

DEVELOPMENT OF PAPER-BASED COLOR TEST-STRIP FOR PARACETAMOL DETECTION IN JAMU

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ABSTRACT

Chemical medicine were either synthetic chemicals or isolation products must not be added to herbal medicine, that was standared by Indonesian Head of the Drug and Food Control Agency in statement regarding Criteria and Procedure for Registration of Traditional Medicines, Standardized Herbal Medicines, and Phytopharmaka. This study aims to develope prototype of paper-based color test-strip to identify paracetamol trace in several types of Indonesian herbal medicine, more familiar as Jamu. Study conducted with make paper test-strip prototype by immobilizing reagents into Whatman's filter paper, then it used to identify paracetamol in extracted Jamu samples. The paracetamol qualitative test uses 10 Jamu samples that distributed in Samarinda area. were also carried out on. Comparison method with qualitative color reaction tests using specific reagents in the laboratory were also carried out beside the paper-based color test-strip method. In addition with positive control tests using 5000 ppm paracetamol solution to make sure both method used were valid. The results of this study showed that two prototype paper-based color test-strips containing 10% iron (III) chloride reagent and folin ciocalteu were able to detect the presence of paracetamol chemicals in Jamu samples with color reaction.

Keywords: Test-strip; Paper-based; Paracetamol, Jamu, Indonesian Herbal Medicine

INTRODUCTION

According to Indonesian Health Law No. 36 of 2009, the definition of traditional medicine is an ingredient or ingredients in the form of plant material, animal ingredients, mineral materials, extracted preparations, or mixtures of these materials which have been used for generations for treatment, and can be applied in accordance with norms applies in the community. Indonesian traditional medicine or native Indonesian medicine, better known as Jamu, is generally made from a mixture of herbal medicines, which are medicines derived from plants. Plant parts used can be in the form of roots, stems, leaves, tubers, or all parts of the plant.

Chemical medicine which are synthetic chemicals or isolation products must not be added to herbal medicine, that was standared by Indonesian Head of the Drug and Food Control Agency in statement number: HK.00.05.41.1384 regarding Criteria and Procedure for Registration of Traditional Medicines, Standardized Herbal Medicines, and Phytopharmaka. Indonesia National Agency of Drug and Food Control pers confrence on 2006 stated many aching rheumatic pain Jamu which defiled chemical medicines like phenylbutazone, methampyrone, diclofenac sodium, piroxicam, paracetamol, prednisone, or dexamethasone.¹ The chemical medicines identified as being mixed in so many Jamu products found

on 2015 was dominated by painkillers and antirheumatic agents, such as Paracetamol, Phenylbutazone and Dexamethazone.^{2,3} The results of the qualitative analysis on 2019 with TLC method were obtained

from two of the five aching rheumatic pain Jamu samples obtained from the Kibin industrial area of Serang Regency positively containing paracetamol.⁴

Acetaminophen or paracetamol (N-acetyl-paraaminophenol) is the most widely used antipyretic class of drugs worldwide and also one of the most common medicinal chemicals illegally founded on Jamu in Indonesia.^{5,3} Chemicals medicine were added by the producers of Jamu with the intent may be to increase the efficacy of Jamu and provide more instant effect, regardless the cumulative side effect from the medicine to human body if consumed continuously every day.⁶ Paracetamol can causes hepatotoxicity in high dose and in multiple daily doses.⁵ The indicator strip is one of the alternative tests for detecting a compound or diagnosing a parameter of a particular disease, which is made to facilitate the diagnosis and analysis of a compound more easily, since it does not require complicated equipment and the test time is not too long.⁷ The qualitative test of analgesic material on Jamu using a prototype test-strip is able to identify the chemical medicine paracetamol. According to the research that using a prototype test-strip containing the Nata De Coco-Al₂O₃ membrane is able to detect the chemical medicine paracetamol and mefenamic acid in stiff aches and bone flu.⁸

The present work proposes test-strip papers aiming at the detection or semi-quantitative determination of organic drugs by visual comparison of color changes, in a similar analytical procedure to that of pH monitoring by universal pH paper.⁹ Simple paper-based color test-strips can be made from the impregnation of 10% iron (III) chloride solution in Whatman's filter paper and can be used for qualitative analysis of paracetamol. This method is cheaper and does not need to use hazardous combine solvents as well as chloroform, acetone, methanol and amonia eluent that used in qualitative testing using the Thin Layer Chromatography method.¹⁰ This study is developing a prototype of paper-based color test-strip to detect chemical paracetamol in 10 Jamu samples in the Samarinda region.

MATERIAL AND METHOD

Material

The materials used in this study were Whatman's filter paper, stirring rods, glass cups, erlenmeyers, measuring cups, watch glass, magnetic stirrers, glass funnels, porcelain cups, test tubes, analytical balance sheets, volume pipettes, oven, iron (III) chloride 10%, folin ciocalteu, concentrated nitric acid, paracetamol, aquadest, ethanol, and 10 Jamu samples.

General Procedure

Making a Prototype Test-Strip

Whatman filter paper number 1 is prepared with a size of 1 x 5 cm² and then each filter paper is dropped with a reagent of iron (III) chloride 10% and folin ciocalteu. Filter paper is allowed to stand until it produces clear colors from each reagent. For iron (III) 10% chloride reagents they are yellow and folin ciocalteu reagents are light green. A clear color change on filter paper indicates the reagent particles are optimally bound or absorbed into the filter paper.

Jamu's Sample Preparation and Extraction

In this study the samples used were gout Jamu, aching rheumatic pains Jamu, bones flu Jamu, and toothache Jamu that were collected from several herbal stalls in the Samarinda region. The

sampling technique used in this study is purposive sampling, taken herbs which are often consumed by people in the region.

Samples of Jamu weighed approximately 2 grams put into a beaker. Samples were extracted by maceration using 100 mL of ethanol 95% solvent, then shaken out using magnetic stirrer for 30 minutes, the liquid extract from the herbal medicine sample was filtered then collected in a beaker.

Preparation of Paracetamol Comparative Solutions

Paracetamol was weighed as much as 50 mg and then dissolved with 95% ethanol as much as 10 ml in a beaker, stirred until dissolved.

Detection Method

1) Qualitative Color Reaction Test

a) 10% Iron (III) Chloride Reaction

The extracted Jamu sample was taken as much as 1 ml and then put in a test tube. The sample was dripped with 2 drops of iron (III) chloride solution of 2 drops, a greenish-brown color was formed indicating paracetamol.

b) Folin Ciocalteu's Reaction

The extracted herbal sample was taken as much as 1 ml and then put in a test tube. Samples were added 2 drops of folin ciocalteu reagent, formed a light green color and formed a white precipitate indicating paracetamol.

2) Qualitative Test with using the prototype of paper-based color test-strip

Filter paper that has been impregnated with the reagent is dropped with a sample of the herbal extract that has been previously extracted. The prototype test-strip which contains a yellow iron (III) chloride reagent of 10% will turn grayish green and the prototype test-strip which contains a light green focal ciocalteu reagent will turn to dark green. After that a positive control test was done with a comparison solution of paracetamol and a negative control using aquadest on each prototype test-strip.

Analytical Discussion

The analysis used is descriptive in the form of qualitative data based on the results of research at the Samarinda College of Health Sciences Laboratory. The data that has been obtained from the results of research conducted then analyzed and presented in tabular form.

RESULTS AND DISCUSSION

Making a Prototype Test-Strip

The test-strip was made from Whatman filter paper number 1 with a size of 1 x 5 cm² which was impregnated with iron (III) chloride reagents and folin ciocalteu. While test-strips

with nitric acid reagents were not effective because they can not dry at room temperature or by heating using an oven, so the test-strips are used only two reagents, first with 10% iron (III) chloride reagents and second test-strips with folin ciocalteu reagents.

Test-strips are made using immobilization techniques, which include reagents in a material or device. Test-strips made by immobilizing specific reagents such as iron (III) chloride and folin ciocalteu have the ability to identify and distinguish specifically the presence of paracetamol. Use of the term immobilization because the reagents used are reagents that are not moving actively.

Test-strips that have been dropped with reagents are then dried. The test-strip with the reagent of Iron (III) Chloride is dried at room temperature, while the test-strip with the Folin Ciocalteu reagent cannot be dried at room temperature so it is warmed up using an oven at 70°C for 30 minutes.

Feasibility Test of Prototype Test-Strip

The feasibility test of prototype test-strip test begins by dripping with a standard solution of paracetamol with a concentration of 5 mg / ml on the test-strip and visual changes are observed. The solvent used to dissolve paracetamol is 95% ethanol because paracetamol is soluble in 7 parts of 95% ethanol.¹¹ Paracetamol in ethanol solvent has a faster in reaction than paracetamol in aquadest solvent.¹²

Test-strips with iron (III) chloride reagents which were initially yellow will turn greenish brown after drops with paracetamol solution and test-strips with folin ciocalteu reagents which were initially light green will turn dark green after drops with paracetamol solution.

Testing Herbal Samples

The process of detecting with the test-strip method can be happen because of the reaction between the sample and the reagent that was immobilized.¹³ The advantage of a test-strip compared to other detection devices is that it provides a fast response of about 60 to 120 seconds after being reacted with a sample.¹⁴

Testing to determine the presence or absence of the content of paracetamol in herbal medicine samples was carried out with a qualitative color reaction test and using a prototype test-strip. Jamu samples with codes A, B, C, D, E, F, G, H, I, and J. were made by maceration using ethanol 95% solvent and shaken out with magnetic stirrer for 30 minutes. The purpose of extraction is to pull out the active substance and separate it from the herbal compilation mixture. The active substance to be extracted in this extraction is paracetamol because it has a high solubility with ethanol.¹¹

Based on the results of the study obtained the following observations:

Table 1. Observation and Qualitative Results with Chloride Iron (III) Reagents

Sample	Qualitative Color Reaction Test with Iron (III) Chloride Reagents		Qualitative Test with Prototype Test-Strip Impregnated with Iron (III) Chloride Reagents	
	Observation Results	Qualitative Results	Observation Results	Qualitative Results
A	Dark brown	(-)	Light yellow	(-)
B	Brown	(-)	Light yellow	(-)
C	Dark brown	(-)	Light yellow	(-)
D	Greenish brown	(+)	Greenish brown	(+)
E	Dark brown	(-)	Light yellow	(-)
F	Greenish brown	(+)	Greenish brown	(+)
G	Dark brown	(-)	Yellow	(-)
H	Brown	(-)	Yellow	(-)
I	Greenish brown	(+)	Greenish brown	(+)
J	Dark brown	(-)	Yellow	(-)
Positive Control (Paracetamol)	Greenish brown	(+)	Greenish brown	(+)

Table 1's Information :

1. Qualitative Color Reaction Test

(+) If the observation results are visually greenish brown.

(-) If the observations are visually brown or dark brown.

2. Qualitative Test with Prototype Test-Strip

(+) If the observation results are visually greenish brown.

(-) If the visual results are visually yellow or bright yellow.



Figure 1 . Observation after Prototype Test-Strip Impregnated with Iron (III) Chloride Reagents dropped with Extracted Sample show visually greenish brown if paracetamol presence



Figure 2 . Observation after Prototype Test-Strip Impregnated with Iron (III) Chloride Reagents dropped with Extracted Sample show visually yellow or bright yellow if paracetamol absence

Table 2. Observation and Qualitative Results with Folin Ciocalteu Reagents

Sample	Qualitative Color Reaction Test with Folin Ciocalteu Reagents		Qualitative Test with Prototype Test-Strip Impregnated with Folin Ciocalteu Reagents	
	Observation Results	Qualitative Results	Observation Results	Qualitative Results
A	Turbid yellow	(-)	Light green	(-)
B	Turbid yellow	(-)	Light green	(-)
C	Greenish yellow	(-)	Light green	(-)
D	Light green and sediment formed	(+)	Dark green	(+)
E	Turbid yellow	(-)	Yellowish green	(-)
F	Light green and precipitate formed	(+)	Dark green	(+)
G	Turbid yellow	(-)	Light green	(-)
H	Pale Orange	(-)	Light green	(-)
I	Greenish yellow and sediment formed	(+)	Dark green	(+)
J	Pale yellow	(-)	Yellowish green	(-)
Positive Control (Paracetamol)	Light green and precipitate formed	(+)	Dark green	(+)

Table 2's Information :

1. Qualitative Test Color Reaction

- (+) If the observation results are visually light green and a precipitate is formed.
- (-) If the visual results are visibly turbid yellow, greenish yellow, pale yellow, or pale orange.

2. Qualitative Test Prototype Test-Strip

- (+) If the observation results are visually dark green.
- (-) If the observations are visually light green or yellowish green.



Figure 3 . Observation after Prototype Test-Strip Impregnated with Folin Ciocalteu Reagents dropped with Extracted Sample show visually dark green if paracetamol presence

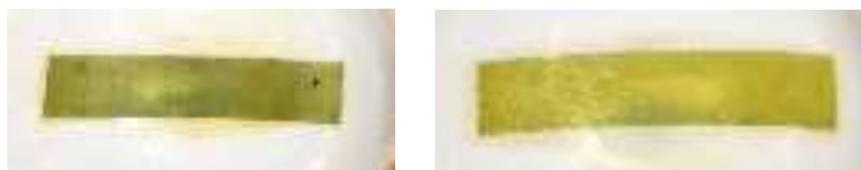


Figure 4 . Observation after Prototype Test-Strip Impregnated with Folin Ciocalteu Reagents dropped with Extracted Sample show visually light green and yellowish green if paracetamol absence

The level of paracetamol used as a positive control was 5000 ppm of 95% ethanol solvent. Based on the results of Dirgantara's research, the detection limit of the test strip prototype was good enough for paracetamol in the range of 125 - 5000 ppm, so in this study paracetamol levels were used as much as 5 ppm.⁸ The solvent used in this research was 95% ethanol because the solubility of paracetamol was higher than the solubility with water solvent. Research conducted by Nugraha shows that the color produced is more intense when using 95% ethanol solvent because the concentration in ethanol solution is higher and reacts faster with reagents.¹²

The color formed in the qualitative test of the color reaction with a 10% iron (III) chloride reagent when dropped with a standard solution of paracetamol is greenish brown. The same color was shown by 3 of the 10 samples studied, namely samples D, F, and I when dropped with 10% iron (III) chloride reagent, so it can be concluded that all three samples contained the chemical drug paracetamol. Samples that do not contain paracetamol produce colors that do not match the colors produced by the standard solution of paracetamol which is dark brown or brown.

The same treatment was carried out using the folin ciocalteu reagent. The color formed in the qualitative test of the color reaction with the folin ciocalteu reagent when drops of the paracetamol standard solution is light green with white precipitate. The same color was shown by 3 of the 10 samples studied, namely samples D, F, and I when dropped with folin ciocalteu reagents, so it can be concluded that all three samples contained the chemical drug paracetamol. Samples that did not contain paracetamol produced colors that did not match the colors produced by the standard solution of paracetamol, namely turbid yellow, greenish yellow, pale orange, and greenish yellow with white precipitate.

Testing of the sample was continued using a test strip that had been made using a 10% iron (III) chloride reagent and folin ciocalteu. Test strips containing 10% iron (III) chloride reagents initially yellow in color will turn greenish brown if dropped with a standard solution of

paracetamol. Test strips containing folin ciocalteu reagents which were initially light green will turn dark green if dropped with a standard solution of paracetamol. The same color was shown by 3 out of 10 samples namely samples D, F, and I when dropped into a test strip containing 10% iron (III) chloride reagents and folin ciocalteu. The observations made using the test strip are the same as the qualitative test of the color reaction. This shows that the test strips made are able to identify the presence of paracetamol contained in herbal samples that have been tested with qualitative color reaction tests. The minimum detection limit of the test strips made is unknown, so the paracetamol contained in the herbal sample is not known how large the levels are. However, the more concentrated color produced by the test strip when testing shows that the higher levels of paracetamol contained.

The success of this immobilization can be seen from membran changing color after additional reagent and there is no leeching after additional sample. The advantage of using this test strip is that it makes it easier to analyze medicinal chemicals in herbal medicine, so that if research is conducted in remote areas far from a laboratory it can be done using a prototype test

strip.¹⁵ The material used is also very economical and easy to obtain, namely by using whatman filter paper and not using a membrane like the Nata De Coco- Al_2O_3 membrane in making a prototype test-strip. Short duration of time in produce and simple preparation is also one of the advantages of this prototype test strip because it does not require days. A test strip containing 10% iron (III) chloride reagent only requires 3 hours of drying time at room temperature in its manufacture while a test strip containing a folin ciocalteu reagent only takes 30 minutes with a heating temperature of 70°C.

CONCLUSION

Based on the results of research conducted, it can be concluded that the prototype test-strip with 10% iron (III) chloride reagent and folin ciocalteu was able to detect the presence of paracetamol drug chemicals in Jamu samples.

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