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by Nugrahaningsih Wh

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Nugrahaningsih Wahyu Harini, Fitta Permata Putri, Ary Yuniastuti, Lisdiana, and Ely Rudyatmi

Excretion of Cassava (*Manihot esculenta* Crantz) Leaves Extract After Oral Administration in Rat

Nugrahaningsih Wahyu Harini[1, a)], Fitta Permata Putri[2, b)], Ary Yuniastuti[1, c)], Lisdiana[1, d)] and Ely Rudyatmi [1, e)]

[1] *Biology Department, Mathematics and Science Faculty Universitas Negeri Semarang*
[2]*Student of Graduate Program Biology Department, Mathematics and Science Faculty Universitas Negeri Semarang.*

[a)] Corresponding author: nugrahaningsihwh@mail.unnes.ac.id
[b)] fittapermataputri79@gmail.com
[c)]ariyuniastuti@mail.unnes.ac.id
[d)]lisdiana@mail.unnes.ac.id
[e)]elyrudy@mail.unnes.ac.id

Abstract. The development of new drug needs pharmacokinetic understanding. Cassava leaf is one of traditional herb used for many purposes of disease. This study was conducted in order to find out the level of flavonoid urine, excretion rate, and the total of flavonoid excreted after oral administration. The time series experiment was done to measure the level of flavonoid urine and excretion rate of flavonoid. A total of six adult male Wistar rats weight ranging between 180-200 g were randomly selected from population. The single oral dose of 600 mg/rat extract was administered. Urine was collected at 6, 12, 24 and 48 hours after oral administration. The level of flavonoid was measured by High Performances Liquid Chromatography (HPLC) Nova-Pak C18 Column (4,6 3 250 mm) at 347nm wavelength. The flavonoid levels of urine were 0.077 mg/mL (6 hours), 0.113 mg/mL (12 hours), 0.145mg/mL (24 hours), and 0.185 mg/mL (48 hours) respectively. The total flavonoids were excreted 0.259 mg (0-6 hours), 0.313 mg (6-12 hours), 0.564 mg (12-24 hours). (24-48 hours). The excretion rates were 0.043 mg/h (0-6 hours), 0.052 mg/h (6-12 hours), 0.047 mg/h (12-24 hours) and 0.036 mg/h (24-48 hours). It was found out that the flavonoid excretion of cassava leaves extract was more than 48 hours suggesting. The fastest excretion rate was 6-12 hours after oral administration.

INTRODUCTION

Cassava leaves generally consumed as vegetables and processed into traditional food in Indonesia. Cassava leaves contain several compounds such as protein, some minerals, vitamin B1, vitamin B2, vitamin C and carotene [1]. Flavonoid in high level plant has the function as an antioxidant [2]. Cassava leaves contain alkaloid compound, flavonoid, tannin, anthraquinone, phlobatannin, saponin, protein, vitamins A, C and E and mineral such as calcium, magnesium, phosphor, iron ions, sodium, and chloride [3]. High content of flavonoids and saponins in cassava leaves act as anti-inflammatory and antibacterial, in which both played a role in inhibiting cyclooxygenase and lipoxygenase cycles.

Ethanol extract from cassava leaves contains carotenoids, flavonoid, tannin and terpenoidss which act as anti-allergy and anti-inflammatory by pressing cyclooxygenase and lipoxygenase activity, lipid peroxidation, capillary permeability and platelet aggregation [4]. The development of cassava leaves as a medicine needs the data about its effectiveness and safety. Excretion rate was important to know how long the cassava leaves extract on the body. This study was conducted in order to find out the excretion rate of flavonoid containing cassava leaves extract after oral administration.

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MATERIALS AND METHOD

Preparation of extract and dose

One kilogram of cassava leaves was dried using oven at 50ºC, producing 262 g of dried cassava leaves. The dried leaves were mashed followed by distilled water until the volume reached 525 mL. The suspension was mixed for 30 minutes and incubated at room temperature for five days. The supernatant was evaporated used rotary evaporator and dried in the oven at temperature 50ºC. Preliminary study was conducted to get the dose for oral administration. The single dose was 600 mg/rat.

Animals

A total of 6 male adult Wistar rats, between 2-3 months old with weight ranging between 180 g and 200 g were used throughout this investigation. Each rat was caged in metabolic cage. Urine were collected for 0-6 hours, 6-12 hours, 12-24 hours and 24-48 hours after oral administration. The urine sample was filtered by a membrane filter size 45µm. The filtrate was taken for HPLC analysis [5]. An amount of 20µl filtrate each was injected into column of HPLC.

Standard Curve

The standard solution (flavonoid) made by weighing 1.04 g of Rutin and dissolved into 100 mL methanol. The solution was diluted into five different concentrations of 12.5 ppm, 25 ppm, 50 ppm, 100 ppm and 200 ppm for preparing the standard curves (Figure 1).

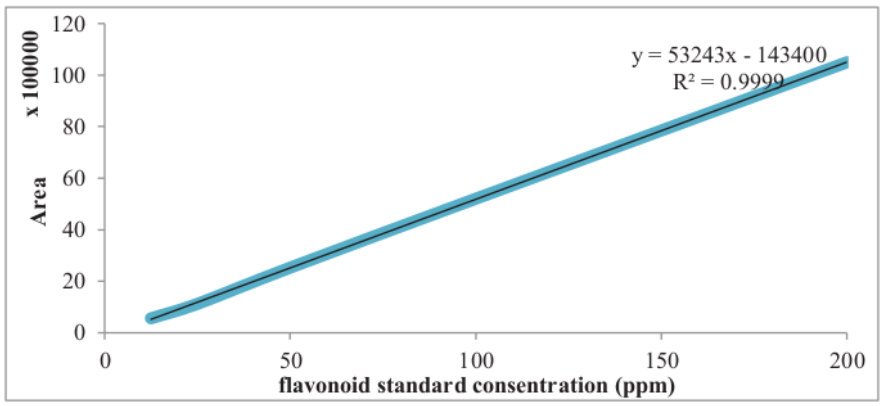

FIGURE 1. Standard flavonoid curve of cassava leaf extract

HPLC analysis of flavonoid urine

HPLC analysis was performed using the reversed phase Nova-Pak C18 Column (4,6 3 250 mm) at 347 nm wavelength. The mobile phase used in this study consisted of 27% eluent A (acetonitrile) and eluent B (methanol: water: acetic acid) with an 8%: 87%: 5% ratio, the pH was adjusted to 3.64 used acetic acid with a motion phase stream of 0, 5 ml/min, the column temperature was set at 25ºC.

RESULTS

The flavonoid levels after oral administration of cassava leaf extract at 0-6 hours, 6-12 hours, 12-24 hours, and 24-48 hours was obtained by calculating the mean flavonoid levels of each time series. The flavonoid level was calculated

with the standard flavonoid curve obtained by y = 53243x - 143400. The level of flavonoid urine was presented in Table 1.

TABLE 1. Mean of flavonoid content of cassava leaf extract in rat urine

Period (hours)	Average of urine volume (ml) n = 6	Level of flavonoid (mg/ml)	Total flavonoid in excretion Du (mg)
0-6	3.370	0.077	0.259
6-12	2.770	0.113	0.313
12-24	3.900	0.145	0.564
24-48	4.620	0.185	0.857
SUM	14.660	0.520	1.993

The results of flavonoids measurement of cassava leaf extract in rat urine showed an increase in each measurement time. Flavonoid levels of cassava leaf extract were the lowest at 0-6 h and increased at 12 h after oral administration. The highest level of flavonoid was observed at 24-48 h after oral administration. The dimension (Du) was the mount of flavonoids of cassava leaves extract excreted. The amount of Du was obtained from the calculation of flavonoid levels in rat urine (mg/mL) multiplied by urine volume (ml). Using the Du, the rate of flavonoid excretion of cassava leaf extract was calculated with the time (dt) being the periods of collecting urine. The rate of flavonoid excretion of cassava leaf extract (Du/dt) is shown on semi logarithmic graph in Figure 2. The rate of flavonoids excretion of cassava leaf extract was found the highest at 6-12 hours. Flavonoid excretion rate of cassava leaf extract started to decrease at 24 hours and 48 hours.

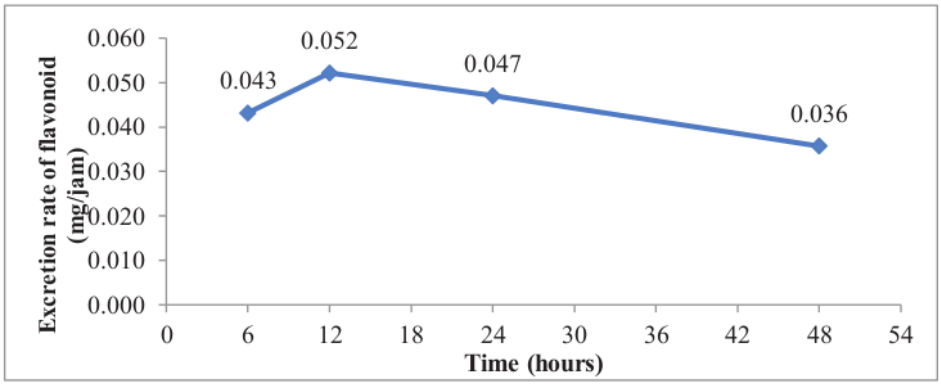

FIGURE 2. Flavonoid excretion rate of cassava leaf extract in rat urine.

DISCUSSION

Drugs or ingredients ingested orally undergo process of absorption, distribution, metabolism, and excretion (ADME), and provide the effects of drug action on the body [6]. Metabolism and excretion are the process of elimination of drugs or medicinal ingredients from the body, and it can be through the lungs, kidneys, feces and bile. Urine in pharmacokinetics studies is used to measure the bioavailability of drugs or drug ingredients.

Natural compounds commonly used as drug ingredients are flavonoids, the most common phenolic compounds in plants [7]. Flavonoids commonly found in green plants, except algae. The main flavonoid of cassava leaves is Rutin, a flavonoid glycoside group consisting of flavanol quercetin and disaccharide [8]. Rutin hydrolyzed by *Lactase Phlorizin Hydrolase* (LPH) inside the intestinum and it becomes aglycone flavonoid in the shape of *isoquercetin (quercetin 3-glucoside)* [9]. Aglycone is absorbed and transported to the liver through the hepatica vein to further metabolism. Inside the liver, aglycone is metabolically converted into *O*-methylated, sulphates, and glucuronide. O-methylated, sulphates, and glucuronide goes to the blood to the target organ or tissue and undergoes urine excretion [10, 11]. Small part of metabolite goes to small intestine along with bile and excreted through the feces. Conjugate of

glucuronide and sulfate is excreted with bile and urine within 48 hours [12]. Unabsorbed flavonoids in intestinum is excreted through feces.

Excretion is influenced by many factors such as kidney function, liver function, and urine volume. Disturbed kidney and liver functions would cause slow excretion. It is because the medicine hardly continues to the kidney to be excreted but accumulated in the organ and the tissue. The urine volume would also affect the excretion process; the more volume of urine, the more efficient the excretion would be.

In this study, the lowest flavonoid of cassava leaves extract appears at 6 hours after oral administration of flavonoids. Flavonoids levels of cassava leaves extract in urine increased 12 hours, 24 hours, and 48 hours after oral administration. This condition indicated that Rutin has slow distribution in the circulation than quercetin. Rutin must be hydrolyzed by enzymes from bacteria in the intestine to be easily absorbed by the intestine [9]. This causes the cassava leaves extract flavonoids likely to be excreted beyond 48 hours. However, different subtances may have different excretion rates, depending on their types and molecular structures.

Another study used six major flavonoids of *Fructus Sophorae* extract. The six major flavonoids of *Fructus Sophorae* extract such as sophoricoside, genistin, genistein, rutin, quercetin, and kaempferol were analyzed from urine and bile samples. The study reported that in bile sample six flavonoid of *Fructus Sophorae* extract were excreted at 36 hours. Six flavonoids of *Fructus Sophorae* extract in urine sample excreted completely in 96 hours. Their results suggested that the excretion six flavonoid of *Fructus Sophorae* extract via bile and urine was low. This observation suggests that six flavonoids of *Fructus Sophorae* extract excreted through other excretion or changed into their metabolites [13].

In this study, the percentage of flavonoid of cassava leaf extract that was excreted within 48 hours was 5.56% from the total of 35.85mg/ml flavonoid. Flavonoids of cassava leaf extract experienced intestinal excretion through feces and bile. The flavonoid's excretion of cassava leaves though urine was less from the total 35.85mg/ml is only 0.520 mg/mL

Excretion rate described how fast a substance is removed from the body. The data of excretion rate are important to understand the clearance of flavonoid from the blood. The clearance data needed to determine how often a substance or drug must be given. The clearance of substance was influenced by several factors included renal function, molecule weight, solubility and other physicochemist of substance. Rutin, the main flavonoid of cassava leaves has molecular weight 610.521 g/mol and solubility 1.25 mg/L. These physicochemist may cause the substance to stay longer in the tissue or blood.

Flavonoid of cassave leaves' exctract was slow released through urine. It was suggested that flavonoids of cassava leaves extract remained in the blood and tissues more than 48 hours. The probability is that, flavonoid of cassava leaves extract has been converted to a metabolite undergoing excretion through the feces. The study explored the distribution and elimination profile of α mangostin compounds reported that after 6 hours of single oral dose of 20 mg/kgBW in rats, the means α mangostin compound were 9.12 µg/g (0.3%), 7.83 µg/g (0.26%), 7.43 µg/g (0.24%), 3.32 µg/g (0.11% %) and 2.23 µg/g (2.23%) in the cardiac, lungs, liver organ, kidney organ and intestine, respectively. Elimination analysis of α mangostin compound was performed on urine and feces samples measured at 0, 6, 12, 24, 36, and 48 hours after oral administration; α mangostin level in urine after 6 hours remained not detected. Levels of α mangostin compound began to be detected in 12 hours of urine by 0.019% and undetectable at 24, 36, and 48 hours after oral administration. In the feces sample, the α mangostin compound was not detected at 6 h, while detectable at 12 h (0.107%), 24 h (0.257%) and 36 hours (0.091%). The compound was then and undetectable at 48 h after oral administration. α mangostin compound was detected higher in feces than in urine, indicating its conversion into a metabolite, undergoing excretion through the feces [14]

Study on wogonin (5,7-dihydroxy-8-methoxyflavone) which is the main flavonoid extract of *Scutellaria baicalensis* root at a single oral dose of 20 mg/kg. It was reported that of 21% of extracts given, 16.33 % was excreted through feces, 4.13% through urine, and 0.41% via bile. This suggests that wogonin (5.7-dihydroxy-8-methoxyflavone) undergoes intestinal excretion along with its metabolism in conjugated form [15].

CONCLUSION

The urinary excretion rate of cassava leaves extract was slow release from body (>48 hours) or perhaps its excreted through other excretion routes, in which allowed the accumulation within organs and tissues. Another possibility was the flavonoids extract of cassava leaves were undergo the excretion through feces and bile. Further research is needed on the levels of flavonoids in feces and bile to complement the flavonoid excretion data of cassava leaf extract in rat urine beyond 48 hours.

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