



In Vitro Test of ChiveLeaves Infuse (*Allium schoenoprasum*, L.) on Calcium Oxalate Solubility using Atomic Absorption Spectrophotometry

Iksen*, Ginda Haro, Siti Morin Sinaga

Faculty of Pharmacy, University of Sumatera Utara Jalan Tri Dharma No.5
Pintu 4 Kampus USU, Medan, Indonesia, 20155

Abstract : Chives (*Allium schoenoprasum* L.) is a long-lived plants which are very easy to grow. Chives can also be used fresh or boiled as part of spice in cooking. Chives leaves contained a variety of phytochemical compounds included alkaloids, flavonoids, glycosides, steroids, tannins and various minerals such as potassium, magnesium and sodium, which high potassium contains is believed to dissolve calcium oxalate in kidney stones, where one of the main factors affecting solubility calcium is potassium. This study aims to determine the effect of potassium to the calcium oxalate solubility in chive leaves infuse solution. This study using fresh and dried chive leaves infuse solution and divided into 2 groups. Group 1 (S1) was fresh chive leaves infuse solution and group 2 (S2) was dried chive leaves infuse solution. Both of these groups will be added by calcium oxalate and incubated on 37⁰C for four hours. Then the amount of potassium absorption level and calcium solubility assayed using atomic absorption spectrophotometry respectively at wave length 766.5 nm and 422.7 nm. These results indicated that potassium in fresh and dried chive leaves infuse solution significantly affect the solubility of calcium oxalate. Based on the above results, it can be concluded that both of the fresh and dried chive leaves infuse solution can dissolve calcium oxalate. Levels of dissolved and solubility percent of calcium oxalate in dried chive leaves solution was higher than fresh chive leave solution.

Keywords : Chives; Infuse; Calcium Oxalate; Atomic Absorption Spectrophotometry.

Introduction

Chives (*Allium schoenoprasum* L.) is a long-lived plants which are very easy to grow. This plant is known as the vegetable of the Liliaceae family . All parts of the chives edible. Chives can also be used fresh or boiled as part of spice in cooking ¹.

Indonesian society have used chives for treatment for a long time, such as vaginal discharge, constipation and infectious germs in the intestines, speed up the blood flow, prevent blood clotting, antioxidants, breast cancer and antihypertensive^{1,2,3,4}. Chives leaves contained a variety of phytochemical compounds included alkaloids, flavonoids, glycosides, steroids, tannins and various minerals such as potassium, magnesium and sodium^{5,6}, which contains high potassium is believed to dissolve calcium oxalate in kidney stones, where one of the main factors affecting solubility calcium is potassium⁷.

Infusion technique has several advantages when compared with the manufacture of the extract that is cheaper, faster and a tool used quite simple⁸, while measuring the levels of calcium and potassium using atomic

absorption spectrophotometry⁹ due to the advantages of having a high sensitivity and the implementation was quick and simple^{9,10,11}.

Experimental

Materials

Ammonium oxalate, aquabidest, calcium chloride, fresh and dried chive leaves, nitric acid 65%, and the standard solution of potassium and calcium 1000 mg/mL

Methods

Preparation of fresh and dried chive leaves infuse solution

Chives leaves infusion solution was made with two kinds of which are fresh chives leaves infuse solution and dried chives leaves infuse solution. The dose used was 10% w/ v in accordance with the infuse criteria in the Indonesian Pharmacopoeia Edition IV¹².

Research Design

In this study, samples were divided into 2 groups, namely of S1 and S2. Each group was treated as follows:

S1 :Fresh and dried chive leaves infuse solution

S2 : Dried chive leaves infuse solution.

All of these group will be added by calcium oxalate and then incubated at 37 °C for 4 hours.

Preparation of Sample

Preparation of Potassium's Calibration Curve

A total of 5 mL of 1000 ppm potassium (the mother liquor) was added to a 100 mL volumetric flask and then added aquabidest right to mark boundaries, the obtained raw potassium 50 ug/mL. Each of 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL pipette potassium standard solution 50 ug/mL in a 50 mL volumetric flask to obtain successive concentration of 2 ppm; 4 ppm; 6 ppm; 8 ppm and 10 ppm and measured by atomic absorption spectrophotometry at a wavelength of 766.5 nm. Then obtained a calibration curve of potassium.

Preparation of Calcium's Calibration Curve

A total of 5 mL of 1000 ppm calcium (the mother liquor) was added to a 100 mL volumetric flask and then added aquabidest right to mark boundaries, the obtained raw potassium 50 ug/mL. Each of 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL pipetted calcium standard solution 50 ug/mL in a 50 mL volumetric flask to obtain successive concentration of 2 ppm; 4 ppm; 6 ppm; 8 ppm and 10 ppm and measured by atomic absorption spectrophotometry at a wavelength of 422.7 nm. Then obtained a calibration curve of calcium

Destruction of Samples

100 mL for each S1 and S2 samples was added by 10 mL nitric acid 65 %.and heat on a hot plate until the infuse solution become tranpicuous.

Determination of Potassium and Calcium Levels in Sample

The sample solution that has been prepared with the absorbance measured using flame atomic absorption spectrophotometry at a wavelength 766.5 nm for potassium and 422.7 nm for calcium. Absorbance values obtained should be within the range of the calibration curve of potassium and calcium standard solution. Levels of potassium and calcium are calculated based on the regression equation of the calibration curve.

Result and Discussion

Calibration Curve of Potassium and Calcium

Both of these calibration have the same range for the concentration which were measured on concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm.

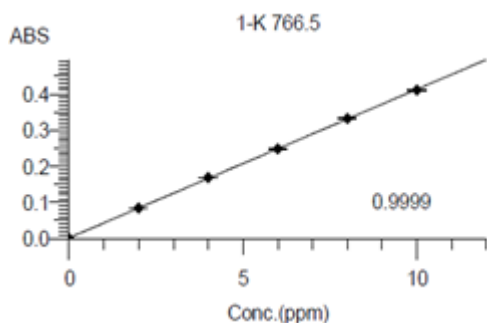


Fig.1 The calibration curve of potassium standard solution

The correlation coefficient obtained from these two metals can be accepted as the appropriate requirements for the correlation coefficient which should not smaller than 0,995. Coefficient above suggested a linear relationship between the concentration of the metal and absorbance⁹.

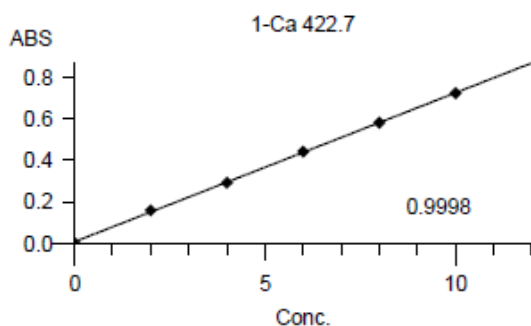


Fig2.The calibration curve of calcium standard solution

The Effect of Calcium Oxalate Solubility in Fresh and Dried Chives Leaves Infuse Solution.

The solubility testing of calcium oxalate by the infusion of the chives leaves of conducted with a view to determine the potential of early anticalculi effect by immersing 100 mg of calcium oxalate in 100 mL chives leaves infuse which the concentration of infuse was according to the Indonesian Pharmacopoeia Edition IV..

Table 1. Level of Potassium and Calcium Level in Sample

No	Infuse Group	K's Level (µg/mL)	Early Ca's Level (µg/mL)	Ca's Level After Incubation (µg/mL)	Solubility Level (µg/mL)	% Of Solubility
1	S1	190.04	46.207	71.93	25.723	2.57
2	S2	951.77	122.412	179.208	56.796	5.68

According to the table 1 we can see that the level of calcium oxalate dissolved in S1 is equal to 25 723 µg / ml with a percentage of 2.57% solubility. Levels of calcium oxalate dissolved in S2 is equal to 56.796 µg / ml with a percentage of 5.68% solubility. Percent solubility of calcium oxalate in the group S2 was higher than S1. This is likely due to the levels of potassium in the S2 group was higher than S1 group.

Dissolving power of potassium to calcium oxalate precipitate caused by the location of potassium in the volta row to the left side from calcium, so it will get rid of potassium calcium oxalate compound to join, and will dissolve calcium compounds. The more to the left of an element in volta row, the reductant power will getting stronger. This means that the element will be able to reduce ions of the elements from its leftside⁸.

Conclusion:

From the anticalculi in vitro test result, it can be concluded that fresh and dried chives leaves infuse solution have the potential as anticalculi. It can be seen from the solubility percentage of calcium oxalate at 2.57% and 5.68% per four hours of treatment.

References:

1. Andarwulan, N., dan Faradilla, R.H.F., Sayuran Fenolik Pada Beberapa Sayuran Indigenous Dari Indonesia, 2012, Bogor: SEAFASST Center, IPB : 57-60.
2. Amalia, L., Sukandar, E.Y., Roesli, R.M.A., and Sigit, J.I., The Effect of Ethanol Extract of Kucai (*Allium schoenoprasum* L.) Bulbs on Serum Nitric Oxide Level in Male Wistar Rats. International Journal of Pharmacology, 2008, 4(6): 487-493.
3. Jalkumar, B, and Jasmine, R, A Review on a few medicinal plants possessing anticancer activity again human breast cancer, International Journal of PharmTech Research, 2016, 9(3): 333-365.
4. Sachinm U.R., Priyanka, R.P., and Sagar, R.M., Use of Natural Antioxidants to Scavenge Free Radicals: A Major Cause of Diseases, International Journal of PharmTech Research, 2010, 2(2): 1074-1081.
5. Iksen, Determination of Potassium, Calcium, And Sodium Level In Chive (*Allium schoenoprasum* L.) Fresh And Boiled In Atomic Absorption Spectrophotometry, Thesis , Faculty of Pharmacy, University of North Sumatera, 2015.
6. Al-Snafi, A. E. ,Pharmacological Effects Of *Allium* Species Grown In Iraq. International Journal of Pharmaceuticals and Health care Research., 2014,1(4) : 132-155.
7. Tripathi, A., Gopalji, S., Iswar, D., and Sashank, S., To Study The Composition of Human Stones in Gorakhpur Region, North-East Uttar Pradesh, India, International Journal of PharmTech Research, 2013, 5(2): 526-535.
8. Alvin, Y, Analysis of Calcium Oxalate and Calcium Carbonate Solubility in Infuse of Fresh Sow Thistle Leaves (*Sonchus arvensis* L.) and Sow Thistle Leaves Extract Capsules Dosage By Atomic Absorption Spectrophotometry, Thesis, Faculty of Pharmacy, University of North Sumatera, 2015.
9. Ermer, J., Method Validation in Pharmaceutical Analysis, Weinheim: Wiley-Vch Verlag GmbH & Co. KGaA, 2005, 171.
10. Nadia, S., Jansen, S., and Muchlisyam, The Effect of Calcium to The Absorption Lead In Male Mice (*Mus musculus* L.), International Journal of PharmTech Research, 2016, 9(3): 193-197.
11. Settle, F., Handbook of Instrumental Techniques For Analytical Chemistry. United States of America, 1997, 398.
12. Indonesia Pharmacopoeia, Indonesia's Ministry of Health, 1995, Fourth Edition : 1126.
