

The Effect of Chives (*Allium schoenoprasum* L.) Leaves Ethyl Acetate and Hexane Fractions on Human Calcium Kidney Stones Solubility by In Vitro Method

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Abstract: The aim of this research was to find out whether the ethyl acetate and hexane fractions had an effect to dissolve the level of calcium in human kidney stones. The solubility activity of chives leaves ethyl acetate and hexane fraction were carried out by using the in vitro method which was to test the solubility of the calcium components of human kidney stones in various concentrations (1%, 2.5% and 5%). The fractions were added by human kidney stones and incubated on 37°C for four hours. The amount of calcium solubility was measured by using atomic absorption spectrophotometer at 422.7 nm. The results showed that ethyl acetate fraction had a dissolving effect of calcium on the human kidney stone which are respectively 18.98 µg/mL, 32.84 µg/mL and 26.22 µg/mL better than hexane fraction which are respectively 2.02 µg/mL, 2.45 µg/mL and 3.03 µg/mL. In these results, the concentration of 2.5% of ethyl acetate fraction showed the highest dissolving level which is 91.99%. Based on the research, it can be concluded that ethyl acetate fraction of chives leaves is a new potential for herbal treatment of kidney stones.

1 INTRODUCTION

Kidney stones are one of the most common diseases in the kidneys. Generally, kidney stones occur because the body lacks fluids resulting in blockages in the channels from the kidneys to the bladder. The stones in the kidney are mainly formed from chemicals that are usually found in urine such as calcium, uric acid, phosphate, cystine and others chemicals compound. Different types of treatments are applied for kidney diseases such as medicine, changing of life style, treatment by surgery etc. (AUA, 2014; Mugni, 2013).

According to Al-Snafi (2013) and Iksen (2015), chives are believed to be a multifunctional medicine for various diseases. Chives contain various phytochemical compounds including alkaloids, flavonoids, glycosides, steroids, tannins and various minerals such as potassium, calcium, magnesium and sodium. Previous research showed that infuse water of chives has a calcium oxalate solubility [5]. However, until now there is no scientific proof of human calcium kidney stone of ethyl acetate and hexane fractions of chives leaves. The purpose of

this study is to study the effect of chives leaves ethyl acetate and hexane fractions on human calcium kidney stones solubility.

2 MATERIALS AND METHODS

2.1 Materials

Distilled water, human calcium kidney stones, fresh chives leaves, nitric acid 65% (Merck), ethanol solvent (Merck), ethyl acetate solvent (Merck), hexane solvent (Merck) and standard solution of calcium 1000 ppm.

2.2 Plant Material Preparation

Fresh chives leaves was collected from local area of Simalungun distric (North Sumatera, Indonesia) and authenticated by Herbarium Medanese (MEDA) Universitas Sumatera Utara. Voucher specimen was collected and deposited in Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara.

2.3 Preparation of Fraction

Extraction was done by maceration method using ethanol solvent. 200 g of powdered chives leaves were *marcerated* in 1 L ethanol solvent 24 hours, then filtered, do it continuously until the filtrate obtained is clear and colorless. 10 g concentrated ethanolic extract, then fractioned using ethyl acetate and hexane solvents to get the ethyl acetate and hexane fractions (Ditjen POM, 1995; Depkes RI 1995).

2.4 Experimental Design

In this research, samples were divided into 6 groups, which were namely EA1 (1% ethyl acetate fraction), EA2 (2.5% ethyl acetate fraction), EA3 (5% ethyl acetate fraction), H1 (1% hexane fraction), H2 (2.5% hexane fraction), and H3 (5% hexane fraction). All of these groups were added by human calcium kidney stone and incubated at 37°C for four hours. Calcium level was measured before and after incubation by using atomic absorption spectrophotometry method at 422.7 nm wavelength.

2.5 Calcium Calibration Curve

Calcium calibration curve was prepared by using 6 different concentration which were 0 ppm, 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1,0 ppm. All of these concentrations will be measured by using atomic absorption spectrophotometry method at 422.7 nm wavelength.

2.6 Calcium Determination in Sample

Before measurement of calcium level, the organic compounds should be destructed by using nitric acid 65% which heated on a hot plate until the sample solutin become transpicious. After that, the sample solution measured using air-acetylene flame at 422.7 nm wavelength.

3 RESULTS AND DISCUSSIONS

3.1 Calcium Calibration Curve

Calcium calibration curve is obtained by measuring the absorbance of the standard solution at a wavelength of 422.7 nm for calcium. Calibration curve of calcium standard solution is as shown in the figure below (Fig. 1). From the calculation results obtained the correlation coefficient (r) of 0.9997 and the regression line equation $Y = 0.101443X +$

0.000795. These results indicate that there is a linear relationship between concentration and absorbance, which is indicated by the value of the correlation coefficient that is close to 1. The value of the correlation coefficient obtained meets the predetermined requirements, namely the value of the correlation coefficient of not less than 0.995.

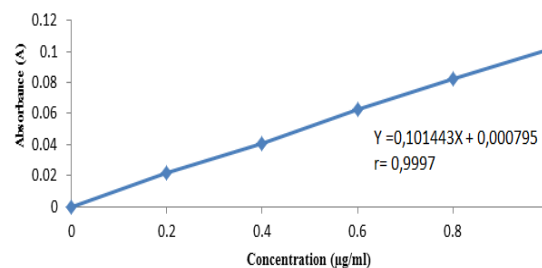


Figure 1: Calibration curve of calcium

3.2 The Effect of Chives Leaves Ethyl Acetate and Hexane Fractions on Solubility of Human Calcium Kidney Stones

The level of of human calcium kidney stone solubility is presented in Table 1. Based on the results, it can be seen that ethyl acetate fraction within dose 2.5% gave the highest activity in dissolving the kidney stone compared to other fraction. This can happen due to the potassium which could happen because potassium is able to push the calcium bond in kidney stone and can be remove through urine (Putra et al., 2018; Iksen, 2015).

Other possibility is because of the phytochemical compound especially flavonoid compounds which are the main compound in ethyl acetate fraction. The mechanism by flavonoid maybe caused by its improving the dissolving effect by making a complex bond with the calcium from kidney stone. Calcium complex bond with flavonoid will be free and can be remove through urine (Haro et al., 2017; Sinaga et al., 2018).

4 CONCLUSIONS

Chives leaves ethyl acetate fraction solution with 2.5% concentration gives the highest activity in dissolving calcium kidney stone. This can happen due to the potassium and flavonoids compound from chives leaves. It is hoped that this study could be an alternative for the treatment of kidney stones disease.

Table 1: The Effect of Chives Leaves Ethyl Acetate and Hexane Fractions on Solubility of Human Calcium Kidney Stones

No	Treatments	Calcium Level (µg/mL)		Solubility	
		Before Incubation	After Incubation	(µg/mL)	(%)
1	EA1 (1%)	1.81	20.79	18.98	91.30
2	EA2 (2.5%)	2.85	35.70	32.84	91.99
3	EA3 (5%)	3.74	29.97	26.22	87.49
4	H1 (1%)	11.78	13.80	2.02	14.63
5	H2 (2.5%)	13.71	16.17	2.45	15.15
6	H3 (5%)	20.88	23.91	3.03	12.67

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