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Original Article

Antioxidant Activity and Isolation of Xanthine Oxidase Inhibitor from *Ruellia tuberosa* L. Leaves

Aktsar Roskiana Ahmad[1,2], Berna Elya[1], Abdul Mun'im[1]*

ABSTRACT

Introduction: The leaves of *Ruellia tuberosa* L. have been known to demonstrate strong antioxidant and xanthine oxidase (XOD) inhibitory activities. The aim of this study was to isolate antioxidant and XOD inhibitor from the leaves of the plants. **Methods:** Isolation of antioxidant and XOD inhibitor were conducted using chromatography techniques. The structure of the isolated compound was elucidated by spectroscopic methods. **Results:** In this study, a flavonoid was isolated and characterized as methoxylated flavonoid based on the spectral data including UV, IR, GC-MS, and NMR. The compound demonstrated DPPH free radical scavenging activity with IC_{50} of 28.79 µg/ml, and XOD inhibitory with IC_{50} of 0.67 µg/mL. **Conclusion:** The isolated compound was determined as 5-hydroxy-3,7-dimethoxy-2-(4-((3*S*,4*S*,5S,6R)-4,5,6-trihydroxy-2(hydroxymethoxy)-tetrahydro-2H-pyrane-3-iloxy) phenyl)-4H-chromen-4-on or camarosids. The isolated compound demonstrated strong DPPH free radical scavenging and XOD inhibitory activity

Key words: Antioxidant, DPPH, *Ruellia tuberosa*, Xanthine Oxidase, Flavonoid.

Aktsar Roskiana Ahmad[1,2], Berna Elya[1], Abdul Mun'im[1]*

[1]Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok 16424 West Java, INDONESIA
[2]Faculty of Pharmacy, Indonesia Muslim University, Makassar-INDONESIA

Correspondence

Abdul Mun'im, Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas INDONESIA, Kampus UI Depok 16424 West Java, INDONESIA

Phone no: +628111184550

E-mail: munim@farmasi.ui.ac.id

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INTRODUCTION

Genus *Ruellia* consist of 250 species, flowering plants, is commonly known as Ruellia. This genus distributed in the tropical country. Some of them are used as traditional medicinal plantsas anti-gonorrhea, anti-asthma, anti-fever and anti bronchitis.[1] One of the species is *Ruellia tuberosa* L.[2] This plant was believed to originate from South America.[3] In Indonesia, this plant distributed all over the country, mostly on the humid area. The plant is well known as medicinal plant and to have some biological activities, such as antinociceptive and anti-inflammation,[3] antimicrobial,[4] antioxidant and antihyperlipidemic,[5] antianthelmintic,[6] and anticarcinogenic.[7,8]

The phytochemical constituent of the plant has been investigated revealing the presence of flavonoid, phenolic compounds, sterol, saponin and alkaloids, triterpenoids, saponins.[9] Some flavonoids have been isolated from this plant.[10] The leaf, stem, root, fruit, and flower of this plant contained volatile oil.[11]

In the previous study, the butanol fraction of this plant demonstrated strong antioxidant and xanthine oxidase inhibitory activity.[9] This plant contained phenol and flavonoid.[4] Flavonoids are the mayor compound in leaves of this plant, such as cirsimaritin, cirsimarin, cirsiliol 4′-glucoside, sorbifolin, pedalitin and luteolin-7-*O*-glucoside.[10] Flavonoid is a polyphenolic compound which widely produced in nature. Some of researchers reported that the secondary metabolites such as flavonoid are potential to decrease the activity of xanthin oxidase and to scavenge the free radicals.[12-14] Flavonoid can give significant inhibition ability to xanthine oxidase and scavenging ability to peroxide.[15] This compound has some pharmacological activities as anti bacterial, antiviral, antioxidant, antimutagenic, and inhibits some of enzymes. Therefore this study presented isolation of flavonoid from the leave of the plant and to evaluate the xanthine oxidase inhibitory activity.

METHODS

Plant materials

The plant materials were obtained from Bogor, West Java. The authentification of sample was conducted in The Indonesian Academy of Science, Cibinong, and the voucher specimen was deposited in Herbarium of Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Indonesia.

Extraction and isolation

The leaves powder was macerated successively using dichloromethane and methanol. The methanolic extract was dispersed using warm water, then was partitioned with ethyl acetate and n-buthanol, successively. Organic layers were concentrated using rotary vacuum evaporator and dried to give ethyl acetate and buthanol fraction. Butanol fraction (50g) was chromatographed on a Sephadex LH-20 with 50% methanol. The fraction was collected in 10 ml aliquots in the test

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tube, and then was monitored by UV at 250, 300 and 350 nm, and then the absorbance were plotted with test tube number. The test tube under the same curve were combined and evaporated to give 6 fractions. Fraction 1 was chromatographed on a silica gel G 60 column with methanol: chloroform (3:1), to give 4 fractions (A-D). The precipitate of fraction B was recrystallized using methanol to get crystal.

Structure elucidation

The compound was identified by analyzing the spectroscopic data of UV-Vis, IR, GC-MS spectrometry, Nuclear magnetic resonance proton (^{1}H-NMR), carbon (^{13}C-NMR) and NMR-2D technique which included HMBC, DEPT, H-H COSY,and C-H COSY. UV spectrum of the compound was performed using Shimadzu UV-265. FT-IR spectrum was recorded in Shimadzu FTIR-8400S.^{1}H-NMR and ^{13}C-NMR spectra of the compound were recorded using DMSO-d_6 as the solvent system (JEOL α400 MHz NMR spectrometer).

Antioxidant activity assay

The isolate was assayed the antioxidant activity using DPPH method according to the method reported by Mun'im et al with slight modification.[16] Briefly, sample solution in methanol (2 ml) was diluted with 2 ml methanol and then was added DPPH solution in methanol (0.5mM). The reaction mixture was shaken homogenously, incubated at 37°C for 30 minutes, and the absorbances were measured at 517 nm (Shimadzu, Japan). Quercetin and BHT were used as positive control. The DPPH free radical-scavenging activity was expressed in terms of IC_{50} (the concentration of a sample requires to reducethe DPPH radical concentration by 50%).

Xanthine oxidase inhibitory assay

Xanthine oxidase inhibitory activity was determined according to Umamaheswari method with modification.[17] Briefly, sample solution test (1.0 ml) and 2.9 ml of phosphate buffer solution pH 7.8 and xanthine solution (2.0ml, 0.15mM)) were mixed and pre-incubated at 30°C for 10 min. The reaction was initiated by adding xanthine oxidase (0.1 ml, 0.1 U/ml). The reaction mixtures were incubated at 30°C for 30 min. The reaction was stopped by adding 1.0 ml of HCl 1 N, the absorption was measeured at 284 nm using a UV-Vis spectrophotometer.

Enzyme inhibition mechanism

The assay was performed in the absence and presence of the isolated compound with varying concentrations of the substrate, using the xanthine oxidase assay. The mode of inhibition mechanism of the compound was determined using Lineweaver-Burk plot. The data were calculated followingMichaelis-Menten formula.[18]

RESULTS

Structure of isolated compound

Isolated compound was a yellowish-white powder, melting point 150-152°C. Determination of the structure of isolated compound was conducted by analyzing the spectroscopic data including UV spectroscopy, infrared spectroscopy (IR), mass spectroscopy (MS), proton nuclear magnetic resonance (^{1}HNMR), carbon nuclear magnetic resonance (^{13}CNMR), DEPT spectroscopy, NMR-including 2D H-H COSY, C-H COSY and HMBC. UV λ_{max}: 229, 278 and 327. FT-IR ν_{max} (cm^{-1}): 3294, 2929.97, 1654,98, 835.21. ^{1}H-NMR (400 MHz, DMSO-d_6) δ*H*: 3.74 (*s*, 3H); 3.93 (*s*, 3H): 4.57 (*s*, 1H); 5.03 (*t*, 1H); 5.11 (*d*); 5.36 (*d*), 6.95 (*s*, 1H); 6.97 (*d*, 1H); 7.15 (*d*, 2H, *J*=8.8 Hz), and 8.07 (*d*, 2H, *J*=7.2 Hz).[13] C-NMR (100 MHz, DMSO) δ*C*:56,4; 60.0; 60.6; 69.6; 73.1; 76.5; 77.1; 91.6; 99.8; 103.6; 105.1; 116.5; 123.8; 128.1; 131.9; 151.9; 152.6; 158.6; 160.3; 182.2 and 163.3.

Antioxidant activity

The antioxidant activity of the isolate obtained was then tested again using the method of DPPH free radical scavenging. Test result of antioxidant compounds with IC_{50} value of the isolate is 28.79 µg/ml. DPPH free radical scavenging activity of isolated compound was presented in Table 1.

Xanthine oxidase inhibitory activity

Inhibition of the xanthine oxidase assay results shows that the isolated compound with IC_{50} values of 0.21 µg/ml. The XOD inhibitory activity of the isolated compound was lower than thatof allopurinol (Table 2).

Mode of inhibition

Lineweaver-Burk plot it shows point on the y-axis, which means having a competitive type of Mode of inhibition of the isolated compound was determined by Lineweaver-Burk plot. Based on the result, the inhibition was competitive inhibition (Figure 2).

DISCUSSION

UV-Vis spectrum of the isolated compound showed the maximum absorbance at a wavelength of 229, 278 and 327 nm which showed the presence of conjugated double bond. The FT-IR spectrum at ν = 3294 cm-1 indicated the presence of OH groups, cm^{-1} = 2929.97 cm^{-1} showed the C-H stretching vibration, ν = 1654.98 cm^{-1} indicated the presence of C=O stretching vibration, ν = 835.21 cm^{-1} indicated the presence of a substituted aromatic ring group. Spectra ^{1}H- and ^{13}C-NMR showed the characteristic of flavonoid glycoside. The isolated compound was identified bearing two methoxy groups at δ*C* 60.0 (3.70 ppm, 3H, *s*) and 56.4 ppm (3.93ppm, 3H, *s*) base on the ^{1}H- and ^{13}C-NMR spectra. This was supported by H-H and C-H COSY. An anomeric proton at 5.03 ppm correlated with C-4' (160.3 ppm) on HMBC (Heteronuclear Multiple Bond Connectivity) spectra. Detail HMBC correlation was presented in Figure 1. Base on the spectral data, the structure of isolated compound was determined as 5-hydroxy-3,7dimethoxy-2-(4-((3*S*,4*S*,5*S*,6*R*)-4,5,6-trihydroxy-2-(hydroxyl methyl)-tetrahydro-2H-pyran-3-yloxy)phenyl)-4H-chromen-4-one or was known as Camarosid (Figure 1). This compound has been isolated for the first time from *Calotropis procera*.[19] Until now, there is no report about the isolation of this compound from *Ruellia tuberosa*.

Flavonoid is polyphenolic compound, and well known to have antioxidant activity. Hydroxyl group in aromatic ring play a role as the proton donor to the free radical, and have chelating properties.[20] The number and position of the phenolic groups determine free radical scavenging activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method is based on the measurement of scavenging ability of antioxidant on stable free radical, DPPH.[21] Hydroxyl (phenolic) position or configuration is the most significant factorforfree radical scavenging activity. The hydroxyl groups donate hydrogen to radicals, stabilizing them.[19] *Ortho*-dihydroxy

Table 1: DPPH free radical scavenging activity

Name	IC_{50} (mg/ml)
Isolated compound	2.88
Quercetin	3.17
BHT	2.87

Table 2: Xanthine oxidase inhibitory activity

Name	IC_{50} (mg/ml)
Isolated compound	0.21
Allopurinol	0.066

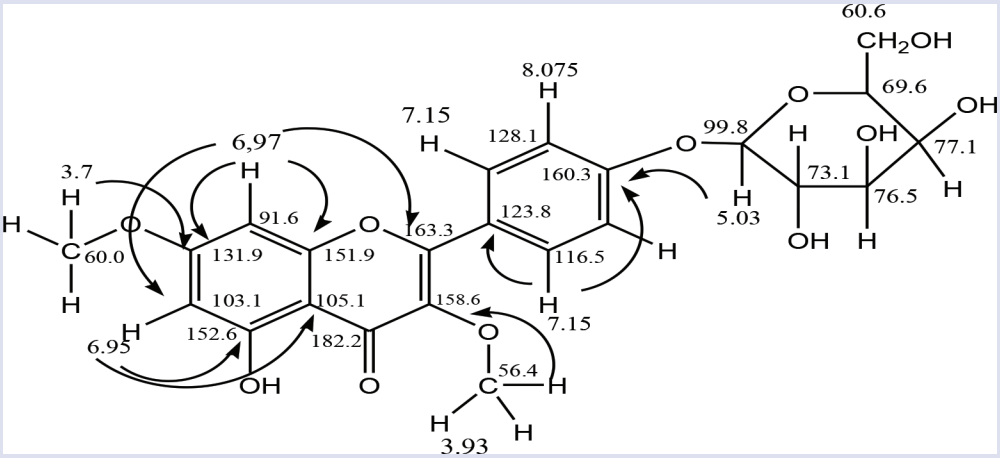

Figure 1: HMBC correlation of isolated compound

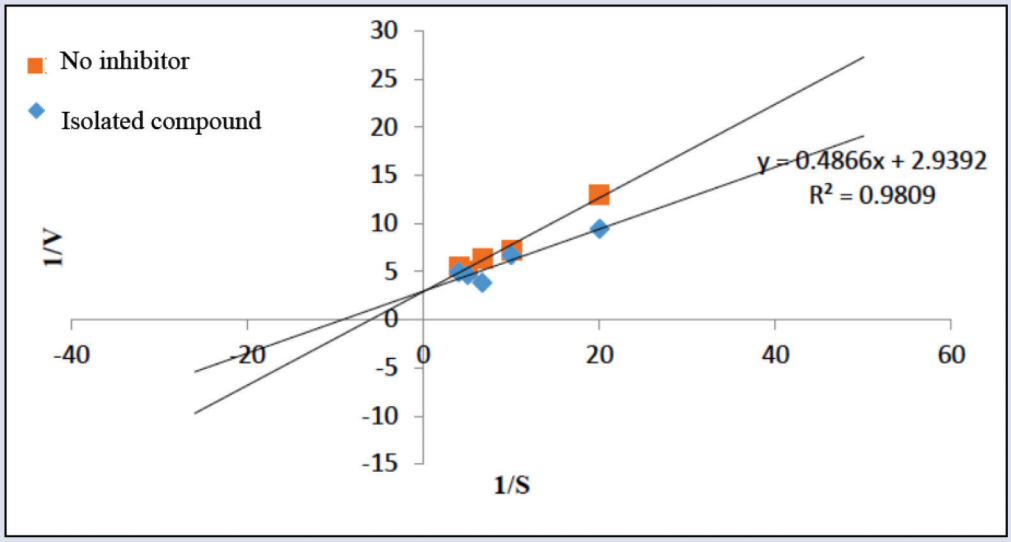

Figure 2: Lineweaver-Burk plot of xanthine oxidase inhibition of the isolated compound

in the B-ring of flavonoid is the most potent of peroxyl radicals scavengers.[20] Flavonoids with a hydroxyl grup at C-3 and 3',4'-dihydroxyare reported to be more potent than the 3',4'-catechol. Methylation or glycosylation of hydroxyl substituent abolishes the scavenging of DPPH.[20,21]

Flavonoid compounds, phenols, and tannins potentially inhibit xanthine oxidase.[12] Flavonoid contributes to reduce oxidative stress through inhibition of activation of the regulatory enzyme, such as the enzyme xanthine oxidase. The isolated compound is flavonoid glycosides with a hydroxyl group at the C-5 position can provide good inhibition against xanthine oxidase. The hydroxyl group at position C-5 and C-7 showed a positive contribution as the enzyme xanthine oxidase inhibitor, while the C-2' position, C-8, and C-3 will negatively contribute to the inhibition of xanthine oxidase.[18,14]

Lineweaver-Burk plot and isolate obtained Michaelis-Menten constant constants (Km) of isolated compound was 9.17. These results indicated the existence of a competitive inhibition type inhibitor. From the data analysis of Lineweaver-Burk and Michaelis-Menten constants, the isolated compound showed a competitive type inhibition mechanism. The compound will compete with the substrate on the active site of the enzyme, and as a result decreased enzyme activity.

CONCLUSION

The phytochemical investigation of *Ruellia tuberosa* leaves yielded a flavonoid, Camarosid. The isolated flavonoid exhibited strong xanthine oxidase inhibitory and antioxidant activities with IC_{50} of 0.21 and 2.88 µg/ml, respectively.

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CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATIONS USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl, XOD: Xanthine oxidase, UV: Ultraviolet, IR: Infra red, GC-MS: Gas Chromatography-Mass spectrometry, NMR: Nuclear magnetic resonance.

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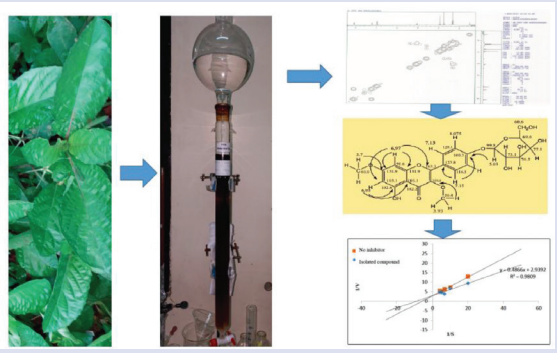
GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- This paper reported the isolation of antioxidant and xanthine oxidase inhibitor from *Ruelliat uberosa* leaves.
- A flavonoid, Camarosid was isolated using combination of chromatography techniques. The structure was elucidated based on spectral data.
- The isolated compound demonstrated free radical scavenging activity against DPPH, and xanthine oxidase inhibitory activity.

AUTHOR PROFILE

Akstar Roskiana Ahmad: Lecturer of Laboratory of Natural Products, Faculty of Pharmacy, Indonesia Muslim University, Makasar, Indonesia.

Berna Elya: Professor in Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia. Her research focused on Phytochemical and activity of Genus Garcinia.

Abdul Mun'im: Ph.D., Associate Professor at Departement Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia (UI) Depok, West Java, Indonesia. He has experience in the area of Pharmacognosy and Phytochemistry, working in drugs discovery of herbal plants, extraction technology, Metabolite profiling, Structure Elucidation, and Degenerative Diseases (such as diabetes mellitus, hypertension, and cholesterol)

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