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DEXAMETHASONE ANALYSIS ON SIX BRANDS OF PACKAGED HERBAL MEDICINE USING THIN LAYER CHROMATOGRAPHY METHOD

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ABSTRACT

Herbal medicine is used for generations because of its safety and free from adverse side effects, but the healing process takes a long time. This is the background of herbal medicine manufacturers to improve their performance by adding dexamethasone compounds. This compound is a synthesis of corticosteroid hormones whose use must be under the supervision of a doctor because it has side effects of cardiovascular disease, Cushing's syndrome, osteoporosis, hypertension, gastrointestinal ulcers, infections, and cataracts. The presence of this compound can be detected using the Thin Layer Chromatography method by comparing the Rf value of the sample with the standard solution of dexamethasone. The eluents used are methanol, ethyl acetate, and toluene with a ratio of 1:45:55. This method was used to detect dexamethasone content in six samples of packaged herbs circulating in the Mojokerto region, East Java province. Of the six samples analyzed, five of them showed positive results, namely samples A, B, C, D and F with a difference in the Rf value of the sample and standard of each 0.01; 0.01; 0.00; 0.00; and 0.02. Sample E does not contain dexamethasone indicated by the difference in the sample's Rf value against the 0.5 standard.

Keywords: *Herbal medicine, herbal packaging, dexamethasone, corticosteroids, Thin Layer Chromatography (TLC).*

INTRODUCTION

Traditional medicine is commonly used in Indonesia and Southeast Asia such as Singapore, Malaysia, Philippines, and the Thailand. Traditional medicine is immensely popular in Indonesia known as jamu (Kuswandi et al., 2021). According to the world health organization (WHO), herbal medicine is defined as a practice that includes herbs, herbal ingredients, herbal preparations, and finished herbal products that contain active plant ingredients or other ingredients, or even combinations (WHO, 2013). Herbal medicine or herbal medicine has been used for generations because it is free from adverse side effects, safer, and harmless. However, herbal medicine cannot be cured immediately and takes longer for the healing process. Because of this, many herbal medicine manufacturers add certain chemicals to improve the work of herbal medicine. The addition of these chemicals can increase quite serious adverse side effects such as phototoxic reactions, contact urticaria, allergic contact dermatitis, angioedema, and anaphylaxis. One chemical added to increase the effectiveness of herbal remedies is dexamethasone (Kuswandi et al., 2021).

Dexamethasone has the IUPAC name disodium;[2-[8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16octahydrocyclopentane[a]ph-enanthren-17-yl]-2-oxoethyl] phosphate, this compound is soluble in water (Al-Janabi et al., 2020). Dexamethasone is a synthetic corticosteroid hormone commonly used for immunology and anti-inflammatory drugs (Qin et al.,

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2022). In addition, this compound has other pharmacological effects such as anti-toxic, anti-allergic and anti-rheumatic activity (Li et al., 2021). This compound is used to treat various diseases, such as allergies, autoimmune, cancer, eye disorders, covid-19, and many others. The medical use of this compound is limited due to its low solubility in water (Madamsetty et al., 2022). The use of dexamethasone should also be as prescribed by a doctor, as it has side effects such as cardiovascular disease, Cushing's syndrome, osteoporosis, hypertension, gastrointestinal ulcers, cataracts, and infections (Huscher et al., 2009; Daniel & Newell-Price, 2017; Davis et al., 2007; Saag et al., 1994). Therefore, mixing these drugs in herbal medicines is illegal, so it must be monitored closely to ensure the safety of herbal medicines (Primpray et al., 2019).

Analysis of dexamethasone as a mixture of herbal medicines uses chromatographic methods. The chromatographic methods used include HPLC, LC-MS/MS and UHPLC-Q-Orbitrap HRMS. However, the chromatography method takes a long time, must be destroyed, expensive, requires personal skills in operating the tool, and requires many dangerous reagents (Kuswandi et al., 2021). The kinetic spectrophotometry method can also be used for analysis of dexamethasone content in herbal medicines with a detection limit of 0.14 mg / L. The weakness of this method is that it is less selective for the presence of contamination substances (Akhoundi-Khalafi & Shishehbore, 2015). Ananto et al., 2020 used iodometric titration methods and HPLC for dexamethasone content analysis, but this method requires expensive costs and a long time. Because the addition of dexamethasone to herbal medicine is illegal, it is necessary to analyze the content of these compounds in traditional herbal medicine. One method that can be used is the qualitative method of Thin Layer Chromatography (KLT).

Thin-layer chromatography (TLC) is a method of separating components based on differences in adsorption or partitioning by the stationary phase influenced by the movement of the developer solvent mixture (14). This method is simpler, cheaper and can be used to detect the presence of dexamethasone compounds. This method involves a mobile phase that is a mixture of solvents saturated in a chamber and a stationary phase that is stuck on the KLT plate. While the results of the analysis can be known from the calculation of the Rf value of the sample with the standard standard, the following is the formula for calculating the Rf value:

$$Rf = \frac{Distance\ traveled\ by\ the\ substance}{Distance\ traveled\ by\ the\ eluent}$$

Therefore, simple qualitative methods such as KLT can be used to detect dexamethasone content mixed in herbal medicines or herbs.

RESEARCH METHOD

This research is descriptive research. Meanwhile, the analysis of dexamethasone mixture in packaged herbs was carried out at the Food and Beverage Analysis laboratory of the Institut Ilmu Kesehatan Bhakti Wiyata in August 2022. The analysis method used is thin-layer chromatography (TLC).

Sample Preparation. Samples of herbs from six varied brands weighed 7 grams and were included in 250 ml Erlenmeyer. Dissolved in 49 ml of chloroform-methanol solvent mixture (9:1) and soaked for 3 hours for maceration process. Next shake using a shaker for 30 minutes. After that it is filtered to separate the residue from its filtrate. The filtrate from the filtrate is evaporated at a temperature of 70 ^{o}C to dry using a water bath. The remaining evaporation is dissolved in 10 ml methanol (Khoirunnisa et al., 2017).

Preparation of Reagents $FeCl_3$ 0.5% and potassium hexacyanorate(III) 0.5%. The 0.5% $FeCl_3$ reagent is made by dissolving 50 mg of $FeCl_3$ in 10 ml of distilled water. 0.5% potassium hexacyanoferrate (III) is made by dissolving as much as 50 mg of potassium hexacyanoferrate (III) in 10 ml of distilled water (Singh and Verma, 2008).

Color Test Execution. Six samples of herbs A, B, C, D, E, and F that have been extracted are inserted in test tubes of 2-3 ml. Then added 1 ml of H_2SO_4 4N and 1 ml of $FeCl_3$ 0.5% further

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shaken. Then 0.25 ml of 0.5% potassium hexacyanoferrate (III) is added and shaken again. A positive reaction is shown from the formation of a brownish color that slowly changes to green (Singh and Verma, 2008).

Preparation of Dexamethasone Comparison Raw Solution. BPFI dexamethasone solution 0.5% w/v is prepared by means of raw dexamethasone weighing 50 mg and put in a 10 ml measuring flask. Add methanol to the limit mark, then shake until homogeneous (Permatasari et al., 2021).

Manufacture of Mobile Phase and Stationary Phase. The mobile phase in the KLT method is made by mixing methanol, ethyl acetate, and toluene with a ratio of 1:45:55 and a total volume of 25 ml into a chromatographic vessel. The mobile phase is saturated using filter paper (Khoirunnisa et al., 2017). KLT plate measuring 10 x 10 cm with a propagation distance of 8 cm is activated using an oven at a temperature of 100-105 ^{o}C for 30 minutes.

KLT Testing. The KLT plate spotted by the stain is inserted into a chromatographic vessel containing a previously saturated mobile phase mixture. Wait until the mobile phase solution propagates on the KLT plate.

Spotting Detection and Rf Counting. Detection of stains on KLT plates is observed under UV light at a wavelength of 254 nm, then marked on the stains formed. The sample Rf value is calculated and compared with the standard standard Rf value of dexamethasone.

RESULTS AND DISCUSSION

Analysis of the dexamethasone compound content in packaged herbal medicine was carried out using two methods, namely a color test as a preliminary test and a test using the thin layer chromatography method. The test results of the two methods are shown in **Table 1** and **Figure 2**.

1. Preliminary Test of Dexamethasone

This study conducted preliminary tests to detect the presence of dexamethasone in six samples of packaged herbs using color tests using reagents H_2SO_4, $FeCl_3$ 0.5%, and potassium hexacyanoferrate (III) 0.5%. The results of this preliminary test are shown in **Table 1**.

Table 1. Dexamethasone Preliminary Test on Six Herbal Medicine Samples

Sample	Color Test
A	+
B	+
C	+
D	+
E	+
F	+

Table 1. for the preliminary test results showed that all samples of positive herbal medicine contained dexamethasone which was characterized by a change in the color of the reagent to bluish green.

Testing the presence of dexamethasone content in six samples of packaged herbs sold in the market showed positive results through preliminary tests. In preliminary tests using reagents H_2SO_4, $FeCl_3$ 0.5%, and potassium hexacyanoferrate (III) 0.5%, where positive action will be shown by a change in color to green to bluish. This color change occurs due to a reduction-oxidation reaction between corticosteroid compounds (dexamethasone) and $FeCl_3$. In this reaction, dexamethasone reduces $FeCl_3$ to iron (II) ions under acidic conditions. In this reaction iron (III) acts as an oxidizing agent that oxidizes two electrons in the side chain of dexamethasone ketol. The resulting iron (II) ion then reacts with potassium hexacyanophrate(III) to form a bluish-green iron (II) ferricyanide complex, according to the reaction mechanism in **Figure 1** (Singh and Verma, 2008).

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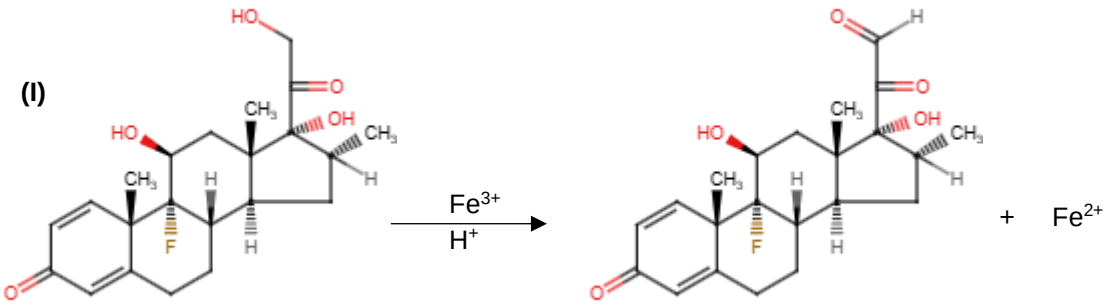

(II) $K_3Fe(CN)_6 + Fe^{2+} \rightarrow K[Fe(CN)_6] + 2K^+$

Figure 1. (I) Redox reaction of dexamethasone with $FeCl_3$. (II) Formation of a complex of bluish-green color

2. Dexamethasone Analysis Using Thin Layer Chromatography

The thin layer chromatography (KLT) test uses the BPFI dexamethasone standard of 0.5%. In this analysis method, the Rf value of the standard dexamethasone is 0.25 shown in **Figure 2**.

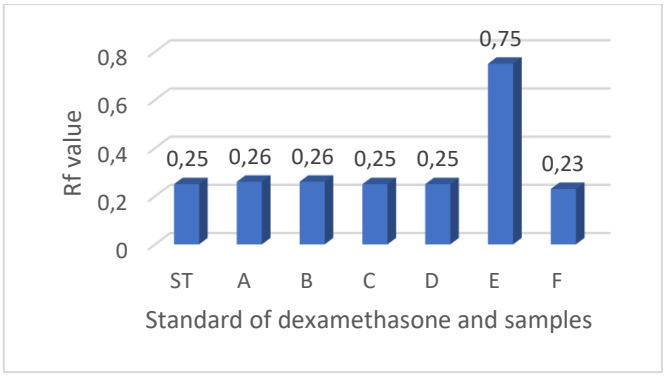

Figure 2. Rf Value Curve of Dexamethasone from Six Herbal Medicine Samples

This value will later be used as a comparison of the Rf value of the six samples tested. Five of the six samples tested showed Rf values that were the same as the standard and three of them only differed by 0.01.

Curves Figure 1. Shows that the KLT method is quite effective in detecting dexamethasone content in packaged herbal medicine samples. Methanol, Ethyl acetate, and toluene (1:45:55) can be used for dexamethasone content analysis as evidenced by the difference in Rf values of samples and standards that are not too far between 0 to 0.02. Of the six samples tested using KLT, five of them were positive for dexamethasone with a difference in the Rf value of samples with a standard of less than 0.2 (Samosir et al., 2018). The five samples that showed positive results were samples A, B, C, D and F with each Rf value difference repectivly 0.01; 0.01; 0.00; 0.00; and 0.02. While sample E showed negative results with a difference in Rf value of 0.5, because the difference was more than 0.2, then sample E did not contain dexamethasone compounds.

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CONCLUSION

Dexamethasone is a corticosteroid hormone synthesis compound that can increase the work activity of packaged herbs. This compound can be detected by color test in preliminary tests using reagents H_2SO_4, $FeCl_3$ 0.5%, and potassium hexacyanoferrate (III) 0.5%. Six packaging samples showed positive results, as evidenced by the change in the color of the reagent to bluish green. Dexamethasone was also analyzed using the KLT method using methanol, ethyl acetate eluent, and toluene (1:45:55). Five samples showed positive results seen from the difference in the Rf value of the sample with the standard in the range of 0.00 to 0.02. The five samples were samples A, B, C, D, and F. Only sample E showed negative results with a difference in Rf value of 0.5.

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