MICROBIAL CONTAMINATION IN MIXED SYRUP CONTAINING ERDOSTEIN AND CETIRIZIN STORED AT DIFFERENT STORAGE TEMPERATURE

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ABSTRACT

Pharmaceutical services include drug dispensing activities, including drug compounding. Drug compounding is the process of mixing or changing ingredients to produce drugs that suit patient needs. Unmonitored storage conditions for compounded drugs in the community can affect the evaluation of the physical, chemical, and microbial contamination stability. The aim of this study was to determine the effect of storage temperature on the results of the evaluation of microbial contamination in compounding syrup pharmaceuticals, so it can provide education to the public about the storage patterns that have been carried out so far. Evaluation of microbial contamination in this study included contamination of the Total Yeast and Mold Count (TYMC) and Total Plate Count (TPC). The objective of this study was a mixture of Erdostein syrup and cetirizine tablets. The research materials used for the microbial contamination test were mineral water, sterile water, Plate Count Agar (PCA) media, and Potato Dextrose Agar (PDA). The results of the study in the form of TYMC and TPC on day 0 were 0 cfu/mL. After 7 days of storage, microorganisms, namely TYMC, grew at room temperature (<30°C) $4.98x10^2$ cfu/mL and cold temperature (2-8°C) $2.2x10^2$ cfu/mL. The TPC results at room temperature storage (<30°C) were $8.33x10^2$ cfu/mL and at cold temperatures (2-8°C) were $3.67x10^2$ cfu/mL. Based on the results of this study, it can be concluded that during the storage process of the compounded preparation for 7 days, there was an increase in the number of microorganism colonies, so that the microbial contamination did not meet the requirements of the United States Pharmacopeia (USP) 46 edition, with the number of microorganism colonies in the TYMC and TPC evaluations for room temperature storage being higher than cold storage temperatures.

Keywords: Total Yeast and Mold Count, Total Plate Count, Compounding syrup

INTRODUCTION

Drug compounding is the process of combining, mixing, or changing ingredients to create a drug tailored to the patient's needs (U.S. Food and Drug Administration, 2017). Compounding is part of dispensing, which is an activity of preparing, delivering, and providing drug information to patients based on a prescription. by a doctor (Ministry of Health, Republic of Indonesia, 2014).

An evaluation of the preparation was carried out at one of the pharmacies in the Cimahi area that handles prescriptions for Erdostein syrup with added cetirizine tablets according to a doctor's prescription. Erdostein is a drug with thinner sputum or mucolytic agent that can be used in patients with chronic bronchitis on person adults (Moustafa et al., 2014). Cetirizine is an antihistamine that blocks histamine production during an allergic reaction.

Storage drugs that are not in accordance with their characteristics influence health consequences and consume drugs (Mubarok et al., 2023). Storage drugs that are affected by several factors, such as light, temperature, and humidity, affect the

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stability and quality of medicine. In public condition storage, drugs are not monitored and carried out in accordance with the knowledge and conditions of the environment. Many people believe that keeping the drug syrup in the cupboard cooler will make drugs more durable and long-lasting. The results of a survey study (Sari et al., 2021) show that respondents still do not have sufficient knowledge about the method of storing drugs in syrup form or solution. Only 35.6% knew the storage drug in syrup form or solution, which was in accordance with the label. Storage drugs can influence the effectiveness of drugs, which affects their quality and use. Part of the big drug is stored in the temperature room, and no rays may be exposed directly to the sun and dry. Drug quality is produced in accordance with standard regulations; however, there is contamination due to improper storage conditions that can cause the growth of microorganisms (Salaeh, 2018).

Similar to other pharmaceutical preparations, compounded preparations also require evaluation to determine the stability characteristics of the preparation after the compounding and storage process to determine its safety and effectiveness. These evaluations can include physical, chemical, and microbiological evaluations. Based on research on the stability of Erdostein syrup mixtures under 2 storage conditions at cold temperature (4°C) and room temperature (20°C), changes in physical and chemical stability occurred (Kurnia, 2017). Differences in storage temperature can also affect the results of microbiological evaluations of microbial contamination, namely the Total Yeast Mold Count (TYMC) and Total Plate Count (TPC). The optimal temperature for microorganism growth is in the range of 23-26°C. Outside this temperature range, the growth of microorganisms is reduced.

In the United States Pharmacopeia 46th edition, it states that stock oral with water carrier has a maximum limit of pollution microbes for TYMC of 10^1 cfu/g or cfu/mL and TPC is 10^2 cfu/g or cfu/mL if the product exceeds the limit determined, then the no fulfil condition pollution microbes (USP, 2023). Contamination microbes that exceed the standard limits that have been set can lower the quality of stock medicine, so that they become unstable and can result in a decline in the effectiveness of drugs (Ratajczak et al., 2015).

The purpose of this study was to determine the effect of storage temperature on the results of the evaluation of microbial contamination in syrup preparations made by a pharmacy in Cimahi City. The results of this study are expected to provide educational material or information from health service providers to the public that drug storage temperature is not the only condition that guarantees drug quality.

Research on the physical and chemical evaluation of Erdostein concoction has been conducted in previous studies (Kurnia, 2017), but the evaluation of contamination of Erdostein concoction drug preparations has not been conducted. The research conditions were also the same as most drug storage in the community, namely at room temperature and cold storage temperatures.

RESEARCH METHODS

Tools and Materials

Incubator (Memmert, Germany), Autoclave (All American 50X, United States); Micropipette 100-1000 µL (DragonLab, Indonesia), and colony counter (BCC 116 B-One, China). Erdostein dry syrup (GKL1315715538A1); Cetirizine 10 mg tablets (GKL0408511617A1), Potato Dextrose Agar Media (Merck), and Plate Count Agar (Merck).

Research Procedures

1. Erdostein and Cetirizine Syrup Mixture

The preparation of Erdostein and Cetirizine syrup mixture as a sample was made by pharmaceutical personnel at a pharmacy in Cimahi under conditions that were the same as when providing prescription services. First, Erdostein dry syrup was reconstituted with mineral water. Three tablets of cetirizine 10 mg were ground in a mortar until they were smooth. Part of the reconstituted Erdostein dry syrup was taken to dissolve the cetirizine

tablets and then put into the Erdostein syrup bottle. Shake until homogeneous. In this study, the concoction samples were stored at different temperatures and opened twice daily for 7 days.

2. Plate Count Agar (PCA) Media

Plate Count Agar (PCA) (11.25 grams was suspended in 500 mL of the aquadest solution in an Erlenmeyer flask. The PCA media solution was sterilized in an autoclave at 121 °C for 15 minute.

3. Potato Dextrose Agar (PDA) Media

Potato Dextrose Agar (PDA) amounting to 19.5 grams were suspended in a 500 mL aquadest solution in an Erlenmeyer flask. The PDA media solution was sterilized in an autoclave at 121 °C for 15 minute.

4. Evaluation of Yeast Mold Number (YMC)

One milliliter of Erdostein syrup mixture was resuspended in 9 mL of sterile distilled water in a sterile test tube to obtain a solution with a dilution of 10^{-1} is obtained. Make up to a dilution of 10^{-2} and 10^{-3}. From each dilution, 1 mL of the solution was taken using a micropipette and placed into a sterile petri dish, and then PDA media was added and homogenized. After the media solidified, the Petri dish was incubated in an inverted position at 30°C for 72 hours.

5. Total Plate Count (TPC) Evaluation

One milliliter of the Erdostein syrup concoction sample was resuspended in 9 mL of sterile distilled water in a sterile test tube to obtain a solution with a dilution of 10^{-1} is obtained. Make up to a dilution of 10^{-2} and 10^{-3}. From each dilution, 1 mL of the solution was taken using a micropipette and placed into a sterile petri dish, and then PCA media was added and homogenized. After the media solidified, the Petri dish was incubated in an inverted position at 37°C for 18-24 hours.

Data analysis

This research was conducted using the experimental method with type taking data quantitative using research design posttest only two group design with 2 group tests consisting of cold temperature (2-8°C) and room temperature (<30°C) storage test groups.

Quantitative data for TYMC and TPC were descriptively and quantitatively analyzed. The results of the TYMC and TPC evaluations will be described according to the limits of microbial contamination requirements stated in the United States Pharmacopeia edition 46. The data will be processed statistically using the Mann Whitney test to determine the differences in the results of the microbial contamination evaluation between the two storage temperatures.

RESULTS AND DISCUSSION

Based on experimental research conducted on the Erdostein-Cetirizin syrup mixture, the following results were obtained:

Table I. Results of Evaluation of Yeast Mold Numbers and Total Plate Counts of Erdostein and Cetirizine Syrup Mixtures on Day 0 and Day 7

Storage Period	Yeast Mold Number (cfu/mL)						Total Plate Count (cfu/mL)					
	Temperature (<30°C)			Temperature (2-8°C)			Temperature (<30°C)			Temperature (2-8°C)		
	1	2	3	1	2	3	1	2	3	1	2	3
Day 0	0	0	0	0	0	0	0	0	0	0	0	0
Average	0			0			0			0		
Day 7	$2.95x10^2$	$1x10^3$	$2x10^2$	$9.5x10^1$	$5.25x10^2$	$1.4x10^2$	$1x10^3$	$6x10^2$	$9x10^2$	$1x10^2$	$3x10^2$	$7x10^2$
Average	4.98×10^2			2.2×10^2			8.33×10^2			3.67×10^2		

Note:
Yeast Mold Number Requirements $<10^1$ cfu/mL, Total Plate Count $<10^2$ cfu/mL.

Figure 1. Yeast Mold Number of Erdostein – Cetirizine Syrup Mixture Preparation with Room Temperature (A) and Cold Temperature (B) storange for 7 days

Figure 2. Total plate count of Erdostein – Cetirizine Syrup Mixture stored at room temperature (A) and cold temperature (B) for 7 days.

The object of this study was a sample of Erdostein-Cetirizine syrup concoction prepared at a pharmacy in Cimahi, West Java. Three bottles of the concoction samples were prepared at each storage temperature. Evaluation of the concoction sample was performed on day 0 immediately after the sample was made. No colonies of mold, yeast, or bacteria were found after microbial contamination on day 0. Storage of the concoction sample was carried out at room temperature (<30°C) and cold temperature (2-8°C). During 7 days of storage, the concoction bottle was opened and closed 2 times a day. On the 7th day, the concoction sample was re-evaluated, and it was found that microbial contamination in the old yeast number and total plate count exceeded the required limits.

Research related to the evaluation of compounded preparations is mostly conducted on physical and chemical stability, which is considered *urgent* because it has a direct impact on the efficacy of the preparation and its organoleptic appearance by consumers. The evaluation of pharmaceutical preparations should be conducted comprehensively, including physical, chemical, and microbiological evaluations. Evaluation of microbiological contamination that exceeds this limit can result in damage to the drug preparation.

Microbial contamination after 7 days of storage exceeded the TYMC and TPC evaluation requirements, but the growth of these microbes did not differ significantly based on the Mann Whitney statistical test. Based on this, the storage temperature did not significantly affect the growth of microorganism colonies, although the number of microorganisms grown was higher at room temperature than at cold temperatures. These results can be used in conjunction with a chemical stability test to determine the stability of the drug at a certain storage temperature. Cold storage can damage some drugs and reduce their concentrations in preparations for storage over a certain period (Ponsonby et al., 2024). Research on the stability of drug levels in compound preparations at different storage temperatures has been conducted and shown that there is an effect of the length of time the drug is stored, even though it has been stored under the recommended conditions. The compounding technique and conditions when preparing the syrup compound also need to be considered (Anslem et al., 2024).

Microbiological evaluations included microbial contamination of the Total Yeast Mold Count (TYMC) and Total Plate Count (TPC). The research stages began with sterilization of the equipment used, making Potato Dextrose Agar (PDA) and Plate Count Agar (PCA) media. PDA media is used for KPK evaluation with dextrose content, which acts as a source of sugar and energy, and each component among these ingredients has a function and role in supporting the growth of microorganisms, especially fungi, and agar components that solidify the media. PCA media was used for TPC evaluation, with glucose content and yeast extract used to grow all types of bacteria and other nutritional content obtained from tryptone, vitamins from yeast extract, and glucose as a source of bacterial energy. In this study, negative controls (-) were used to ensure that the media were free from microorganism contamination.

The Erdostein–Cetirizine syrup preparation was performed at a pharmacy in Cimahi. The preparation was evaluated for TYMC and TPC at the Microbiology Laboratory of Santo Borromeus University. On day 0 after preparation, TYMC and TPC evaluations showed 0 colonies. Based on these results, the preparation on day 0 met USP requirements. The fulfillment of these requirements can be attributed to the effects of preservatives usually present in the finished product preparations of each drug included in the formulation.

The storage of compounded preparations was carried out at 2 different temperatures: room temperature (<30°C) and cold temperature (2-8°C). Both storage temperatures are conditions that are often used by the public to store drug preparations without paying attention to the instructions on packaging or asking health workers in pharmaceutical services. The results of the evaluation of microbial contamination of TYMC and TPC showed that compounded preparations that had been stored for 7 days had contamination that exceeded the requirements under both storage conditions. Microorganism contamination can occur because of poor handling of the mixture during compounding and repackaging processes.

Compounded preparations in pharmacies are not sterile preparations, but if they experience microbiological contamination, it can also affect the physical and chemical properties of the drug.

Several factors that cause increased growth of microorganisms can be categorized into internal and external factors. Internal factors that can influence it are the effectiveness of preservatives that are unable to inhibit the growth of microorganisms optimally within a certain period of time. The addition of appropriate preservatives, such as sodium benzoate, to syrup preparations at effective levels can inhibit the growth of microorganisms (Nurjanah et al., 2020). The longer storage time of drug preparations will also increase the number of microorganism colonies (Salaeh, 2018).

External environmental factors that can affect the growth of microorganisms include temperature, humidity, light intensity, and oxygen. The number of colonies of mold, yeast, and bacteria in the concoction samples stored at cold temperatures was less than those stored at room temperature. This is because at cold temperatures, the growth of microorganisms can be inhibited. Optimal growth of mold and yeast occurs at room temperature, namely 25-30°C, and water media can also increase the growth of mold and yeast colonies (Prest et al., 2016), which is used as a solvent in Erdostein syrup and is a good medium for the growth of mold and yeast when stored for a long time. The mesophilic bacteria that appeared in the TPC evaluation had an optimal growth temperature of 20-45°C (Fauzi et al., 2017). The growth of aerobic bacteria can be increased in the presence of oxygen (Couvert et al. 2019). During the storage period of the concoction preparation, the bottle opening and closing procedure was carried out according to the frequency of drug use, allowing oxygen and other contaminants to enter. Light intensity can affect the growth of mold and yeast colonies because it can kill microorganisms. This can occur because during storage, both at room temperature and cold temperatures, there is no continuous exposure to light, either from sunlight or lamps. The lower the light intensity received, the greater the number of colonies grown. Light intensity is related to indoor humidity because if natural or artificial light is not distributed properly, it can affect the humidity level of the room. As a result, high humidity in a room can increase the growth of microorganisms (Lestiani & Pawenang, 2018). Several studies have also shown that microbial contamination of finished drug preparations circulating in several countries can exceed the threshold requirements (Agustin, 2019; Jazmati et al., 2022; Musa et al., 2023).

CONCLUSION

Based on the research conducted, it can be concluded that the results of the Erdostein-Cetirizin syrup preparation made in one of the pharmacies located in Cimahi met the requirements of Yeast Mold Number and Total Plate Count on day 0 after being mixed. In storage for 7 days at different storage temperatures, the Yeast Mold Number and Total Plate Count exceeded the standard limits of the United States Pharmacopeia 46 edition. The growth of microorganism colonies was higher at room temperature (<30°C) than at cold temperatures (2-8°C).

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