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Total plate count and yeast mold count in liquid traditional medicine (Jamu) sold in the sukoharjo region market

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ABSTRACT

Jamu is the name for traditional medicine from Indonesia. This liquid traditional medicine is made by boiling all the ingredients, then squeezing the juice and mixing it with boiled water. This simple process of making liquid traditional medicine can cause undesirable impacts, microbial contamination which can cause diseases such as *Escherichia coli* bacteria which can cause diarrhea. In Sukoharjo, in several areas, there are still many sellers of liquid traditional medicine selling their wares in the market. There is no information regarding the quality of this liquid traditional medicine. This research aims to determine microbial contamination in traditional medicinal liquids sold at the Sukoharjo Regional Market. Research methods include total plate count to determine bacterial contamination and yeast mold count to determine fungal contamination in the kencur rice liquid traditional medicine. The results of calculating the total plate count showed that 3 samples exceeded the contamination limit with the highest total bacterial value of $4.1x10^5$ Colony Forming Unit (CFU)/ml. The results of the research showed that from the calculation of yeast mold count, 9 samples exceeded the contamination limit with the highest total yeast mold value of $1.1x10^6$ CFU/ml. This results can provide information to the public to increase awareness of consuming herbal medicine and to the local government to provide education to herbal medicine traders. There are still several traditional medicine quality standards that have not been observed in this study, so it is recommended that the quality of the types of microorganisms contamination, be further analyzed.

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1. INTRODUCTION

Traditional medicine has been embedded in the cultural life of the Indonesian people [1]. Jamu is the name for traditional medicine from Indonesia. Consumption of traditional medicine continues to increase as people become more aware of returning to nature [2]. Traditional herbal medicine is used as alternative medicine [3]. The use of herbal medicine as a means of treatment is based on experience passed down from ancestors [4]. Traditional medicine consists of various plant parts that work together to help care for and prevent disease [4]. Jamu is made from natural ingredients. The process of making liquid traditional medicine is still very simple [4],namely boiling all the ingredients, squeezing the juice, and mixing it with boiled water [5], [6]. This simple processing does not rule out the possibility that the liquid traditional medicine is contaminated by microorganisms which can cause poisoning, infection, or disease [7], [8].

Efforts are needed to prevent the circulation of traditional medicines that do not meet safety requirements. One of the requirements for good traditional medicine is that it must be free from microbial contamination [9]. Drug safety parameters include total plate count test and mold/yeast count test [10], [11]. Each preparation whose raw materials come from nature has a bacterial contamination limit of total plate count $<10^3$ CFU/ml, and for fungal contamination limit the yeast mold count is $<10^2$ CFU/ml. Previous research still found microorganism contamination in herbal medicine that did not meet the safety requirements for traditional medicine, including microbial contamination in temu ireng traditional medicine [12], and kunir tamarind traditional medicine, and also found the bacteria *Staphylococcus aureus* and *Escherichia coli* [13][14] . Other research states that fungi were found, namely Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Penicillium digitatum, Penicillium brevicompactum, and Acremonium sp. in herbal medicine [15]

One form of traditional medicine is liquid, which is usually called jamu gendong [16] hich is a traditional medicine that is popular with many people. Jamu gendong is made fresh to sell it directly to consumers, not preserved and distributed without marking [17]. Jamu gendong traditional medicine is made and processed using simple equipment, so traditional medicine can be contaminated with microbes, either due to poor sanitation or personal hygiene in making traditional medicine [18]. At this time you can find traders selling liquid traditional medicine by carrying them, using wheelbarrows, on motorbikes, and some in the market. In Sukoharjo, in several areas, you can still find many sellers of liquid traditional medicine selling their wares in the market. Liquid traditional traders are still very traditional in processing herbal medicine so they are assumed to pay little attention to hygiene and sanitation, both products and the environment. So there is a need for information about the quality and safety of liquid traditional so that consumers avoid diseases caused by microbial contamination.

Based on this description, research was carried out on Total Plate Count and Yeast Mold Count on Liquid Traditional Medicine (Jamu) Sold in the Sukoharjo Regional Market. This research was carried out to determine bacterial contamination using the total plate count method and fungal contamination using yeast mold count method, as well as their feasibility based on the safety requirements set by Regulation of The Food and Drug Supervisory Agency of the Republic of Indonesia. With the research carried out, it is hoped that it can increase awareness and vigilance among people who like to consume herbal medicine, as well as protect the public from the dangers of microbial contamination.

2. RESEARCH METHOD

2.1. Materials.

The materials used included samples of liquid traditional medicine (jamu) sold at the Sukoharjo Regional Market, PCA (Plate Count Agar) media, PDA (Potato Dextrose Agar) media, spirits, 70% alcohol, distilled water, and tissue. The equipment used includes laminar air flow, autoclave, incubator, vortex, oven, petri dish, volume pipette, dropper pipette, ball pipette, test tube, Erlenmeyer, glass beaker, measuring cup, analytical balance, spirit lamp, magnetic stirrer, one needle, colony counter, cool box, micropipette, tip.

The research procedure was carried out through several stages, namely preparation and sampling of liquid traditional medicine, homogenization and dilution of liquid traditional medicine samples, total plate count test, and yeast mold count test. The advantages of this methods are that living microbial cells can be counted, several types of microorganisms can be counted at once, and can be used for the isolation and identification of microbes, because the colonies formed may come from a microbe that has a specific growth appearance. There are many types of traditional liquid herbal medicines sold in the community, including rice kencur, tamarind turmeric, betel leaves, sambiroto, temu lawak juice, turmeric juice, and lempuyang chili. In this study, the samples used were liquid traditional medicine, the type of rice kencur, which is the traditional liquid herbal medicine that is most often consumed by children, teenagers, and adults because the taste is not bitter and can be found in various areas, especially in traditional markets in the Sukoharjo region.

2.2. Sampling Preparation.

Preparation begins with preparing the tools used in the research. Sampling was carried out in the morning at which time the market was busy with buyers. The liquid traditional medicine sample was put into herbal packaging bottles from liquid traditional medicine traders in the market according to conditions in the field. This aims to represent the real situation that the liquid traditional medicine purchased uses packaging from liquid traditional medicine sellers in the market. Liquid traditional medicine samples were brought to the laboratory using a cool box containing an ice pack. Cool boxes containing ice packs are used to reduce bacterial growth and air pollution during the trip to the laboratory. Sample analysis is carried out immediately after the liquid traditional medicine sample is in the laboratory. The samples tested in this study were liquid traditional medicines such as kencur rice which were sold at the Sukoharjo Regional Market. Codes up to 1-12 indicate the sample comes from market areas 1-12 from all regions in Sukoharjo.

2.3. Homogenization and Dilution of Samples.

The 1 ml liquid traditional medicines sample was pipetted aseptically, then put into a sterile tube containing 9 ml diluent solution, then homogenized to obtain a 10^{-1} dilution. The diluent solution was put into 4 sterile tubes with 9 ml of each sterile tube. The 10^{-1} dilution from the results of homogenization in the sample preparation was pipetted 1 ml in an aseptic manner and put into the first tube which was filled with 9 ml of diluent until a 10^{-2} dilution was obtained, then homogenized using a vortex. Subsequent dilutions were made up to 10^{-5}.

2.4. Total Plate Count Test.

The dilution of the sample that had been made previously was pipetted 1 ml each aseptically into a sterile petri dish and made in duplicate. 15 ml of thawed PCA (Plate Count Agar) media at 45 ± 1°C, poured into each petri dish. The petri dish is shaken gently so that the sample is evenly distributed on the media and allowed to solidify. All Petri dishes were incubated upside down at 37°C for 24 hours to 48 hours. The number of growing colonies was observed and counted. The total plate count was calculated in 1 ml of a sample by multiplying the average number of colonies in the dish with the dilution factor used [19], [20].

2.5. Yeast Mold Count Test.

The dilution of the sample that had been made previously was pipetted 1 ml each aseptically into a sterile petri dish and made in duplicate. 15 ml of PDA (Potato Dextrose Agar) media that has been thawed at a temperature of 45 ± 1°C is poured into each petri dish. The petri dish is shaken gently so that the sample is evenly distributed on the media and allowed to solidify. All Petri dishes were incubated at 20 - 25°C and observed from day 3 to day 5. Yeast colonies (yeast) have a small round shape, white, almost like bacteria. Meanwhile, mold colonies have fibers like cotton on the surface of the colony. The number of growing colonies was observed and counted. The yeast mold count was calculated in 1 ml of a sample by multiplying the average number of colonies in the dish with the dilution factor used [21], [22].

3. RESULTS AND DISCUSSIONS

This research was conducted with the aim of knowing the contamination rate of total plate count and yeast mold count of liquid traditional medicine (jamu) which is sold in the Sukoharjo Regional Market. The results of the study can be seen Yeast Mold Count Test in table 1 and table 2.

Table 1. Total Plate Count for Liquid Traditional Medicine (Jamu) Sold in the Sukoharjo Regional Market

No	Sample	Total Plate Count Sample (CFU/ml)	Limits of Microbial Contamination [9]	Conclusion	Explanation
1	Sample 1	$1x10^4$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
2	Sample 2	Negative	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
3	Sample 3	$1.4x10^5$	$1x10^5$	$> 1x10^5$	Exceeding the limit of contamination

No	Sample	Total Plate Count Sample (CFU/ml)	Limits of Microbial Contamination [9]	Conclusion	Explanation
4	Sample 4	$3.9x10^5$	$1x10^5$	$> 1x10^5$	Exceeding the limit of contamination
5	Sample 5	Negative	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
6	Sample 6	$1.2x10^4$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
7	Sample 7	$1.8x10^4$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
8	Sample 8	$3x10^3$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
9	Sample 9	$4.1x10^5$	$1x10^5$	$> 1x10^5$	Exceeding the limit of contamination
10	Sample 10	$2x10^3$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
11	Sample 11	$3x10^3$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit
12	Sample 12	$2x10^3$	$1x10^5$	$< 1x10^5$	Not beyond the contamination limit

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

Total Plate Count test aims to determine the number of bacteria present in the liquid herbal medicine of the kencur rice type which is sold at the Sukoharjo Regional Market. The Total Plate Count test was carried out using the pour plate method at five dilutions. The medium used was PCA media and incubated at 37 °C for 24 hours. Done in Duplo (replication twice). PCA media containing bacterial growth and those without growth can be seen in Figure 1. The results of the Total Plate Count calculation can be seen in Table 1. From these results, it can be seen that 9 samples met the requirements, and 3 samples showed the presence of bacterial contamination with varying amounts. Samples that met the requirements were taken from regions 1, 2, 5, 6, 7, 8, 10, 11, and 12. Meanwhile, samples that did not meet the requirements were obtained from regions 3, 4, and 9. The highest Total Plate Count was found in samples taken from region 9 with a value of $4{,}1x10^5$ CFU/ml. Previous research also showed that Total Plate Count exceeded the limit, Total Plate Count in herbal medicine in the Bata Bantaeng area found in 3 of 4 samples exceeded the limit [22].The highest Total Plate Count in rice kencur herb at the Badung Bali Kedonganan Market was found to be $267.6x10^8$CFU/ml [23]. Research of [24], shows that all herbal samples from markets in Kebumen district obtained Total Plate Count that exceeded the maximum contamination limit. The Total Plate Count of herbal turmeric tamarind sold in the Talang sub-district does not meet quality requirements. They said that the growth of microorganisms in the herbal medicine could be due to hygiene factors that were not maintained by the liquid traditional medicines sellers starting from the process of selecting raw materials to serve the herbal medicine.

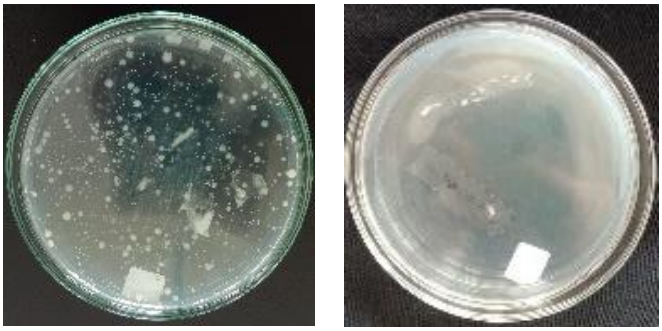

Figure 1. Plate Count Agar Media, bacterial colonies (left) and no bacterial colonies (right)

Table 2. Yeast Mold Count for Liquid Traditional Medicine (Jamu) Sold in the Sukoharjo Regional Market

No	Sample	Yeast Mold Count Sample (CFU/ml)	Limits of Microbial Contamination [9]	Conclusion	Explanation
1	Sample 1	$1.5x10^5$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
2	Sample 2	$5.9x10^4$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
3	Sample 3	$5.2x10^4$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
4	Sample 4	$1.1x10^6$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
5	Sample 5	$1.3x10^4$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
6	Sample 6	$3.5x10^3$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination

No	Sample	Yeast Mold Count Sample (CFU/ml)	Limits of Microbial Contamination [9]	Conclusion	Explanation
7	Sample 7	$2.4x10^4$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
8	Sample 8	Negative	$1x10^3$	$< 1x10^3$	Not beyond the contamination limit
9	Sample 9	$2.3x10^5$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
10	Sample 10	$2.1x10^3$	$1x10^3$	$> 1x10^3$	Exceeding the limit of contamination
11	Sample 11	$0.4x10^3$	$1x10^3$	$< 1x10^3$	Not beyond the contamination limit
12	Sample 12	$0.8x10^3$	$1x10^3$	$< 1x10^3$	Not beyond the contamination limit

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

Furthermore, The Yeast Mold Count test aims to determine the amount of fungi contained in the liquid traditional medicine of the kencur rice type which is sold in the Sukoharjo Regional Market. The Yeast Mold Count test was carried out using the pour plate method at five dilutions. The media used was PDA media and incubated at room temperature +25 ºC for 3 days. Done in Duplo (replication twice). PDA media containing yeast mold growth and those without growth can be seen in Figure 2. The results of the Yeast Mold Count calculation can be seen in Table 2. From these results, it can be seen that only 3 samples met the requirements, while the other 9 samples showed varying amounts of yeast contamination. Samples that met the requirements were taken from areas 8, 11, and 12. Meanwhile, samples that did not meet the requirements were obtained from areas 1, 2, 3, 4, 5, 6, 7, 9, and 10. The highest Yeast Mold Count was found in samples taken from region 4 with a value of $1.1x10^6$ CFU/ml. Previous research also showed that the Yeast Mold Count exceeded the limit, fungi were found in rice kencur with a total colony of $107x10^5$ CFU/ml. This contamination can be caused by washing raw materials that are less clean, as well as contamination of microorganisms from the soil. One of the raw materials for rice kencur traditional medicine is the rhizome of kencur which is the part of the plant that is underground [25]. The soil environment around plant roots allows microorganisms to grow due to the presence of nutrients that can fulfill growth.

Figure 2. Potato Dextrose Agar Media, yeast mold colonies (left) and no yeast mold colonies (right)

Regulation of The Food and Drug Supervisory Agency of the Republic of Indonesia Number 32 of 2019 states that traditional medicine is included in liquid traditional medicine, with requirements for microbial contamination, Total Plate Count $< 10^5$ CFU/ml, and Yeast Mold Count $< 10^3$ CFU/ml. Based on the analysis of microbial contamination using the Yeast Mold Count and Total Plate Count methods, it can be seen that the quality of the kencur rice traditional medicine samples that meet these two requirements are samples taken from regions 8, 11, and 12. Meanwhile, those that do not meet the requirements are samples taken from regions 3, 4, and 9. For samples taken from regions 1, 2, 5, 6, 7 and 10, they only meet the Yeast Mold Count requirements. According to Tivani research, the presence of a liquid traditional medicine sample that does not meet the requirements or contains contamination by microorganisms indicates that the level of hygiene is poor in liquid traditional medicine. This can be caused by the herbal processing process that is less hygienic, washing the rhizomes that are not clean, the liquid traditional medicine having direct contact with other herbal preparations, the equipment used is not properly cleaned, and the place used in the liquid traditional medicine

manufacturing process is not kept clean so that the herbal preparations are easily contaminated by bacteria in the environment.

Samples of the liquid traditional medicine of the kencur rice type taken from regions 8, 11, and 12 met the requirements and had good quality so the quality could be said to be good. This shows that the process of making herbal medicine for presentation and environment is good because the results of the Yeast Mold Count and Total Plate Count tests show a safe limit for use. These results indicate that the kencur rice type liquid herbal medicine taken from areas 8, 11, and 12 are safe for consumption because it complies with the requirements set by Regulation of The Food and Drug Supervisory Agency of the Republic of Indonesia. Based on observations made by researchers on liquid traditional medicine sellers from areas with samples that meet the requirements, it is known that microbial values that do not exceed the limit can be influenced by several factors [25], including the cleanliness of the raw materials used, the place for processing the herbs, the processing of the herbs, good packaging, how to store the herbs that have been produced, the process of washing the raw materials until they are clean using running water.

Meanwhile, samples of the liquid traditional medicine of the kencur rice type taken from regions 1, 2, 5, 6, 7, and 10 only partially fulfilled the requirements. And samples of liquid traditional medicine for rice kencur taken from regions 3, 4, and 10 did not meet the requirements. The traditional medicine is not of good quality so the quality can be said to be not good. Liquid traditional medicine samples that do not meet the requirements are caused by the number of microbial colonies growing beyond the maximum limit set by The Food and Drug Supervisory Agency of the Republic of Indonesia. Observation results show that the process of serving herbal medicine is less hygienic, and the environment where herbal medicine is sold is not clean. According to [26], the habit of traders who homogenize liquid traditional medicine using their hands can lead to the presence of microorganisms because after serving buyers the traders hold money from buyers and touch other items, rags that are not clean and touch other objects that are in a bad condition, not clean. So there may be microorganisms attached to the skin which then cause bacteria to grow in the herbal medicine. Hands and clothes that are not cleaned by herbal medicine makers and processing places that are not kept clean can increase the level of contamination of herbal medicine [27]. Apart from the source of microorganism contamination in liquid traditional medicine originating from hands, one source of contamination can also come from the use of containers and processing tools that are dirty and contain high amounts of microbes [28], [29]. The use of containers and processing equipment repeatedly without washing them can cause the growth of putrefactive and pathogenic microorganisms that are harmful to health. Processing is the main factor in the occurrence of bacterial contamination in liquid traditional medicine. Whereas the processing of liquid traditional medicine only uses hands. A large number of microbial growth can also be obtained from the process of making liquid traditional medicine. In the process of making liquid traditional medicine, heating is not carried out until it boils, for the reason is that if it is heated to boiling, the efficacy of the liquid traditional medicine will be lost or reduced [30]. This process can lead to the presence of bacteria that are still alive. According to [31], [32], some bacteria can live at temperatures of 55-60°C and relatively low oxygen conditions at temperatures of 45-90°C.

Traditional medicine that are contaminated with bacteria in small amounts may not cause disease directly if consumed, but if consumed excessively or continuously can cause disease in the future [33]. Bacterial content that exceeds predetermined limits is at risk of causing various diseases, including infections and foodborne diseases or food poisoning [34]. In addition, bacteria can produce toxins that can cause fever in the body, up to death. Infectious disease is one of the most common types of disease suffered by people in developing countries, including Indonesia. One of the causes of infection is bacteria [35]. The pathogenesis of bacterial causes includes the initiation of the infectious process and the mechanisms leading to the appearance of the signs and symptoms of the disease. The initial stage is the entry of bacteria into the body, then attached or attached to the host cell. The ability of microorganisms to increase pathogenesis is highly dependent on the virulence factors of microorganisms which include invasiveness and toxigenicity. Invasiveness is the ability of

microorganisms to penetrate host tissues, overcome the host's defenses, multiply, and spread throughout the body [36], [37].

Based on the results of calculating the number of yeast molds, there were samples of herbal medicine from several areas produced by herbal medicine sellers that exceeded the applicable provisions. So it is necessary to watch out for and carry out further research because if what grows is a colony of pathogenic fungi, it can be harmful to the health of the body. The content of yeast that exceeds predetermined limits has the risk of causing various diseases such as allergies, infections, and poisoning due to excessive exposure to mycotoxins from mushrooms [38], [39]. One of the pathogenic yeasts is *Candida albicans* which can cause mouth infections (sprue) [40], [41], [42], [43]. One example of a mold that is pathogenic is *Aspergillus flavus* [44], which can produce a mycotoxin called aflatoxin [45]. Aflatoxin is carcinogenic and hepatotoxic [46]. In liquid traditional medicine that is found to have a high Yeast Mold Count value, it is necessary to improve the processing process and to pay attention to the cleanliness of the environment. It would be better for herbal medicine sellers, especially in markets in the Sukoharjo area, to improve the cleanliness of raw materials, especially the process of washing raw materials. So even though the raw materials for making liquid traditional medicine are taken from soil which is a habitat for mold and yeast, with a good and correct washing process, the mold and yeast contained in the raw herbal medicine materials can be eliminated, so that the mold and yeast do not contaminate the herbal medicine.

4. CONCLUSION

The results of calculating the total plate count of liquid traditional medicine (jamu) sold at the Sukoharjo Regional Market found that 3 samples that exceeded the contamination limit with the highest Total Plate Count value of $4.1x10^5$ CFU/ml. The results of calculating the number of yeast molds in liquid traditional medicine (jamu) sold at the Sukoharjo Regional Market found that 9 samples exceeded the contamination limit with the highest Yeast Mold Count value of $1.1x10^6$ CFU/ml. The results of this research can provide information to the public to increase awareness of consuming herbal medicine, especially in terms of hygiene, as well as provide information to the local government to provide education to herbal medicine traders in processing and serving traditional medicine to avoid microbial contamination. There are still several traditional medicine quality standards that have not been observed in this study, so it is recommended that the quality of the types of microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus* sp. contamination, be further analyzed.

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