

KAEMPFEROL FROM
STELECHOCARPUS BURAHOL, (BL.)
HOOK F. & TH. LEAVES AND
XANTHINE OXIDASE INHIBITION
ACTIVITY

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ABSTRACT

Objective: *Stelechocarpus burahol*, (Bl.) Hook f. & Th. is a plant widely distributed in Java Island of Indonesia. The aim of this study is to identify compounds from the leaves of *S. burahol* that exhibited activity as xanthine oxidase inhibitor (XOI).

Methods: The leaves were extracted with aqueous ethanol and hydrolyzed with HCl methanol, then, partitioned sequentially with chloroform and ethyl acetate. The ethyl acetate fractions were separated by column chromatography with cellulose as stationary phase and methanol 50% as mobile phase.

Results: Purification from this extracts afforded three compounds with one compound identified, namely kaempferol. The four compounds possessed as XOI with IC50 values ranging from 0.27 to 0.45 µg/ml.

Conclusion: Kaempferol exhibited the highest inhibition of 0.27 µg/ml.

Keywords: Kaempferol, Xanthine oxidase inhibitor, *Stelechocarpus burahol*, (Bl.) Hook f. & Th

METHODS

Plant material, extraction, and isolation

The leaves of *S. burahol* were collected from Sleman District, Yogyakarta Province, Indonesia, in August 2013 and identified in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Gadjah Mada University. A voucher specimen was deposited in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Muhammadiyah University of Purwokerto. Leaves of *S. burahol* were dried and pulverized. Water layer separated and followed by addition of ethyl acetate, shaken and allowed to stand for 6 hrs. The ethyl acetate fraction was collected and then separated using column chromatography method with cellulose as stationary phase and methanol 50% as mobile phase. The stationary phase used is 300 g using a column with a diameter of 6.5 cm manufacture of a column using a wet method, by mixing the stationary phase with a mobile phase with a ratio of 1:1 and then poured in a column chromatography, left overnight. The stationary phase was mixed with ethyl acetate fraction (28 g) with a ratio of 1:1, mixed homogeneously, placed on a stationary phase that

has compacted in a column chromatography. Subfractions collected every 30 ml, 130 subfractions thus obtained. During the subfraction taking is monitored using a ultraviolet (UV) lamp in the column. There fluorescence of flavonoids in the column because the flavonoid compound fluoresces under UV light. After separation of active fractions by column chromatography, three compounds were obtained, and one was identified based on spectra data using ^1H - and ^{13}C -NMR.

XO inhibitory (XOI) activity assay

1 A well-known XOI, allopurinol (100 $\mu\text{g}/\text{ml}$) was used as a positive control for the inhibition test. XO activity determined by adding 200 mL of substrate (xanthine) 0.15 mM in a mixture of 100 mL of XO 100 mU/mL and 724 mL of phosphate buffer pH7.5. XO activity determined by observing the rate of formation uric acid from xanthine by spectrophotometry at wavelength (λ) 290 nm from minute 0 up to 3 minutes at a temperature of 25°C. The difference, at this stage the addition of 200 mL **1** allopurinol at a concentration of 10 $\mu\text{g}/\text{mL}$ to 100 $\mu\text{g}/\text{mL}$ into a mixture of phosphate buffer, xanthine, and XO. In similar way, also determined the XOI activity by 200 mL of test solution (carried out using the orientation of concentration 10 $\mu\text{g}/\text{mL}$ to 100 $\mu\text{g}/\text{mL}$).

RESULT AND DISCUSSION

1 The inhibitory effect of ethanolic extract of *S. burahol* leaves, chloroform fraction, ethyl acetate fraction, allopurinol, and isolates have been performed in Graphs 1 and 2. Determination of the enzyme XOI activity using spectrophotometric method by observing t**1**Thus, the determination of uric acid formation carried out during the first 4 minutes **1** he formation of uric acid at a wavelength of 290 nm. Thus, ethyl acetate fraction was fractionated by column chromatography. The stationary phase used was cellulose and 50% methanol as mobile phase. Subfractions 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 test thin layer chromatography (TLC), using 3 times eluated acetic acid 30% as a mobile phase and cellulose as the stationary phase. Subfraction 50-79 was separated using preparative TLC to obtain three isolates (B1, B2, B3). Further isolates tested activity as inhibitors of the enzyme XO, the results obtained (Table 1) that all isolates had activity to the enzyme XO, from the largest to the smallest activity were B1, B3, and B2 with IC₅₀ values of 0.27, 0.30, 0.45 $\mu\text{g}/\text{mL}$. Respectively, B1 isolates have IC₅₀ similar to the ethyl acetate fraction (0.31 $\mu\text{g}/\text{mL}$), but lower than allopurinol (IC₅₀ values 4.59 $\mu\text{g}/\text{mL}$) as positive control. Thus, the study continued to identify B1 isolates.

B1 isolates structure elucidation

^1H -NMR (acetone- d_6 . 400 Hz) of the isolates B1 is used to identify the number and position of H contained in the molecular structure. In these data, there are several groups of signals consisting of

CONCLUSION

In summary, the most active isolate which inhibits the XO (B1) with IC50 of 0.27 $\mu\text{g/ml}$ is kaempferol. Moreover, three compounds isolated from the active fractions showing different XOI activities in vitro may contribute to antihyperuricemic effect. These result supported the antihyperuricemic activity of *S. burahol* leaves as health dietary candidate.

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