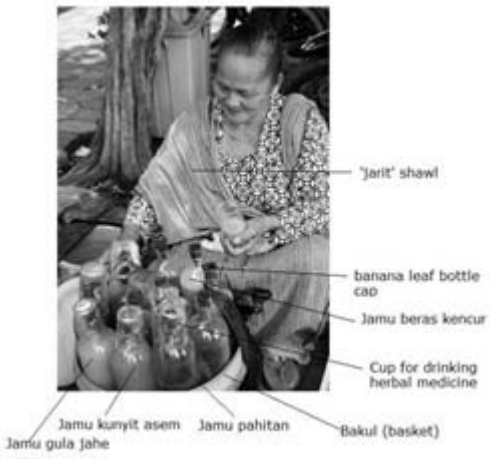

Fig. 1. Women who sell jamu gendong
Source: Modification from https://id.m.wikipedia.org/wiki/Berkas:Jamu_Gendong.JPG

stages need to be carried out through biochemical tests to confirm the *E. coli* content in the samples.

Figure 2 (A) illustrates the normal growth of *E. coli* ATCC 25922 used as a control while Figure 2 (B) shows that the *MacConkey Agar* (MCA) media contains lactose, neutral red, bile salt and several other ingredients. The colony shape of suspected *E. coli* on MCA is pink/pink to red with or without precipitates or reddish zones. The characteristics and shape of the colonies shown by the isolation results are in accordance with the morphological characteristics proposed by Akter et al. (2018), Effendi et al. (2019), Damayanti and Purwantisari (2020), and Khoiriyah et al. (2022). This happens because *E. coli* has the ability to ferment lactose contained in MCA, so that the pH size decreases. *Escherichia coli* that ferments lactose will grow with a pink colony colour (Khoiriyah et al. 2022). This situation occurs because the neutral red indicator substance if it is in an acidic medium turns into a pink colour while in an alkaline atmosphere it is colourless (Suarjana et al. 2017). This condition is in accordance with what Neogen (2011) said that the colour of the MCA medium will change from brick red to pink because the MCA medium contains lactose as a carbohydrate source for *E. coli* and neutral red as a colour indicator in the medium. Meanwhile, the results of lactose fermentation in the form of acid will react with bile salts contained in the *MacConkey Agar* medium and will form a cloudy sediment around it.

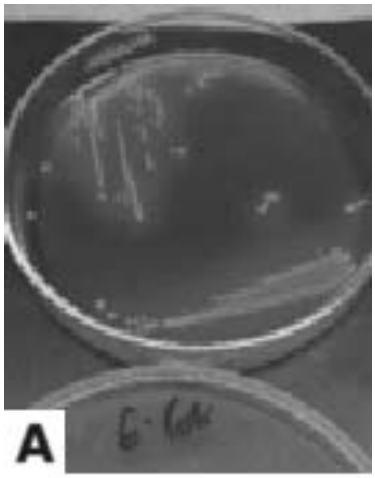

Fig. 2 A. Positive control of *E. coli* ATCC 25922

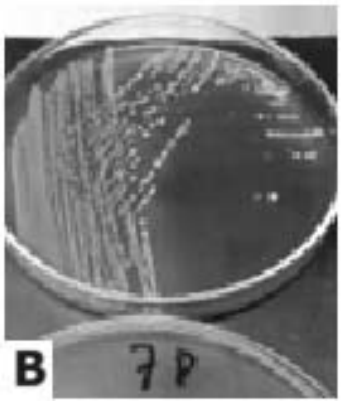

Fig. 2 B. Sample of jamu pahitan code number 7P on MCA media
Source: Private Document

Table 2 is a confirmation test from the observation of several samples suspected of containing *E. coli* on MCA media at the isolation stage (Table 1). The confirmation test in question is by conducting biochemical tests and Gram staining on samples that are indicated to contain *E. coli.* The following are presented observations of the confirmation test results, which can be seen in Table 2. Of the 5 colonies in code number 7P that were tested for confirmation by biochemical tests (In-

dol, MR, TSIA, Oxidase) and Gram staining, the results showed that only 1 colony did not contain *E. coli*, while 4 other colonies tested positive for *E. coli*. Following the details of the information, the results of the confirmation test of the sample with code 7P obtained data wherein the 1st, 2nd, 4th, and 5th colonies gave the same results as the positive control of *E. coli*, namely Indol (+), MR (+), TSIA yellow on slant, yellow on butt, there is gas, H_2S (-), oxidase test (-), and Gram stain Gram bacillus (-). It was concluded that the sample with code number 7P was positive for *E. coli*.

Fig. 3. Indole test on sample number 7P starting from E. coli control, 1st, 2nd, 3rd, 4th, 5th (read: from right to left)
Source: Private Document

In other sample code numbers, namely 2J, 6BK, 10BK (4 colonies), and 7BK, it was declared negative for not containing *E. coli* because based on the data in Table 2, it is known that the confirmation test results obtained are opposite to the *E. coli* control, namely Indol (-) and MR (-). In the TSIA test there are yellow samples on the slant and there are also red samples on the butt, only 1 sample contains gas but the other samples do not have gas, H_2S (-), oxidase test (-), and Gram bacillus Gram stain (-).

Here are some biochemical test results that strengthen the evidence that sample 7P is contaminated with *E. coli*, among others. The appearance of a red ring on the surface of the culture during the indole test, the red ring is not formed in the 3rd sample 7P (Fig. 3). This is in accordance with the opinion of Sari et al. (2019) and Kristiawan et al. (2022) that if the indole reaction is positive, the surface of the media will be characterised by the formation of a cherry red ring. According to Cappuccino and Sherman (2005), the formation of a red ring in the indole test on the surface of the culture occurs because *E. coli* has a tryptophanase enzyme that can hydrolyze tryptophan amino acids into indole and pyruvic acid. The indole formed will react with p-dimethylamino benzaldehyde contained in Kovac's reagent and form quinoidal compounds in the form of a red layer on the surface of the culture.

Evidence from the results of other biochemical tests that indicate that sample 7P is contaminated with *E. coli* is the change in the colour of the culture to red after being tested with MR reagent in the MR test, the yellow colour formed in sample 7P 3rd means negative, does not contain *E. coli* (Fig. 4).

This is in accordance with the opinion of Kartikasari et al. (2019) that through the MR test on *Musculus pectoralis* samples from Broiler chicken meat, it is known to contain *E. coli* which is characterised by a red solution. Glucose contained in the MRVP medium will be fermented to form acid by *E. coli* until the pH drops to 4.4. (Merck Microbiology Manual 12th Edition). The acid formed is detected by the addition of a methyl red indicator. Under pH 4.4 conditions, methyl red will be red, while at pH 6.2 methyl red will be yellow, and under conditions between these pH, methyl red will be graded to orange (Leboffe and Pierce 2011).

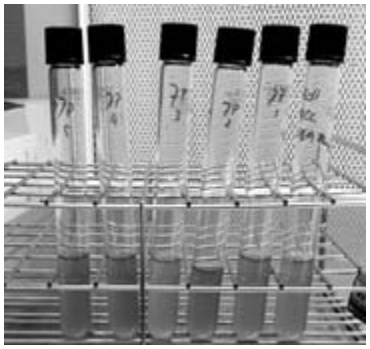

Fig. 4. MR test on sample number 7P starting from E. coli control, 1st, 2nd, 3rd, 4th, 5th (read: from right to left)
Source: Private Document

Other evidence that reinforces that sample 7P is contaminated with *E. coli* is the colour change in the TSIA slant agar during the TSIA test (Fig. 5). Both the *slant* and *butt* (bottom) become yellow in colour, indicating an acidic state. In addition, there was also gas accumulation at the bottom of the

Fig. 5. TSIA test on sample number 7 P starting from *E. coli* control, 1st, 2nd, 3rd, 4th, 5th (reada: from right to left)
Source: Private Document

tube and some of the agar cracked. This condition is in accordance with the opinion of Ummamie et al. (2017), Saidah and Susilawati (2018) and Kristiawan et al. (2022), during the TSIA test both the butt and slant are yellow. It was also confirmed by Leboffe and Pierce (2011) and Mahon (2015) that the colour change to yellow indicates the ability of *E. coli* to ferment lactose, glucose and or sucrose on TSIA media. This carbohydrate fermentation causes a decrease in pH, which turns the phenol red indicator in the media yellow (Sari et al. 2019; Khoiriyah et al. 2022). In addition, this fermentation also forms gas, which causes gas at the bottom of the tube and cracks in the media.

Other evidence that confirms the presence of *E. coli* in sample 7 P is that no purple colour formed on the bactident oxidase strip in the oxidase test (Fig. 6). This happens because *E. coli* does not

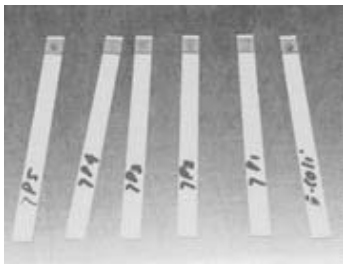

Fig. 6. Oxidase test on samples numbered 7P 1st, 2nd, 3rd, 4th, 5th, E. coli control (read: from right to left)
Source: Private Document

have the enzyme cytochrome oxidase, which with tetramethyl-p-phenylenediamine oxidase reagent forms a purple colour (Leboffe and Pierce 2011). Usually in the use of oxidase test to detect the enzyme cytochrome oxidase, the enzyme is responsible for catalysing the transport of electrons between cells in bacteria, so that at the end of the electron transport chain it is expected that oxygen has been reduced (Cahyani et al. 2020).

The final evidence that confirms that sample 7P contains *E. coli* is that the Gram stain of the sample found Gram bacilli (-), which is the same as the staining of the *E. coli* positive control (Fig. 7A and 7B).

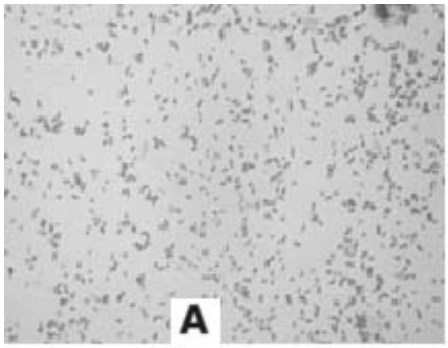

Fig. 7A. Gram stain test Positive control *E. coli* ATCC 25922
Source: Private Document

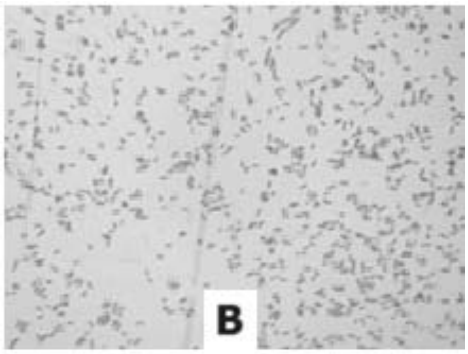

Fig. 7B. Gram stain test of sample code number 7P
Source: Private Document

Some of the possible causes of sample number 7 P of Jamu Gendong herb being contaminated with *E. coli* contamination are due to lack of care for hygiene factors, among others:

1. In general, the process of washing glass bottles as a place for herbal concoctions after washing is not rinsed with boiling hot water, because boiling hot water is very effective in killing *E. coli*.
2. If the water used for making herbal concoctions has been contaminated by *E. coli* and the boiling process is not perfect, then it is likely that the boiled herbal concoctions that are ready for sale still contain *E. coli*.
3. Lack of concern for the cleanliness of the water in the bucket used to wash the glass used when drinking Jamu. It is better to use a water source that is guaranteed to be clean and healthy, and to be diligent in renewing the water in the bucket when it feels cloudy.
4. Lack of attention to personal hygiene factors for Jamu sellers, such as hand hygiene, cleaning clothes used to dry Jamu drink cups.

Various alternatives for preventing and mitigating are possible, so that *E. coli* contamination does not occur in herbal medicine consumed by the public. These include:

1. There is a need to educate herbal medicine sellers about hygienic factors in preparing tools and sources of materials used, the manufacturing process, storing them in glass bottles before selling, and cleanliness of the body and clothes worn by herbal medicine sellers. This educational activity should be carried out periodically under the supervision and responsibility of the Food and Drug Supervisory Agency, Republic of Indonesia.
2. It is necessary to select the ingredients used in making herbal concoctions that are free from mould, free from insects, not rotten, and fresh so that overall the ingredients appear to be of high quality.
3. The need for training and practice in making herbal medicine hygienically and correctly in determining the dosage of ingredients used according to the recipe. This training is organised by the Food and Drug Supervisory Agency for beginner herbal medicine sellers, so that they gain knowledge and clean quality herbal products (the content of the substances in them does not change).
4. There is a need to monitor the quality of herbal medicine that is marketed in the community from the Food and Drug Supervisory Agency periodically to sellers of herbal medicine, so that it is hoped that this can prevent the occurrence of herbal concoctions being contaminated with *E. coli*.
5. There is a need to collect data on the number of herbal medicine sellers in each region by the Food and Drug Supervisory Agency, which can be used to determine the accuracy of the data for various purposes.

CONCLUSION

Jamu Gendong has been an important part of Indonesia's cultural heritage for centuries, and this study focusing on Jamu Gendong sellers in the Jati Adabiah area of Padang City introduces a new perspective on the integration of traditional knowledge and modern microbiological techniques. This approach not only enriches the understanding of the composition and potential benefits of Jamu Gendong but also addresses contemporary health concerns, making it relevant and valuable to both traditional medicine enthusiasts and the wider community. On the other hand, this study is able to bridge the gap between traditional knowledge and modern science, by combining advanced microbiological tests such as indole test, MR test, TSIA test, oxidase test, and Gram stain, with traditional herbal knowledge, thus offering an interdisciplinary perspective especially in improving the understanding of the safety and efficacy of herbal remedies.

Referring to the benefits of Jamu Gendong as a traditional herbal medicine that is able to maintain and increase the stamina of the body to stay healthy and energetic, as well as the relatively small percentage of contaminated samples (10%) due to various factors related to hygiene, it can be concluded that there are 5 results of the study of Jamu Gendong herb recipes, namely, *jamu kunyit asem*, *jamu pahitan*, *jamu beras kencur*, *jamu kunyit sirih*, and *jamu gula jahe*. Overall, it can be said that the quality of processed Jamu Gendong sold in the Padang City area is relatively safe and hygienic for consumption by the community, but it is necessary to continue to socialise and monitor hygienic factors in order to ensure the safety and quality of herbal medicines.

RECOMMENDATIONS

To prevent *E. coli* contamination in Jamu Gendong, it is very necessary to always have guid-

ance, socialisation, and monitoring of the cleanliness of equipment and the process of making Jamu by the Coordinator of the Office of Cooperatives and Micro, Small and Medium Enterprises and Industry.

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