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Microbiological Profiling of Online-Market Moringa (Moringa oleifera) Leaf Powder: Total Plate Count and Yeast–Mold Analysis

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ABSTRACT: Moringa is a traditional medicinal plant with proven efficacy for the community. This plant has high antioxidant activity and contains active substances that are beneficial for health. Microbial contamination is often present in traditional medicinal plants, one of the causes of product damage is due to microbial growth in the product. The purpose of this study was to determine whether the Total Plate Count (TPC) and Yeast Mold Count (YMC) in Moringa leaf herbal powder met the requirements of the Indonesian Food and Drug Supervisory Agency Regulation Number 32 of 2019 concerning the safety and quality requirements for traditional medicines. The sampling technique used in this study is random sampling. The method used to calculate the number of bacteria in this study was the Total Plate Count (TPC), while the calculation of the number of yeast molds was the Yeast Mold Count (YMC). The independent variable in this study was moringa leaf powder, which is a product of several online shop Banyumas Regency (X), Blora Regency (Y), and Central Jakarta (Z). The results of the study using the TPC method showed that the average number of bacteria in Moringa leaf herbal powder samples X, Y, and Z was 1.8×10^4 colonies/g, 4.6×10^4 colonies/g, and 5.7×10^5 colonies/g, respectively. YMC method showing the average values of samples X, Y, and Z are 1.3×10^2 colonies/g, 2.8×10^2 colonies/g, and 4.6×10^2 colonies/g. Total Plate Count and Yeast Mold Count of Moringa leaf herbal powder meets the requirements of the Indonesian Food and Drug Supervisory Agency Regulation Number 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines, namely microbial contamination of Total Plate Count (TPC) $\leq 5 \times 10^7$ colonies/g and Yeast Mold Count (YMC) $\leq 5 \times 10^5$ colonies/g.

KEYWORDS: TPC, YMC, Herbal Powder, Moringa Leaves, Online Shop

I. INTRODUCTION

As technology develops, it is becoming easier to search and getting what you want, as well as looking for herbal products or traditional medicines that are in high demand during the pandemic era. Online shops one example of a community solution to buy a product without having to direct contact with the seller using only electronic devices just by communicating, you can get the desired product. Herbal medicine products which is sold freely in online shop of course, buyers also have to be smart when choosing. Studies of commercial Moringa products have shown that some samples have bacterial and fungal contamination exceeding standard limits (e.g., European Pharmacopeia). Emphasizing the importance of quality control will have an impact on consumer safety [1].

Herbal medicine products sold freely in online shops require buyers to be discerning when choosing herbal medicines online, as not all herbal medicines are halal-certified and contain safe ingredients. Therefore, it is necessary to test herbal products sold freely in online shops to determine the quality of the herbal medicine and whether it meets the safety and quality standards of traditional medicine. Herbal medicine is a traditional medicine made from natural ingredients, a cultural heritage that has been passed down from generation to generation for health.

Herbal medicine preparations are inextricably linked to bacteria and fungi. Herbal medicine made without Good Traditional Medicine Manufacturing Practices is feared to have an inadequate level of safety, as the herbal medicine may have a Total Plate Count (TPC) and Yeast Mold Count (YMC) that do not meet requirements. The Total Plate Count (TPC) indicates the number of microbes in a product, while the Yeast Mold Count (YMC) indicates the number of mold and yeast colonies in a product. However, this risk can

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be mitigated when the powder is stored under the appropriate conditions to ensure there is no ingress of moisture and then processed in a hygienic manner to reduce contamination and/or cross-contamination by following hazard analysis critical control point or similar procedures (even in a home setting) including a heat treatment, i.e., boiling, to further reduce microbial hazards [2]. Based on previous study, the real-time stability test was carried out at room temperature (28.5–33.5°C) with 50–75% relative humidity, and the sampling interval was once every third month (Month-0, Month-3 and Month-6) of moringa tea products. The qualities of moringa tea bags and brewed moringa teas stored at room temperature (28.5–33.5°C) and 50–75% relative humidity showed good physicochemical properties and stability in the test during six months of observation [3]. Another study also stated that there was no significant relationship between the total microbial content of packaged and unpackaged herbal medicine [4].

Moringa leaves (Moringa oleifera) are a multifunctional plant that provides natural supplements, antioxidants, essential amino acids, multivitamins and minerals, and other high-quality natural nutrients for the body. Therefore, Moringa leaf herbal powder is currently in high demand for boosting the immune system. The selection of Moringa leaf herbal powder sold in several online shops is based on the high number of products sold and high product ratings. Therefore, it is necessary to conduct TPC and YMC tests to determine the microbiological quality of the herbal medicine. Microbial contamination contained in the water extract of Moringa leaves are Total Plate Count value of 0 colonies/g and Mold/Yeast count value of 0 colonies/g and contain nutritional values such as water (75.85%), ash (3.87%), protein (6.27%), fat (2.20 %) and carbohydrate (14.01%) [5]. Based on this background, the author is interested in conducting a study entitled "Total Plate Count and Yeast Mold Test on Moringa (Moringa oleifera) Herbal Powder Brands X, Y, and Z Sold Openly in Online Stores". Rachmawati and Suriawati [5] dan Jayani et al [6] points out that processing and storage conditions significantly determine the level of microbial contamination. This is important when comparing different brands of "X, Y, Z."

II. MATERIAL AND METHOD

a. Sample

The sample used in this study was Moringa leaf herbal powder, a product from several online shops from Banyumas Regency, Blora Regency, and Central Jakarta, Indonesia.

Figure 1. Sample of Moringa leaves

b. Chemical and Medium

0.9% NaCl solution (sodium chloride), PCA (Plate Count Agar) medium, PDA (Potato Dextrose Agar) medium, and distilled water.

c. Total Plate Count (TPC)

Moringa leaf powder samples X, Y, Z were weighed as much as 1 gram, placed into a test tube containing 9 ml of saline solution to obtain a dilution of 10-^{1}. The sample was diluted to 10^{-6} using saline solution. As much as 1 ml of each dilution from 10^{-1} to 10^{-6} was inoculated into a petri dish using the pour plate technique and 25 ml of PCA media was added. After solidifying, the media was incubated at 27 C for 48 hours in an incubator.Two replicates were performed. Observe and count bacterial colonies using a colony counter. Observe and count yeast and mold colonies using a colony counter [3], [7], [8].

d. Yeast Mold Count (YMC)

Moringa leaf powder samples X, Y, Z were weighed as much as 1 gram, placed into a test tube containing 9 ml of saline solution to obtain a dilution of 10^{-1}. The sample was diluted to 10^{-4} using saline solution. As much as 1 ml of each dilution from 10^{-1} to 10^{-4} was inoculated into a petri dish using the pour plate technique and 25 ml of PDA media was added. Two replicates were performed [3], [9].

e. Data Analysis

Moringa leaf herbal powder X, Y, and Z meet the requirements of the Indonesian Food and Drug Monitoring Agency Regulation Number 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines, namely microbial contamination with a Total Plate Count

(TPC) of $\leq 5 \times 10^7$ colonies/g and a Yeast Mold Count (YMC) of $\leq 5 \times 10^5$ colonies/g.

III. RESULT AND DISCUSSION

This study aimed to determine the TPC and YMC in Moringa leaf powder sold in online shops. The results of this study were assessed by measuring the bacterial colony count after a 48-hour incubation period and the yeast colony count after a 5-day incubation period. This study used Moringa leaf powder herbal medicine obtained from three online shops in Banyumas Regency, Blora Regency, and Central Jakarta. These three locations were selected as samples because they had high product sales and product ratings. In Banyumas Regency, 473 products were sold and received a 4.9/5 rating, in Blora Regency, 1.2,000 products were sold and received a 4.9/5 rating, and in Central Jakarta, 1.7,000 products were sold and received a 4.8/5 rating. The calculation results and average Total Plate Number values for the three samples are shown in Table 1.

Table 1. Total Plate Count of Sample

Sample	TPC (colonies/g)
X	1.9×10^4
Y	4.6×10^4
Z	5.7×10^5

The results obtained from the calculations show that all three samples of Moringa leaf powder herbal medicine (X, Y, and Z) showed bacterial colony growth in Petri dishes. In sample X, one replicate bacterial colony grew at a dilution of 10^{-1} until 10^{-4}, while the second replicate bacterial colony grew at a dilution of 100,000. In sample Y, one replicate and the second replicate bacterial colony grew at a dilution of 10^{-1} until 10^{-6}. In sample Z, one replicate bacterial colony grew at a dilution of 10^{-1} until 10^{-6}, while the second replicate bacterial colony grew at a dilution of 10^{-1} until 10^{-5}. Based on the data obtained, the Total Plate Count (TPC) values for samples X, Y, and Z met the ALT requirement for traditional powdered medicinal preparations, namely $\leq 5 \times 10^7$ colonies/g. This bacterial growth likely resulted from the manufacturing process, such as inadequate hygiene in washing raw materials and equipment. Microbial contamination in samples is closely related to hygiene factors, including the seller, the equipment used, and the ingredients. .

According to the Indonesian Food and Drug Monitoring Agency Regulation Number 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines, the recommended yeast count for powdered herbal preparations is $\leq 5 \times 10^5$ colonies per gram. The calculation results and average yeast count values for the three samples are shown in Table 2.

In sample X, one replicating yeast colony grew at a dilution of 10^{-1} until 10^{-4}, while a second replicating yeast colony grew only at a dilution of 10^{-1}. In sample Y, one replicating yeast colony grew at a dilution of 10^{-1} until 10^{-2}, while a second replicating yeast colony grew at a dilution of 100. In sample Z, both the first and second replicating yeast colonies grew at a dilution of 10^{-1} until 10^{-3}.

Table 2. Yeast Mold Count of Sample

Sample	YMC (Colonies/g)
X	1.3×10^2
Y	2.8×10^2
Z	4.6×10^2

Based on the results obtained during the study, all three samples of Moringa leaf powder herbal medicine (X, Y, and Z) from the first and second replications showed the growth of yeast colonies in petri dishes. Based on the data obtained, the Yeast Mold Number (YMC) values for all samples met (Adequate Intensity Index) requirements for traditional herbal medicine powder preparations, namely $\leq 5 \times 10^5$ colonies/g. Yeast mold growth can be caused by storing raw materials in humid conditions in plastic packaging, which can trigger yeast growth. Several other factors that can trigger yeast growth include storage room temperature, storage period, and a less than hygienic herbal medicine powder manufacturing process.

To prevent yeast growth in the Moringa leaf powder manufacturing process, it is necessary to pay attention to the temperature and humidity of the raw material storage area, and the storage time before processing, as warm and humid environments can affect yeast growth [10]. The data obtained from these three samples all meet the requirements of the Indonesian Food and Drug Monitoring Agency (BPOM) Regulation Number 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines, namely microbial contamination with a Total Plate Count (TPL) of $\leq 5 \times 10^7$ colonies/g and a Yeast Mold Count (YMC) of $\leq 5 \times 10^5$ colonies/g.

These results suggest that purchasing Moringa leaf herbal powder online is not a cause for concern, as it has been proven to meet the quality requirements for traditional medicines.

IV. CONCLUSION

All three samples X, Y, and Z meet the requirements of the Food and Drug Monitoring Agency (BPOM) Regulation Number 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines. All three samples are safe for consumption as traditional medicines.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

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