**INTRODUCTION**

Stelechocarpus burahol leaves were used to overcome gout and are capable of lowering cholesterol levels [1]. Fruits of S. burahol also have high vitamin C as efficacious as antioxidants and leaves are now believed to address diabetes [2]. Antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl of ethyl acetate extract of the burahol fruits was higher than the isolates of the active fraction from n-butanol extract of burahol flowers [3]. Antihyperuricemia activity of ethanol and hexane extract from burahol leaves were reported equivalent with allopurinol on the xanthine oxidase inhibition test using mice (*in vivo*) [4]. Water extract of *S. burahol* leaves possesses the potential of lowering the uric acid level of rats [5] and chicken [6].

Phytochemical analysis of *S. burahol* leaves obtained from some areas reported some chemical constituents such as saponin, alkaloid, tannin, phenolic, flavonoid, triterpenoid, steroid, and glycoside. Tannin content of the *S. burahol* leaves originating from West Java (Bogor and Garut) was undetectable, while *S. burahol* leaves from Central Java all contain tannins [2]. Batubara et al. (2012) [7] reported that *S. burahol* fruits had pharmacological activity as an oral deodorant composed of some volatile compounds that were obtained by gas chromatography of ethyl acetate fraction of ethanolic extract of *S. burahol* leaves and models of XO inhibitor downloaded via Protein Data Bank with code 3BDI. The five compounds was performed molecular docking against xanthine oxidase enzyme target using Pyrx.

**RESULTS**

The research was conducted by *in silico* analysis of xanthine oxidase (XO) inhibitors of volatile compounds from ethyl acetate fraction of ethanolic extract of *S. burahol* leaves and models of XO inhibitor downloaded via Protein Data Bank with code 3BDI. The five compounds was performed molecular docking against xanthine oxidase enzyme target using Pyrx.

The extract (170 g) was hydrolyzed with 400 ml 1 N HCl:methanol (1:1) at 100°C for 30 minutes. The hydrolyzate was fractionated using chloroform and ethyl acetate. The volatile compounds in ethyl acetate were identified by gas chromatography.
XO inhibitory activity assay

The inhibitory effect on XO was measured spectrophotometrically at 290 nm under aerobic condition, with some modifications, following the method reported by Ernawati and Susanti (2001) [14]. A well-known XO, allopurinol (100 µg/ml), was used as a positive control for the inhibition test. Xanthine oxidase activity was determined by adding 200 ml of substrate (xanthine) 0.15 mM in a mixture of 100 µl of xanthine oxidase 100 mU / ml and 724 µl of phosphate buffer pH 7.5. XO activity was determined by observing the rate of formation of uric acid from xanthine by spectrophotometry at a wavelength (λ) of 290 nm from minute 0 up to 3 minutes at a temperature of 25°C. Data were obtained in the form of rate (Δ A290 minutes) [15].

XO inhibitory activity of allopurinol was determined by adding 200 µl of allopurinol at a concentration of 10 µg / ml to 100 µg / ml into a mixture of phosphate buffer, xanthine and xanthine oxidase. In the similar way, also the XO inhibitory activity by 200 ml of test solution (carried out using the orientation of concentration 10-100 ug/ml) was determined.

GC/MS analysis

Gas chromatography was determined with 30 m × 0.25 mm agilent HP 5 MS column. Injection was in the split mode with a 1:33 ratio. The column oven and injector temperatures were 120°C and 310°C, respectively. The linear flow rate of helium gas was 23.7 cm/seconds. GC/MS analysis was carried out using GC/MS QP010S Shimadzu in the AQQ mode with a mass range of m/z 28-600.

Processing and data analysis

The mass spectra from peak chromatogram were analyzed by comparing with the library (NIST62.LIB, WILEY229.LIB) of mass spectra.

Molecular docking

Ligand reference: A set of reference compounds were obtained by screening an online 3D-mimic database with the following procedure: The target protein (the crystal structure of XO the crystal structure of xanthine oxidase (3bdj) obtained from wwwpdb.org, obtained from www.pdb.org [16]. Compounds that would be docked can also be downloaded. Then, they were inputted using Vina.

Molecular screening procedure: All the selected compounds that have a 3D-optimized geometry of the ligand-binding energy is determined with reference to XO in bovine milk source (3BDJ) by the way of docking using Vina. This is done by loading compounds in the table openbabel in PyRx program and by converting all the files into the extension. pdbqt and then Vina wizard was executed by maximizing grid box to obtain the binding energy in kcal/mol units.

RESULT AND DISCUSSION

*S. burahol plants used in the research were determined in the Laboratory of Biology, Faculty of Pharmacy, University of Gadjah Mada. Determination goal is to ensure that the plant specimen is true. The determination was based on the book Flora of Java Vol III [17].

In this study, the leaves were collected at the Yogyakarta District, Yogyakarta Province, Indonesia. Extraction method used was maceration, by soaking the crude drug powder with a liquid solvent. In this study, the solvent used is 70% ethanol. Ethanol 70% was selected due to a more selective, non-toxic, and neutral ability to prevent the growth of mold and bacteria. The extract obtained was evaporated over a water bath until thick consistency (20.12%) was obtained.

The inhibitory effects of ethanolic extract of S. burahol leaves, chloroform fraction, ethyl acetate fraction, and allopurinol are shown in Table 1.

Determination of the activity of xanthine oxidase inhibitory by using spectrophotometric method. XO activity was determined by observing the rate of formation of uric acid from xanthine at a wavelength of 290 nm. Xanthine has an absorption rate in the ultraviolet wavelength of 260 nm [19] over a 6-minute uric acid formation reaction kinetics which is linear [20]. In this study, the rate of formation of uric acid linear was 5 minutes. Thus, the determination of uric acid formation was carried out during the first 4 minutes. Ethanol extract possessed inhibiting activity toward XO enzyme (52.11 µg/ml). Antihyperuricemic activity of ethanolic extract of burahol leaves was reported (4). Antioxidant activity of melatonin extract of burahol leaves was reported (1). Ethyl acetate fraction had the higher activity with IC50 of 0.31 µg/ml than chloroform fraction with IC50 of 9.78 µg/ml. Ethyl acetate fraction had lower activity of 0.31 ug/ml than chloroform fraction of 9.78 ug/ml. Thus, ethyl acetate fraction was separated and detected by GC/MS. The chromatogram of the separation of the compound of the ethyl acetate fraction is shown in Fig. 1.

Table 1: The xanthine oxidase inhibition of ethanolic extract of S. burahol leaves, chloroform fraction, ethyl acetate fraction, and allopurinol

| Concentration (µg/ml) | % inhibition | IC<sub>50</sub> (µg/ml) |
|-----------------------|--------------|------------------------|
| Allopurinol           |              |                        |
| 0.57                  | 37.91        | 4.59                   |
| 1.43                  | 53.59        | 4.29*                  |
| 2.86                  | 77.12        | 3.16**                 |
| Ethyl acetate fraction|              |                        |
| 0.57                  | 49.12        | 0.31                   |
| 1.14                  | 71.93        |                        |
| 2.29                  | 80.70        |                        |
| Chloroform fraction   |              |                        |
| 10.86                 | 45.61        | 9.78                   |
| 54.31                 | 74.56        |                        |
| 108.62                | 81.58        |                        |
| Ethanolic extract     |              |                        |
| 39.81                 | 45.67        | 52.11                  |
| 79.63                 | 60.54        |                        |
| 119.44                | 76.54        |                        |

*Septiningsih et al., 2012 [18] **Ernawati and Susanti, 2014 [14].

S. burahol: Stelechocarpus burahol, IC<sub>50</sub>: Inhibitory concentration 50%

Fig. 1: Gas chromatogram of ethyl acetate fraction of ethanolic extract of Stelechocarpus burahol leaves

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**Fig. 1:** Gas chromatogram of ethyl acetate fraction of ethanolic extract of Stelechocarpus burahol leaves

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The separation of ethyl acetate fraction obtained five compounds: (1) 3-heptene, 2,2,4,6,6-pentamethyl (2.15%), (2) methyl hexadecanoate (4.16%), (3) ethyl tridecanoate (34.63%), (4) methyl octadecanoate (7.26%), and (5) ethyl hexadecanoate (51.8%). The major compound was ethyl hexadecanoate, which was an ester compound. Fig. 1 shows the structure of 5 volatile compounds from the ethyl acetate fraction of ethanol extract of *S. burahol* leaves.

Binding energy of five compounds against XO enzyme were showed in Table 3 below. The retrieved 3 compounds have lower free energy than oxipurinol, so it is suspected that these compounds have inhibitory activity of the enzyme XO better than oxipurinol (Fig. 2). The three-dimensional structure of the enzyme 3 BDJ is shown in Fig. 3.

From the five of the volatile compounds from the fraction of ethyl acetate, it can be observed that the four compounds are esters (methyl hexadecanoate [4.16%], ethyl tridecanoate [34.63%], methyl octadecanoate [7.26%], and ethyl hexadecanoate [51.8%]. Ethyl tridecanoate, methyl octadecanoate, and ethyl hexadecanoate have Gibbs free energy lower than oxipurinol with average free energy (ΔG) -10.7, -7.6, -7.1 kcal/mol. The ethyl acetate fraction has potent compounds as inhibitors of the enzyme XO in the form of ester where the compounds ethyl tridecanoate, methyl octadecanoate, ethyl hexadecanoate have a large percentage, ie respectively 34.63%, 7.26%, 51.8% (Table 2). Previous research reported that ethyl acetate extract and ethyl acetate fraction of ethanolic extract from *Sonchus arvensis* leaves inhibited XO with inhibitory concentration 50% (IC50) 15.29 g/ml and 16.20 g/ml, respectively, more potent than ethanolic extract with IC50 23.64 g/ml [21]. Purwatiningsih et al. (2010) [4] reported that ethanolic extract and hexane extract of *S. burahol* leaves almost have equivalent activity as XO inhibitor with allopurinol. Haddi and Marouf (2015) [22] reported that the crude and ethyl acetate extracts of *Pistacia lentiscus* leaves had XO inhibitory activity (ethyl acetate fraction with 60.2%, necessary IC50 of XO (IC50 = 2.50).

![Fig. 2: Oxypurinol](image)

**Table 2: Some volatile constituents of the ethyl acetate fraction of ethanolic extract of *S. burahol* leaves identified by GC/MS**

| Peak | Retention time | Area % | Name structure | Similarities' index | Library |
|------|----------------|--------|----------------|---------------------|---------|
| 1.   | 17.71          | 2.15   | 3-Heptene, 2,2,4,6-pentamethyl | 78                  | NIST62.LIB |
| 2.   | 22.60          | 4.16   | Methyl hexadecanoate | 94                  | NIST62.LIB, WILEY229.LIB |
| 3.   | 23.96          | 34.63  | Ethyl tridecanoate | 92                  | NIST62.LIB |
| 4.   | 26.57          | 7.26   | Methyl octadecanoate | 94                  | NIST62.LIB |
| 5.   | 27.80          | 51.8   | Ethyl hexadecanoate | 93                  | NIST62.LIB, WILEY229.LIB |

GC/MS: Gas chromatography/mass spectrophotometry, *S. burahol*: Stelechocarpus burahol

![Fig. 3: Xanthine oxidase from bovine milk source (3BDJ)](image)
extracts of Pistacia lentiscus leaves had X0 inhibitory activity (ethyl acetate fraction with 60.2 %, necessary concentration to inhibit 50% of xanthine oxidase enzyme (IC50) = 2.50, the crude with 55.3 %, IC50 = 2.57).

CONCLUSION

The volatile contents of the ethyl acetate fraction of S. burahol leaves' ethanolic extract are 3 heptene, 2,2,4,6,6-pentamethylhexa-2,4-dien-3-olate, methyl octa-decanoate, and ethyl hexadecanoate. There are three chemicals that have free energy lower than oxy-purinol and which inhibit the activity of the enzyme X0, i.e., ethyl tridecanoate, methyl octadecanoate, and ethyl hexadecanoate with free energy as (ΔG)−10.7,−7.6,−7.1 kcal/mol.

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Table 3: Binding energy between 5 volatile compounds from the ethyl acetate fraction with BDJ enzyme

| Compound                  | Binding energy (kcal/mol) | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  |
|--------------------------|--------------------------|----|----|----|----|----|----|----|----|----|
| Heptene, 2,2,4,6,6-penta-metil | −5.8                     | −5.7| −5.7| −5.7| −5.5| −5.5| −5.5| −5.3| −5.1| −5.1|
| Methyl heptadecanoate    | −7.3                     | −7.3| −6.9| −6.9| −6.8| −6.7| −6.6| −6.2| −6.2| −6.1|
| Ethyl tridecanoate       | −12.1                    | −11.5| −11.2| −11.2| −11.1| −10.4| −10.4| −9.9| −9.9| −9.8|
| Methyl octadecanoate     | −8                       | −7.9| −7.8| −7.8| −7.7| −7.7| −7.4| −7.3| −7.2| −7.2|
| Ethyl hexadecanoate      | −7                       | −7.5| −7.4| −7.4| −7.2| −7.2| −7.1| −7.0| −6.6| −6.5|
| Oxy-purinol              | −7.4                     | −7.3| −6.5| −6.4| −6.3| −6.2| −6.2| −6.6| −6.6| −6.6|

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