The Journal Of Muhammadiyah Medical Laboratory Technologist
No.6 No.1, Mei 2023
P-ISSN : 2597-3681
E-ISSN : 2614-2805

The Potential of *Averrhoa bilimbi* Juice As An Alternative Reagent in Proteinuria Examination

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Tanggal Submit:
31 Oktober 2021

Tanggal Review:
7 November 2022

Tanggal Publish Online:
10 Mei 2023

ABSTRACT

Proteinuria indicates a kidney disorder and provides important prognostic information in diagnosing kidney disease. To determine the presence of protein in the urine, a urinalysis examination is carried out. Proteinuria examination generally uses the heating method with acetic acid, sulfosalicylic acid, and concentrated nitric acid as reagents. In Indonesia, the use of acetic acid for proteinuria examination is still the primary choice. An alternative reagent is needed that can replace the function of acetic acid. The objective of this study is to determine the potential of Averrhoa Blimbi juice as an alternative reagent for proteinuria examination.

This was a Pre-experimental study with a completely randomized design. This study used urine samples from a patient with chronic kidney disease. Urine samples were examined using 6% acetic acid reagent as a control and using Averrhoa bilimbi juice with various concentrations. Each juice concentration was examined twice so that the experimental units in this study were 40 units. The Kruskal-Wallis test was used to determine the potential of Averrhoa bilimbi as an alternative reagent.

At concentrations of 5%-20% showed the same positive results as 6% acetic acid, namely +2, concentration 25%-35% resulted in +3, and other concentration resulted in +4. The results of data analysis showed p=0.04 (p<0.05). This shows that Averrhoa bilimbi has the potential as an alternative reagent for proteinuria examination.

Averrhoa bilimbi juice with a 5%-20% concentration has the potential as an alternative reagent for 6% acetic acid to check proteinuria levels.

Keywords : *Averrhoa bilimbi ; alternative reagents; proteinuria; acetic acid; urinalysis*

INTRODUCTION

Urine as a metabolic product contains various substances, including nitrogen, urea, and ammonia. The urine content indicates different physiological functions in the body related to metabolism and excretion, including the kidneys, liver, and pancreas. The presence of substances that are still

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useful for the body in the urine indicates an error in kidney function. Protein is one of the substances still beneficial for the body, often found in urine (proteinuria) (Barrett et al., 2010; Rayner et al., n.d.; Shier et al., 2012). Proteinuria indicates a kidney disorder and provides important prognostic information in diagnosing kidney disease (Jumaydha et al., 2016; Lamb et al., 2009; Leung et al., 2017). To determine the presence of protein in the urine, a urinalysis examination is carried out. Proteinuria examination generally uses the heating method with acetic acid, sulfosalicylic acid, and concentrated nitric acid as reagents. In Indonesia, the use of acetic acid for proteinuria examination is still the primary choice.

Indonesia's vast territory and in the form of an archipelago, resulting in an uneven distribution of laboratory tools and materials. The unavailability of proteinuria test reagents in remote areas can result in delayed diagnosis of several diseases that require rapid results of proteinuria examination, for example, in patients with eclampsia or preeclampsia.

Therefore, an alternative reagent is needed that can replace the function of acetic acid. Alternative reagents used must be easy to find and derived from natural ingredients. Several previous studies used lime as a reagent for proteinuria examination. Lime contains citric acid, which can clump protein in the urine. In addition to lime, another natural ingredient that can be an alternative to chemical reagents in proteinuria analysis is *Averrhoa bilimbi*. *Averrhoa bilimbi* juice is acidic with a pH of 0.9-1,5 (Lim, 2012; Lima et al). This study wanted to determine the potential for the best acid content and concentration in *Averrhoa bilimbi* juice to be used as an alternative reagent for proteinuria examination.

METHODS

Participants and sample size

This study was carried out in February 2020 at the Biochemistry Laboratory of the Health Analyst Department of the Mataram Health Polytechnic.

This study used a urine sample from a patient with chronic kidney disease. Urine samples were examined using 6% acetic acid reagent as a control and using *Averrhoa bilimbi* juice with concentrations of 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, and 5%. Each juice concentration was examined twice so that the experimental units in this study were 40 units.

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Design and procedure

This was a pre-experimental study with a completely randomized design. The procedures of this study include:

a. *Averrhoa bilimbi* juice is made into 20 different concentrations, including concentrations of 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, and 5%.
b. Urine samples were first examined using a 6% acetic acid reagent to determine the level of proteinuria positivity.
c. Urine samples were divided into 20 groups, and each group was examined using one concentration of *Averrhoa bilimbi* juice for two times examination.
d. Examination of urine samples with *Averrhoa bilimbi* juice by mixing 5 ml of urine with three drops of juice solution in a test tube. The tube was then heated using a water bath at 100ºC for 5 minutes. The protein deposits/clumps formed were assessed.

Data analysis

Data on the potential of *Averrhoa bilimbi* as an alternative reagent was analyzed bivariate. The Kruskal-Wallis test was used to determine the effect or potential of *Averrhoa bilimbi* as an alternative reagent. This research has $\alpha <$ 0.05, 95% CI, and 80% power of a study.

RESULT

This study used urine samples from a patient with chronic kidney disease. On the first examination using 6% acetic acid reagent as a control, the result was proteinuria +2. Furthermore, an examination was carried out using *Averrhoa bilimbi* juice using 20 different concentrations, and each concentration was repeated two times. The results can be seen in table 1.

Table 1. Proteinuria Examination Results Using Several Concentrations *Averrhoa bilimbi*

Averrhoa bilimbi Concentrations	Proteinuria Positivity	
	1st Replication	2nd Replication
5%	+2	+2
10%	+2	+2
15%	+2	+2
20%	+2	+2
25%	+3	+3
30%	+3	+3
35%	+3	+3
40%	+4	+4
45%	+4	+4
50%	+4	+4
55%	+4	+4
60%	+4	+4
65%	+4	+4
70%	+4	+4
75%	+4	+4
80%	+4	+4
85%	+4	+4
90%	+4	+4
95%	+4	+4
100%	+4	+4

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Based on table 1, it can be seen that the urine samples examined using Averrhoa bilimbi juice with concentrations of 5%, 10%, 15%, and 20% had the same positivity value (+2) with 6% acetic acid. Statistical test to determine the potential of Averrhoa bilimbi as an alternative reagent for proteinuria examination was carried out using the Kruskal-Wallis test. Based on the results of this statistical analysis, it is known that $p = 0.004$ ($p < 0.05$), which indicates that Averrhoa bilimbi has the potential as an alternative reagent for proteinuria examination.

DISCUSSION

Averrhoa bilimbi is a medicinal plant that belongs to the *Oxalidaceae* family. This plant belongs to the genus *Averrhoa* and is named after the Arab philosopher, doctor, and Islamic jurist Ibn Rushd, often known as Averroes. *Averrhoa bilimbi* is a plant used in traditional medicine to treat various diseases and maintain one's health. Multiple studies have been carried out over the years to prove the scientific basis for using the leaves and fruits of *Averrhoa bilimbi* as a therapy in the treatment of various diseases (Alhassan and Ahmed, 2016; Mokhtar and Abd Aziz, 2016; Saini, 2016; Setyawan et al., 2021; Valsan and Raphael, 2016). Apart from being used as a treatment, *Averrhoa bilimbi* is also used as a cooking spice or vegetable, cleaning clothes stains, giving a shine, brass goods, food preservatives, and various other functions (Lim, 2012).

This study was conducted to determine the potential of *Averrhoa bilimbi* juice as an alternative reagent for proteinuria examination. *Averrhoa bilimbi* contains aliphatic acid (acetic acid and citric acid) of 47.8% (Lim, 2012). The content of *Averrhoa bilimbi* can be used as an alternative reagent for proteinuria examination. Study related to the potential of *Averrhoa bilimbi* as a reagent for proteinuria examination has not been widely carried out.

Before conducting proteinuria examination using several concentrations of *Averrhoa bilimbi* juice, proteinuria was first examined using a 6% acetic acid reagent as an initial observation/control. The aim was to determine the true positivity of urine protein. After that, proteinuria examination was carried out using several concentrations of *Averrhoa bilimbi* juice, where the concentrations used were 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, and 5%. Each concentration was examined two times.

The proteinuria examination results using *Averrhoa bilimbi* juice with

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several concentrations increased proteinuria positivity. The higher the concentration of *Averrhoa bilimbi* juice given to the urine sample, the higher the positivity of the proteinuria produced. Previously, urine samples that were examined using 6% acetic acid reagent as initial observations showed the results of proteinuria +2. While the urine samples examined using several concentrations of Averrhoa bilimbi resulted in an increase in protein positivity, this can be seen at concentrations of 20%, 15%, 10%, 5% yielded +2, while at concentrations of 35%, 30 %, 25% resulted in +3, then at concentrations of 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% resulted +4.

Proteinuria examination using *Averrhoa bilimbi* juice with concentrations of 20%, 15%, 10%, 5% resulted in +2, where the results of protein positivity were the same as those using 6% acetic acid reagent. It's because the concentration of 20%, 15%, 10%, 5% has the same level of acidity with 6% acetic acid reagent (pH = 2,3). While at concentrations of 35%, 30%, 25%, it resulted in a +3, where the results were different from the previous concentration because they had a higher acidity level (pH = 2,1). Then at concentrations of 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% resulted in a +4, where the results were different from the concentration previously, because it has a higher acidity level (pH = 2.0).

The presence of protein clumps indicates positive results, and the precipitate formed can be caused by coagulation and protein denaturation. One of the properties of protein is that it can undergo denaturation. Denaturation can change the nature of the protein to be difficult to dissolve in water. This clumping can be caused by heating as well as the addition of acid. Further heating and the addition of this acid will cause denaturation and damage to the protein structure so that the protein will precipitate. Protein solubility will increase if given an excess of alkaline treatment. It's happening because the base's positive ions cause the initially neutral or zero protein to become positively charged, which increases its solubility. The further the difference in pH from the isoelectric point, the more the solubility will increase. The isoelectric point is when the acid pH is in an amphoteric form (zwitterion). At this isoelectric point, the protein solubility decreases and reaches the lowest number. The protein will precipitate and agglomerate (Nelson and Cox, 2017).

Further research is needed with better research methods to determine the

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possibility of Averrhoa bilimbi juice as a substitute for acetic acid reagent in proteinuria examination.

CONCLUSION

Averrhoa bilimbi juice with a 5% - 20% concentration has the potential as an alternative reagent for 6% acetic acid to check proteinuria levels.

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