

IN VITRO AND IN VIVO STUDIES OF ANTIHYPERURICEMIC AND ANTIOXIDANT ACTIVITY FROM BULBS OF BAWANG TIWAI (*ELEUTHERINE PALMIFOLIA* (L.) MERR.) FROM INDONESIA

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Received: 31 October 2018, Revised and Accepted: 14 December 2018

ABSTRACT

Objectives: This study would like to investigate the *in vitro* antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl assay and *in vitro* xanthine oxidase activity of the bulbs. This study performs *in vivo* assays to study the antihyperuricemic activity and antioxidant in the hyperuricemic rat through plasma malondialdehyde measurement.

Method: The study was conducted by testing the fresh bulbs of bawang tiwai (*Eleutherine palmifolia* (L.) Merr. with chemical solvent of ethanol 70% to extract the bulbs. Allopurinol and Vitamin C were used as positive control for the antihyperuricemic assay and antioxidant assay, respectively. Other chemical substances were also used in this study. This study used chicken extract (Brands) 20 ml/kg/body weight to induce the level of uric acid in the blood serum, and potassium oxonate (Sigma 156124) to inhibit the uricase in rats.

Results: The results show that the levels of uric acid were measured using spectrophotometer with dichloro-hydroxybenzen sulfonate (Biolabo) as a reagent. The ethanol extract of bawang tiwai (EBT) (*E. palmifolia* (L.) Merr) was potential to reduce uric acid level at 140, 280, and 560 mg/kg body weight, but possibly without inhibition against xanthine oxydase activity.

Conclusion: All doses of EBT could inhibit lipid peroxidation in hyperuricemic condition caused by high purine diet in 14 days.

Keywords: Antihyperuricemic, Antioxidant activity, Bulbs of bawang tiwai, *In vitro*, *In vivo*.

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INTRODUCTION

Hyperuricemia is a metabolic disorder in the human body with an excess of uric acid in blood plasma. It leads to gouty arthritis, characterized by inflammatory joints due to the deposition of the crystals. Although the risk of mortality is relatively low, gout affects the patient's quality of life. The metabolic pathway for uric acid involves xanthine oxidase, an enzyme that catalyzes the oxidation of xanthine to uric acid. High activity of xanthine oxidase induces not only the level of uric acid in blood but also side products and free radicals. The accumulation of free radicals that overwhelms the capacity of endogenous antioxidant potentially gives harmful effects to human body.

Flavonoids, isolated from plants, have been reported as a potential inhibitor for xanthine oxidase [1-4]. Morin, kaempferol, isorhamnetin, and baicalein have been reported to have inhibitory activity against xanthine oxydase *in vitro* [5]. Other reports show that flavonoids (quercetin, morin, myricetin, puerarin, and kaempferol) given at 50 and 100 mg/kg body weight in 3 days with potassium oxonate as an inductor are able to reduce the level of hyperuricemia significantly up to 35% [6,7].

Bawang Tiwai, also known as bawang dayak (*Eleutherine palmifolia* (L.) Merr.), is a bulbous plant that numerously grows in Borneo. People in Borneo consume the bulbs as an alternative medicine to treat common diseases. Unlike other bulbous plants, bawang tiwai has less odor and distinct flavor compared to red onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Moreover, it holds a stronger red pigment than the red onion. Bawang Tiwai contains eleutherin, eleutherol, and isoeleutherin which have been reported to show antifungal activity [8,9], wound healing activity by stimulating Smad-mediated collagen production [10] and α -glucosidase inhibitory [11]. To date, there is no empirical report on antioxidant, xanthine oxidase inhibitory and antihyperuricemic activity

of bawang tiwai bulbous. Therefore, this study would like to investigate the *in vitro* antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DDPH) assay and *in vitro* xanthine oxidase activity of the bulbs. Then, this study performs *in vivo* assays to study the antihyperuricemic activity and antioxidant in the hyperuricemic rat through plasma malondialdehyde measurement.

MATERIALS AND METHODS

Animal

A total of 36 of Sprague Dawley rats with weight 150–250 g were obtained from Bogor Agricultural University. They were acclimatized for a week in light and temperature-controlled room with 12 h dark-light cycle. They were fed with standard rat pellet and water, under strict hygienic conditions.

Sample, drug, and other chemical substances

Fresh bulbs of bawang tiwai were obtained directly from Borneo. The bulbs were determined by Herbarium Bogoriense of Indonesian Science Institute (LIPI) Bogor as *E. palmifolia* (L.) Merr. The chemical solvent used to extract the bulbs was ethanol 70%. Allopurinol and Vitamin C were used as positive control for the antihyperuricemic assay and antioxidant assay, respectively. Other chemical substances used in this study were xanthine oxidase (Sigma 4376), xanthine (Sigma 4002), phosphate buffer (pH 7.5), DPPH (1,1-difenil-2-pikrilhidrazil) (Sigma), malondialdehyde standard (TEP), thiobarbituric acid (TBA) (Merck), ethers, ethylenediaminetetraacetic acid, and trichloroacetic acid (TCA). We used chicken extract (Brands) 20 ml/kg/body weight to induce the level of uric acid in the blood serum, and potassium oxonate (Sigma 156124) to inhibit the uricase in rats. The levels of uric acid were measured using spectrophotometer with dichloro-hydroxybenzen sulfonate (Biolabo) as a reagent.

Preparation of extract

About 400 g of dried bulbs of bawang tiwai were extracted with ethanol 70% by maceration process for 24 h. The suspension was filtrated, and the precipitate was re-macerated 4 times subsequently. Then, the extract was concentrated using rotary evaporator at 50–60°C to obtain dense ethanol-extract of bawang tiwai (EBT). The percentage of crude extract (relative to dried plant material) obtained was 17.2%.

Phytochemical screening

Phytochemical screening of EBT was performed using Farnsworth method. It included the identification of alkaloid, flavonoid, saponin, tannin, quinone, steroid/triterpenoid, essential oil, and coumarin.

In vitro assay of xanthine oxidase inhibitory activity [12]

The *in vitro* assay was performed to investigate xanthine oxidase inhibitory activity of EBT. First, the absorbance of xanthine, remain from uric acid metabolism, was measured at 265 nm. A half milliliter of EBT with concentrations of 100, 200, 300, 400, 500, and 600 ppm was added to 1.45 ml of phosphate buffer 50 mM, pH 7.5. Then, each mixture was added to 1 ml of 0.3 mM xanthine in phosphate buffer. The tubes were incubated at 25°C for 15 min. Then, 0.05 ml of 0.3 U/ml xanthine oxidase was added to each tube and incubated for 45 min. The reaction was stopped by adding 0.5 ml HCl 1N. The absorbance of each mixture was measured using spectrophotometer at 265 nm. Allopurinol, as a positive control, was measured with the same procedure. The standard curve was made using the absorbance of xanthine with known concentrations. The absorbance of the mixture of substrate and enzyme was used as "blank," and the absorbance of substrate was used as negative control. The percentages of inhibitions were calculated using this equation:

$$\% \text{inhibition} = \frac{(\text{Activity of xanthine oxidase without extract} - \text{Activity of xanthine oxidase with extract})}{\text{Activity of xanthine oxidase without extract}} \times 100$$

In vitro DDPH antioxidant assay [13]

As stock, 10 mg sample was dissolved in 10 ml methanol (1000 ppm). Then, stock solution was diluted into 10, 20, 30, 40, 50, and 60 ppm. Each mixture was added to 1 ml of DPPH 0.4 mM and methanol. The mixtures were incubated at 37°C for 30 min. Their absorbances were measured at 515 nm using spectrophotometer ultraviolet (UV)-Visible.

In vivo assay of antihyperuricemic and antioxidant activity [14]

A total of 36 of male Sprague Dawley rats were divided into six groups: Normal, negative (hyperuricemic) control, positive control (allopurinol), experimental groups given with low dose (140 mg/kg) of EBT (EBTL), experimental groups given with middle dose (280 mg/kg) of the extract (EBTM), and experimental groups given with high dose (560 mg/kg) of the extract (EBTH). Normal group was given standard lab diet, while other five groups were each given high-purine diet from chicken extract, 20 ml/kg body weight for 14 days. The extracts with referred dose were given 1 h after the daily meal. On the 14th day, 1 h after the main treatments, the rats were given potassium oxonate 250 mg/kg body weight, intraperitoneally. 3 h after the induction, all rats were anesthetized with ether, and the blood was collected, intracardially. Plasma was separated from blood cells by centrifugation at 3000 rpm for 10 min. Uric acid levels were measured by dichloro-hydroxybenzene sulfonate method using Biolabo kit and spectrophotometer (RD-60 Semi-Auto Biochemistry) at 520 nm. Furthermore, malondialdehyde in the plasma was also measured using spectrophotometer UV-Visible.

Malondialdehyde (MDA) measurement [15]

About 200 ml plasma was added to 1.0 ml of trichloroacetate (TCA) (20%) and 2.0 ml of TBA (0.67%). The homogenized mixture was heated on water bath for 10 min. After cooling the room temperature, it was centrifuged at 3000 rpm for 10 min. The red filtrate's absorbance was measured at 532 nm using spectrophotometer UV-Visible. Concentration of MDA was calculated from a standard curve prepared from known concentration at 0 to 1.6 nmol/ μ l.

RESULTS AND DISCUSSION

Phytochemical screening of EBT

Based on the Farnsworth method, EBT contained steroids, triterpenoids, alkaloids, flavonoids, quinones, and tannins. However, essential oils, coumarins, and saponins were not detected in EBT.

In vitro xanthine oxidase inhibitory activity and antioxidant activity from EBT

The extract yield obtained from the maceration process with ethanol 70% was 17.2%. The result of the assay of the xanthine oxidase inhibitory activity was presented in Table 1. EBT at 600 ppm gave 73.52% inhibition to the enzyme's activity, while allopurinol gave 73.74% inhibition only at 2 ppm concentration. The IC₅₀ of EBT was 447.86 ppm, while IC₅₀ of allopurinol was 2.78 ppm.

The scavenging ability of DPPH free radical was used to analyze the antioxidant potential of EBT. The DPPH radical scavenging potential of the different EBT and Vitamin C (as positive control) is represented in Table 2. The IC₅₀ of EBT was 14.05 μ g/mL, while IC₅₀ of Vitamin C was 2.71 μ g/mL.

In addition, the antioxidant activity index (AAI) indicates the success of a compound in the effects of antioxidation. Đurđević *et al.* (2014) characterized compounds from its antioxidant activity as very strong (AAI >2), strong (AAI >1–2), moderate (AAI >0.5–1), and poor (AAI <0.5) [16]. Based on the criteria, EBT was categorized as a very strong antioxidant (AAI = 2.3623) as well as Vitamin C (AAI = 11.9809).

The experimental groups, fed with chicken essence (20 ml/kg) for 14 days along with potassium oxonate on the 14th day, significantly have higher uric acid levels (6.93 mg/dL) than the normal group (4.41 mg/dL), $p < 0.05$. After the extract administration for 14 days, the uric acid levels on day 14 from EBTL, EBTM, and EBTH (140 mg/kg BW,

Table 1: Percent inhibition of xanthine oxidase from EBT and allopurinol

Sample	Concentration (ppm)	Mean of percent inhibition \pm SD (n=3)
EBT	100	13.24 \pm 0
	200	42.44 \pm 0.52
	300	30.88 \pm 2.08
	400	37.13 \pm 1.56
	500	63.97 \pm 6.24
	600	73.52 \pm 1.04
Allopurinol	0.1	34.67 \pm 5.85
	0.25	41.74 \pm 6.54
	0.5	44.47 \pm 6.63
	1	67.16 \pm 3.86
	2	73.74 \pm 10.79
	5	85.78 \pm 1.48

EBT: Ethanol extract of Bawang Tiwai, SD: Standard deviation

Table 2: DPPH scavenging potential of EBT and Vitamin C

Sample	Concentration (ppm)	Mean of percent inhibition \pm SD (n=3)
EBT	10	47.63 \pm 3.02
	20	53.77 \pm 3.13
	30	57.55 \pm 1.43
	40	60.66 \pm 2.20
	50	68.00 \pm 3.55
	60	72.31 \pm 3.45
Vitamin C	2	48.12 \pm 2.99
	4	53.64 \pm 2.76
	6	61.57 \pm 2.11
	8	69.49 \pm 1.33
	10	77.45 \pm 2.15
	12	83.17 \pm 2.30

EBT: Ethanol extract of bawang tiwai

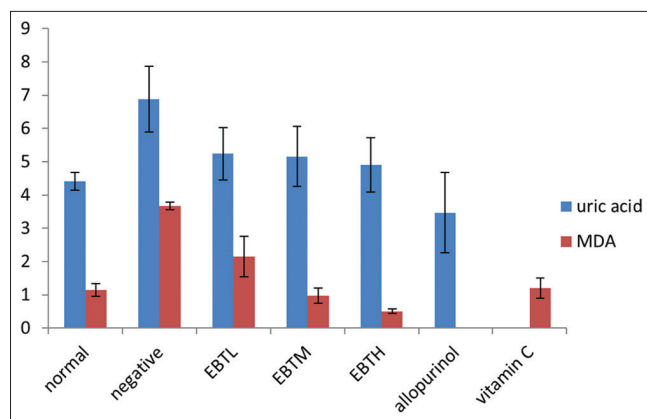


Fig. 1: Effect of the ethanol extracts of bawang tiwai on the uric acid level in rat plasma and malondialdehyde concentration as a product of lipid peroxidation (mean±SD, n=6)

280 mg/kg BW, and 560 mg/kg BW) were 24 mg/dL, 5.16 mg/dL, and 4.9 mg/dL, respectively. Statistically, EBT at these three doses could inhibit the increase of uric acid level in rats compared to the negative group. On day 14, all experimental and allopurinol groups had similar uric acid level to the normal group (Fig. 1).

EBT could inhibit the increase of uric acid level in hyperuricemic rats as well as inhibit lipid peroxidation, proven by significant lower MDA level than negative groups.

This research showed that the hyperuricemic condition is occurred along with the increase of MDA level in plasma. The negative group has a significant higher MDA level in plasma (3.67 ± 0.12 nmol/mL) than normal group (1.15 ± 0.19 nmol/mL), $p < 0.05$. MDA is the product of lipid peroxidation in the human body caused by the occurrence of free radicals (Liu *et al.*). In many studies, the product of the peroxidation becomes the parameter to measure the oxidative stress (Alves *et al.*, 2010) [8]. High intake of dietary purine stimulates xanthine oxidase to catalyze the oxidation of purine into uric acid. The process produces reactive oxygen species that cause peroxidation of membrane lipid. EBT, EBTM, and EBTH have capability to reduce the MDA level in plasma significantly. On day 14, the MDA levels of EBT, EBTM, and EBTH were 2.16 ± 0.61 , 0.97 ± 0.23 , and 0.51 ± 0.07 nmol/mL, respectively, while the MDA level of positive control (Vitamin C) was 1.21 ± 0.30 nmol/mL. Statistically, EBTM and Vitamin C have similar MDA level with the normal group.

DISCUSSION

According to the fact that each year the prevalence of gout and hyperuricemia increases rapidly, thus, we studied the EBT (*E. palmifolia* (L.) Merr) to investigate its potential to reduce the uric acid level in human body. In previous reports, flavonoid shows inhibition to xanthine oxidase activity. Cos *et al.*, in his *in vitro* study, approved that isorhamnetin, baicalein, kaempferol, and morin had inhibition to the enzyme's activity with IC_{50} 2.51, 2.79, 1.06, and 10.1 μ M, respectively [5]. While Mo *et al.* performed *in vivo* assays of flavonoid including quercetin, morin, myricetin, kaempferol, and puerarin which significantly reduced the uric acid level in mice 26.94%, 28.99%, 30.59%, 35.39%, and 31.28%, respectively [6]. Bulbous plants are cultivars that contain flavonoid, especially kaempferol and quercetin. We hypothesized that bawang tiwai possess these flavonoids as the other bulbous plants. Moreover, the flavonoids content of bawang tiwai has not been empirically reported. The amount of quercetin in bawang tiwai was still unknown. Thus, we suggest a further research investigating the qualitative and quantitative study of flavonoids contained in bawang tiwai.

Apaya and Chichioco-Hern (2011) in the previous report categorized extract as strong inhibitor if it inhibits more than 50% of the enzyme's

activity at 50 ppm concentration [17]. Based on the results of the *in vitro* assay, EBT has low inhibition to xanthine oxidase activity. For 50% inhibition, EBT required 44,786 ppm concentration. However, the *in vivo* assay provides expected result. Statistically, EBT in all doses (140, 280, and 560 mg/kg body weight) has significant lower uric acid level than the negative control. EBT has capability to reduce the uric acid level in hyperuricemic rats. The incoherent results of *in vitro* and *in vivo* assays are probably occurred because the antihyperuricemic activity of EBT was based on different mechanism than inhibition of xanthine oxidase. In future research, the uricosuric and uricostatic of EBT could be explored that it could contribute to reduce the uric acid level.

Based on the results, it is important to consume antioxidant as well as antihyperuricemic compounds to suppress the symptoms gout and hyperuricemia. Bawang tiwai has both antihyperuricemic and antioxidant activities; therefore, it could be further investigated as alternative herbs to overcome hyperuricemia.

CONCLUSION

The ethanol extracts of bawang tiwai (*E. palmifolia* (L.) Merr) were potential to reduce uric acid level at 140, 280, and 560 mg/kg body weight, but possibly without inhibition against xanthine oxidase activity. All doses of ethanol extracts of bawang tiwai could inhibit lipid peroxidation in hyperuricemic condition caused by a high purine diet in 14 days. Thus, we conclude that EBT had antihyperuricemic as well as antioxidant activities.

AUTHORS' CONTRIBUTIONS

Dian Ratih Laksmiawati and Rininta Firdaus conceived of research conception/design, manuscript preparation, experiment, data acquisition, and data analysis/interpretation.

Mediana Astika Zein verified the analytical method, data analysis, supervised the findings, and final approval.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Chang WS, Lee YJ, Lu FJ, Chiang HC. Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Res* 1993;13:2165-70.
- Hudaib MM, Tawaha KA, Mohammad MK, Assaf AM, Issa AY, Alali FQ, *et al.* Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. *Pharmacogn Mag* 2011;7:320-4.
- Kostić DA, Dimitrijević DS, Stojanović GS, Palić IR, Dordević AS, Ickovski JD. Xanthine oxidase: Isolation, assays of activity, and inhibition. *J Chem* 2015;2015:8.
- Lin S, Zhang G, Liao Y, Pan J, Gong D. Dietary flavonoids as xanthine oxidase inhibitors: Structure-affinity and structure-activity relationships. *J Agric Food Chem* 2015;63:7784-94.
- Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, *et al.* Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J Nat Prod* 1998;61:71-6.
- Mo SF, Zhou F, Lv YZ, Hu QH, Zhang DM, Kong LD, *et al.* Hypouricemic action of selected flavonoids in mice: Structure-activity relationships. *Biol Pharm Bull* 2007;30:1551-6.
- Yu Z, Fong WP, Cheng CH. The dual actions of morin (3,5,7,2',4'-pentahydroxyflavone) as a hypouricemic agent: Uricosuric effect and xanthine oxidase inhibitory activity. *J Pharmacol Exp Ther* 2006;316:169-75.
- Upadhyay A, Chattopadhyay P, Goyary D, Mazumder PM, Veer V. *Eleutherine indica* L. Accelerates *in vivo* cutaneous wound healing by stimulating smad-mediated collagen production. *J Ethnopharmacol* 2013;146:490-4.
- Ieyama T, Gunawan-Puteri MD, Kawabata J. A-glucosidase inhibitors from the bulb of *Eleutherine americana*. *Food Chem* 2011;128:308-11.
- Boussoulaim N, Baghiani A, Krache I, Trabsa H, Kenouf S, Arrar L.

- Inhibitory effects of *Anchusa azurea* extracts on xanthine oxidase activity and its hypouricemic effects on mice. *Int J Pharm Pharm Sci* 2015;7:195-9.
11. Fidrianny I, Alvina A. Antioxidant capacities from different polarities extract of three kinds ginger using DPPH, frap assays and correlation with phenolic, flavonoid, carotenoid contents. *Int J Pharm Pharm Sci* 2014;6:521-5.
 12. Laksmiawati DR, Nurhidayati L, Ningsih NN. Establishment of hyperuricemia mouse model with oxonic acid potassium salt and essence of chicken. *Res J Pharm Biol Chem Sci* 2017;64:64-9.
 13. Gholizadeh F, Mokarram P, Dastgheib S, Rahpeima Z. The effect of the aquatic extract of stevia on the mda level and catalase activity in the testicular tissue of streptozotocin-nicotinamide-induced diabetic rats. *Shiraz E Med J* 2018;19:e61044.
 14. Apaya KL, Chichioco-Hernandez CL. Xanthine oxidase inhibition of selected Philippine medicinal plants. *J Med Plant Res* 2011;5:289-92.
 15. Alves TM, Kloos H, Zani CL. Eletherinone, a novel fungitoxic naphthoquinone from *Eleutherine bulbosa* (Iridaceae). *Mem Inst Oswaldo Cruz* 2003;98:709-12.
 16. Đurđević J, Vasić S, Radojević I, Đelić G, Čomić L. Antibacterial, antibiofilm and antioxidant activity of *Potamogeton nodosus* Poir. Extracts. *Kragujevac J Sci* 2014;36:137-44.
 17. Liu XJ, Yan XQ, Wang NL. Studies on chemical constituents of *Eleutherine americana*. *Zhong Yao Cai* 2009;32:55-8.