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Indonesian Journal of Pharmaceutical and Clinical Research

In Vitro Test of Anticalculi Effect from Ethyl Acetate Fraction of Chives Leaf (*Allium schoenoprasum* L.)

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Abstract. The purpose of this study was to analyze the ability of chives leaves of ethyl acetate fraction to dissolve calcium in human kidney stones. This research starts from making the ethyl acetate fraction, calibration curve for calcium and measuring dissolved calcium levels in kidney stones by using atomic absorption spectrophotometry. The results showed that the ethyl acetate fraction with a concentration of 2.5% had the greatest anti-calculi capability of 92.02%. The conclusion of this study is that the ethyl acetate fraction of chives leaf has the potential to be anticalculi in the treatment of kidney stones.

Keyword: Chives, Atomic absorption spectrophotometry, Anticalculi, In vitro

Abstrak. *Tujuan dari penelitian ini adalah untuk menganalisis kemampuan fraksi etil asetat daun kucai untuk melarutkan kalsium pada batu ginjal manusia. Penelitian ini dimulai dari pembuatan fraksi etil asetat, pembuatan kurva kalibrasi kalsium serta pengukuran kadar kalsium terlarut pada batu ginjal dengan menggunakan spektrofotometri serapan atom. Hasil penelitian menunjukkan bahwa fraksi etil asetat dengan konsentrasi 2,5 % memiliki kemampuan antikalkuli yang terbesar yakni 92,02%. Kesimpulan penelitian ini adalah bahwa fraksi etil asetat daun kucai berpotensi sebagai antikalkuli pada pengobatan batu ginjal.*

Kata Kunci: *Kucai, Spektrofotometri serapan atom, Antikalkuli, In vitro*

Received 30 November 2019 | Revised 16 December 2019 | Accepted 19 December 2019

1. Introduction

Kidney stones are one of the most common diseases in the kidney. Generally, kidney stones occur because the body lacks fluids so that there is a blockage in the channel from the kidneys to the bladder. The stones in the kidneys are formed from chemicals that are usually present in urine such as calcium, uric acid, phosphate and other chemicals [1], [2]. One of the medicinal plants that are widely used people in Indonesia is Chives (*Allium schoenoprasum* L.). Chives many health benefits such as overcome vaginal discharge, constipation and and antimicrobial. In addition, chives are also efficacious improving blood flow and prevent blood clots. Chives

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leaves contain various phytochemical compounds including alkaloids, flavonoids, glycosides, steroids, tannins and various minerals such as potassium, calcium, magnesium and sodium [3]. Previous studies have shown that infuse and extracts (ethanol and ethyl acetate) of chives leaves have anticalculi activity [4], [5], [6], [7]. Therefore, this study aims to examine the continuation of anticalculi effect using the ethyl acetate fraction of chives leaf to help the treatment of kidney stones by using traditional medicine.

2. Materials and Methods

2.1 Materials

Materials used in this research were chives (*Allium schoenoprasum* L.) leaves (obtained from Pasar Baru, Perbaugan regency, North Sumatera, Indonesia), human kidney stones (obtained from Permata Bunda Hospital, Medan), nitirc acid 65% (Merck), demineralizd water (Brataco), standard solution of calcium (BOECO), ethanol (Merck), and ethyl acetate (Merck). The instrument used were Atomic absorption spectrophotometry (Hitachi Zeeman-2000) with air-acytylene gas and cathode lamp of calcium. Chives leaf was identified at Herbarium Medanense (Universitas Sumatera Utara Number 928/MEDA/2017).

2.2 Preparation of Ethyl Acetate Fraction of Chives Leaves

40 g of chives leaf ethanol extract was fractioned using ethyl acetate solvent, homogenized and partitioned by using separatory funnel. Then the ethyl acetate fraction were concentrated with a rotary evaporator at 50^{o}C [8].

2.3 Research Design

In vitro anticalculi effect of the ethyl acetate fraction of chives leaves divided into 6 groups 1% without and with incubation with human kidney stones, 2.5% without and with incubation with human kidney stones, 5% without and with incubation with human kidney stones. All of these groups (50 ml for each group) will be added with 50 mg of human kidney stones and then incubated at 37^{o}C for 4 hours.

2.4 Quantitative Analysis by Using Atomic Absorption Spectrophotometry

Before the quantitative analysis, all the sample preparation were added by 10 mL of nitric acid 65% and heat on hotplate until the solution become tranpicious. After this, the calcium level from each groups were measured by using atomic absroption spectrophotometry. The absorbance value of calcium should be within the range of standard calcium calibration curve (0.2; 0,4; 0.6; 0.8 and 1 ppm) at 422.7 nm wavelenght [4], [9]. Calcium values were expressed as mean ± SD with six replicates measurements.

3. Results and Discussions

3.1 Calibration Curve of Calcium

Calcium calibration curves were obtained by measuring the absrobance of standar solutions at wavelenght of 422.7 nm for calcium. From the calibration curve measurements (Figure 1), the regression Y=0.101443X+0.000795 was obtained. The calcium correlation cofficient obtained could be accepted according to Harmita (2004) and Putra *et al* (2018) [10], [11], [12].

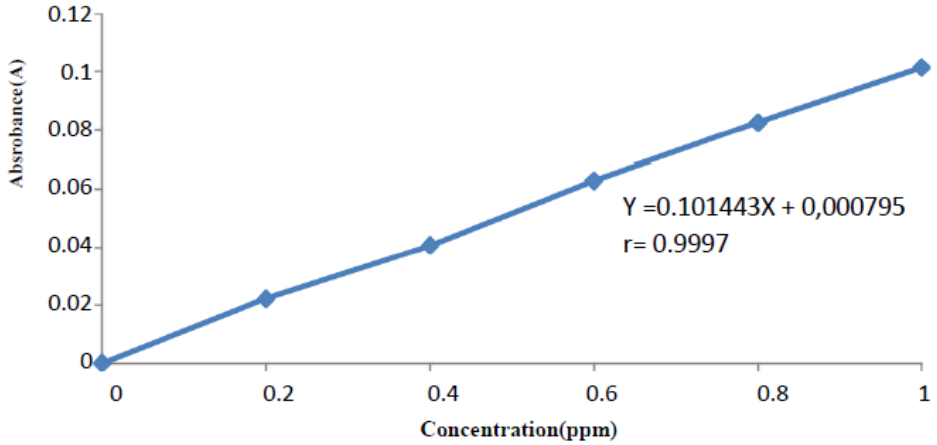

Figure 1. Calibration curve of calcium

3.2 In Vitro Anticalculi Effect

In vitro anticalculi testing was using chives leaves ethyl acetate fraction was aimed to determine the solubility of calcium in human kidney stones. The level of calcium solubility in fraction solution is presented in Table 1. Based on Table 1 it can be seen that the higher the concentration of ethyl acetate fraction, the calcium concentration will increase as well. The highest percentage of calcium solubility is at concentration of 2.5 % while at the concentration of 5% there is a decrease in the calcium solubility of kidney stones.

Table 1. In vitro anticalculi result

Concentration	Calcium Level Before Incubation (ppm)	Calcium Level After Incubation (ppm)	Level of Dissolved Calcium (ppm)	Percentage of Calcium Solubility (%)
1%	1.8176±0.0351	20.7120±0.0351	18.89	91.22
2.5%	2.8510±0.0239	35.7204±0.0351	32.87	92.02
5%	3.7448±0.0464	29.9125±0.0351	26.17	87.48

The ability to dissolve kidney stone calcium is caused by the extracts which have important roles in calcium solubility in kidney stones such as flavonoids, potassium compounds and the acidity (pH) of the extract. The formation of calcium in kidney stones is inhibited by flavonoids, potassium, alkaloids and citric acid. Flavonoid compounds contained in the extract are important factors in the calcium solubility of kidney stones. This is because the carboxylic

hydroxyl groups of flavonoid compounds react with calcium in kidney stones to form Ca-flavonoid chelate complexes that are easily soluble in water [13].

4. Conclusion

Chives leaves ethyl acetate fraction solution showing a potential anticalculi effect. The results of this study are in line with previous studies in which infuse and extract had anticalculi activity.

Acknowledgement

The authors are gratefully thank to Faculty of Pharmacy Universitas Sumatera Utara and Sekolah Tinggi Ilmu Kesehatan Senior Medan for providing the facilities.

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