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 acid levels increase it will cause discomfort and it’s associated with hypertension, and cardiovascular 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 gout arthritis (Martillo et al., 2014; Suresh, 2005). Normal levels of uric acid in 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).

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 (EES) was tested for phytochemical screening and anti-hyperuricemia activity using potassium oxonate. Mice were divided into five groups (Allopurinol, Na-CMC, EES 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 EES has potential compounds in the treatment of gout. Tests to reduce uric acid levels showed that EES 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.

Received: April 24th, 2020
Accepted: July 5th, 2020
Published: August 31st, 2020

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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.

![Shallot peels](image1.png)

**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) | 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 ± 0.338*** | 1.34 ± 0.114*** |

Result are expressed as mean ± SD; n = 5; *p <0.05; **p <0.01; ***p <0.001

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.

ACKNOWLEDGMENT

The authors would like to thank Sekolah Tinggi Ilmu Kesehatan Senior Medan for providing the laboratory.

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