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The Influence of Ethanol Extract of Sambiloto and Sambung Nyawa Leaves to Decrease Concentration of Uric Acid

Agus Suprijono*. Vicky Selta Agatha Z, Anang Budi Utomo

Sekolah Tinggi Ilmu Farmasi, Yayasan Pharmasi, Semarang, Indonesia 50193

**Corresponding Author: agussuprijono1967@gmail.com*

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Abstract: *Hyperuricemia is a condition of high concentration of uric acid in the blood that occurs because of a formation of uric acid in the body. Uric acid is formed as a residue of the protein metabolism of foods containing purine. Several active compounds in the sambiloto leave (Andrographis Paniculata (Burm.F.) Nees) and sambung nyawa leave of allegedly being able to decrease the concentration of uric acid. The aims of this research is to understand the influence of the difference between the ability of ethanol extract of sambiloto leaves and ethanol extract of sambung nyawa leaves* (*Gynura Procumbens* (Lour.) Merr.) *against the concentration of uric acid in vitro as well as understand the effective concentration of ethanol extract of sambiloto leaves and extract ethanol of sambung nyawa leaves against decreasing concentration of uric acid in vitro. The extraction method applied in this research is the method of extraction re-maceration by solvent ethanol 70% in 5 days, the sampling technique applied was purposive sampling. The determination of concentration of uric acid is determined by the Spectrophotometry method ABX Pentra. Series of concentrations used was 250, 500, 750, 1000, 1250, 1500, and 1750 ppm. The results of the research, the ability is effective in decreasing the concentration of uric acid in vitro on the extract of sambiloto leaves and extract of sambung nyawa leaves is 250 ppm. Statistical results revealed that there is an influence of the ethanol extracts of sambiloto leaves and ethanol extracts of sambung nyawa leaves against decreasing the concentration of uric acid in vitro. And there is no difference between ethanol extracts of sambiloto leaves with ethanol extracts of sambung nyawa leaves against a decreased in the concentration of uric acid in vitro.*

Keywords*: hyperuricemia, Andrographis Paniculata, Gynura Procumbens, in vitro*

INTRODUCTION

Uric acid is a by-product of purine metabolism which comes from foods that contain protein consuming every day. Purines are also by product of the breakdown of cells in the blood. Purines are abundant in animal origin (Shrimps, squid, clams, mussels, anchovies, sardines and offal) and can also be found in vegetables (cauliflowers, spinach, asparagus, beans, ear mushroom, cassava leaves, papaya leaves, kale) and fruits (pineapple, coconut, and durian)(Astuti, Prayoga, Firmansyah, & Renaldi, 2018; Dungga, 2022). Purines are also produced from the results of the destruction of body cells that occur. The excess uric acid will accumulate in the joints, causing pain or swelling. Symptoms of gout include tingling and pain, joint affected by gout look swollen, red and hot (Faesal Yatim, 2006).

Uric acid is the end product of purine metabolism which can precipitate in tissues and can cause inflammation known as gout. The concentration of uric acid is very closely related to the way of living, incorrect food consumption patterns, and alcohol abuse that

occurs in most population (Annita & Handayani, 2017; Rahmi, Kumolosasi, Jalil, & Buang, 2022). Purines and pyrimidine released by nucleotide breakdown may be reused or catabolized. Pyrimidine is catabolized to CO_2 and NH_3 and purines are converted to uric acid (Delita Septia Rosdiana, Ali Khomsan, 2018; Mubarak, 2022)

Everyone can experience gout. Generally, this disease is mostly experienced by men, whereas in women the percentage is smaller and only appears after menopause. In male, concentration of uric acid tend to increase with age. Currently, gout does not only experienced by the elderly, but people who are still relatively young can also be affected by gout (Delita Septia Rosdiana, Ali Khomsan, 2018; Irdiansyah, Saranani, & Putri, 2022; Zhu, Y, Pandya, B.J. and Choi, 2011)

The use of medicinal plants for the treatment of gout has recently been widely applied, because apart from being cheap they are also relatively safer compared to drug therapy. This has encouraged various efforts to find alternatives to the use of traditional medicines derived from medicinal plants(Dalimarta Setiawan, 2002)

Sambiloto and Sambung Nyawa contain active compounds from the polyphenol group (a class of flavonoid compounds and group of the phenolic compound) and minerals. These substances are required by these two plants to become part of their own defense system against external attacks.(Hidayah et al., 2023; Rais, 2015; Septianingsih, Susanti, & Widyaningsih, 2012)

Flavonoid activity as a decreasing of concentration of uric acid through inhibition of the action of the xanthine oxidase enzyme. Several flavonoids besides being able to inhibit the xanthine oxidase enzyme also act as superoxide radical scavenging antioxidants (Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, Pieters L, Vlietinck AJ, 1998). As a medicine, the efficacy of Sambiloto has been known for a long time. All parts of the bitter plant, such as leaves, stems, flowers and roots, taste very bitter if eaten or boiled to drink. The bitter taste is caused by the presence of andrographolid compounds which are abundant in the Sambiloto plant, especially the leaves and stems. Allegedly, this compound is an active compound in Sambiloto leaves which contains a lot of mineral elements such as potassium, sodium, calcium, and grit acid. Several research have also proven that the bitter plant contains alkanes, ketones, aldehydes, resins, and essential oils (Sakinah, Tarwaca, Putra, & Rogomulyo, 2018; Septianingsih et al., 2012; TOBING, 2022)

The ABX Pentra Spectrophotometer is a spectrophotometer commonly used in health laboratories or hospitals. The advantage of this tool is that it already contains various reagents and when the sample to be tested is put into it, the tool will automatically add the necessary reagents according to the input data that we previously set. Parameter that can be examined include albumin, total protein, SGPT, SGOT, Triglycerides, Uric acid, urea, LDH, creatinine, glucose, amylase, lipase, cholesterol, HDL, total bilirubin, specific protein, magnesium, sodium, potassium, chloride, calcium. The method applied is uricase (Tinder modification), (Coudène, Pascal, Marson, Benjamin, Badiou, Stéphanie, Flavier, Sébastien, Anelli, Sébastien, Cristol, Jean Paul and Dupuy, 2005; Release & Use, 2020)

RESEARCH METHODS

The independent variable in this research were the concentration of the ethanol extract of sambiloto leaves, the ethanol extract of the sambung nyawa leaves used were 250, 500, 750, 1000, 1250, 1500 and 1750 ppm. The dependent variable was the uric acid-reducing activity of ethanol extract of sambiloto leaves, the ethanol extract of the sambungnyawa leaves. The control variable was the uric concentration of 17 mg/dL, re-

maceration extraction method, the sample weight of sambiloto leaves and sambungnyawa leaves are 100 grams.

Materials and Tools

The test materials used are sambiloto and sambungnyawa leaves powder, uric acid crystal p.a, aquadest and uric acid reagent FS TBHBA (4-aminoantipyrine and 2,4,6-tribomo-3 hydroxybenzoic acid) p.a

The tools used were the ABX Pentra Spectrophotometer, analytical balance, volume pipette, measuring flask, dropping pipette, and test tube**.**

Procedures

Re-maceration of 100 grams of sambiloto leaves powder and sambungnyawa leaves powder macerated with 1 L of 70% etahnol for 5 days, the extractor is replaced 1x24 hours. The filtrate obtained for 5 days was concentrated to obtain a thick extract of sambiloto leaves and thick extract of sambungnyawa leaves.

Extract qualitative test

Qualitative identification of extract carried out in this research were 3 flavonol glycoside shinoda (flavones, chalcones, aurons), taubeck (flavones), phenolics, polyphenols, flavonoids, minerals (sodium, magnesium, potassium and calcium), alkaloids, tannins and saponins.

Preparation of uric acid solution 17 mg/dL

A total of 85 mg of uric acid crystals was weighed carefully and put into a 500 mL measuring flask. Dissolve in 400 mL of hot distilled water if necessary used heating until dissolved. Put until it reaches room temperature, then add distilled water up to the 500 mL mark. Obtained uric acid solution with concentration of 17 mg/dL

Determination of the concentration of Sambiloto leaf extract and Sambungnyawa leaf extract. The concentrations of Sambiloto leaf extract and Sambungnyawa extract used in this research were 250, 500, 750, 1000, 1250, 1500 and 1750 ppm

Sample grouping

This reseach used 7 groups of extract samples with concentrations of 250, 500, 750, 1000, 1250, 1500 and 1750 ppm and 1 aquadest negative control tube. The concentration standard of uric acid was measured first, then the concentrations were measured again after being treated with the addition of Sambiloto leaf extract and Sambungnyawa leaf extract. The experiment was carried out 5 times replication

How to measure concentration of uric acid

Measurements were made by means of 500 µl of uric acid standard solution plus 500 µl of sambiloto extract and sambungnyawa extract then added with uric acid reagent on the ABX Pentra Spectrophotometer. Measurement concentration of uric acid used a reagent kit which consist of 2 kinds of reagent contains pH 7, 4-aminoantipyrine phosphate buffer, $K_4[Fe(CN)_6)$, peroxidase (POD) and uricase (Coudène, Pascal, Marson, Benjamin, Badiou, Stéphanie, Flavier, Sébastien, Anelli, Sébastien, Cristol, Jean Paul and Dupuy, 2005).

How to calculate (%) decreased uric acid:

$$\%\ decreasing\ concentration = \frac{initial\ concentration - final\ concentration}{initial\ concentration}\ x\ 100\%$$

RESULTS AND DISCUSSION

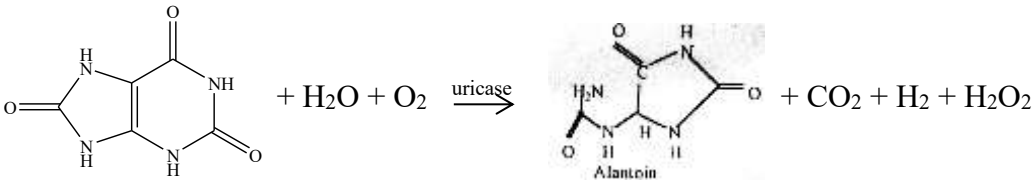
The initial step in this research was the identification/determination of test plants which was carried out to avoid errors in collecting research materials, as well as to ensure that the test plants used in this research were sambiloto (*Andrographis paniculata* (Burm .f.) Nees) and sambungnyawa. (*Gynura procumbens* (Lour.) Merr). The leaves of Sambiloto and sambungnyawa that have been washed are then dried in the sun by covering them with a black cloth until the leaves are dry and the water content in the leaves is less than 10%. The drying process is carried out with the aim of preventing enzymatic processes, maintaining the stability of the compounds and minimizing the water content that can be used as a medium for growing microorganisms, which reduce the quality of the active compounds contained in the simplicia. After drying, then grind it using a blender. To uniform the particle size of the simplicia powder, it was sifted using a 30/40 mesh sieve.(Hidayah et al., 2023)(TOBING, 2022)

(The compound withdrawal process is carried out using the remaceration method). Re-maceration is a maceration method that is carried out repeatedly, generally every day the solvent is replaced with a recent one. The principle of re-maceration is soaking simplicia which has a certain degree of fineness with a solvent. The filtered fluid will penetrate the cell wall and then enter the cell cavity containing the active substance, thereby dissolving the active substance. The difference in concentration between the solution of the active substance inside and outside the cells will push out the more concentrated solution of the active substance inside the cell. The solvent used is 70% ethanol. Ethanol is a universal solvent, which means it can dissolve a wide variety of active ingredients.

Qualitative test was conducted to determine the content of active compounds contained in the extract. Qualitative tests performed included tests for magnesium, potassium, calcium, sodium, phenolics, polyphenols, flavonoids, alkaloids, saponins, tannins, 3-flavonol glycosides, Shinoda and Taubeck. Based on the results of the qualitative tests that have been carried out, it can be concluded that the ethanol extract of Sambiloto leaves contains compounds of potassium, magnesium, sodium, flavonoids, phenolics, polyphenols, alkaloids, and saponins. Meanwhile, the ethanol extract of the leaves of sambungnyawa contains compounds of potassium, magnesium, sodium, flavonoids, phenolics, polyphenols, tannins, alkaloids, and saponins. Based on the results of the preliminary tests that have been carried out, it can be concluded that the ethanol extract of Sambiloto leaves contains active compounds in the form of flavonoids, phenolics, polyphenols, saponins, tannins, and alkaloids. As for the ethanol extract of the leaves of Sambungnyawa, it contains active compounds of flavonoids, phenolics, polyphenols, saponins, tannins and alkaloids.(Tobing, 2022)(Rahmi et al., 2022)

Measurement of concentration of uric acid used a reagent kit which consists of 2 kinds of reagents. The first reagent contains pH 7, 4-aminoantipyrine phosphate buffer, K_4[Fe(CN)6], peroxidase (POD) and uricase. The principle of the TBHBA photometric enzymatic reaction is that uric acid which reacts with water will be oxidized to allantoin in the presence of uricase, then hydrogen peroxide as a by-product of the reaction will react with 4-aminoantipyrine and 2, 4, 6 – tribromo – 3 hydroxybenzoic acid (TBHBA) to form quinimine which is pink in color with the help of peroxidase, the color formed is then measured for its absorbance with a UV Visible spectrophotometer at the maximum

wavelength. Measurement based on the intensity of the color resulting from the reaction of uric acid with *Uric Acid FS TBHBA* reagent as below:



$$\text{TBHBA} + 4\ \text{aminoantipirin} + 2H_2O_2 \longrightarrow \text{Quinimine} + 3H_2O$$

Figure 1. Reaction of Uric Acid with Uric Acid FS TBHBA Reagent

Measurement of concentration of uric acid 1 in this test was preceded by measuring the initial concentration of uric acid so that the concentration of uric acid solution was known quantitatively before adding the Sambiloto leaf extract ang Sambungnyawa extract. The concentration of uric acid solution used in thus in this research was 17 mg/dL.

Table 1. The results of measuring the average concentration of uric acid before and after the addition of Sambiloto and Sambungnyawa extracts.

Concentration (ppm)	Extract sambiloto	Extract Sambung Nyawa
Control	24,38 %	24,74 %
250	46,57 %	45,94 %
500	48,32 %	47,52 %
750	49,67 %	48,83 %
1000	51,26 %	50,29 %
1250	52,49 %	51,15 %
1500	53,05 %	51,75 %
1750	53,83%	52,36 %

The results of measuring concentration of uric acid can be seen in the bar chart below:

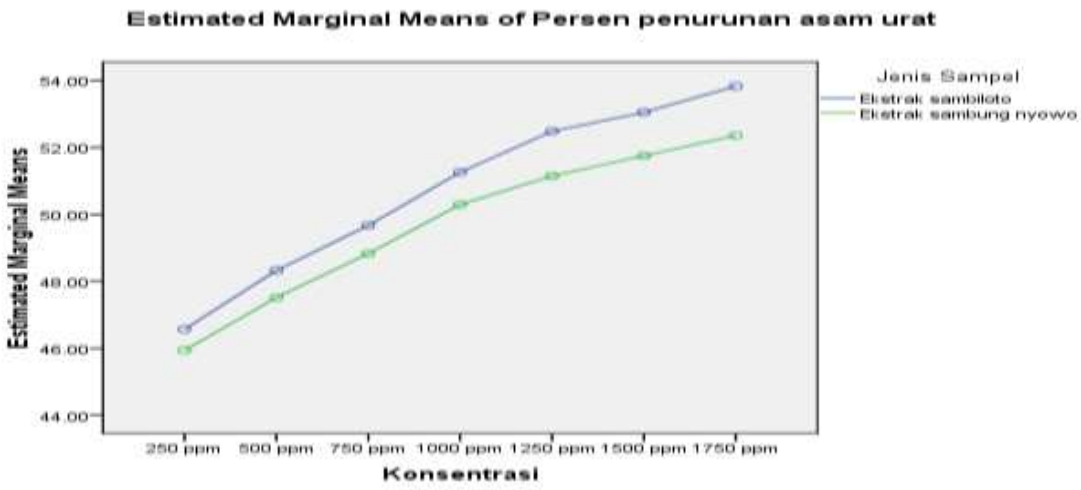


Figure 2. Graph of the average percentage decrease in concentration of uric acid after give of Sambiloto leaf extract and Sambungnyawa leaf extract

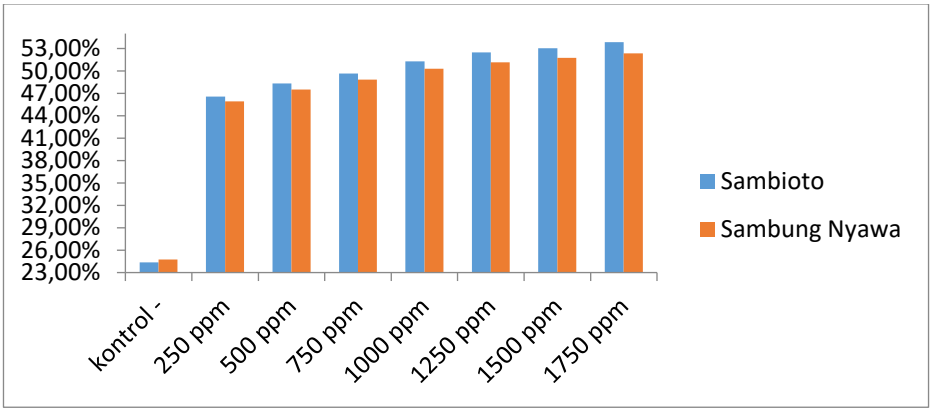


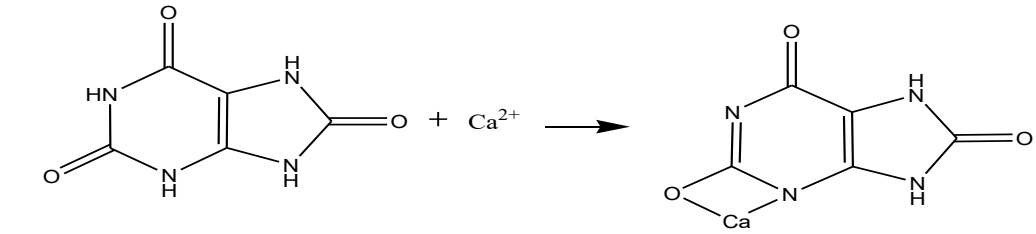
Figure 3. Bar chart of the percentage of decreased concentration of uric acid

In the group with the addition of Sambiloto extract and Sambungnyawa extract, the concentration of uric acid were significantly reduced. This is probably due to the presence of polyphenols and minerals dissolved in the extract preparations.

The decrease in the concentration of uric acid is due to ionization after the addition of Sambiloto extract and Sambungnyawa extract which contain flavonoids. The ionized uric acid will then bind to mineral ions to form salt compounds that are easily soluble in water. This is based on the nature of uric acid which is a weak acid. The uric acid at normal pH will ionize into urate ions with the existing cations, urate ions will form urate salts. Compounds that can reduce concentration of uric acid are flavonoids. Uric acid will bind to flavonoids to form complexes so that the uric acid structure here will be damaged and its activity will decrease. The results revealed a decrease due to a bond between uric acid and flavonoids which caused uric acid to no longer bind to the TBHBA color reagent so that the concentration of uric acid that was read was the residual concentration of uric acid.(Rahmi et al., 2022)(A.K. Ratty, 1988)

Sambiloto leaf extract and Sambungnyawa leaf extract may have a role in other compounds that play a role in the formation of urate salts which are more soluble in water

so the reading of the ABX Pentra Spectro results reveals a decrease from the previous concentration. The ionized uric acid will then bind to mineral ions to form salt compounds. This is based on the nature of uric acid which is a weak acid. The uric acid at normal pH will ionize into urate ions. The complex compound formed between uric acid and K^+ cations is urate salt or potassium uric. Uric salts are much more soluble in water than uric acid (Septianingsih et al., 2012)(Nagao A, Seki M, 1999)(C. Rinaudo, 1982) (Veramida, 2011). Uric acid, apart from forming complexes with potassium cations, also forms complexes with other cations such as calcium and magnesium. Metals tend to lose electrons and non-metals tend to gain electrons. The more electronegative an atom, the more valence electrons the atom and therefore will become an anion. Atoms that are less electronegative will lose one or more valence electrons and will become cations (Satyajit, 2009).



Uric Acid Potassium Urate

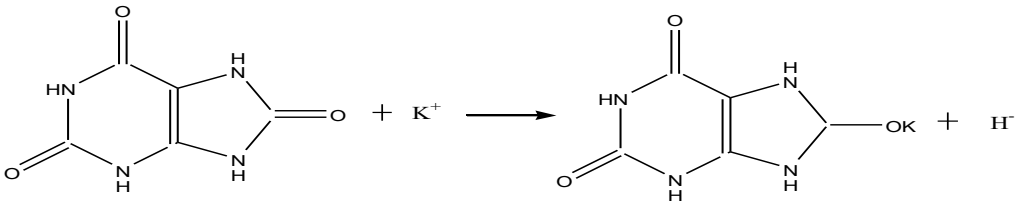
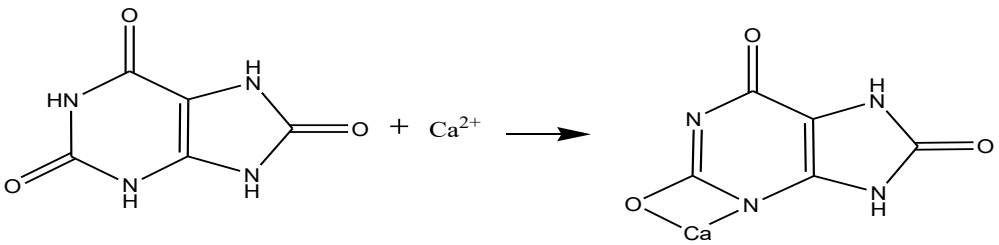
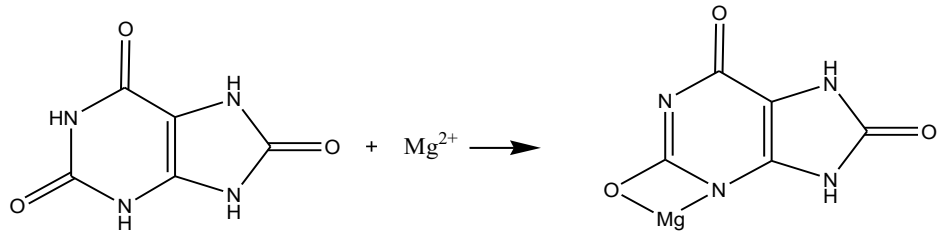


Figure 4. The reaction between uric acid and K+ ions produces potassium urate (Allen RN, Shukla MK, Burda JV, 2006)



Uric Acid Urate Calcium

Figure 5. The reaction between uric acid and Ca2+ ions produces calcium urate (Allen RN, Shukla MK, Burda JV, 2006)



Uric Acid Magnesium Urate

Figure 6. The reaction between uric acid and Mg2+ ions produces magnesium urate (Allen RN, Shukla MK, Burda JV, 2006)

Nucleophilic interactions with metal cations at the uric acid position will be different. Nucleophiles will interact with metal cations in coordination. The most favorable position for K+ cation interactions is the bond between the N7 and O8 positions. While all cations with a valence of 2 such as Mg and Ca are more binding at the N3 and O6 positions. The results reveal a decreased concentrationcompare to the initial measurement. This is because the amount of uric acid oxidized by uricase has decreased, because some of it has become urate salts. If urate salt is reacted with uric acid reagent, the reaction between the two will not produce allantoin and hydrogen peroxide which will react with 4-aminoantipyrine and 2,4,6-tribromo-3 hydroxybenzoic acid (TBHBA), thus a colored quinimine compound will not be pink, which will be read as concentration of uric acid.

To find out a significant difference in the percentage value of the ability to decreased concentration of uric acid between the Sambiloto extract and the Sambungnyawa Extract, which is significant or not, a road ANOVA test was carried out using the SPSS version 23 method. In the data normality test, the significance results were 0.09 and 0.1 greater than the requirements. (α) 0.05 which reveals the results of the data are normally distributed. In the homogeneity test, the result is significance of 0.346 or greater than the requirement (α) 0.05 which indicates homogeneous data results. Anova statistical results can be observed by looking at the significance value which is equal to 0.000 which is smaller than the value of 0.05. This reveals that there are differences in the concentration groups, which means that the concentration groups of Sambiloto extract and Sambungnyawa Extract can decrease concentration of uric acid. So that the effective concentration obtained is 250 ppm for Sambiloto extract and Sambungnyawa extract.

After the Anova test was carried out, then it was compared whether or not there was an effect between the two extractions, namely Sambiloto Extract and Sambungnyawa extract in their ability to reduce concentration of uric acid. The test results with the "t" test results obtained a significance of 0.07 or greater than the requirement (α) 0.05, meaning that there is no difference in the data results, which indicates that between the two extractions, namely Sambiloto extract and Sambungnyawa extraction, there is no effect in reduced concentration of uric acid.

CONCLUSIONS

From the results of the research that has been conducted, it can be concluded there is an effect of the ethanol extract of Sambiloto leaves and the ethanol extract of Sambungnyawa leaves on decreased concentration of uric acid in vitro. The effective concentration of the ethanol extract of Sambiloto and ethanol extract of Sambungnyawa is 250 ppm. There is no difference between the ethanol extract of Sambiloto leaves and the ethanol extract of Sambungnyawa leaves on the activity of reduced concentration of uric

acid in vitro. Both Sambiloto and Sambungyawa extracts can be consumed as capsule preparations or water extract.

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