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# Most Probable Number (MPN) Coliform of Jamu Gendong Beras Kencur in Traditional Markets in Sukoharjo Region

**Makhabbah Jamilatun*), Pradea Indah Lukito, Eka Nanda Safitri**
Department of Pharmaceutical and Food Analysis, Health Polytechnic of the Health Ministry, Surakarta, Jl. Kesatriyan 2, Danguran, Klaten Selatan, Klaten, Indonesia

*Corresponding author:makhabbah.j@gmail.com

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**Abstract:** One of the traditional medicines that is very popular with the public is the jamu gendong beras kencur. The safety of traditional medicine products (herbal medicine) is a demand that has been put forward since the emergence of human health problems due to microorganism contamination, such as coliform. This study aims to determine Coliform bacteria contamination in jamu gendong beras kencur in traditional markets in the Sukoharjo area. The method used in this study is the MPN (Most Probable Number) method, which consists of two stages, namely the Presumptive Test and the Confirmed Test. The study results showed that samples with codes 1, 2, 5, 6, 7, 8, 11, and 12 had an MPN value of 0/100 ml. Furthermore, samples with codes 3, 4, 9, and 10 showed an MPN value >0/100 ml. Based on the research results, samples that meet the quality of good drinking water based on the Regulation of the Minister of Health of the Republic of Indonesia in 2010, are samples with codes 1, 2, 5, 6, 7, 8, 11, 12 while samples with codes 3, 4, 9, 10 do not meet the requirements. There needs to be education on how to make good herbal medicine, environmental sanitation, and clean herbal medicine sales locations so that herbal medicine sellers can improve the quality of the herbal medicine they sell and ensure it is safe to consume.

**Keywords:** coliform bacteria, jamu gendong, beras kencur, MPN (most probable number)
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## 1. INTRODUCTION

The biological wealth owned by the Indonesian people is one of the greatest biological riches in the world. Indonesia has more than 30,000 species of higher plants. It is known that 7000 plants have been used as raw materials for the pharmaceutical industry regularly (Setiawan, 2022). WHO notes that 68% of the world's population still relies on traditional healing systems involving plants. More than 80% of the world's population uses herbal or traditional medicines to support health. This treatment aims to cure and prevent the disease (Emilda et al., 2017).

Jamu is one of the traditional medicines that is easy to obtain, especially from herbal medicine sellers often found in cities and villages. Jamu gendong is the ancestral heritage of the Indonesian people and is a well-known characteristic (Kusumo et al., 2020). Among the various types of herbal medicine sold to the public, one is the herbal medicine for beras kencur (Monita et al., 2021; Santika et al., 2021). Beras kencur herbal medicine is claimed to have several health benefits, including eliminating aches, warming the body, and being used as a drink to maintain stamina (Army, 2018; Silalahi, 2019). Beras kencur herbal medicine can be found in various areas, including the traditional markets of the Sukoharjo region.

Beras kencur herbal medicine is made by boiling all the ingredients or extracting them by squeezing the juice contained in the main ingredient, then mixing it with water. The composition of the main ingredients used to make beras kencur is rice and kencur. The concoction ingredients are prepared by slicing or crushing them first (Jalil, 2019; Isnawati & Sumarno, 2021). The requirements for the water used are that it must be safe, free from pathogenic microbes and harmful substances, and acceptable in

terms of colour, taste, odour, and turbidity. If you don't pay attention, during the process of making beras kencur herbal medicine, there is the potential for bacterial contamination to occur. Contamination of jamu gendong can occur, among other things, due to a lack of attention to cleanliness, both cleanliness of raw materials, cleanliness of the environment (Makhabbah Jamilatun, 2022), equipment used (Makhabbah Jamilatun, 2022), (Makhabbah Jamilatun & Safitri, 2023), and cleanliness of manufacture (Priamsari & Susanti, 2020), (Listi et al., 2022).

Product safety, whether food, medicinal preparations, or traditional medicine (herbal medicine), must be fulfilled since human health problems arise from microorganism contamination (Lukman, 2020). Products contaminated with these microorganisms can cause disease (Brooks et al., 2018; Setiyanti et al., 2022). The requirement for drinking water must be safe to drink (Rumondor et al., 2014), so it is necessary to pay attention to the source of the water used to make herbal medicine. The problem currently facing is the increasingly high level of water pollution (A. A. Aminah & Jamilatun, 2017; Makhabbah Jamilatun & Aminah, 2017) from household wastewater and industrial waste. New efforts continue to be made to obtain water sources, especially to provide drinking water that meets established requirements (Anisafitri et al., 2020). In its management, drinking water is susceptible to contamination by microorganisms, especially coliform bacteria (A. Aminah & Jamilatun, 2016). The higher the level of coliform bacterial contamination, the higher the risk of other pathogenic bacteria that usually live in human and animal waste (Fatiqin et al., 2019; Saputri & Efendy, 2020).

Prevention of water pollution consumed by humans can be determined by microbiological examination of the water. The method often used is MPN (Most Probable Number), which is a method for determining the number of coliform bacteria in drinks (Listi et al., 2022). The MPN method is used to count the number of bacteria, especially to detect the presence of coliform bacteria using the fermentation tube method, which is compared with the MPN table (Makhabah Jamilatun & Aminah, 2016; Putri & Kurnia, 2018). The results of calculating the number of bacteria using the MPN method must meet the requirements of the Regulation of the Minister of Health of the Republic of Indonesia Number 492 of 2010; the requirement for MPN coliform drinking water quality is 0/100 mL (Permenkes RI, 2010).

Observations carried out by researchers show that traditional markets in the Sukoharjo region have poor sanitation and hygiene during the sales process, and are based on previous research, namely the discovery of total plate count and yeast mold count contamination in beras kencur jamu gendong in several traditional markets in the Sukoharjo region (Makhabbah Jamilatun & Lukito, 2024). So, research was carried out on the Most Probable Number (MPN) Coliform of Jamu Gendong Beras Kencur in Traditional Markets in the Sukoharjo Region. The advantages of the MPN method are that it is quite easy to do, can determine the specific number of certain microbes using appropriate media, is more sensitive, and can detect coliforms in very low numbers.

## 2. METHODS

### 2.1. Materials

The materials used include beras kencur herbal medicine, LB (Lactose Broth) media, BGLBB (Brilliant Green Lactose Bile) media, spirits, ethanol, distilled water, and tissue. Equipment used includes laminar air flow, incubator, oven, autoclave, vortex, analytical balance, magnetic stirrer, ball pipette, petri dish, volume pipette, erlenmeyer, glass beaker, measuring cup, dropper pipette, spirit lamp, durham tube, waterbath, tube needle, test tube, cool box, micropipette, tip.

### 2.2. Preparation and Sampling

Research begins by preparing the tools and materials used. The sampling technique used in this research for jamu gendong beras kencur was the cluster sampling technique (Jirna, 2019). Samples were taken

from each sub-district, with 12 sub-districts in the Sukoharjo Region. In each sub-district, one market was selected that had the most visitors, and from that market, one herbal medicine trader was selected that had the most customers. Sampling was carried out by placing the jamu gendong beras kencur sample into a glass bottle with a volume of 100 ml and taking it to the laboratory using a cool box. Jamu gendong beras kencur samples were tested for MPN.

### 2.3. MPN Test

The herbal medicine samples were tested for MPN with a 3 3 3 series with simplo repetition. Media control was used to determine that the Coliform contamination was from the sample. There are two stages of examination of the MPN method used in this study, namely the presumptive and confirmatory tests. To determine the amount of MPN coliform, it was then adjusted to the requirements of the Regulation of the Minister of Health of the Republic of Indonesia Number 492 of 2010, the requirements for drinking water quality MPN Coliform.

a. *Presumptive Test*

Prepare nine culture tubes. Each tube was filled with 10 mL of sterile LB (Lactose Broth) liquid media, and each tube was equipped with a Durham tube. The liquid media is filled with three tubes containing LBDS (Lactose Broth Double Strength) and six tubes containing LBSS (Lactose Broth Single Strength). A total of 10 mL samples of jamu gendong beras kencur were poured using a sterile pipette into three culture tubes containing the first three series of LBDS. A total of 1 mL of the jamu gendong beras kencur sample was poured using a sterile pipette into three culture tubes containing the second 3 series of LBSS. A total of 0.1 mL of the jamu gendong beras kencur sample was poured using a sterile pipette into three culture tubes containing the third LBSS 3 series. All culture tubes containing samples were incubated at 37ºC for 1 × 24 hours. Observed for turbidity and air bubbles in the Durham tube. The code for tubes that are positive for producing gas is recorded (Makhabah Jamilatun & Aminah, 2016; Hendiana et al., 2022)

b. *Confirmed Test*

Inoculate 1-2 cycles of positive cultures for gas in Lactose Broth (LB) from the presumptive test into a confirmation test tube containing BGLBB media, including a Durham tube. For coliform bacteria, incubate at 37°C for 1 × 24 hours. Observe for turbidity and air bubbles in the Durham tube, then note the positive tube code for emitting gas. The amount of coliform bacteria content can be seen by counting the positive tubes on BGLBB media and comparing with the MPN table; these results describe the number of colonies per 100 mL of sample (Listi et al., 2022), (Utami & Miranti, 2020).

## 3. RESULTS AND DISCUSSION

This research aimed to determine coliform contamination in the jamu gendong beras kencur sold at the Sukoharjo Region Traditional Market using the MPN (Most Probable Number) method. There are two stages of examination of the MPN method used: the presumptive and confirmed tests. First, the presumptive test using LB (Lactose Broth) media, is used to determine the presence or absence of coliform bacteria based on the formation of acid and gas caused by lactose fermentation (Putri & Kurnia, 2018), (Nurjannah & Novita, 2018), (Listi et al., 2022). The test is declared positive if gas is formed, which can be seen by the presence of an empty cavity at the top of the Durham tube, and acidic properties by changing the colour of the medium to cloudy yellow in the tube, as shown in Figure 1. The respiration activity of microorganisms causes gas bubbles to be produced in the Durham tube. The results of the confirmation test in this study can be seen in Table 1, where there are 11 samples with positive tubes. However, lactose fermentation or the formation of gas in the Durham tube on LB media does not always indicate coliform bacteria, because lactose is also fermented by other microbes, for example, lactic acid bacteria (Irianto, 2013), (Richard Hendarto et al., 2021). Therefore, the presumptive test is continued with a confirmation test (Susanti & Aprilliyani, 2018).

Table 1: Results of Positive Tube Observations on Jamu Gendong Beras Kencur from Presumptive Tests on LBDS and LBSS Media

| No | Sample Code | LBDS (10 mL) | | | LBSS (1 mL) | | | LBSS (0,1 mL) | | | Positive Tube Combination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | | 3 | | | 3 | | | |
| 1 | 1 | + | + | + | + | + | + | + | - | - | 3-3-1 |
| 2 | 2 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 3 | 3 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 4 | 4 | + | + | + | + | + | + | + | + | + | 3-3-3 |
| 5 | 5 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 6 | 6 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 7 | 7 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 8 | 8 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 9 | 9 | + | + | + | + | + | + | + | - | - | 3-3-1 |
| 10 | 10 | + | + | + | + | + | + | - | - | - | 3-3-0 |
| 11 | 11 | + | + | + | - | - | - | - | - | - | 3-0-0 |
| 12 | 12 | - | - | - | - | - | - | - | - | - | 0-0-0 |

Description: (+) = there is gas in the Durham tube and there is turbidity, (-) = there is no gas in the Durham tube and there is no turbidity. Sample codes 1 to 12 indicate that the sample comes from market areas 1 to 12 from all areas in Sukoharjo.

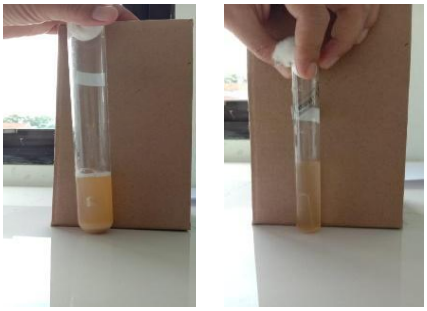

Figure 1. Presumptive test on LB media (positive (left) and negative (right)

Table 2: Results of Positive Tube Observations on Jamu Gendong Beras Kencur from the Confirmative Test on BGLBB Media

| No | Sample Code (S) | BGLBB (10 mL) | | | BGLBB (1 mL) | | | BGLBB (0,1 mL) | | | Positive Tube Combination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | | 3 | | | 3 | | | |
| 1 | 1 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 2 | 2 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 3 | 3 | + | - | - | - | - | - | - | - | - | 1-0-0 |
| 4 | 4 | + | + | + | + | + | + | + | + | + | 3-3-3 |
| 5 | 5 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 6 | 6 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 7 | 7 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 8 | 8 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 9 | 9 | + | + | + | + | + | + | + | - | - | 3-2-1 |
| 10 | 10 | + | + | + | + | + | + | - | - | - | 3-3-0 |
| 11 | 11 | - | - | - | - | - | - | - | - | - | 0-0-0 |
| 12 | 12 | - | - | - | - | - | - | - | - | - | 0-0-0 |

Description: (+) = there is gas in the Durham tube and there is turbidity, (-) = there is no gas in the Durham tube and there is no turbidity. Sample codes 1 to 12 indicate that the sample comes from market areas 1 to 12, from all regions of Sukoharjo.

Second, a confirmatory test was carried out to confirm the presence of coliform bacteria, using BGLBB (Brilliant Green Lactose Bile Broth) media (Susanti & Aprilliyani, 2018). BGLBB is a selective medium that contains bile salt and brilliant green. These two substances can inhibit the growth of gram-positive bacteria, so only gram-negative bacteria that ferment lactose and produce gas can grow, including coliforms (Irianto, 2013; Utami & Miranti, 2020). Samples that are positive for containing coliform bacteria are marked by gas formation in the Durham tube. The colour changes to cloudy on the BGLBB media negative samples are marked by no gas formation at the top of the Durham tube, and

there is no change in the colour of the media to become cloudy on the BGLBB media, as shown in Figure 2. The results of the confirmation test in this study can be seen in Table 2. Based on the confirmation test results, there were four positive and eight negative samples.

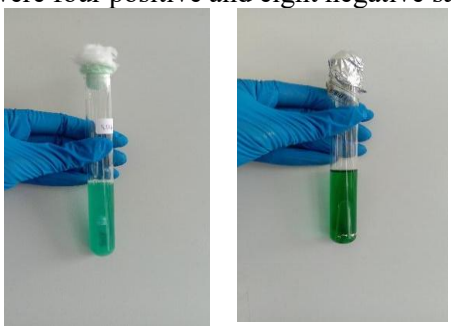

Figure 2. Confirmation test on positive (left) and negative (right) BGLBB media

Table 3: MPN Results of Jamu Gendong Beras Kencur and Compliance with Requirement

| No | Sample Code (S) | MPN Index Per 100 mL of Jamu Gendong Beras Kencur | Quality Requirements of Drinking Water (Permenkes RI, 2010) | Conclusion |
|---|---|---|---|---|
| 1 | 1 | 0 | 0 / 100 ml | eligible |
| 2 | 2 | 0 | 0 / 100 ml | eligible |
| 3 | 3 | 4 | 0 / 100 ml | not eligible |
| 4 | 4 | ≥1898 | 0 / 100 ml | not eligible |
| 5 | 5 | 0 | 0 / 100 ml | eligible |
| 6 | 6 | 0 | 0 / 100 ml | eligible |
| 7 | 7 | 0 | 0 / 100 ml | eligible |
| 8 | 8 | 0 | 0 / 100 ml | eligible |
| 9 | 9 | 271 | 0 / 100 ml | not eligible |
| 10 | 10 | 190 | 0 / 100 ml | not eligible |
| 11 | 11 | 0 | 0 / 100 ml | eligible |
| 12 | 12 | 0 | 0 / 100 ml | eligible |

Note: Sample codes 1 to 12 indicate the sample comes from market areas 1 to 12 from all regions of Sukoharjo.

The coliform bacteria content can be seen by counting the positive tubes on the BGLBB media and compared with the MPN table. The results of the coliform MPN calculation can be seen in Table 3. From these results, it can be seen that four samples do not meet the drinking water requirements, the highest MPN is found in the sample taken from area 4, with a value >1898. These results describe the number of colonies / 100 mL sample. Meanwhile, the other eight samples met the MPN requirements for drinking water quality. Coliform bacteria in water greatly influence the quality of drinking water. Based on the requirements of the Regulation of the Minister of Health of the Republic of Indonesia Number 492 of 2010, the requirement for MPN Coliform drinking water quality is 0/100 mL. If there are no coliform bacteria in the sample, the sample is suitable for consumption.

Based on observations, in 8 samples that were negative or did not contain coliforms, the jamu gendong carrying beras kencur was sold by bicycle and around the market, the hygiene and sanitation of the herbal medicine seller was good, and bottles or containers of herbal medicine were washed with clean water. In contrast, the four positive samples were found to have coliform bacteria. Based on observations, the herbal medicine seller stays in one place, the area used to sell herbal medicine is close to the location where there are piles of rotten vegetables, and the herbal medicine seller does not handle the container properly, namely, the herbal medicine seller rinses the herbal container with a reservoir of water in a bucket that has been used several times, The herbal medicine bottles used are used bottles of mineral water or syrup with the color starting to turn black. Also added, during the interview, the seller of jamu gendong said that during the processing of herbal medicine, the water used to make jamu gendong beras kencur is not boiled, or when making herbal medicine, the water is not boiled until it boils.

Several previous studies showed the same results. Research conducted by Susanti & Aprilliyani (2018)

(Susanti & Aprilliyani, 2018), found that 60% of the jamu gendong samples were positive for coliform bacteria. The research results by Hendiana et al. (2022) (Hendiana et al., 2022), stated that 70% of herbal medicine sellers in East Karawang District did not meet the requirements for permitted contamination limits. A similar thing was found in research conducted by Saputro (Saputro, 2019) on a sample of Jamu Gendong in Semarang. Several factors that can influence the quality of herbal medicine products containing beras kencur include the raw water used when making herbal medicine carrying beras kencur, cleanliness during manufacture, handling of the buyer's container, and the conditions of the selling place or selling environment (Listi et al., 2022). He also added that herbal medicine sellers should wash their hands to reduce the possibility of contamination. Apart from what has been mentioned, coliform contamination can occur due to the use of water that is not boiled or the heating temperature during processing does not reach 100 $^0$C, sellers lack hygiene in processing herbal medicine, unclean presentation and sales on the roadside so it is easily contaminated, as well as poor sanitation hygiene, for example, when serving, the glass used is not washed with soap and running water so that bacteria still stick to the glass (Tivani, 2018), (Makhabbah Jamilatun & Lukito, 2024). Poor manufacturing processes can cause herbal medicine to be contaminated with microbes in terms of sanitation and hygiene, and contamination by microorganisms originating from other external factors (Hendiana et al., 2022).

Based on the research results, several samples contained coliform contamination. The discovery of coliforms in drinking water, in this case, the jamu gendong beras kencur, can endanger health and cause diseases, such as typhus, diarrhea, and dysentery (Putri & Kurnia, 2018; Waangsir et al., 2022). According to the Regulation of the Minister of Health of the Republic of Indonesia Number 492 of 2010, good drinking water quality includes microbiological requirements, one of which is the absence of coliforms as an indicator of pollution in every 100 ml of water sample. Based on the results of this research and previous research Jamilatun & Lukito, 2024; Makhabbah Jamilatun & Lukito, 2024), there is a need for education regarding how to make herbal medicine that is good, safe, and avoids bacterial contamination, as well as environmental sanitation when processing and clean sales locations for herbal medicine to herbal medicine sellers in traditional markets. The Sukoharjo area, especially where samples were found with microbial contamination exceeding the regulatory limits. In this way, herbal medicine sellers can improve the quality of the herbal medicine they sell and make it safe to consume.

## 4. CONCLUSION

Based on the research results, it can be concluded that four samples of jamu gendong kencur beras at the Sukoharjo Region Traditional Market were found to be positive for containing coliform bacteria. Coliform bacteria were found in Sample 3 (4/100 ml), Sample 4 (>1898/100 ml), Sample 9 (271/100 ml), and Sample 10 (190/100 ml). Meanwhile, eight other samples were negative for containing coliform, namely samples 1, 2, 5, 6, 7, 8, 11 and 12. There needs to be education on how to make good herbal medicine, environmental sanitation, and clean herbal medicine sales locations so that herbal medicine sellers can improve the quality of the herbal medicine they sell and make it safe to consume.

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