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Research Article

Evaluation of Xanthine Oxidase Inhibitory and Antioxidant Activities of Three Organs of Idat (*Cratoxylum glaucum* Korth.) and Correlation with Phytochemical Content

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ABSTRACT

Introduction: Idat (*Cratoxylum glaucum* Korth.), belonging to the genus Cratoxylum, is known to contain xanthone, quinone, flavonoids, and other phenolic compounds. **Objectives:** to analyze total phenolic, flavonoid, antioxidant activity, and inhibitory xanthine oxidase activities of leaves, stem, and cortex of idat. **Methods:** Extraction of leaves, stem, and cortex of idat was carried out by reflux using n-hexane, ethyl acetate, and ethanol as a solvent. Antioxidant activity was tested by the DPPH method and calculated to get the antioxidant activity index (AAI). Determination of total phenolic and flavonoid levels by ultraviolet-visible spectrophotometry. **Results:** Spectrophotometers measured the inhibitory activity on xanthine oxidase in 96-well plates with allopurinol as standard. Total phenolic and flavonoid content from *C. glaucum* extracts varied from 6.62 to 48.77 g GAE/g extract and 1.54 - 25.96 g QE/100 g extract, respectively. The ethanol extracts of leaves, stem, and cortex were very strong antioxidant activity with Antioxidant Activity Index (AAI) values 3.89; 4.55; 10.50, meanwhile AAI of ascorbic acid and quercetin 9.46 and 14.81 respectively. The n-hexane of stem extract had a strong xanthine oxidase inhibitory activity with the IC_{50} was 36.64 µg/ml, while allopurinol was 5.02 µg/ml. **Conclusions:** Total phenolic content contributed to antioxidant activity. Phenolic compounds in leaves extracts led to the xanthine oxidase inhibitory and antioxidant activities. The extract of *C. glaucum* was active as an antioxidant and potentially an inhibitor of xanthine oxidase agents.

Key words: Antioxidant, *Cratoxylum glaucum*, Xanthine oxidase inhibitory.

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INTRODUCTION

Uric acid is formed endogenously. It is the end product of purine catabolism formed in the liver, the primary excretion in the kidney, and a small portion in the digestive system.[1] Uric acid is a marker of oxidation conditions in the body, such as ischemic conditions, atherosclerosis, diabetes, and chronic liver disorders.[2,3] The xanthine oxidase enzyme catalyzes the oxidation of xanthine to uric acid and hypoxanthine to xanthine. The oxidation is producing superoxide radicals like hydrogen peroxide and reactive oxygen species (ROS).[4]

An increase in the number of superoxide radicals can cause oxidative stress causing macro and micro complications in metabolic syndrome.[5,6] Gout is a condition of high uric acid levels (hyperuricemia) in the blood that lasts for a long time.[7] Allopurinol is used clinically to treat hyperuricemia and gout. But it has an undesirable effect like nephropathy, hypersensitivity, hepatitis, and skin rash.[8]

Cratoxylum glaucum belongs to the Hypericaceae family. *C. glaucum* is traditionally used to lower blood pressure, while young leaves are used in cuisine.[9] Pharmacological activities showed by the genus Cratoxylum were cytotoxic,[10-13] antidiabetic activity,[14] antimalarials,[15,16] antibacterial,[17,18] anti-HIV-1,[19] and antioxidant.[20-24] Information related to the evaluation of antioxidant activity and xanthine oxidase inhibitory activity of *C. glaucum* is very limited. The present research aimed to evaluate the antioxidant and xanthine oxidase inhibitory activities of leaves, stem, and cortex of *C. glaucum* Korth and their correlation with total phenolic and flavonoid content.

MATERIALS AND METHODS

Materials

Xanthine, xanthine oxidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid were obtained from Sigma-Aldrich Chemicals (St. Louise, MO, USA). The ascorbic acid buffer solution was obtained from Merck (Darmstadt, Germany). Allopurinol was purchased from TCI (Tokyo, Japan), all other reagents were analytical grade.

Preparation of plant extract

Fresh leaves, stems, and cortex of *C. glaucum* Korth., were collected in May 2018 from Bangka Belitung Island, Indonesia. The plant was determined at Herbarium Bogoriense of Research Center for Biology-Indonesian Institute of Sciences, Indonesia. The collected material was washed in tap water, cut, and dried at 50°C for two days for leaves (L) and three days for stems (S) and cortex (C). Then

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the crude drug (CD) was ground into powder before extraction. The phytochemical test was formed on crude drugs and extracts such as n-hexane, ethyl acetate, and ethanol using Farnsworth and Sarker's modified method.[25,26] Powdered sample 100 g was extracted by reflux employing three solvents with increasing polarity. Extraction was started with n-hexane and repeated three times for each of them. The residue was subsequently refluxed by a similar method using ethyl acetate and ethanol. The extract was evaporated at 50°C under vacuum (Rotavapor R-3, Butchi®, Switzerland). So, there were three n-hexane extracts (LE-1, SE-1, and CE-1), three ethyl acetate extracts (LE-2, SE-2, and CE-2), and three ethanol extracts (LE-3, SE-3, and CE-3). The extract yield percentage was determined based on the crude drug.

Total phenolic content (TPC)

The total phenolic content was evaluated with a Folin-Ciolcalteu reagent adapted to Pourmorad's method.[27] Each extract of 0.5 mL was piped into 5 mL Folin-Ciolcalteu reagent 10% and 4 mL of sodium carbonate 1M and incubated the mixtures for 15 min. The absorbance was measured at wavelength 765 nm. For each extract, the analysis was performed in triplicate. The results were reported as g gallic acid equivalents (GAE) per 100 g extract (g GAE/100 g) according to the calibration curve of gallic acid 40-100 µg/mL as standard.

Total flavonoid content (TFC)

The total content of flavonoids was measured using an adapted method from Chang.[28] Each extract of 0.5 mL was piped into 0.1 mL aluminum chloride 10%, 0.1 mL sodium acetate 1M and 2.8 mL aqua dest. The mixture was diluted with 1.5 mL ethanol and 15 min incubated. At wavelength 415 nm, the absorbance was read. For each extract, the analysis was performed in triplicate. The results were presented as g equivalents quercetin (QE) per 100 g extract (g QE/100 g) according to the calibration curve of quercetin 50-100 µg/mL as standard.

Antioxidant activity (AAI)

The 2,2-diphenyl-1-picrylhydrazyl evaluated the antioxidant activity (DPPH) adapted from the modification of Blois's method.[29] Different concentrations of each sample were mixed with DPPH solution 50 µg/mL (volume 1:1) to start the reaction to get a calibration curve. Using blank methanol and DPPH was used as a control. The standard was used as ascorbic acid and quercetin. Evaluation of absorbance was conducted at wavelength 516 nm by using spectrophotometer UV-Vis (Beckman® Coulter DU 720) after 30 min of incubation. The experiment was done with tri replication for standard and sample.

The radical scavenging activity was calculated according to the equation; $I\% = [(Abs_0 - Abs_1)/Abs_0] \times 100$, where Abs_0 is the control absorbance and Abs_1 is the absorbance of the sample at various concentration. The IC_{50} (concentration that inhibits 50% of DPPH) is calculated using the calibration curve between concentration and percent inhibition. The antioxidant activity index (AAI) was determined using the equation; AAI = [final concentration of DPPH (µg/mL)]/ IC_{50} (µg/mL).[30]

Xanthine oxidase inhibitory activity (XOI)

The inhibitory activity on xanthine oxidase was measured by spectrophotometer in 96-well plates (Corning®, UV-Transparent Clear Microplates) below the aerobic conditions, following the method reported by Owen and Duong with some modification.[31,32] Dissolved the extracts in dimethyl sulfoxide (DMSO) then dilute with buffer. The final DMSO concentration was not higher than 0.5%. Allopurinol was used as a standard. The test solution consisted of 50 µL of the sample of extract, 35 µL of phosphate buffer (pH 7.5), 30 µL of freshly made solution for the enzyme (0.2 unit/mL xanthine oxidase in phosphate buffer pH 7.5), then 60 µL of substrate solution (0.15 mM of xanthine in phosphate buffer pH 7.5). The test solution was incubated at 25°C for 5 min. The absorbance was measured at 15 minutes using a microplate reader (Tecan infinite® M200 pro) at λ 290 nm. In the same procedure, a blank was prepared, but the enzyme was substituted by phosphate buffer pH 7.5. The analysis for allopurinol and each extract was performed in triplicate. The inhibitory activity of xanthine oxidase was determined using the formula: $I\% = [(A - B)/A] \times 100$, where A = absorbance of enzyme xanthine oxidase without test extract – blank of A (absorbance without XO and test extract), B = absorbance of the test extract – blank of B (absorbance without XO). The IC_{50} value was determined from the calibration curve between concentration and percent inhibition.

Statistical analysis

The results reported were the means ± SD of at least three independent tests, using MS Excel Software, to evaluate the IC_{50} value. Analysis of the statistic (one-way ANOVA and post hoc Tukey) was carried out by SPSS 23. Using Pearson's method, correlations were made between the total phenolic and flavonoid content with antioxidant and xanthine oxidase inhibitory activities.

RESULTS

Phytochemical screening

Phytochemical screening was conducted to determine secondary metabolites in the leaves, stems, and cortex of *C. glaucum*. The screening was performed on the crude drug and extracts. The results of the phytochemical analysis were shown in Table 1. Alkaloid was not detected in all crude drugs and extracts. Flavonoids, quinones, phenols tannins, saponins, and steroids/triterpenoids were detected in the leaves and cortex's crude drug.

Extraction

The reflux method was used to extract the chemical content of *C. glaucum*, using solvents with increasing polarity. Determination of extract density was done to determine the level of viscosity of the extract used. Measurement of density was carried out on 1% w/v extract, and the results indicated the density of the extracts had in the range 0.62 - 0.91. Table 2 showed the yield and density of extracts.

Total phenolic and flavonoid content

Total phenol content of the extract was calculated as gallic acid equivalent using the calibration curve equation $y = 0.0054x + 0.057$; $R^2 = 0.994$. Total phenol levels for each organ of *C. glaucum* differed in the range of 6.62 - 48.77 g GAE/100 g extract (Table 2). Ethanol extract for each part of the plant organ had the highest concentration than the other solvents in the range of 29.51 - 48.77 g GAE/100 g extract. The total phenolic content of the extract was calculated as quercetin using a calibration curve equation $y = 0.0067x + 0.1121$, $R^2 = 0.995$. Total flavonoid levels for each extract varied from 1.54 to 25.96 g QE/100 g extract. SE-2 (ethyl acetate extract of the stem) had the highest total flavonoid levels (25.96 g QE/100 g), and the lowest levels (1.54 g QE/100 g) was CE-3 (ethanol extract of cortex). The results of total flavonoid content were shown in Table 2.

Antioxidant activity of the extract

Evaluation of antioxidant activity used free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), which was soluble in methanol or ethanol. It had a maximum wavelength of λ 515-520 nm. The antioxidant activity was expressed in the antioxidant activity index (AAI) showed in Table 3.

Xanthine oxidase inhibitory activity

Evaluation of xanthine oxidase inhibitory activity (XOI) from different parts of *C. glaucum* gave IC_{50} values in the range of 36.64 - 96.96 µg/ml with the IC_{50} of allopurinol was 5.02 µg/ml. The XOI activity of each extract can be seen in Table 3.

Table 1: Phytochemical screening of crude drug and extracts of *C. glaucum*.

Phytoconstituents Tested	Leaves of *C. glaucum*				Stem of *C. glaucum*				Cortex of *C. glaucum*			
	CD	LE-1	LE-2	LE-3	CD	SE-1	SE-2	SE-3	CD	CE-1	CE-2	CE-3
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Quinones	+	-	+	+	+	-	-	+	+	-	-	+
Phenols	+	-	+	+	+	-	-	+	+	-	+	+
Tannins	+	-	-	+	-	-	-	-	+	-	-	+
Saponins	+	-	-	+	+	-	-	+	+	-	+	+
Steroids/ Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+

CD = crude drug; (+) detected; (-) not detected, LE-1= n-hexane leaves extract, LE-2 = ethyl acetate leaves extract, LE-3 = ethanol leaves extract, SE-1= n-hexane stems extract, SE-2 = ethyl acetate stems extract, SE-3 = ethanol stems extract, CE-1= n-hexane cortex extract, CE-2 = ethyl acetate cortex extract, CE-3 = ethanol cortex extract

Table 2: Total phenolic and total flavonoid content of *C. glaucum* extracts.

Sample	Yield (%)	Density of Extract	TPC (g GAE/100 g)	TFC (g QE/100 g)
LE-1	4.61	0.62	8.89 ± 0.02	8.89 ± 0.04
LE-2	7.63	0.87	27.78 ± 0.07	25.28 ± 0.03
LE-3	16.12	0.76	35.95 ± 0.04	8.60 ± 0.01
SE-1	3.29	0.64	8.68 ± 0.02	20.36 ± 0.06
SE-2	3.26	0.91	17.88 ± 0.04	25.96 ± 0.09
SE-3	3.18	0.82	29.51 ± 0.04	4.92 ± 0.02
CE-1	2.33	0.69	6.62 ± 0.02	13.96 ± 0.05
CE-2	4.25	0.86	18.31 ± 0.02	17.89 ± 0.02
CE-3	12.84	0.87	48.77 ± 0.11	1.54 ± 0.01

the reported values are mean ± SD (n=3)

Table 3: Xantin oxidase inhibitory activity and antioxidant activity in various extracts of *C. glaucum*.

Sample	IC_{50} (µg/ml) (XOI)	IC_{50} (µg/ml) (DPPH)	AAI (DPPH)
LE-1	87.06 ± 1.47[a]	16.24 ± 0.29	1.54 ± 0.03
LE-2	83.64 ± 1.73[a]	7.33 ± 0.01	3.41 ± 0.01
LE-3	56.16 ± 1.16	6.24 ± 0.01	3.90 ± 0.01
SE-1	36.64 ± 0.68[b]	16.69 ± 0.66	1.50 ± 0.06
SE-2	69.62 ± 2.10[c]	12.32 ± 0.12	2.03 ± 0.02
SE-3	96.96 ± 2.52	5.50± 0.02	4.55 ± 0.02
CE-1	37.69 ± 4.28[b]	76.78 ± 0.06	0.33 ± 0.00
CE-2	65.42 ± 2.51[c]	8.70 ± 0.06	2.88 ± 0.02
CE-3	64.88 ± 3.61[c]	2.38 ± 0.01	10.50 ± 0.03
Allopurinol	5.02 ± 0.04	-	-
Ascorbic Acid	-	2.64 ± 0.00	9.46 ± 0.01
Quercetin	-	1.69 ± 0.00	14.81 ± 0.02

the reported value is mean ± SD (n=3), a - c with the same letter is not significant differences at $p<0.05$

Correlation between total phenolic, flavonoid, antioxidant activity index, and xanthine oxidase inhibitory in various extracts of *C. glaucum*

The high and positive correlation between AAI and total phenolic content was shown by the LE sample ($r = 0.995$, $p < 0.01$) and also for SE and CE samples (Table 4). Total phenolic content (TPC) of leaves extract (LE) had negative and significant correlation with xanthine oxidase inhibitory activity ($r = -0.795$, $p < 0.01$). The same thing was shown by stem extract (SE), which had a negatively significant correlation with total flavonoid content ($r = -0.668$; $p < 0.05$) (Table 5). The antioxidant activity of leaves extract (LE) was negatively significant correlation with IC_{50} XOI ($r = -0.728$, $p < 0.05$) (Table 6).

DISCUSSION

Idat (*Cratoxylum glaucum* Korth.) included in the Hypericaceae family, traditionally used for joint pain, lowering blood pressure, and young leaves are used in cuisine. Based on the results of phytochemical screening did not contain alkaloids. The difference in the crude drug content was caused by the distribution of different compounds in plant organs. The solvent which is used in extraction will affect the chemical content contained in the extract. *Cratoxylum* genus is known to have xanthone, flavonoids, quinones, anthraquinone, steroids/triterpenoids, and phenolic compounds.[20,33]

Extraction was carried out by reflux with different polarity solvents. The compound in the crude drug will be derived based on polarity. For leaves and cortex, ethanol extract had the highest yield, showing its high polar compound content (Table 2).

The higher antioxidant activity is demonstrated by lower IC_{50} DPPH or higher AAI of DPPH. The LE-1 with AAI value 1.54 and SE-1 (1.50) were classified in the category of strong antioxidant activity (AAI = 1 - 2), and n-hexane cortex extract (CE-1) was poor antioxidant with AAI value 0.33 (AAI < 0.5), meanwhile ethyl acetate and ethanol

Table 4: Pearson's correlation coefficient of total phenolic, flavonoid, and AAI of DPPH.

Antioxidant parameter	Pearson's coefficient correlation (r)	
	TPC	TFC
AAI DPPH LE	0.995**	0.298ns
AAI DPPH SE	0.958**	-0.910**
AAI DPPH CE	1.000**	0.889**

Table 5: Pearson's correlation coefficient of total phenolic, flavonoid, and xanthine oxidase inhibitory (IC_{50} XOI).

Xanthine oxidase inhibitory parameter	Pearson's coefficient correlation (r)	
	TPC	TFC
IC_{50} XOI LE	-0.795*	0.432ns
IC_{50} XOI SE	0.991**	-0.668*
IC_{50} XOI CE	0.686*	-0.264ns

IC_{50} XOI = xanthine oxidase inhibitory, TPC = total phenolic, TFC = total flavonoid, LE = sample LE, SE = sample SE, CE= sample CE, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

Table 6: Pearson's correlation coefficient of AAI DPPH and xanthine oxidase inhibitory (IC_{50} XOI).

Antioxidant parameter	Pearson's coefficient correlation (r)
	IC_{50} XOI
AAI DPPH LE	-0.728*
AAI DPPH SE	0.914**
AAI DPPH CE	0.666*

AAI = Antioxidant activity index of DPPH scavenging activities, IC_{50} XOI = xanthine oxidase inhibitory, LE = sample LE, SE = sample SE, CE = sample CE, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

extracts such as LE-2, LE-3, SE-2, SE-3, CE-2, CE-3 were very strong antioxidant activity (AAI > 2) with AAI values in the range of 2.03 - 10.50. Ethanol cortex extract (CE-3) with AAI value 10.50 had stronger antioxidant activity than ascorbic acid with AAI value 9.46. Besides, quercetin had an AAI value of 14.81. This result is in line with research by Scherer and Godoy.[30]

TPC and TFC had correlated with AAI DPPH when higher TPC exposed higher AAI DPPH. A positive correlation between AAI and total phenolic content was shown by the LE sample ($r = 0.995$, $p < 0.01$) following SE and CE samples (Table 4). In general, phenolic compounds have antioxidant activity due to the ability to donate electrons or as chelating metals.[34] *C. glaucum* is known to have antioxidant activity.[20] The previous researches stated that the other species (*C. formosum*) had antioxidant activity.[35,36] Dulcisxanthone B and cochinxanthone D had an antioxidant activity that was isolated from *C. cochinchinense*.[22,37] Phenolic compounds included tannins, flavonoids, and other compounds. Flavonoids with OH substitution on rings A and or B belong to the phenol group.[38] The antioxidant activity of flavonoids was influenced by the degree of hydroxylation and other substituents found in flavonoids.[34]

Phenolic compounds are known to have antioxidant activity [39,40] and xanthine oxidase inhibitory activity.[41] Flavonoid compounds have antioxidants and an anti-inflammation.[42] Total phenolic content (TPC) of leaves extract (LE) had negative and significant correlation with xanthine oxidase inhibitory activity ($r = -0.795$, $p < 0.01$). this revealed that high phenolic content would lower IC_{50} XOI, which exposed stronger xanthine oxidase inhibitory activity. The same thing was shown by stem extract (SE), which had a negatively significant correlation (Table 5) with total flavonoid content ($r = -0.668$; $p < 0.05$). The antioxidant activity of leaves extract (LE) was a negatively significant correlation with IC_{50} XOI ($r = -0.728$, $p < 0.05$), indicating that higher antioxidant activity in line with increases in xanthine oxidase inhibitory activity (Table 6). Phenolic compounds and flavonoids have xanthine oxidase inhibitory activity, [43,44,] like quercetin, myricetin, morin, kaempferol, and puerarin.[45]

The results showed that each organ of the plant gave a different pattern of inhibitory activity depending on the solvent used. This can be due to the differences in chemical content in each organ of *C. glaucum*. According to the result, it can be presumed that the total phenolic content of leaf extracts contributed to its antioxidant activity and xanthine oxidase inhibitory activity.

CONCLUSIONS

Leaves, stem, and cortex of *Cratoxylum glaucum* Korth. were potential as sources of natural antioxidants. The ethanol cortex extract had the most powerful AAI of DPPH than the other extracts. Total phenolic content of leaves extract gave significant and positive correlation with AAI of DPPH and significant and negative correlation with IC_{50} XOI. The n-hexane stem extract had the strongest xanthine oxidase inhibitory activity with an IC_{50} value of 36.64 µg/mL. Total phenolic content in leaves extracts contributed to antioxidant and xanthine oxidase inhibitory activities.

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CONFLICTS OF INTEREST

None.

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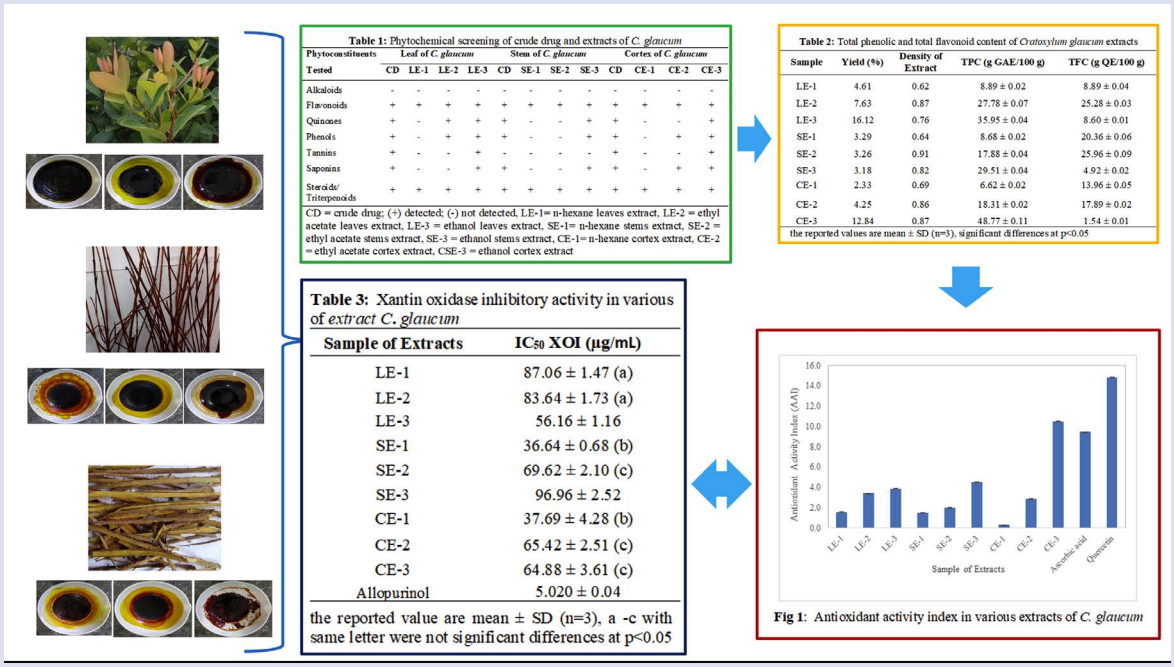
GRAPHICAL ABSTRACT



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