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Research Article

CONSTITUENT AND ANTIHYPERURICEMIC ACTIVITY OF *STELECHOCARPUS BURAHOL* LEAVES SUBFRACTIONS

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ABSTRACT

Objective: The goal of this research was to evaluate antihyperuricemic activity of *Stelechocarpus burahol* leaves subfractions and isolate its chemical constituent of active subfraction.

Methods: Ethyl acetate fraction from *S. burahol* extract was subfractionated by vacuum liquid chromatography, and the active subfractions were further subfractionated by classic column chromatography using isocratic eluent, followed by isolated chemical constituent from active subfraction. Hyperuricemic rat model was induced by given potassium oxonate intraperitoneally. The inhibitory effect of subfractions on the xanthine oxidase (XO) activity was determined using ultraviolet-visible spectrophotometry method.

Results: Subfractions E.3, E.4, and E.5 significantly ($p<0.05$) reduced the serum uric acid (UA) level 43%, 46%, and 33%, respectively. The E.3, E.4, and E.5 have showed very weak XO inhibitory activity. Subfraction E.3.2 and E.4.3 significantly ($p<0.05$) reduced the serum UA level 29% and 38%, respectively, however still very weak effect on XO activity. Chemical constituent which was isolated from subfraction E.4.3 was kaempferol-3-O-rhamnoside.

Conclusion: The subfractions of ethyl acetate fraction had antihyperuricemic activity *in vivo* but less effect on XO activity *in vitro.* Isolated compound in active antihyperuricemic of subfraction E.4.3 was kaempferol-3-O-rhamnoside.

Keywords: *Stelechocarpus burahol*, Subfraction, Antihyperuricemic, Xanthine oxidase inhibitory activity, Kaempferol-3-O-rhamnoside.

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INTRODUCTION

Hyperuricemia means high levels of uric acid (UA) in the blood, a condition considered to be closely associated with increasing risks for developing gout, cardiovascular diseases, hypertension, and metabolic syndrome [1,2]. The risk of developing gout is closely related to the serum UA level. The 5-year risk of gout varied from 0.6% for those with a serum UA level >7 mg/dl to 30.5% for those with a level >10 mg/dl [3]. UA is the end-product of purine metabolism in human with the loss uricase. Xanthine oxidase (XO) converts hypoxanthine to xanthine and xanthine to UA [4]. The XO activity derived species reactive oxygen species/reactive nitrogen species (ROS/RNS). ROS and RNS can generate ischemic-reperfusion (IR) injury [5].

Currently available antihyperuricemic agents for chronic gout can be classified into three categories: (i) uricostatic (XO inhibitor), for example, allopurinol and febuxostat, (ii) uricosuric, for example, benzbromarone, sulfinpyrazone, and probenecid, and (iii) uricolytic, for example, uricozyme and rasburicase [6,7]. Despite advances in the use of antihyperuricemic agents for hyperuricemia and gout treatment, however, adverse effects such as severe hypersensitivity reactions associated with allopurinol and severe hepatotoxicity risk of benzbromarone limit their therapy usage [8].

The medicinal use of botanical plants is gaining renewed interest in connection with the sustaining of health and clinical disorder. Scientists have studied to explore the potent antihyperuricemic from a wide variety of traditional herbal plants. Many plants have potential as medicinal plants for gout treatment [9]. *Stelechocarpus burahol* (Bl.) is a traditionally plant for antihyperuricemic and gout [10]. The trees are widely distributed in Thailand, Vietnam, Malaysia, Indonesia (Java, Bali, Sumatra, and Borneo) [11]. The local name in Indonesia is burahol, turalak (Sunda) or kepel (Jawa) [12]. A previous study revealed that ethanolic extract from *S. burahol* leaves was one of the samples which had a potent antihyperuricemic *in vivo* compared to *Annona squamosa, Annona muricata,* and *Annona reticulata* [13]. N-hexane and ethyl acetate fractions of *S. burahol* leaves decreased serum UA level in rats [14]. Furthermore, the *S. burahol* leaves contained flavonoid and terpenoid [10,15]. Many researchers reported that some of flavonoid compounds have strong XO inhibitory and antihyperuricemic *in vivo* activity. However, it is not clear which compounds are active in the extract, fraction and subfraction. This study aimed to evaluate the potent of subfractions of *S. burahol* in lowering of UA. The activity was evaluated by *in vivo* potassium-oxonate-induced acute hyperuricemia and *in vitro* XO inhibitory activity assay. Furthermore, phytochemical compound of active subfraction was isolated and identified.

METHODS

Materials

Xanthine, XO, potassium dihydrogen phosphate, dimethyl sulfoxide (DMSO), potassium oxonate were purchased from Sigma Chemicals Co. Allopurinol was obtained from Ifars Pharmaceutical Laboratories. The UA kit was purchased from Sclavo Diagnostic. Silica gel 60 H for thin-layer chromatography and silica gel 60 (0.063 – 0.200 mm) for column chromatography from Merck were used.

Instrument and reagent

Nuclear magnetic resonance (NMR) spectra (^{1}H-NMR and ^{13}C-NMR spectra taken at 500 MHz and 125 Hz of Agilent 500 MHz by Consol Sistem) were recorded in deuterated solvent using methanol. MS spectra were measured using ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry/mass spectrometry (Waters) ESI positive with mobile phase A (H_2O, 0.1% formic acid) and

B (acetonitrile, 0.1% formic acid), gradient method. Ultraviolet (UV) spectra were identified using spectrodensitometry (Camag Thin-layer Chromatography Scanner).

Animal

Male Wistar rats weighed 170-200 g (age, 7-8 weeks) were obtained from the animal laboratory (School of Pharmacy, Bandung Institute of Technology). The rats were allowed to adapt to their environment at a constant temperature of 25°C for a week before being used. They were given free access to feed standard pellets and water during the study. All the procedures were approved by Animal Ethics Committee, Bandung Institute of Technology.

Plant materials

Leaves of *S. burahol* was collected from Central Java, Indonesia, and determined in School of Life Science and Technology, Bandung Institute of Technology. The materials were washed, wet sortation, dried, and grinded into powder.

Preparation of the extract and fractionation

The dried leaves powder of *S. burahol* (6 kg) was macerated with 30 L ethanol 96% for 5 days at room temperature. After 5 days, the mixture was filtered and the residue washed using ethanol and treated for 5 days of the same treatment as before. The extract was concentrated under reduced pressure to yield a residue (9.2%). The ethanolic extract was then suspended with Aquadest and extracted successively with equal volume of n-hexane and ethyl acetate. Each fraction was then concentrated under reduced pressure to obtain n-hexane fraction (33%), ethyl acetate fraction (12%), and aqueous fraction (32%). The ethyl acetate fraction was subfractionated by vacuum liquid chromatography. The column was packed with silica gel H and then eluted by mobile phase that was fifty eluent system n-hexane, ethyl acetate, and methanol, with increasing polarity (10:0:0→0:0:1 v/v), to divide the fraction into seven subfractions (E.1-E.7). Each subfraction E.3 (1.0 g) and E.4 (0.7 g) was then subfractionated by classical column chromatography by isocratic elution using chloroform-methanol (8:2) to give three and four subfractions (E.3.1-E.3.3 and E.4.1-E.4.4). Subfraction E.4.3 (184 mg) was purified by preparative paper chromatography with mobile phase acetic acid 10% to give three subfractions (E.4.3.1-E.4.3.3). Subfraction E.4.3.2 was then purified by preparative thin layer chromatography with mobile phase chloroform-methanol (8:2) to obtain compound R (5.7 mg).

***In vivo* antihyperuricemic activity**

Animal model and drug administration

Before being used in the experiment, the rats fasted 1 day. The animals were carried to the laboratory at least 1 hr before the experiment begins. Water was withdrawn from the animals 1 hr before drug administration. The experiment was conducted using modified Liu's method [16]. Rats were divided randomly into normal control, hyperuricemic control, standard (allopurinol), and sample groups (n=6 each). Potassium oxonate, subfractions, and allopurinol were dispersed in 0.3% carboxymethylcellulose sodium. The dose of subfraction was calculated from percentage of yielding subfraction. The subfractions were given orally to rats. Allopurinol (10 mg/kg) was used as standard. The volume of the suspension which was administered based on body weight of rat. Before potassium oxonate administration, whole blood was collected for determining serum UA level on 0 h. Briefly, 1 h before the drug administration, all rats were injected intraperitoneally with potassium oxonate 250 mg/kg to increase the serum UA level, except normal control group. Whole blood samples were collected from rats by tail vein bleeding in duration 1st h to 3rd h for obtaining UA level on 1st, 2nd, and 3rd h after drug administration.

UA assay

The blood was allowed to clot for 30 minutes at room temperature and then centrifuged at 10.000 × *g* for 7 minutes to obtain the serum. Serum was stored at −20°C until assayed. Serum UA level was determined by enzymatic colorimetric method using a standard diagnostic kit.

***In vitro* XO inhibitory activity**

XO inhibitory activity was performed based on Umamaheswari [17] and Abdullahi [18] with minor modification. 1 ml fraction (25-200 µg/ml) was mixed 0.9 ml phosphate buffer 50 mM (pH 7.5 at 25°C) and 0.1 ml XO enzyme solution (0.1 unit/ml in phosphate buffer, pH 7.5) and pre-incubated on for 15 minutes at 25°C. 2 ml of xanthine solution 0.15 mM (prepared fresh) was added to the mixture and then incubated for 30 minutes at 25°C. The reaction was then stopped by adding 1 ml 1N hydrochloric acid, and the absorbance was measured at λ 287 nm using UV-Vis spectrophotometer. Different concentrations of the fraction (25-200 µg/ml) were dissolved in DMSO. Allopurinol (0.2-1.6 µg/ml) was used as the positive control. All of the experiments were conducted in triplicate. The XO inhibitory activity of fraction was assessed as the inhibitory percentage (%):

$$Inhibition(\%)=\left\{\frac{(A-B)-(C-D)}{(A-B}\right\}\times 100$$

where A is the activity of the enzyme without the extract, B is the control of A without the extract and enzyme, and C and D are the activity of the extract with and without XO, respectively. The assay was done in triplicate, and IC_{50} values were calculated from the percentage of inhibition 50%.

Statistical analysis

Results showed and represent the mean±standard deviation. The significant difference was statistically calculated by the analysis of variance (one-way), followed by Tukey's post-hoc test. Statistical significant was set at $p<0.1$, 0.05, and 0.01.

RESULTS AND DISCUSSION

Antihyperuricemic activity of subfractions E.1-E.7

The antihyperuricemic activity of subfractions E.1-E.7 on hyperuricemic rats using potassium oxonate is shown in Table 1. In the present study, potassium oxonate, a well-known uricase inhibitor, was used to induce hyperuricemia in rats [19]. The serum UA level of chemically induced hyperuricemic rats was significantly higher ($p<0.01$) than normal control, which indicated that hyperuricemic rat model has been successfully established. Treatment by subfractions E.1-E.7 can reduce serum UA level of hyperuricemic rats, and only E.3 (43%), E.4 (46%), and E.5 (33%) showed significantly different compared to hyperuricemic control ($p<0.05$). The dose of subfraction was calculated using response profile of ethyl acetate fraction and percentage of yielding subfractions. This was assumed that subfractions which obtained from bioactivity-guided subfractionation will contain more bioactive constituents than the crude ethanol extract or ethyl acetate fraction and thus will show inhibition higher than the crude drugs of ethanol extract [13] and ethyl acetate fraction [14].

The XO inhibitory activity of subfractions E.1-E.7 was evaluated by the xanthine-XO enzymatic system. The influence of subfraction on XO activity was calculated by decreasing production of UA, which was measured by UV-Vis spectrophotometry. The XO inhibitory activity of subfractions which were resulted from subfractionation of ethyl acetate fraction by vacuum liquid chromatography is presented in Table 2. Those subfractions E.1-E.7 expressed low and no effect on XO inhibitory activity. Subfractions E.1-E.6 elicited a dose-dependent inhibition of XO enzyme activity. At concentration of 200 g/ml, the highest activity was given by E.3 subfraction, followed by E.4 and E.2 subfractions. These results were compared to allopurinol, which revealed 48.69±1.82% inhibition at 2 µg/ml concentration (Table 1). The all of subfractions had IC_{50} more than 200 µg/ml, which were categorized as very weak activity.

Antihyperuricemic activity of subfractions E.3.1-E.4.4

The antihyperuricemic activity of subfractions E.3.1-E.4.4, which were resulted from subfractionation of subfractions E.3 and E.4, is given in Table 3.

In the present study, the serum UA level of hyperuricemic group increased more than 1.5-fold compared to normal control. The rat which was induced by potassium oxonate can serve as a useful hyperuricemia animal model to evaluate serum UA levels of sample. The oral treatment by subfractions E.3.1-E.4.4 could reduce serum UA level of hyperuricemic rats compared to hyperuricemic control; however, only E.3.2 (20 mg/kg), E.4.2 (8 mg/kg), and E.4.3 (8 mg/kg) subfractions demonstrated antihyperuricemic activity ($p<0.05$). The rats were treated by subfraction E.4.3 had UA levels that were significantly reduced 38% followed by E.3.2 29% compared to hyperuricemic group ($p<0.01$). On the other hand, serum UA level of hyperuricemic group and the rats which were treated by subfractions E.3.1, E.3.3, E.4.1, and E. 4.4 were not significantly different.

All subfractions of E.3 and E.4 presented a dose-dependent inhibition of XO enzyme activity. The results demonstrated that subfractions E.3.1-E.4.4 possessed XO inhibitory activity as shown in Table 4. Among the obtained subfractions, the subfraction E.3.2 at concentration of 200 µg/ml, the highest activity was given by E.3.2 (63.79%), followed E.4.4 (43.20%) and E.4.3 (41.46%). The subfraction E.3.2 gave XO inhibitory activity with IC_{50} 128.39±20.21 µg/ml, while the other subfractions which were resulted by classical column chromatography of E.3 and E.4 gave IC_{50} more than 200 ug/ml. The XO inhibitory activity of subfractions E.3.1-E.4.4 was compared to allopurinol, which is clinically used as a drug for the XO inhibitor [20]. Allopurinol as standard showed very strong inhibitory activity with IC_{50} 1.08±0.04 µg/ml (Table 4).

The subfractions E.3.1-E.4.4 which were resulted from subfractionation of E.3 and E.4 subfractions by classical column chromatography had antihyperuricemic activity *in vivo* but had less XO inhibitory activity *in vitro.* The previous studies also found that ethanolic extract of *S. burahol* exposed lower XO inhibitory activity but had significant antihyperuricemic activity *in vivo* [14,21].

Table 1: UA level of hyperuricemic rats before and after giving subfractions (E.1-E.7)

Group	Dose (mg/kg)	Serum UA level (mg/dl)			
		0 h	1 h	2 h	3 h
Normal		2.62±0.33	2.42±0.37^{r}	2.34±0.28^{r}	2.46±0.39^{r}
Hyperuricemic		2.53±0.19	3.61±0.21^{cz}	3.92±0.22^{cz}	3.86±0.17^{cz}
Allopurinol	10	2.46±0.36	1.82±0.40^{r}	1.93±0.16^{r}	2.20±0.51^{r}
E.1	35	2.67±0.26	3.05±0.40^{z}	3.46±0.15^{cpz}	3.54±0.20^{bz}
E.2	14	2.37±0.49	2.59±0.25^{pz}	3.14±0.20^{cz}	3.16±0.46^{cz}
E.3	8	2.49±0.39	2.07±0.14^{r}	2.72±0.13^{bqz}	3.00±0.67^{az}
E.4	8	2.42±0.14	1.94±0.82^{r}	2.72±0.61^{bqz}	2.89±0.60^{y}
E.5	8	2.40±0.59	2.42±0.43^{rz}	3.37±0.35^{bz}	2.93±0.78^{x}
E.6	7	2.21±0.38	2.60±0.21^{pz}	2.96±0.56^{cz}	2.77±0.40^{y}
E.7	10	2.30±0.40	2.70±0.64^{az}	3.18±0.37^{cz}	2.83±0.40^{ax}

$^{a}p<0.1$; $^{b}p<0.05$; $^{c}p<0.01$ compared to normal control. $^{p}p<0.1$; $^{q}p<0.05$; $^{r}p<0.01$ compared to hyperuricemic control. $^{x}p<0.1$; $^{y}p<0.05$; $^{z}p<0.01$ compared to allopurinol.
UA: Uric acid

Table 2: *In vitro* xanthine oxidase inhibitory activity of subfractions (E.1-E.7)

Group	Percentage of xanthine oxidase inhibition (µg/ml)				IC_{50} (µg/ml)
	25	50	100	200	
E.1	2.93±2.5	6.56±3.47	7.50±2.67	10.10±1.87	>200
E.2	7.90±2.20	12.55±3.96	13.83±4.57	18.22±7.78	>200
E.3	13.00±3.50	21.36±4.01	28.22±3.61	42.02±4.05	>200
E.4	3.46±2.06	8.38±5.91	13.82±3.84	19.84±4.90	>200
E.5	2.82±2.78	4.53±2.36	4.88±2.93	9.10±3.70	>200
E.6	6.70±1.82	6.35±2.40	6.95±2.99	9.27±3.51	>200
E.7	12.08±7.08	5.55±7.76	−5.84±2.78	3.47±22.50	-
Allopurinol	Percentage of xanthine oxidase inhibition (µg/ml)				2.16±0.53
	0.2	0.5	1	2	
	10.62±1.39	16.73±2.97	27.69±7.95	48.69±10.82	

-: No activity

Table 3: UA level of hyperuricemic rats before and after giving subfractions (E.3.1-E.4.4)

Group	Dose (mg/kg)	Serum UA level (mg/dl)			
		0 hr	1 hr	2 hr	3 hr
Normal		2.62±0.33	2.42±0.37^{r}	2.34±0.28^{q}	2.46±0.39^{r}
Hyperuricemic		2.80±0.43	3.61±0.32^{cz}	4.43±0.68^{cx}	4.16±0.95^{cz}
Allopurinol	10	2.46±0.36	1.82±0.40^{qx}	1.93±0.16^{q}	2.20±0.51^{r}
E.3.1	12	2.83±0.24	2.99±0.45^{z}	3.79±0.65^{cz}	3.78±0.68^{y}
E.3.2	20	2.87±0.28	2.55±0.32^{ary}	3.37±0.18^{pz}	3.56±0.89^{y}
E.3.3	5	2.72±0.65	2.99±0.37^{z}	3.97±0.48^{cz}	3.89±0.57^{bz}
E.4.1	12	2.98±0.14	2.96±0.42^{z}	3.74±0.72^{cz}	4.04±0.92^{bz}
E.4.2	8	2.82±0.28	2.80±0.45^{qz}	3.75±0.84^{cz}	4.08±0.60^{cz}
E.4.3	8	2.57±0.40	2.23±0.38^{r}	3.53±0.48^{bz}	3.72±0.63^{y}
E.4.4	6	2.76±0.30	2.93±0.26^{pz}	4.13±0.51^{bz}	4.08±0.75^{cz}

UA: Uric acid. Keterangan: $^{a}p<0.1$; $^{b}p<0.05$; $^{c}p<0.01$ compared to normal control. $^{p}p<0.1$; $^{q}p<0.05$; $^{r}p<0.01$ compared to hyperuricemic control. $^{x}p<0.1$; $^{y}p<0.05$; $^{z}p<0.01$ compared to allopurinol

Structure elucidation

The subfraction E.4.3 of *S. burahol* leaves was purified by preparative paper chromatography and repurified by preparative thin layer chromatography. Compound R was obtained as a pale yellow amorphous powder. The UV spectrum of R showed λmax at 268 nm and 348 nm which was suggested as flavonoid [22]. Table 5 revealed the chemical shift value of ^{1}H and ^{13}C-NMR for compound R. The ^{1}H and ^{13}C-NMR values of all carbons were assigned on the basic of heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC).

Based on the characterization and identification by UV-visible spectrophotodensitometry, ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC demonstrated that isolate was predicted as kaempferol-3-O-rhamnoside ($C_{21}H_{20}O_{10}$). It was also confirmed by mass spectrum which presented molecular ion [M+H^{+}] at 433.38, while the molecular weight of kaempferol-3-O-rhamnoside ($C_{21}H_{20}O_{10}$) is 432.38 g/mol. Therefore, it can be concluded that isolate was kaempferol-3-O-rhamnoside (Fig. 1).

The isolate of active subfraction, kaempferol-3-O-rhamnoside, may be active compound. The structure–activity relationships exhibited that flavones and flavonols with a-7-hydroxyl group such as chrysin, luteolin, kaempferol, quercetin, myricetin, and isorhamnetin inhibited XO activity at low concentration. The previous research presented that kaempferol showed XO inhibitory activity at IC_{50} value 0.67 μM [23]. Ahmad [24] denoted that kaempferol had XO inhibitory activity at IC_{50} 1.87 μg/ml whereas the IC_{50} of kaempferol-3-O-ß-D-glucopyranoside and kaempferol-3-O-(4-O-galloyl)-α-L-arabinopyranoside was 11.74 and 13.96 μg/ml, respectively. The main inhibition mechanism of

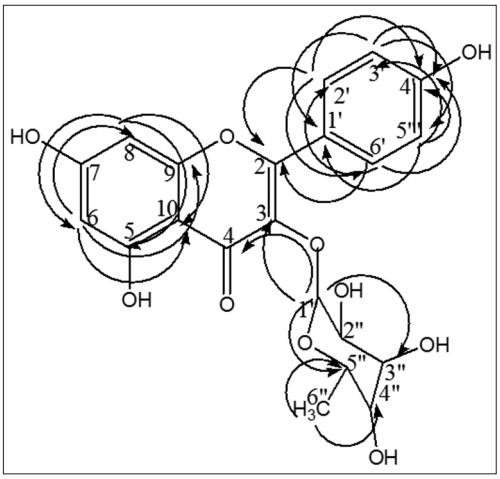

Fig. 1: Heteronuclear single quantum coherence and heteronuclear multiple bond correlation of compound R

Table 4: *In vitro* xanthine oxidase inhibitory activity of subfractions (E.3.1-E.4.4)

Group	Percentage of xanthine oxidase inhibition (μg/ml)				IC_{50} (μg/ml)
	25	**50**	**100**	**200**	
E.3.1	15.91±2.69	18.68±2.42	30.01±6.04	33.22±4.17	>200
E.3.2	26.93±2.42	34.86±2.34	46.70±2.24	63.79±4.28	128.39±20.21
E.3.3	15.88±1.88	22.51±6.69	25.44±4.16	28.67±1.84	>200
E.4.1	5.98±2.87	13.54±5.31	15.72±6.89	15.47±7.07	>200
E.4.2	7.29±6.53	6.65±4.60	11.09±5.99	19.60±5.56	>200
E.4.3	22.35±4.53	25.33±6.22	32.14±3.93	41.46±7.19	>200
E.4.4	17.59±4.21	22.38±6.95	31.38±3.50	43.20±9.03	>200
Allopurinol	**Percentage of xanthine oxidase inhibition (μg/ml)**				1.08±0.04
	0.2	**0.5**	**1**	**2**	
	21.93±4.67	35.28±6.80	47.66±3.38	78.02±2.93	

Table 5: ^{1}H and ^{13}C-NMR chemical shift value for compound R

Atom position	δ ^{1}H (bpj)	δ ^{13}C (bpj)	HSQC (H↔C)	HMBC (H↔C)
2	-	158.58		
3	-	136.16		
4	-	179.58		
5	-	163.19		
6	6.20 (1H, *d*, 2.1 Hz)	99.95	C-6	C-8, C-9, C-10
7	-	166.24		
8	6.38 (1H, *d*, 2.2 Hz)	94.83	C-8	C-6, C-5, C-10
9	-	161.62		
10	-	105.82		
1’	-	122.60		
2’, 6’	7.77 (2H, *d*, 8.5 Hz)	131.88	H-2’↔C-2’ H-6’↔C-6’	C-4’, C-6’, C-2 C-4’, C-2’, C-2
3’, 5’	6.94 (2H, *d*, 8.5 Hz)	116.54	H-3’↔C-3’ H-5’↔C-5’	C-4’, C-5’, C-1’ C-4’, C-3’, C-1’
4’	-	159.26		
Ar-OH	4.2	-	-	-
1”	5.37 (1H, s)	103.49	C-1”	C-4, C-3, C-3”, C-5”
2”	3.72 (1H, *m*)	72.04	C-2’	
3”	3.71 (1H, *m*)	73.17	C-3”	
4”	3.68 (1H, *m*)	-		
5”	3.65 (1H, *m*)	71.91	C-5”	
6”(CH3)	0.95 (3H, *d*, 4.5 Hz)	17.65	C-6”	C-5”, C-4”

HSQC: Heteronuclear single quantum coherence, HMBC: Heteronuclear multiple bond correlation, NMR: Nuclear magnetic resonance

kaempferol on XO activity may be due to the insertion of kaempferol into the active site of XO occupied the catalytic center of the enzyme to avoid the entrance of the substrate and induced conformational changes of XO [25]. Kaempferol is presented antihyperuricemic activity in mice hyperuricemic [26].

CONCLUSION

The subfractions of ethyl acetate fraction which were resulted from subfractionation by a vacuum liquid chromatography and further subfractionation by a classical column chromatography had antihyperuricemic activity *in vivo* but gave less XO inhibitory activity *in vitro.* Compound R in active antihyperuricemic subfraction E.4.3 was kaempferol-3-O-rhamnoside.

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