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The Activity of Purple Sweet Potato Leaves (*Ipomea batatas* Ver.) Extract to Calcium Oxalate Concentration of Male Rat (*Rattus novergicus*)

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ABSTRACT

Purple sweet potato leaves (*Ipomea batatas* Ver.) has been proven to have anti-lithiasis effects in vitro in treating kidney stone disease. This is due to the high content of potassium in the leaves. The study aimed to analyze the effect of purple sweet potato leaf extract on kidney stone decay in male white rats induced by ethylene glycol 0.75% and ammonium chloride 2% for 10 days. In this study, 24 mice were divided into 6 groups: normal or without induction group, positive control, negative control, dose group 300 mg/200-gram body weight, 400 mg/200-gram body weight, and 500 mg/200-gram body weight. The parameters observed were calcium levels in the urine tested using Atomic Absorption Spectrophotometry, oxalate levels in urine tested using UV VIS Spectrophotometer. The results of this study indicate that purple sweet potato leaf extract at a dose of 500 mg/200-gram body weight can increase the levels of calcium and oxalate in the urine of rats compared with negative controls.

Keywords: *Ipomea batatas* Ver.; calcium; oxalate; ethylene glycol; ammonium chloride

INTRODUCTION

Kidney stone disease is the most common disease after kidney failure and prostate enlargement. The worst effect of this disease is permanent kidney damage (Wijaya and Darsono, 2005). Kidney stones are small stones that form in the kidneys due to precipitation that occurs in the urine moves down to the urinary pipe (ureter). This stone can clog the urinary tract (urethra) and during urination cause pain and difficulty to get out (Nisma, 2011).

Kidney stones are one of the diseases that are found throughout the world. Even in continental Europe and Australia have a high prevalence of kidney stone disease. In Asia, the incidence of urinary tract stones reaches 1-5%. Besides, many cases of urinary tract stones are also found in developing countries such as India, Thailand, and Indonesia, where the incidence reaches 2-15% (Sja'bani, 2009).

Currently, kidney stone healing can be done in several ways including ESWL (Extracorporeal Shockwave Lithotripsy), PCNL (Percutaneous Nephro Litholapaxy), open surgery, conservative therapy (Fauzi & Marco, 2016). In addition to modern treatments, kidney stone healing can use medicinal plants (Sumarno & Mayangsari, 2016).

In Indonesia, purple sweet potato (*Ipomea batatas* Ver.) is widely known as a staple food in various regions. Sweet potato is a staple food in certain areas, while leaves and stems are used as vegetables. The major component of purple sweet potato leaves is potassium compounds, in general, sweet potato leaves contain 508 mg/100 g of potassium according to the USDA (United State Department of Agriculture). Besides that, the other biggest content in purple sweet potato leaves is its anthocyanin content.

One mechanism of decay of kidney stones is to use potassium compounds. Where, potassium will shift calcium to join carbonate, oxalate, or urate compounds which form kidney stones. So that the kidney stone deposits will dissolve and drift along with urine. Meanwhile, anthocyanin has long been used to treat various conditions, such as hypertension, abnormalities in the liver, dysentery, diarrhea, urinary tract problems such as kidney stones and urinary tract infections, as well as ordinary fevers (Konczak and Zhang, 2004). Besides, in vitro testing as an anti-lithiasis effect of purple sweet potato leaves combined with its roots has been carried out in India and it is proven that purple sweet potato leaves have anti-lytic effects (Sathish and Jeyabalan, 2017).

METHODOLOGY

Tool

The tools in this study used the beaker glass 50 ml and 100 ml (Iwaki, Indonesia), the dropper, the push ball, the petri dish, the analytical balance (Shimaddzu Uni Bloc Japan), the Erlenmeyer (Iwaki Indonesia), the funnel glass, vial, Moisture

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analyzer (Ohaus Indonesia), Rotary Evaporator (IKA RV10 DIGITAL V Germany), UAE (Ultrasonic-Assisted Extraction), Oven (Memmert UN 55), mouse cage, 1 ml size syringe (OneMad Indonesia), Atomic Absorption Spectrophotometer (AAS-AA240 Varian), UV VIS Spectrophotometer (Shimadzu UV-1800).

Ingredients

Materials used in this study were Ethanol 70% (EMSURE Germany) 2 liter, Ethylene Glycol 0.75% (EMPLURA Germany), Ammonium Chloride 2% (EMSURE Germany) as kidney stone inductor, n-hexana (SUPRASOLV Germany) 7 ml, etil asetat (EMSURE Germany) 5 ml, NH_4OH (EMSURE Germany) ± 2ml, KI ± 2 ml, KI 0.5 N ± 2ml, kloroform (EMSURE Germany) 2 ml filter paper, 500-gram purple sweet potato leaves, Aquadest, TLC plate, micropipette.

Ethics

This study was approved by the ethics committee of the Medical Research Ethics Faculty of Medicine and Health Sciences UIN Maulana Malik Ibrahim Malang no 045 / EC / KEPK / FKIK / 2019.

Work procedure

Plant determination

The process of plant determination is carried out in Materia Medika Batu, East Java. The results of determination with the numbers 074 / 303A / 102.7 / 2018 show that the leaf sample used in this study is *Ipomea batatas* Ver with the key determination of 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14b -15b-109b-129b-119b-109b-120b-121a-122b-123b-1b-2b-4b-5b-6b.

Ethanol extract of purple sweet potato leaves

250 grams dried powder of sweet potato leaves put it the Erlenmeyer. Extracted using the ultrasound-assisted extraction (UAE) maceration method with 70 ml of ethanol 70% in 3 stages, with 200 ml, 150 ml and, 150 ml. at each stage carried out for 3x2 minutes. Stirring every 2 minutes. Finally, the solvent is evaporated with a rotary evaporator.

Phytochemical screening

TLC

TLC test was used to determine the presence of compounds in extract qualitatively. As much as 0.5-gram thick extract was dissolved in 10 drops of 70% Ethanol solvent. Then eluted with 7 ml n-hexane solvent and 5 ml ethyl acetate to determine the class of compounds in the extract.

Metal contamination test

The purpose of the metal contamination test is to analyze the presence of contaminants that are suspected to interfere with the results of calcium levels in the urine. This test is important to avoid extracts from metals that can interfere with the test results using atomic absorption spectrophotometry, this is because atomic absorption spectrophotometry is a sensitive tool in testing a metal. Qualitative test of metal contamination using certain reagents to cause reactions and changes or the presence of certain deposits. In the Pb metal contamination test using HCl + NH4OH and NaOH reagents, if it shows positive results it produces a white precipitate in the solution. Then for the Hg metal contamination test using KI 0.5 N reagent and 6 N HCl, if it shows positive results it will be marked by the presence of orange-red and white deposits (Vogel, 1985; Arifiyana, 2018).

Treatment of experimental animals

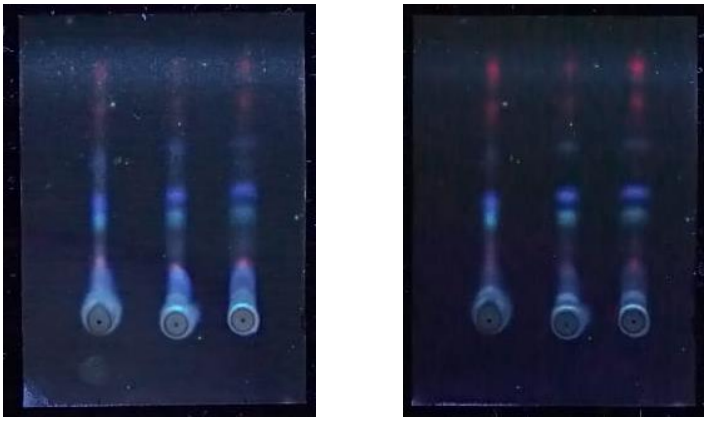
Experimental animals used were 24 male white rats of Wistar strain aged 2-3 months with a weight of 200-300 grams which were adapted for 7 days. Then divided into 6 groups randomly and each group consisted of 4 mice. The group was a normal group without any treatment, negative group, positive group (as a comparison control used Batugin Elixir 0.54 ml / 200 g BW), group I (extract 300 mg / 200-gram body weight), group II (extract 400 mg / 200-gram body weight) and group III (extract 500 mg / 200-gram body weight). All groups were inducted with 0.75% ethylene glycol inductor and 2% ammonium chloride, except the normal group.

The negative control group was given induction. The positive control group was given induction and treated with stone as a comparison. Groups 1, 2 and 3 were given induction and treated with extracts according to group doses. Each group was treated for 10 days. On the 11th day, rat urine was collected and followed by UV VIS Spectrophotometer and calcium oxalate levels with Atomic Absorption Spectrophotometer.

Determination of calcium levels with atomic absorption spectrophotometer.

Making a standard solution

The standard solution used was CaCO3 (1, 2, 3, 4 and 5 ppm), then made a standard curve and measured the absorption of a standard solution with the Atomic Absorption Spectrophotometer at a wavelength of 422.7 nm.



(A) (B)

Figure 1. TLC results with mobile phase n-hexane, Ethyl Acetate (7: 5) and stationary phase Silica Gel F254 (A) before spraying (B) after spraying H2SO4 10%

Samples

Each urine sample was extracted with a 0.5 ml pipette in a 25 ml volumetric flask and was destined to use 5 drops of strong acid in the form of HNO3. then diluted with aqua dest to the boundary mark, measured sample solution uptake with Atomic Absorption Spectrophotometer at a wavelength of 422.7 nm.

Determination of Oxalate Levels with UV-VIS Spectrophotometer.

The standard solution is oxalic acid (1, 2, 3, 4, and 5 ppm) while the urine sample solution is pipetted 0.5 ml in a 10 ml volumetric flask and then diluted with distilled water to mark the limit. The absorption was measured by UV VIS Spectrophotometer at a wavelength of 664.5 nm.

Data Analysis

Research data were statistically analyzed using one-way analysis of variance (ANOVA) and correlation tests.

RESULT AND DISCUSSION

TLC

The solvents in the TLC test were n-hexane and ethyl acetate (7: 5). N-hexane is a type of nonpolar solvent so that n-hexane can dissolve nonpolar compounds (Maulida and Zulkarnaen, 2010). Ethyl acetate is a semi-polar solvent and can dissolve semi-polar compounds in cell walls (Harborne, 1987). Some Rf values from this test can be seen in the following table.

TLC test aims to identify the class of active compounds in purple sweet potato leaf extract (*Ipomea batatas* Ver.) TLC test results obtained 5 spot stains. At Rf 0.28 with orange stain is suspected to be an alkaloid compound, then at Rf 0.46 with blue fluorescence stain is suspected to be a steroid compound, at Rf 0.64 with purple stain is suspected to be a terpenoid compound, at Rf 0.80 with yellow stain is suspected to be a flavonoid group compound and lastly at Rf 0.92 with a red stain, it is thought to be an anthocyanin compound.

Metal Contamination Test

Elemental metals tested qualitatively in purple sweet potato leaf extract (*Ipomea batatas* Ver.) are Hg and Pb. Each was given certain reagents to produce a certain color change or deposition.

The results of Table I show that purple sweet potato leaf extract (*Ipomea batatas* Ver.) is not polluted by heavy metals Pb and Hg. This can indicate that the extract is safe and does not affect the results of calcium testing using atomic absorption spectrophotometry. The activity of purple sweet potato leaf extract against calcium and oxalate urine which was obtained for 24 hours was observed physically. Normal urine color ranges from light yellow and dark yellow, urine that has been obtained for 24 hours on average has a dark yellow or turbid yellow, this is likely due to exposure to chemicals that have been given previously. It is different in the normal test group whose urine color is slightly bright. After testing with AAS and UV VIS the following results were obtained.

From these data, it was found that calcium levels in positive and oxalate controls were higher than negative controls. Thus, indicating the existence of the largest kidney stone decay. From both parameters, it can be seen that the treatment group with a dose of 0.5 g / 200-gram body weight

Table I. Tests for metal contamination

No	Metal	Reagents	Results	Mean
1.	Pb	HCl + NH_4OH	Not formed white precipitate	-
2.	Pb	NaOH	Not formed white precipitate	-
3.	Pb	KI	Not formed yellow precipitate	-
4.	Pb	NH_4OH	Not formed white precipitate	-
5.	Hg	KI 0.5 N	Not formed red-orange precipitate	-
5.	Hg	HCl 6 N	Not formed white precipitate	-

Note: (+) = contains heavy metals; (-) = Does not contain heavy metals

Table II. Calcium concentration result with AAS (mg/L)

Group	Rat 1	Rat 2	Rat 3	Rat 4	Average ±SD
Normal	0.052	0.041	0.048	0.069	0.05±0.011
Negative control	0.052	0.036	0.018	0.014	0.03±0.017
Positive control	0.171	0.028	0.031	0.074	0.07±0.066
Dose of 0.3 g	0.035	0.034	0.053	0.045	0.04±0.008
Dose of 0.4 g	0.051	0.053	0.030	0.044	0.04±0.010
Dose of 0.5 g	0.155	0.045	0.053	0.063	0.07±0.051

Table III. Oxalate concentration result with UV-VIS (mg/L)

Group	Rat 1	Rat 2	Rat 3	Rat 4	Average±SD
Normal	16.345	19.935	21.327	12.607	17.553±3.908
Negative control	30.052	24.438	25.592	22.286	19.344±3.273
Positive control	30.536	34.188	35.396	28.966	32.271±3.020
Dose of 0.3 g	36.442	30.679	31.709	32.943	26.579±2.509
Dose of 0.4 g	29.083	28.803	32.097	24.392	28.593±3.173
Dose of 0.5 g	19.670	35.906	35.946	34.004	31.381±7.860

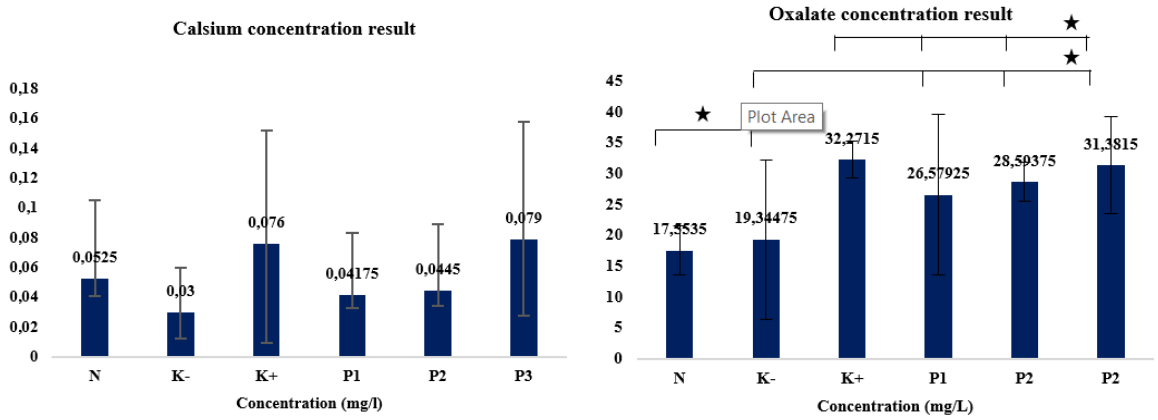
is the most effective because it has the concentration value that is closest to the positive control.

Statistical test results on urine calcium levels indicate no significant difference between group after being tested with one way ANOVA, this is probably due to the limitations of the Atomic Absorption Spectrophotometry tool in analyzing compounds with very little levels. Different results were obtained in the level of oxalate in the urine, which was a significant difference between the negative control group and the normal group and all treatment groups, as well as the positive control group that had a significant difference with the normal group and all treatment groups ($p > 0.05$). Judging from the chart, the most effective dose is a dose of 0.5 g / 200-gram body weight.

Discussion

The purpose of this study was to determine the calcium and oxalate levels in the urine of male rats after being induced with ethylene glycol and ammonium chloride and given purple sweet potato leaf extract (*Ipomea batatas* Ver.). It is hoped that the extract treatment will raise the calcium and oxalate levels which are close to the positive control. From the results obtained, it can be seen that the purple sweet potato leaf extract at all doses has the effect to increase the levels of calcium and oxalate in the urine, and the most effective dose is 0.5 g / 200-gram body weight. The increase in calcium and oxalate levels in urine is due to the role of potassium in the extract.

Potassium content contained in purple sweet potato leaf extract has a mechanism to break the bonds between calcium and oxalate because potassium will get rid of calcium and join calcium oxalate compounds which are forming kidney stones by forming salt compounds that dissolve easily in water, so that kidney stones will dissolve slowly and come out with urine. The ability to dissolve potassium to calcium oxalate deposits is caused by the location of potassium in the voltaic sequence before the location of calcium, so that potassium will get rid of calcium to join carbonate, oxalate, or urate compounds and calcium compounds become soluble (Maharani *et al.,* 2012). The reaction of potassium oxalate formation is as follows:



Note: ★= there is a significant difference (p> 0.05)

Figure 2. (left) Chart of calcium levels after being tested for 10 days (right) Chart of oxalate levels after being tested for 10 days

$$2K^{+} + CaC_2O_4 \longrightarrow K_2C_2O_4 + Ca^{2+}$$

While flavonoids can shed kidney stones by forming complex compounds with the -OH group of Flavonoids to form Ca-Flavonoids. These complex compounds are thought to be more soluble and excreted. Flavonoid diuretic activity can help the removal of stones from the kidneys that are excreted with urine (Nisma, 2011). For example, kejibeling leaf extract which contains flavonoids and is able to shed kidney stones (Dharma, *et al.,* 2014).

From these various data, purple sweet potato (*Ipomea batatas* Ver.) Leaf extract was found to have activity on kidney stone decay. Statistically, the extract is more influential on increasing urinary oxalate levels. Treatment of 0.5 g / 200-gram body weight which has the best activity in dissolving oxalate in the kidney. While for the increase in calcium levels also obtained at a dose of 0.3 treatment. 0.4 and 0.5 of the negative control but not significantly different (p> 0.05).

CONCLUSION

Purple sweet potato (*Ipomea batatas* Ver.) Leaf extract can increase the calcium and oxalate levels of male rats in Wistar strain against negative control. The dose of 500 mg/200-gram body weight is the optimum dose in shed oxalate in the kidney and excreted in rat urine.

REFERENCES

Afriyana, Djamilah dan MA. Hanny Ferry Fernanda. 2018. Analisis Kualitatif Dan Kuantitatif Cemaran Logam Berat Timbal (Pb) Dan Kadmium (Cd) Pada Produk Kosmetik Pensil Alis Menggunakanspektrofotometer Serapan Atom (Ssa). *Journal of Research and Technology,* Vol. 4 No. 1 Juni 2018.

Dharma S., Mimi A. dan Esa F.S., 2014. Pengaruh Ekstrak Etanol Daun Keji Beling (Strobilanthes Crispa (L) Blume) Terhadap Kelarutan Kalsium dan Oksalat Sebagai Komponen Batu Ginjal pada Urin Tikus Putih Jantan. Jurnal Scientia Vol 4 no 1 2014.

Fauzi, A. & Marco, M. A. P., 2016. Nefrolitiasis. *Nefrolitiasis,* 5(2).

Harbone, J.B. 1987. *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan.* Padmawiyata K, Soediro I, Penerjemah. Terjemah dari: *Phytochemical Methods*. Bandung: ITB Press.

Konczak, I., and Zhang, W., 2004, "Anthocyanins–more than Nature's Colours", *Journal of Biomedicine and Biotechnology*, hal. 239–240.

Maharani, E. T., Mukaromah, A. H. & Susilo, J., 2012. Analisis Kalium Dan Prosentase Daya Larut Calsium Oksalat Oleh Kalium Dalam Air Teh Daun Sukun (Artocarpus Altilis). *Seminar Hasil-Hasil Penelitian – LPPM UNIMUS.*

Nisma F., 2011. Pengaruh Penambahan Ekstrak Etanol 70% Buah Anggur Biru (*Vitis vinivera* L.) Terhadap Kelarutan Kalsium Batu Ginjal. Jakarta: *Farmasi FMIPA UHAMKA.*

Sathish, R and Jayebalan, G. 2017. Study Of In Vitro Anti-Lithiatic Effect Of *Ipomoea Batatas* (L) Leaves And Tuberous Roots. *Asian Journal of Pharmaceutical and Clinical Research* Vol 11 Issues 2.

Sja'bani M. 2009. *Batu Saluran Kemih dalam Buku Ajar Ilmu Penyakit Dalam Edisi 5*. Jakarta: Interna Publising. Hal 1025 – 9

Sumarno & Mayangsari, A., 2016. Tanaman Obat Merupakan Komodity Indonesia Masa Depan. *Jurnal Ilmiah Agribios,* 14(2).

Vogel. 1985. *Buku Teks Analisis Anorganik Kualitatif Makro dan Semimikro*. Edisi kelima. Bagian I. PT Kalman Pustaka : Jakarta.

Wijaya, S dan Farida, L. D. (2005). Uji daya antikalkuli perasan buah ketimun *(Cucumis sativus L.)* terhadap tikus putih jantan dengan metode Kalkuli. *Majalah Farmasi Indonesia,* 16(3), 173 – 176, 2005