IN SILICO ANALYSIS OF XANTHINE OXIDASE INHIBITOR OF ETHYL ACETATE FRACTION OF ETHANOLIC EXTRACT OF STELECHOCARPUS BURAHOL (BL.) HOOK F. AND TH. LEAVES

By DINIATIK DINIATIK

WORD COUNT 1312 TIME SUBMITTED 28-DEC-2018 07:15AM
PAPER ID 43117470

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DINIATIK1,2*, SUWIJIYO PRAMONO1, SUGENG RIYANTO1

ABSTRACT

Objective: The research was conducted by in silico analysis of xanthine oxidase (XO) inhibitors of volatile compounds from ethyl acetate fraction of ethanolic extract of Stelechocarpus burahol (Bl.) Hook F. and Th. leaves. The objective of the research was to determine the active compounds as a potential inhibitor of XO using in silico screening method.

Methods: The research was conducted using 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 3BDJ, The five compounds was performed molecular docking against xanthine oxidase enzyme target using Pyrx.

Results: Volatile compounds in the ethyl acetate fraction of ethanolic extract were 3 heptene, 2,2,4,6,6-pentamethyl (2.15%), methyl hexadecanoate (4.16%), ethyl tridecanoate (34.63%), methyl octadecanoate (7.26%), and ethyl hexadecanoate (51.8%). The results showed that three of the chemical compounds have lower free energy value as XO inhibitors than oxypurinol.

Conclusion: They were ethyl tridecanoate, methyl octadecanoate, and ethyl hexadecanoate with free energy (ΔG) −10.7, −7.6, −7.1 kcal/mol.

Keywords: Stelechocarpus burahol (Bl.) Hook F. and Th., Xanthine oxsidase, In silico analysis

INTRODUCTION

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. Therefore, it is crucial to search volatile compounds of these plants that need to be determined using gas chromatography/mass spectrophotometry (GC/MS) method. XO is responsible for metabolizing purine bases. Therefore, this research aims to evaluate the virtual screening as XO inhibitory activity from the compounds in ethyl acetate fraction of ethanolic extract of S. burahol leaves, which was compared to oxypurinol (synthetic XOI) based on energy binding.

METHODS

Plant material preparation

The plant was identified in the laboratory of Plant Morphology and Plant Taxonomy Faculty of Biology, University of Gadjah Mada, Jogjakarta, and was deposited in the Laboratorium of Biology Pharmacy, Faculty of Pharmacy, Muhammadiyah University of Purwokerto. Materials of this research were S. burahol leaves collected from Yogyakarta. The leaves were washed with running water and then dried with natural sunlight under a black cloth. S. burahol leaves were grinded by using a blender.

Extraction

The powder was extracted by maceration technique for 2 days, with 70% ethanol; for the first 24 hrs of maceration, ten parts of the solvent were used for one part of the powder. In the second maceration, four parts of the solvent were used for one part of the powder. The combined macerates were evaporated to obtain a thick ethanolic extract.

Fractionation

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

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 ACQ 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

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 in question was correct. 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 290nm. 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. Ethyl acetate fraction had the higher activity with IC50 was 0.31 ug / ml than chloroform fraction with IC50 was 9.78 ug / 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.

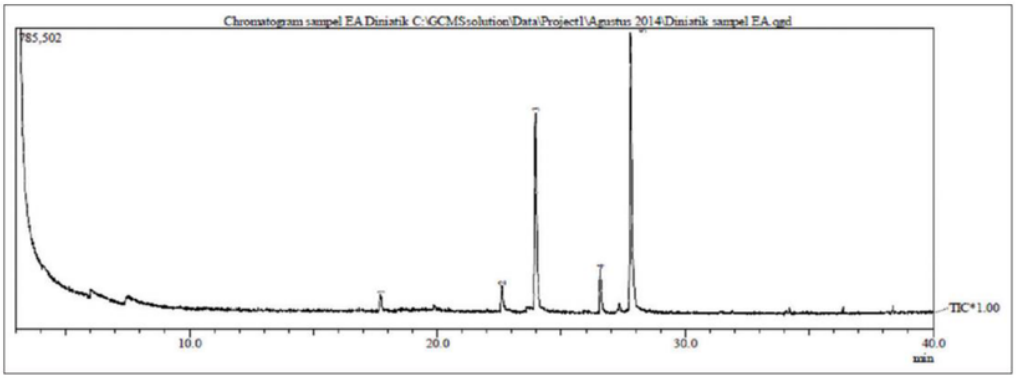

Fig. 1: Gas chromatogram of ethyl acetate fraction of ethanolic extract of Stelechocarpus burahol leaves

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 againts XO enzyme were showed in Table 3 below. The threedimensional 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). acetate extracts of Pistacia lentiscus leaves had XO inhibitory activity (ethyl acetate fraction with 60.2%, necessary IC50 of XO (IC50 = 2.50), Haddi & 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 concentration to inhibite 50% of xanthine oxidase enzyme (IC50) = 2.50, the crude with 55.3 %, IC50 = 2.57).

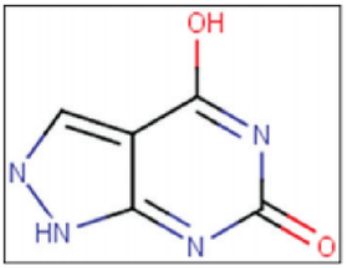

Fig. 2: Oxypurinol

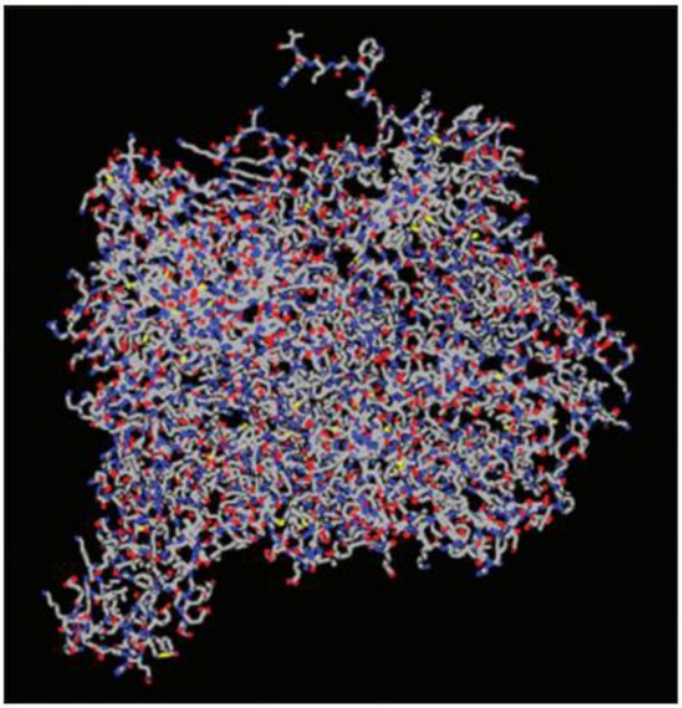

Fig. 3: Xanthine oxidase from bovine milk source (3BDJ)

CONCLUSION

The volatile contents of the ethyl acetate fraction of S. burahol leaves'ethanolic extract are 3 heptene, 2,2,4,6,6-pentamethyl, methyl hexadecanoate, ethyl tridecanoate, methyl octadecanoate, and ethyl hexadecanoate. There are three chemicals that have free energy lower than oxypurinol and which inhibit the activity of the enzyme XO, 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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