The Effect of Ginger *(Zingiber Officinale* Roscoe*)* Fractionation in Decreasing Uric Acid Level of Hyperuricemic White Mice

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Abstract: This study aims to determine the effects of ginger fractionation on uric acid level of hyperuricemic white mice. The study was conducted in 7 days using an in vivo experimental method. Ginger was extracted with 70% ethanol and subsequently fractionated by using n-hexane, ethyl acetate, and water residual solvents. The mice divided into six group treatments. Doses used of the fraction n-hexane, ethyl acetate, and water residual were 100 mg/kg of body weight and allopurinol as a standard was 10 mg/kg of body weight. The examination was carried out on mice with hyperuricemic induced by fresh chicken liver juice 0.5 ml/20g of body weight administered orally. The uric acid levels were measured by NESCO® Multi Check Digital tools. The result of this study showed that ethyl acetate fraction doses 100 mg/kg of body weight are the best fraction to lowering level of uric acid in blood serum.

1 INTRODUCTION

Gout is one of noncontagious disease that has high prevalence in the world. Gout is considered to be primarily a male disease, but there is a more equal sex distribution among elderly patients (Pokhrel et al., 2011). Based on World Health Organization (WHO) survey, Indonesia is the largest state number 4 in the world whose population is suffering from gout and uric acid disease is estimated to occur in 840 people of every 100,000 people. The prevalence of uric acid disease in Indonesia occurs at age below 34 years old by 32% and above 34 years old by 68% (WHO, 2015). In Indonesia, hyperuricemic attacking people under 34 years old with prevalence 1.6-13.6/100.000, and it is predicted keep increasing day by day (Thayibah et al., 2018).

Gout is caused by increased levels of uric acid in the blood (hyperuricemia) exceeding normal levels, giving rise to needle-shaped uric acid crystals and causing stiffness and inflammation in the joints. Hyperuricemia is a condition where the kidneys fail to excrete uric acid, resulting in high uric acid levels. Hyperuricemia is a considered to be closely associated with increased risk for developing gout, cardiovascular diseases, hypertension, and metabolic syndrome (Chen Yu-Chen et al., 2014)

High levels of uric acid are caused by the deposition of monosodium crystals due to the breakdown of purines or combination of both. Normal uric acid levels in the blood range between 3.6 - 8.2 mg / dl in men and 2.3 - 6.1 mg / dl in women. High level of uric acid serum caused by disturbances metabolism of purine in the body, heredity, lifestyle, and consume food containing high purine, for example meat, shells and viscera (Misnadiarly, 2017).

Everyone has uric acid in his body which in normal metabolic condition used to produce uric acid and the amount of uric acid must not exceed normal. Uric acid provided by our body about 85 percent and it is mean that the body only needs about 15 percent purine from food. If the body consume high contain purine from food, it causing gout disease and if can progress in to worse condition into coronary heart disease because crystal of uric acid in endhotelium can blocked blood vessels. Therefore high uric acid levels should be treated so that the impact does not fall in to worse condition (Indriawan, 2010).

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A variety of treatment techniques have been used to treat gout, such as the use of Non-Steroidal Anti-Inflammatory compounds (NSAIDs) and the use of Xanthine Oxidase (XOD) inhibiting compounds to reduce uric acid production. Allopurinol is currently the most effective XOD inhibitor, which is used for treating hyperuricemic and gout by reducing circulating levels of uric acid and vascular oxidative stress. However, serious side effects include skin rashes and allergic reactions that occur in some clinical patients (Chen Yu-Chen et al., 2014). The emergence of side effects and various deficiencies in modern medicine demands to find drugs with the smallest possible side effects, one of alternative to this situation is to use traditional medicine.

Ginger is one of the herbs used for traditional medicine in treating gout. Ginger (*Zingiber officinale* Rosc) is a potential herbal plant according to RISTOJA data in 2015 that is used by Indonesian people for lowering of uric acid levels in the blood. There are 178 plants used in the herb for rheumatism/gout and ginger (*Zingiber officinale* Rosc) is the most widely used herbal plant as a herb to lower uric acid complaints in Indonesia. In addition, ginger extract has also been done a scientific search, get the result that ginger ethanol extract (*Zingiber officinale* Rosc) can be potential as anti hyperuricemia (Lallo, et al., 2018; Yulion, et al., 2017) where the data supports this research.

Jolad et al. (2014) identified more than 60 compounds in fresh ginger grouped into two broader categories; volatiles and non-volatiles. Volatile compounds including sesquiterpene and mono terpenoids hydrocarbons providing the distinct aroma and taste of ginger and non-volatile compounds include gingerols, shogaols, paradols and zingerone.

Ginger contain secondary metabolite such as phenolic compound, flavonoid which is can lowering level of uric acid in the blood serum (Andriani, 2018). The mechanism for lowering uric acid levels is by inhibiting the conversion of xanthine into uric acid. The level of uric acid can be derived from excessive production or less excretion through the kidney (Facchini, et al., 1991). This study aims to determine the effect of ginger fractionation (*Zingiber officinale* Rosc) for 7 days on serum uric acid levels of mice induced by chicken liver juice.

The absence of therapy that was found safe and quick to patients with gout while patients with gout is in reproductive age in productivity and patients with gout is very big amount in Indonesia that based on the above information then researchers interested in doing research ginger (*Z. officinale* Rosc) in lowering uric acid and in the form of fractionate. From this research will be proven that fractionation of ginger (*Z. officinale* Rosc) able to lower the levels of uric acid in 7 day (Lembaga Ilmu Pengetahuan Indonesia, 2016).

The research was conducted to obtain activity extract ethanol on antihiperurisemia in fractionate with ethyl acetate, n-hexsan and fraction of water from ginger (*Zingiber officinale* Rosc) using animal testing male mice (Mus musculus) to be raised levels uric acid first with induced chicken liver juice and to compare positive impact with using allopurinol.

2 RESEARCH METHODOLOGY

Materials and Methods

The materials used in this study were chicken liver juice, ethanol 70% (bratachem), ethyl acetate (bratachem), n-hexsane (bratachem), aqua destilata (bratachem), food standard Squeaky, allopurinol (Kimia Farma), Na-CMC, Nesco®, and test strips uric acid Nesco®.

Plant materials

Gingers (*Z. officinale* Rosc) were collected from Sariak Laweh Sub-district of West Sumatra province Agam Regency. This species was identified by ANDA Herbarium Department of Biology Faculty of Mathematics and Natural Sciences University of Andalas.

Preparation of extracts

Ginger (*Z. officinale* Rosc) were washed well to remove the dust and other foreign materials, dried and grounded into tea size powder. The powder was extracted by maceration with ethanol 70% for overnight at room temperature. This process was repeated two times with the same type and amount of solvent. The extracts were combined and evaporated by using rotary evaporator (Buchi Rotavapor R-200) under reduce pressure to obtain ethanol crude extract (Departemen Kesehatan, 2008).

Fractionation procedures

The ethanol crude extract from *Z. officinale* Rosc was dissolved in distillated water. Then it was extracted successively with different organic

solvents which were hexane and ethyl acetate with ratio 1:1 (v/v) in the separatory funnel. The funnel was shaken for 2 minutes and left at room temperature for 2 hours to settle down. The procedure was repeated for several times to obtain hexane, ethyl acetate, and residual water fractions. All crude extracts were evaporated completely by using rotary evaporator (Buchi Rotavapor R-200) under reduce pressure to obtain dry crude extracts (Arifin, Fahrefi, & Dharma, 2013; Ningdyah, Alimuddin, & Jayuska, 2015; Parwata, Rita, & Yoga, 2009; Tahir, Saleh, & Pasaribu, 2013).

Treatment of experimental animals and dosing design

The mice were divided into six groups containing seven animals in each with different treatments on each group and given variation of fraction with doses 100mg/kgBB in 7 days. A total of 42 male mice with age 3-4 m and weighing 20-30 g were used for this study. The mice adapted for 7 days with the environment at constant temperature before used as experimental objects. They were given feed standard pellets and water during the study. The mice were considered being healthy if the difference of their weight before and after adaptation less than 10% and indicates normal behavior visually (Vogel, 2008).

Male mice were selected for this study based on consideration that male mice are lack of estrogen. This condition makes stress level of male mice lesser than female mice, a factor that might disruptive during the study period. And if they've estrogen, the amount of this hormones exist only in relatively few (Suhendi, Nurcahyanti, Muhtadi, & Sutrisna, 2011).

Making inducers from chicken liver

Chicken liver juice used as inducers to make hyperuricemic condition. The inducers given to mice with oral using sonde with doses 25ml/kgBB (Hayani dan Widyaningsih, 2011). Chicken liver is one of food that have high contain of purine making it suitable for hyperuricemic condition. Chicken liver contain 150-1000 mg purine per 100g liver and able to induce high uric acid levels.

Standard

Standard in this study using allopurinol, which is an antigout drug. Allopurinol used as standard with doses 10 mg/kgBB.

Antihyperuricemic activity

Preliminary study purpose to know the fraction that has the best activity in lowering uric acid levels in experimental animal blood. The mice were divided into six groups containing seven animals in each with different treatments on each group, as seen in the following table:

Table 1. Group Treatments

No	Group	Treatment
1	Negative Control	Administered orally solution of Na-CMC 0.5%
2	Positive Control	Induced by chicken liver juice 0.5 ml/20g bw
3	Hyperuricemia + ginger n-hexane fraction	Induced by chicken liver juice 0.5 ml/20g bw and n-hexane fraction dose 100 mg/kg bw
4	Hyperuricemia + ginger ethyl acetate fraction	Induced by chicken liver juice 0.5 ml/20g bw and ethyl acetate fraction dose 100 mg/kg bw
5	Hyperuricemia + remaining fraction of ginger water	Induced by chicken liver juice 0.5 ml/20g bw and water residual fraction dose 100 mg/kg bw
6	Hyperuricemia + allopurinol	Induced by chicken liver juice 0.5 ml/20g bw and allopurinol dose 10 mg/kg bw

At the early stage, all experimental animals are conditioned to hyperuricemia except negative control, using inducer chicken liver juice doses 0.5 mL/20g of body weight with oral administration of Probe until the level of blood acidity increase. The process of hyperuricemic condition carried out for seven days. Fraction was given 1 hour after induction process of hyperuricemic (Suhendi, Nurcahyanti, Muhtadi, & Sutrisna, 2011) and then uric acid levels measured everyday using NESCO® Multi Check Digital tools it done 2 hour after fraction was given (Muhtadi, Suhendi, W., & Sutrisna, 2013).

Statistical analysis

The statistically significant of difference was calculated by the analysis of variance followed by Duncan's Multiple Range Test.

3 RESULTS AND DISCUSSION

A total of 600 grams of rhizomes have been dried, refined and then extracted with ethanol 70% producing a condensed extract of 160.5 grams. The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction

(Pandey and Tripathi, 2014). The pieces should not be too big, otherwise the solvent will not be able to penetrate the innermost cells. They also should not be reduced to powder, that would result in losing the volatile active ingredients contained inside the plant, and also losing the difficult separation by filtration of the plant material from the liquid used once maceration is completed.

The solvent must be chosen based upon the chemical nature of the compounds contained within the plant. Since the end product will contain traces of residual solvent, the solvent should be nontoxic and should not interfere with the bioassay (Pandey and Tripathi, 2014). Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption (Quy Diem Do et al., 2014). The reasons for the selection solvent consisting of ethanol by 70% and water by 30% because ethanol 70% volatile, cheap, can afford and safe (Azis, et al., 2014). The obtained yield was 26.75%. Results of the result obtained, above the value stated in the Indonesian Herbal Pharmacopeia is not less than 6.6% (Departemen Kesehatan, 2008).

Sample 600 grams dry extracted, with the purpose to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent (Endarini, 2016). Extraction is the separation of medicinally active portions of plant (and animal) tissues from the inactive/inert components using selective solvents through standard procedures. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Sruthi and Indira, 2016).

Technique extraction that used in research it is technique maceration because this technique relatively simple does not require an apparatus complicated and can avoid all compound due to heat components (Efendi, 2018). Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.) inside an airtight container, for a variable time based on the plant material and liquid used. In maceration (for fluid extract), whole or coarsely powdered plant-drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. At the end of the process the solvent is drained off and the remaining miscella is removed from the plant material through pressing or centrifuging (Pandey and Tripathi, 2014; Silva et al, 2017).

A total of 50 grams of condensed extracts of ginger leaves are fractionated consecutively by using polar, semi-polar, and nonpolar solvents. The ethanol extract was fractionated with water and n-hexane to obtain semi-polar fraction. The water layer was fractionated with ethyl acetate solvent to obtain semi-polar fraction of ethyl acetate and polar water fraction. In principle, the polar solvents dissolve polar solutes and nonpolar solvents dissolve non polar solutes that are also called “like dissolve like” (Harborne, 1987; Mariana, Andayani, & Gunawan, 2013). The amount of viscous fraction obtained is n-hexane 4.22 gram, ethyl acetate 5.51 gram, and water remaining 27.5 gram with a yield percentage of 8.44%; 11.01% and 55% respectively.

Hyperuricemia is an elevated uric acid level in the blood. This elevated level is the result of increased production, decreased excretion of uric acid, or a combination of both processes. Hyperuricemia can lead to gout and nephrolithiasis (George and Minter, 2019). Uric acid is formed when purines break down in your body. Purines are chemicals found in certain foods include all meats but spesifically organ meats, game meats and some seafood.

For hyperuricemic condition, the mice were given chicken liver juice as inducers because chicken liver contain high level of purine (≥300 mg/100 g). Chicken liver juice used as inducer of uric acid with the dose is 0.5 mL/20g of body weight for animal experiments until the level of uric acid increase (Juwita et al., 2014).

Allopurinol used as standard in this study because allopurinol is widely known as a synthetic drug widely used for hyperuricemic. Allopurinol is an alternative compound which used to increase uric acid excretion through inhibition of the xanthine oxidase enzyme and 80% is absorbed after oral administration. Like uric acid, allopurinol itself is metabolized by xanthine oxidase to allantoxanthine, maintains the capacity to prevent xanthine oxidase and has along enough effect duration so that the treatment is sufficient once a day (Katzung, 2007).

This study using 42 male white mice with average weight 20-30 grams and average age 3-4 months. The acclimatization procedurs was carried out in 7 days so that the animals can adaptation with the environment. The mice must be healthy, showing normal behaviour and have shining clear eyes. During maintenance mice were feed and drinking enough (Malole, 1989).

After acclimation, treatments animals divided into six groups with each groups consisting of seven mice. Three groups animals given variation of types fraction such as n-hexane, ethyl acetate, and residual water with doses 100mg/kgBB in seven days as testing groups. One group animals as negative control which were not received any treatment, one

group as positive control which were induced by chicken liver juice 0.5 ml/20g bw, and one group given chemical drugs allopurinol as standard. Fraction was given 1 hour after induction process of hyperuricemic, and then uric acid level measured everyday using NESCO® Multi Check Digital tools it done 2 hour after fraction was given.

Table 2. Effects of *Zingiber officinale* Rosc fractions on level of uric acid serum in mice induced chicken liver juice

Group/day	1	2	3	4	5	6	7
Negative	6,8 ± 2,3	6,01 ± 2,0	6,3 ± 1,9	4,4 ± 1,7	5,8 ± 1,4	5,0 ± 1,5	5,3 ± 1,9
Positive	9,4 ± 2,9	9,3 ± 2,8	9,2 ± 2,6	7,4 ± 3,9	6,5 ± 3,3	7,4 ± 2,8	7,1 ± 2,0
n-Heksan	6,9 ± 2,2	6,5 ± 1,6	5,6 ± 1,7	4,4 ± 1,3	4,1 ± 1,0	4,2 ± 1,0	3,9 ± 0,6
Ethyl Asetate	5,7 ± 2,1	5,3 ± 1,2	4,8 ± 1,0	4,1 ± 0,8	3,7 ± 0,5	3,8 ± 0.6	3,9 ± 0,4
Water	6,0 ± 2,4	5 ± 1,5	5,6 ± 0,9	4,7 ± 1,7	4,2 ± 1,7	4,1 ± 1,2	4,4 ± 0,9
Allopurinol	6,7 ± 2,4	5,5 ± 1,6	5,7 ± 1,3	9,1 ± 4,5	5,1 ± 2,2	5,8 ± 2,1	5 ± 1,3

From the above table it can be concluded that the ethyl acetate fraction is the best fraction that can reduce uric acid levels in mice compared to the n-hexane fraction and residual water.

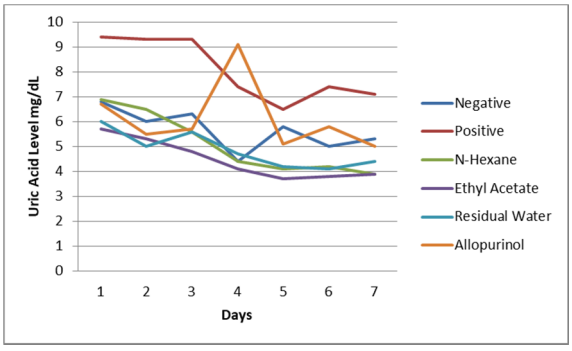

Figure 1. The effect of ginger (*Zingiber officinale* Rosc) fractionation in decreasing uric acid level

The effect of ginger (*Zingiber officinale* Rosc) fractionation in decreasing uric acid level of hyperuricemic white mice show at figure 1. In the Figure 1, we can see the level of uric acid decrease in every fraction.

Ginger (*Zingiber officinale* Rosc) contains over 400 different compounds. The mayor constituents are carbohydrates (50-70%), lipids (3-8%), terpens, and phenolic compounds (Prasad and Tyagi, 2015). Flavonoid is a group of plant phenolic compound that act as antioxidant such as quercetion, rutin, catechin, epicatechin, kaempferol and naringenin (Ghasemzadeh et al, 2010). Due to their importance in human health, ginger can be used to pharmacological activites like anti inflammation, anticancer, antioxidant, antiplatelet, and can be used to reduce cholesterol and uric acid level (Rehman, 2011).

Flavonoid functions as an inhibitor in uric acid formation and able to reduce the level of uric acid by blocking xanthine oxidase, the enzyme responsible for regulating uric acid formation.

In this study, variation of fraction used to knowing which the best fraction to lowering level of uric acid. The fractionation process was carried out in stages based on level of polarity, from polar, semi polar and nonpolar solvents. From the experiment, Duncan's Multiple Range Test used to determine the best fraction for lowering uric acid level.

Table 3. Duncan's Multiple Range Test of Uric Acid Level

Uric Acid Activity						
			Subset			
	Group	N	1	2	3	4
Duncan[a]	Ethyl Acetate 100mg/kgBB	49	4.451			
	Water 100mg/kgBB	49	4.865	4.865		
	n-Hexane 100mg/kgBB	49	5.067	5.067		
	Negative Control	49		5.667	5.667	
	Allupurinol 10mg/kgBB	49			6.135	
	Positive Control	49				8.031
	Sig.		.150	.060	.247	1.000

Test results from analysis Duncan's Multiple Range Test showed that the best fraction for lowering level of uric acid serum is ethyl acetate fraction, because the statistical value of ethyl acetate has different subset with other test groups. It means, suspected active compounds that are given can lower the best of uric acid levels in the blood is in the semi-polar fraction. While on the allupurinol showed that the effect is not different significantly with ethyl acetate. It because the dose of the fraction that used in this study is greater than the dose of Allupurinol.

4 CONCLUSIONS

A total of 600 grams of rhizomes have been dried, refined and then extracted with ethanol 70% producing a condensed extract of 160.5 grams. A total of 50 grams of condensed extracts of ginger leaves are fractionated consecutively by using n-hexane, ethyl acetate, and residual water. The amount of viscous fraction obtained is n-hexane 4.22 gram, ethyl acetate 5.51 gram, and water remaining 27.5 gram with a yield percentage of 8.44%; 11.01% and 55% respectively. From the study can conclude that ethyl acetate fraction of *Zingiber officinale* Rosc is the best fraction for lowering level of uric acid serum. For the future study we can use variation of doses, pathology with other diseases or isolation of pure compound from ginger.

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