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Original Article

SCREENING FOR ANTI-HYPERURICEMIA POTENTIAL OF SOME INDONESIAN MEDICINAL PLANTS THROUGH XANTHINE OXIDASE INHIBITION *IN VITRO* ASSAY

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ABSTRACT

Objective: This study aimed to examine the *in vitro* xanthine oxidase inhibitory activity of 12 plants commonly used as gout medicines by the Indonesian people.

Methods: The measurement of xanthine oxidase enzyme inhibitory activity was using UV spectrophotometry. The *in vitro* assessment of xanthine oxidase inhibition activity was tested on extracts from *Eleutherine bulbosa* (Mill.) Urb. Bulbs, *Pandanus amaryllifolius* Roxb. leaves, *Alyxia reinwardtii* Blume stem barks, *Ruta angustifolia* Pers aerial parts, *Dioscorea hispida* Dennst tubers, *Plantago major* L. leaves, *Symphytum officinale* L. roots, *Euphorbia hirta* L. aerial parts, *Chromolaena odorata* L. leaves, *Solanum torvum* Sw fruits, *Peperomia pellucida* L. Kunth. aerial parts and *Strobilanthes crispa* L. Blume leaves.

Results: The results of this study showed that all tested plant extracts can inhibit xanthine oxidase activity with IC_{50} values varying from 27.80 µg/ml to 47.14 µg/ml. The IC_{50} value of allopurinol, used as positive control, was 1.24 µg/ml. Among all the tested plant extracts, *Strobilanthes crispa* L. Blume leaves extract has the best inhibitory activity against xanthine oxidase enzyme with IC_{50} value of 27.80 µg/ml.

Conclusion: *Strobilanthes crispa* L. Blume leaves extract has the best inhibitory activity against xanthine oxidase, so It has the potential to be developed into herbal medicine to treat hyperuricemia. This study provides scientific support for the anti-hyperuricemia activity of these herbs, which are empirically used to treat gout.

Keywords: Hyperuricemia, Uric acid, Xanthine oxidase, Gout, Plants extract

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INTRODUCTION

Hyperuricemia is a condition that occurs because of the excessive concentration of uric acid in blood over the normal limit, caused by increased xanthine oxidase activity. The risk of developing gout is strongly associated with the degree of hyperuricemia [1]. Excess uric acid results from tissue deposition of monosodium urate (MSU) crystals in joints, bones and some other soft tissues, such as ligaments, tendons and skin or crystallization of uric acid in the renal collecting system (renal tubules and pelvis), resulting in uric acid nephrolithiasis. Normal uric acid levels for adults are no more than 7.0 mg/dl in men and 6.0 mg/dl in women. The removal of uric acid in the urine is regulated by the kidneys. If the production of uric acid becomes very excessive as a result, the level of uric acid in the blood becomes high. This condition is called hyperuricemia, while inflammation of the joints due to uric acid deposition is known as gout. Hyperuricemia is a risk factor for gouty arthritis, kidney stone formation, and atherosclerosis [1-4].

Allopurinol is by far the most commonly used drug for the treatment of hyperuricemia. Allopurinol works by inhibiting the enzyme xanthine oxidase, which converts hypoxanthine into xanthine and xanthine into uric acid. However, allopurinol has many side effects, such as gastrointestinal disorders, allergies and leukopenia; more severe side effects include hepatitis, interstitial nephritis and eosinophilia. In an effort to reduce the side effects of using allopurinol, other types of drugs are needed as alternatives derived from plants that have milder side effects [5-7].

Indonesians have used plants to treat various diseases, including to reduce uric acid levels in the blood. Some selected plants traditionally used as uric acid drugs in Indonesia have been examined [2], but some plants that are also used for gout have not been examined for their activity against xanthine oxidase inhibition *in vitro*. These plants are Dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb.), scented pandan leaves (*Pandanus amaryllifolius* Roxb.), *Alyxia reinwardtii* Blume stem barks, *Ruta angustifolia* Pers aerial parts, *Dioscorea hispida* Dennst tubers, *Plantago major* L. leaves, *Symphytum officinale* L. roots, *Euphorbia hirta* L. aerial parts, *Chromolaena odorata* L. leaves, *Solanum torvum* Sw fruits, *Peperomia pellucida* L. Kunth. aerial parts and Strobilanthes crispa L. Blume leaves [9-16]. These selected plant extracts were examined for their antihyperuricemia activity in this study.

MATERIALS AND METHODS

Plants material

Eleutherine bulbosa (Mill.) Urb. bulbs, *Pandanus amaryllifolius* Roxb. leaves, *Alyxia reinwardtii* Blume stem barks, *Ruta angustifolia* Pers aerial parts, *Dioscorea hispida* Dennst tubers, *Plantago major* L. leaves, *Symphytum officinale* L. roots, *Euphorbia hirta* L. aerial parts, *Chromolaena odorata* L. leaves, *Solanum torvum* Sw fruits, *Peperomia pellucida* L. Kunth. aerial parts and *Strobilanthes crispa* L. Blume leaves. All plant materials were obtained from the Manoko Experimental Garden of the Medicinal Plants Center of the Forestry Department in Lembang, West Java, Indonesia and identified by the Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java, Indonesia. The plant materials obtained were sorted and cleaned of impurities then powdered before extraction.

Chemicals

Ethanol technical grade sodium hydroxide was purchased from Bratachem, Bandung Indonesia. Allopurinol, xanthine, xanthine oxidase was purchased from Sigma Aldrich, Singapore. Potassium dihydrogen phosphate, potassium hydrogen phosphate, dimethyl sulfoxide (DMSO), HCl were from Merck. *In vitro* xanthine oxidase inhibition assay was determined using UV spectroscopy Hp 8452 A Shimadzu.

Phytochemical screening

Phytochemical screening is used to identify the secondary metabolite group found in a plant. Alkaloids, flavonoids, polyphenols, quinones, saponins, steroid-triterpenoids, and monoterpenoids-sesquiterpenoids (essential oils) were among the secondary plant metabolites studied. Phytochemical screening was performed using a conventional approach for plant material [17, 18].

Plants extraction

Each dried powder of plants material (500 g) was extracted by cold maceration in 70% ethanol (5 L x 3 d). The extract solvent was evaporated using a rotary evaporator at 50 °C. The concentrated extracts were collected and stored in a dry condition until analysis [17, 19].

***In vitro* screening of xanthine oxidation inhibition assay**

Activity test has been performed *in vitro* to quantify the activity of an enzyme xanthine oxidase using UV spectrophotometry. Xanthine oxidase, the enzyme from cow's milk was prepared by dilution to a final concentration of 1 Units per ml. In order to enhance the solubility of xanthine, five drops of 1.0 mg NaOH were added to produce 1 million mmol substrate solution of xanthine. The xanthine substrate solution was prepared by dilution to obtain final concentration of 0.15 mmol. The plant's extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and made into a series of dilution to obtain final concentrations of 10-50 µg/ml; allopurinol was used as a positive control with the final concentration of 0.1-2 µg/ml. The amount of 2.9 ml of potassium phosphate buffer (0.05 M, pH 7.5), 1 ml of specimen solution (the plants extracts solution or allopurinol) and 0.1 ml of 0.1 unit/ml xanthine oxidase were added and mixed well, the mixture was pre-incubated for 15 min at 37 °C. After preincubation of the test solution at 37 °C for 15 min, the reaction was initiated by addition of 2 ml of xanthine substrate into the solution and incubated in the dark at 37 °C for 30 min [2, 20-22].

The reaction was stopped by adding 2 ml of 1 N HCl into the solution, and the absorbance was measured at 290 nm using an UV spectrophotometer, suggesting the formation of uric acid. In this study, allopurinol was used as the positive control. All the experiments were performed in triplicate. Inhibition of the xanthine oxidase inhibitory activity was determined by the following formula:

$$\text{Inhibition (\%)} = \frac{[(A_{\text{control}} - A_{\text{control's blank}}) - (A_{\text{sample}} - A_{\text{control's blank}})]}{(A_{\text{control}} - A_{\text{control's blank}})} \text{x100}$$

IC_{50} values were obtained by linear regression analysis of a plot a series of different sample concentrations against percent inhibition [22-23].

RESULTS

All plant extracts were obtained by maceration method using 70% ethanol solvent. The extraction results from each plant can be seen in the table 1.

Table 1: Data of selected medicinal plant's materials extracted along with percentage data on the yield of extraction with ethanol solvent using maceration method

No	Species	Family	Local name	Plant parts	Percentage yields (%)	FHI requirement (%)
1	*Eleutherine bulbosa* (Mill.) Urb	Iridaceae	Bawang sabrang, bawang dayak	Bulbs	15.02	-
2	*Pandanus amaryllifolius* Roxb.	Pandanaceae	Pandan wangi, scented pandan	Leaves	14.69	-
3	*Alyxia reinwardtii* Blume	Apocynaceae	Pulasari	Stem barks	14.53	>12.4
4	*Ruta angustifolia* Pers	Rutaceae	Ingu	Aerial parts	14.57	-
5	*Dioscorea hispida* Dennst	Dioscoreaceae	Gadung, bitter yam	Tubers	9.68	-
6	*Plantago major* L.	Plantaginaceae	Daun sendok, broad-leaved plantain	Leaves	11.45	>8.7
7	*Symphytum officinale* L.	Boraginaceae	Kompri, comfrey	Roots	10.52	-
8	*Euphorbia hirta* L.	Euphorbiaceae	Patikan kebo, asthma weed	Aerial parts	20.98	>18.2
9	*Chromolaena odorata* L.	Asteraceae	Kirinyuh, siam weed	Leaves	14.73	>12.0
10	*Solanum torvum* Sw	Solanaceae	Takokak, pea eggplant	Fruits	14.77	-
11	*Peperomia pellucida* L. Kunth.	Piperaceae	Sasaladaan, suruhan, pepper-elder	Aerial parts	18.35	>13.1
12	*Strobilanthes crispa* L. Blume	Acanthaceae	Kejibeling	Leaves	15.07	>8.5

Notes: FHI is Farmakope Herbal Indonesia 2nd edition; phytochemical screening is carried out on the dried plant parts of selected medicinal plants. The results of phytochemical screening can be seen in the table 2.

Table 2: The results of phytochemical screening of some medicinal plant materials

No	Plant parts	Alk	Flv	Plph	Tan	Mntrp/Sqtrp	Ster	Trtrp	Qui	Sap
1	*Eleutherine bulbosa* (Mill.) Urb. bulbs	+	+	+	-	+	-	-	+	+
2	*Pandanus amaryllifolius* Roxb. leaves	+	+	+	-	+	-	-	+	+
3	*Alyxia reinwardtii* Blume stem barks	+	+	+	-	+	-	-	+	+
4	*Ruta angustifolia* Pers. aerial parts	+	+	+	-	+	-	-	+	+
5	*Dioscorea hispida* Dennst. tubers	-	+	+	-	-	-	-	-	+
6	*Plantago major* L. leaves	-	+	+	-	-	-	-	+	+
7	*Symphytum officinale* L. roots	-	+	+	+	-	-	-	+	+
8	*Euphorbia hirta* L. aerial parts	-	+	+	-	-	-	-	-	+
9	*Chromolaena odorata* L. leaves	-	+	+	-	+	+	-	+	-
10	*Solanum torvum* Sw. fruits	-	+	+	-	+	+	-	+	+
11	*Peperomia pellucida* L. Kunth. aerial parts	-	+	+	-	+	+	-	+	-
12	*Strobilanthes crispa* L. Blume leaves	-	+	+	-	+	+	-	+	_

Notes: +for positive result,-for a negative result, Alk for alkaloids, Flv for flavonoids, Plph for polyphenols, Tan for tannins, Mntrp for monoterpenoids, Sqtrp for sesquiterpenoids, Ster for sterois, Trtrp for triterpenoids, Qui for quinones and Sap for saponins

The results of the xanthine oxidase inhibitory activity test of 12 selected medicinal plants used as gout medicine in Indonesia can be seen in table 3. The percentage inhibition data listed is the average of three repetitions. In this study allopurinol was used as positive

control. The IC_{50}, half maximal inhibitory concentration, was calculated from the concentration-response curve of the respective extracts or specimens. The IC_{50} value of each test plant extract was then visualized in the form of a graph, as shown in fig. 1.

Table 3: Test results of xanthine oxidase inhibitory activity from selected gout medicinal plants

No	Plants extract	The average percentage of Inhibition (%)					IC_{50} (µg/ml)
		Concentration (µg/ml)					
		10	**20**	**30**	**40**	**50**	
1	*Eleutherine bulbosa* (Mill.) Urb. bulbs	15.62+0.63	23.88+0.60	36.98+0.76	47.06+0.19	62.71+0.59	40.86
2	*Pandanus amaryllifolius* Roxb. leaves	12.02+0.63	27.61+0.63	37.80+0,95	45.41+0,43	53.61+0,96	44.57
3	*Alyxia reinwardtii* Blume stem barks	11.26+0.46	27.87+0.55	38.55+0.44	48.24+0.74	57.06+0.45	41.72
4	*Ruta angustifolia* Pers. aerial parts	16.44+0.82	33.56+0.77	43.29+0.88	52.56+0.70	61.09+1.31	37.95
5	*Dioscorea hispida* Dennst. tubers	6.68+0.36	13.26+0.62	30.14+0.84	41.76+0.33	53.49+0.82	47.14
6	*Plantago major* L. leaves	12.51+0,70	29.24+0.63	39.04+0.94	53.44+0.08	62.53+0.58	38.57
7	*Symphytum officinale* L. roots	31.21+1.01	39.02+0.95	42.29+0.53	48.83+1.77	53.49+0.79	42.93
8	*Euphorbia hirta* L. aerial parts	35.52+0.68	42.83+0.51	50.46+0.86	54.91+0.23	61.28+1.46	31.57
9	*Chromolaena odorata* L. leaves	21.47+0.69	29.09+0.82	37.62+0.76	48.03+0.52	62.38+0.40	40.21
10	*Solanum torvum* Sw. fruits	9.05+0.60	24.62+0.48	38.06+0.57	50.60+0.57	74.23+0.54	36.54
11	*Peperomia pellucida* L. Kunth. aerial parts	11.94+0.21	20.16+0.44	30.17+0.72	47.75+1.08	59.51+0.44	43.11
12	*Strobilanthes crispa* L. Blume leaves	23.80+0.50	44.54+0.43	53.92+0.66	65.94+0.63	75.55+0.76	27.80
		0.1	**0.2**	**0.5**	**1.0**	**2.0**	
Allopurinol		15.96+0.46	19.67+0.39	29.30+0.15	47.10+0.21	69.51+0.22	1.24

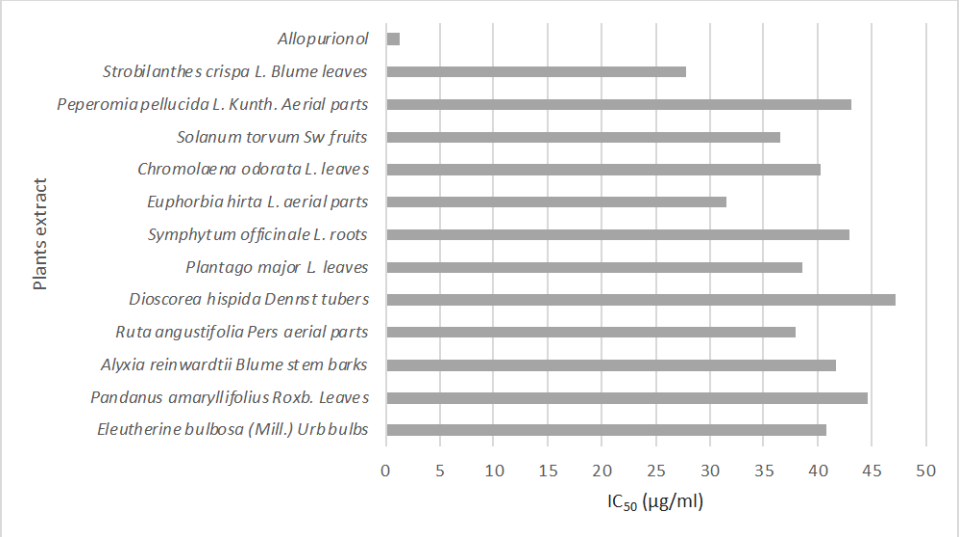

Fig. 1: IC_{50} values of selected medicinal plants extracts and allopurinol

DISCUSSION

Before extraction, all dried plant parts must be grinded first. The purpose of grinding or reducing the size of the dried plants is to expand the surface of materials to contact with the solvent during extraction [17, 24].

All plant parts were extracted by maceration method with 70% ethanol solvent. 70% ethanol is used as an extraction solvent because this solvent is polar so that it can attract almost all metabolites from natural materials; non-toxic so that it is safe for use as a medicinal ingredient, easily separated using a rotary evaporator under vacuum conditions, and relatively affordable [25]. Maceration was chosen as the extraction method in this study because this method uses simple equipment with simple procedures but can withdraw metabolites from plant materials without heating, so this method is also safe to extract metabolites that are not heat resistant. Furthermore, the maceration method is used for the extraction of plant materials because the plant materials may contain some compounds that are not heat resistant require exposure to solvents longer to increase yield and this method is used to make extracts for human consumption [24-26]. Based on table 1, it is known that the extract yield values of some selected gout medicinal plants range from 9.68% to 20.98%. The extract yield is the ratio between the number of metabolites obtained after the extraction process and the weight of the sample used. Extract yield is influenced by the solubility of metabolites from plant material in the solvent. The extract yields for the stems of *Alyxia reinwardtii* Blume, leaves of *Plantago major* L., aerial parts of *Euphorbia hirta* L., leaves of *Chromolaena odorata* L., aerial parts of *Peperomia pellucida* L. Kunth and leaves of *Strobilanthes crispa* L. Blume met the requirements stated in the Farmakope Herbal Indonesia 2nd edition [27]. The extract yield requirements of bulbs of *Eleutherine bulbosa* (Mill.) Urb., leaves of *Pandanus amaryllifolius* Roxb., aerial parts of *Ruta angustifolia* Pers., tubers of *Dioscorea hispida* Dennst., roots of *Symphytum officinale* L., and fruit of *Solanum torvum* Sw. have not been listed in the Farmakope Herbal Indonesia 2nd edition [27]. However, the yield of all extracts from the test plants is in the good category because they are all above 10% or close to 10% for *Dioscorea hispida* Dennst. tubers [17].

Phytochemical screening was conducted on dried parts of the test plants using a common method which has been slightly modified [18]. The results of phytochemical screening as listed in table 2. The selected tested gout medicinal plants contain different classes of secondary metabolites. All tested plant materials contain phenolic and flavonoid groups. Flavonoids or phenolics groups have been reported to inhibit the action of the xanthine oxidase enzyme that converts purines into uric acid [28]. The conversion of hypoxanthine to xanthine by xanthine oxidase and guanase is a process that synthesizes

uric acid through the oxidative pathway. This process is followed by the oxidation of xanthine to uric acid, which also catalyzed by the enzyme xanthine oxidase [1, 4, 8]. Therefore, the pharmacological intervention for hyperuricemia and gout needs to be inhibited by xanthine oxidase. The triterpenoid saponin, riparsaponin, from *Homonoia riparia* Lour stem have been reported to inhibit xanthine oxidase activity [3]. So that, the active substances that play a role in reducing blood uric acid levels in mice can be suspected as flavonoid, phenolic, triterpenoids and saponins groups [18, 29].

The results of *in vitro* xanthine oxidase inhibition assay of some selected gout medicinal plants from Indonesia was summarized in table 2 and fig. 1. *Strobilanthes crispa* L. Blume leaves extract has the smallest IC_{50} values among the tested plant extract (27.8 µg/ml). It indicates that *Strobilanthes crispa* L. Blume leaves extract has the best xanthine oxidation inhibitory activity. However, although the xanthine oxydase inhibitory activity of *Strobilanthes crispa* L. Blume leaves is good enough with an IC_{50} value below 50 µg/ml, this activity is still lower when compared to allopurinol (as a positive control in this study) with an IC_{50} value of 1.24 µg/ml.

In this experiment, the test result of xanthine oxygenase inhibitory activity of ethanolic extract of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) yield the IC_{50} values of 40.86 µg/ml, but the juice of bawang merah or shallot (*Allium cepa* L.) bulbs have better xanthine oxygenase inhibitory activity, with an IC_{50} value of 14.2 µg/ml (with allopurinol (as positive control) IC_{50} value of 10.5 µg/ml). Flavonoids and phenolic compounds from shallots have anti-hyperuricemia activity. Phenolic compounds are inhibitors of several enzymes, including xanthine oxydase, cyclooxygenase and lipooxygenase. Flavonoids can interact with the hydrophobic group of xanthine oxydase therefore it changing the structure of this enzyme and causing the reduction of this enzyme catalytic effect [30]. The two types of onions have different inhibitory activity of xanthine oxydase; this may occur due to differences in extraction methods and solvents. The different extraction methods and solvents can cause variations in the type and amount of metabolites extracted, which can lead to a different activities [17, 30].

Other studies regarding xanthine oxydase inhibition activity screening that have been reported are activity screening of xanthine oxydase inhibitor activity against several Indonesian plants commonly used as traditional gout medicine, such as *Centella asiatica* (L) Urban herbs, *Catharanthus roseus* leaves, *Sida rhombifolia* L. stems, *Physalis peruviana* leaves, *Tinospora crispa* (L.) Miers stems, *Anredera cordifolia* (Ten.) Steenis. herbs, *Annona muricata* L. leaves and *Imperata cyllindrica* stems. In this study, the ethanol extracts *Sida rhombifolia* stems showed the highest inhibition on xanthine oxidase activity with IC_{50} of 21.43 µg/ml, followed by *Sonchus arvensis* leaves extract with IC_{50} of 23.64 µg/ml [8]. These IC_{50} value are not very different when compared to the IC_{50} value of the *Strobilanthes crispa* L. Blume leaves extract (27.8 µg/ml).

The *Strobilanthes crispa* L. Blume leaves, known as keji beling in Indonesia, have been reported to have many pharmacological activities, including as anti-hyperglycemic, anti-oxidant, anti-microbial, wound healing anti-cancer on breast, liver cervical, lung, prostate and nasopharyngeal cancer, anti-inflamatory, anti-trypanosomal, anti-obesity [31-37]. However, the research on the *Strobilanthes crispa* L. Blume leaves as an anti-hyperuricemia has never been studied before, thus, it in necessary to do further research regarding the potential of this plant to be a gout medicine.

Some chemical compounds from *Strobilanthes crispa* L. Blume leaves that have been reported are phenolic compounds such as caffeic acid, ferulic acid, gallic acid, chlorogenic acid, trans-cinnamic acid; flavonoid compounds such as quercetin, rutin, catechin, apigenin, naringenin, kaempferol. Other metabolites of this plant that have also been reported are 1-heptacosanol, tetracosanoic acid; fatty acid groups such as stigmasterol, β-amyrin, taraxerol, taraxerone; fatty acid esters such as taxaxerol and stigmasterol βD-glucopyranoside [32, 38]. Flavonoid compounds and phenolic compounds contained in *Strobilanthes crispa* L. Blume leaves are thought to have a role in inhibiting xanthine oxidase activity [4]. This assumption is based on research that states that flavonoids and phenolic acid can mainly reduce uric acid levels in the serum of hyperuricemia rats by inhibiting xanthine oxydase activity and regulating urate transporters in the kidney. Flavonoids and phenolic acid prevent the pathological process of hyperuricemia by regulating biomarkers related to purine metabolism, amino acid metabolism, and lipid metabolism [28, 30, 39].

Based on this research, plant extracts that also have the potential to be developed as anti-hyperuricemia is aerial parts *of Euphorbia hirta* L. with an IC_{50} value of 31.57 µg/ml, but the xanthine oxidase activity of this plant has been reported. Deep eutectic solvent (DES) has been chosen as the green solvent to be employed in the investigation of a natural inhibitor, *Euphorbia hirta* L., via the green extraction method. The leaves of *Euphorbia hirta* L. were extracted using a molar ratio of 2:1 choline chloride to D-glucose (ChCl-Glu) solvent. This solvent offers various advantages, including low cost and toxicity, biodegradability, and ease of manufacture. *Euphorbia hirta L.* leaves extract possesses xanthin oxydase inhibitory activity with IC_{50} values of 9.40 µg/ml and allopurinol inhibitory activity with IC_{50} values of 6.94 µg/ml. The difference in IC_{50} values with our research is likely due to differences in solvents used in the extraction process and the tested plant parts; however, both studies show that *Euphorbia hirta* L. extract also has great potential to be developed as a gout drug [40].

CONCLUSION

Strobilanthes crispa L. Blume leaves extract has the best inhibitory activity against xanthine oxidase among the tested plant extract, so it has the potential to be developed into herbal medicine to treat hyperuricemia. This study provides scientific support for the anti-hyperuricemia activity of these herbs, which are empirically used to treat gout.

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AUTHORS CONTRIBUTIONS

All authors had significant contributions to the study design and consented to be responsible for all parts of the process. Ami Tjitraresmi and Rini Hendriani conceived of the presented idea. Ami Tjitraresmi, Rini Hendriani, Imam Adi Wicaksono and Yasmiwar Susilawati design the study and supervised the experiment as well as data analysis. Inayah Noviandri, Evariani Dwi Wulandari and Nafrah Hayura Ivan contributed to samples preparation and performed the experiments. Ami Tjitraresmi wrote and edited the manuscript with input from all authors and reviewers.

CONFLICT OF INTERESTS

In this study, we have no conflict of interest to disclose.

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