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Phytochemical Screening and Anti-Hyperuricemia Activity Test In Vivo of Ethanolic Extract of Shallot (Allium cepa L.) Skin Abstract Uric acid is the final product of purine metabolism that will be excreted through urine, feces, and sweat. Excessive production of uric acid can cause hyperuricemia, known as gout. The skin of shallots (Allium cepa L.)

is one of the household wastes that are very rarely used by the community. Ethanol extract of shallot skin (EESS) was tested for phytochemical screening and anti-hyperuricemia activity using potassium oxonate. Mice were divided into five groups (Allopurinol, Na-CMC, EESS 200 mg/kg BW, 300 mg/kg BW, and 400 mg/kg BW) and uric acid levels were observed at 2-hour intervals for six hours. Phytochemical screening shows that EESS has potential compounds in the treatment of gout.

Tests to reduce uric acid levels showed that EESS has better potential than allopurinol at concentrations of 300 mg/kg BW and 400 mg/kg BW after six hours of induction in reducing uric acid levels. Keywords: Allopurinol Hyperuricemia Potassium oxonate Shallot Shallot skin INTRODUCTION Uric acid is a product that produces from genetic excretion and purine metabolism, but the cause of gout is food that is rich in purines, alcohol consumption and being overweight (Tausche et al., 2009; Maiuolo et al., 2016).

When uric aci d increase will discomfort it's with and disease (Kutzing & Firestein, 2008; de Oliveira & Burini, 2012; Avarez-Lario & Macarrón-Vicente, 2011). The increasing uric acid levels can cause urate saturation, the formation of monosodium urate crystals, and the interaction of monosodium urate crystals with leukocytes that lead to acute grout arthritis (Martillo et al., 2014; Suresh, 2005). Normal levels of uric acid in the blood based on sex in adults is 7.0

mg/ dL for men and 6.0 mg/dL for women (Jin et al., 2012; Shani et al., 2016). Gout is often called arthritis gout because it is caused by high levels of uric acid in the blood. High levels of uric acid in the blood that exceeds normal limits can cause the accumulation of uric acid in the joints and other body organs (Avarez-Lario & Macarrón-Vicente, 2011). Uric acid which has accumulated can cause joint pain, pain, and inflammation (Roddy & Choi, 2014).

Further effects of arthritis gout are the inability to walk, joint pain when moving, experiencing damage to the joints, and can even cause people with disabilities (Ragab et al., 2017; Lv et al., 2019; Grygiel-Górniak & Puszczewicz, 2014). This increasing prevalence is associated with gender risk factors, intake of a purine-rich diet (Kanbara & Seyama, 2011), alcohol, obesity, hypertension, impaired kidney function, and genetic factors (Ragab et al., 2017; Benn et al., 2018).

The medications commonly used are allopurinol and febuxostat because due to their mechanism by inhibiting xanthine activity in reducing uric acid levels (Chen et al., 2019; McDonagh et al., 2014). Traditionally, natural compounds are believed to have the potential to reduce uric acid levels (Lv et al., 2019). One of the natural ingredients that are reported to be investigated for its potential activity as an acid-lowering uric acid level is the shallot (Allium cepa L.).

The shallot has flavonoids (quercetin) and other phenolics. Quercetin and phenolics are believed to have the potential to reduce uric acid levels (Rahmat et al., 2018). This study aim is to test the potential of ethanol extract from shallot skin on the uric acid levels which is tested in vivo on male white mice (Mus musculus). MATERIALS AND METHODS Plant collection Fresh shallot (Figure 1) was collected from the local area of Medan, North Sumatra, Indonesia and authenticated by Herbarium Medanense, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara, Indonesia (No.2329/MEDA/2018).

Fresh leaf of shallot skins was dried by aerating at room temperature and avoiding direct sunlight. The dried shallot skin then powdered by mechanical milled. (a) (b) Figure 1. Shallot peels in the form of (a) dried and (b) simplicia powder Preparation of ethanol extract of shallot skin About 1,000 g of the dried shallot skin powder was extracted with 96% ethanol by using the maceration method for five days at room temperature.

The ethanol extract of shallot skin (EESS) solution part was evaporated by using a rotary evaporator at 50 °C to obtain the crude extract (Adawia et al., 2016). Preliminary phytochemical screening The crude EESS was screened by using the standard protocol of phytochemical screening to see the presence of phytochemical compounds. Alkaloids

are detected by Dragendorff's test, flavonoids with Salkowski test, saponins with the frothing test, tannins with ferric chloride test, and steroids as well as terpenoids with Salkowski test (Adawia et al., 2016; Haro et al., 2018).

Animal preparation Healthy adult white male mice (± 25 g) were obtained from Animal House of Sekolah Tinggi Ilmu Kesehatan Senior Medan, Indonesia. Mice were housed in polycarbonate cages at room temperature with 12 hours day-night circle and feed on standard animal pellets and water freely. This study was conducted according to the ethical norms by the National Health Research Ethics Committees and was approved by the National Health Research Ethics Committees, Faculty of Mathematics and Natural Science, Universitas Sumatra Utara (No. 0401/KEPH- FMIPA/2019).

The animals were fasted for 12 hours before experimentation but allowed free access to water. Anti-hyperuricemia activity Test animals were divided into five groups, each consisting of five mice. Determination of dose refers to previous research by modifying previous research by Ningsih and Fahrudin (2018): Group I : Allopurinol suspension with 10 mg/kg BW Group II : 1% Na-CMC Group III : suspension of EESS with 200 mg/kg BW Group IV : suspension of EESS with 300 mg/kg BW Group V : suspension of EESS with 400 mg/kg BW Firstly, the level of uric acid was measured. Then, each mice was induced by potassium oxonate intraperitoneally.

Two hours later, uric acid levels were measured again in each of each group of mice, then oral preparations of each group were given. The uric acid level was measured again after four hours and six hours. Data were analyzed statistically by used ANOVA and Tukey's HSD tests with significantly different ($p < 0.05$).

RESULTS AND DISCUSSION Preliminary phytochemical screening Phytochemical screening is performed to determine the content of the secondary metabolites presented in Table I. It is predicted that the flavonoids contained in the EESS will have the effect of reducing uric acid levels. The ability of this compound to reduce uric acid is inhibited by the mechanism of xanthine oxidase activity in the purine base to inhibit the production of uric acid and prevent inflammation of the joints (Ewadh et al., 2015).

Besides, the presence of various components of bioactive compounds is considered to have uric acid-lowering activity (Bakar et al., 2018). Table I. Phytochemical screening of EESS Phytochemical compounds Results Alkaloids + Flavonoids + Saponins + Tannins + Steroids/Triterpenoids + Anti-hyperuricemia activity In this study, treatment was divided into five groups: the negative control group with 1% Na-CMC suspension, the EESS treatment group with three concentration variations (200, 300, and 400 mg/kg BW), and the positive control group with 10 mg/kg BW of allopurinol. Test animals are induced by

potassium oxonate to develop hyperuricemia.

Potassium oxonate has the function of increasing uric acid levels (Garcia-Arroyo et al., 2018; Oh et al., 2019). The first measurement is taken when the test animal is fasted, followed by the induction of potassium oxonate. The second measurement was taken after two hours of induction. After the uric acid measurement data were obtained, treatment was given to each group.

The third measurement is performed after the next two hours (four hours after induction and treatment). The fourth measurement after the next two hours (six hours after induction) and treatment are shown in Table II. Table II. Uric acid level in various treatment Group Fasting uric acid level (mg/dL) Uric acid level (mg/dL) 2 hours 4 hours 6 hours I 3.14 ± 0.055 4.64 ± 0.602* 4.24 ± 0.568* 3.8 ± 0.604** II 3.14 ± 0.033 4.5 ± 0.4 4.5 ± 0.4 4.48 ± 0.444 III 3.14 ± 0.27 4.42 ± 0.35* 3.74 ± 0.195* 3.16 ± 0.055** IV 3.14 ± 0.114 4.78 ± 0.415* 3.24 ± 0.560*** 2.4 ± 0.071*** V 3.14 ± 0.089 4.68 ± 0.683* 2.86 ± 0.358*** 1.34 ± 0.114*** Result are expressed as mean ± SD; n = 5; *p >0.05; ** p <0.05; *** more significant comparing with allopurinol Observation and measurement of the EESS treatment groups with three concentration variations (200, 300, and 400 mg/kg BW) in animal tests for anti-hyperuricemia activity.

Bioactive content was present in the EESS, such as phenols, flavonoids, flavonoids, and others containing decreasing levels of uric acid, as reported by Benítez et al. (2011). Bioactive components, particularly phenols and flavonoids, are supported by acting as an inhibitor of xanthine enzyme oxidation, which works to reduce uric acid levels (Oskoueian et al., 2011; Kapoor & Saxena, 2016).

Statistical analysis Statistical analysis of ANOVA followed by Tukey HSD analysis showed that there were differences in the effects of EESS treatment on decreased uric acid levels. A significant decrease in uric acid levels occurred after four hours of induction with allopurinol and EESS with concentrations of 300 and 400 mg/kg BW.

Measurement of uric acid levels after six hours of induction there was a significant difference in the administration of allopurinol and EESS at concentrations of 200, 300, and 400 mg/kg BW, where EESS at concentrations of 300 and 400 mg/kg BW showed better activity than allopurinol. The results obtained are different from those reported by Haidari et al. (2008), who reported that A.

cepa skin juice has lower anti-hyperuricemia activity than allopurinol. Based on previous research, the results of the analysis by HPLC showed that phenolic, flavonoid, and flavanol compounds were the most abundant compounds in each part of shallot. Each

section was reported to show antioxidant activity by testing the ability to reduce iron using measurements with UV-Vis spectrophotometry.

The content of flavonoid compounds, phenolics, flavanols, quercetin (Benítez et al., 2011), and polyphenols are responsible for antioxidant activity and lowering uric acid levels from the shallot and has been tested both in vivo as well as clinical trials (Ouyang et al., 2018).

CONCLUSION Phytochemical screening at EESS contains various potential bioactive compounds (alkaloids, flavonoids, saponins, tannins, and steroid/triterpenoids) as anti-hyperuricemia. Ethanol extract of shallot skin showing activity at doses of 300 mg/kg BW and 400 mg/kg BW better than allopurinol to reduce uric acid levels which were tried in male white mice after induction of potassium oxonates.

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