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FULL PAPER

Evaluation of xanthine oxidase inhibitory activity of sinapyl alcohol diacetate and stigmasterol compounds and phytochemical screening stem of *Etlingera rubroloba* A.D Poulsen

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Abstract

Etlingera rubroloba A.D Poulsen is a genus *Etlingera* found in Southeast Sulawesi. This plant is widely used in local communities to relieve joint pain and increase stamina. Previous researchers isolated and identified this plant to obtain the compounds sinapyl alcohol diacetate and stigmasterol. However, these two compounds have never been reported on the Xanthine Oxidase (XO) inhibitory activity in vitro. The results of phytochemical screening have also not been reported in this plant. Therefore, in this study, a phytochemical screening test was conducted using colour reagents, and an in vitro test for XO inhibitory activity was carried out using the XO Kit. The results showed that *E. rubroloba* contains secondary metabolites of flavonoids, alkaloids, tannins, saponins, terpenoids, and the value of the percent XO inhibitory activity at a concentration of 320 µg/mL, namely sinapyl alcohol diacetate 85.52%, stigmasterol 90.42%, and allopurinol 97.63%, as a positive control. This research will be a reference in the development of anti-hyperuricemia medicine of nature.

1. Introduction

Etlingera is a family of Zingiberaceae, which has many species globally, around 150 to 200 species. This plant grows in Indonesia, including Java, Sulawesi, and Kalimantan. In Kalimantan, this plant is consumed daily as vegetables and traditional medicines, such as the species *E. elatior* used by local people as a cooking spice and headache medicine (Poulsen, 2012). In Sulawesi, Wawonii people use it as a nausea medicine (Rahayu, 2006).

Several species of *Etlingera* that have been reported regarding their pharmacological aspects are *E. calophrys* as an antioxidant and contain Yakuchinone A, p-Hydroxybenzoic acid, and stigmasterol compounds (Sahidin *et al.,* 2018). *Etlingera pubescens* contains etlingerin compounds and shows better antibacterial and cytotoxic activity than curcumin (Daniel-Jambun *et al.,* 2019). The stems of *E. elatior* contain stigmasterol, and p-hydroxybenzoic acid compounds, which are active as antioxidants and anti-bacterial (Sahidin *et al.,* 2019). Then the extract of *E. elatior* has intense antioxidant and anticancer activity (Ghasemzadeh *et al.,* 2015). Furthermore, *E. elatior* (ginger flower) can lower uric acid to be used to treat hyperuricemia (Dewi *et al.,* 2016). *Etlingera elatior* flower was extracted as a source of antimicrobial and antifungal agents and did not show toxicity (Lachumy *et al.,* 2010). *Etlingera elatior* (kantan flower) is a potential source of natural antioxidants for food and nutraceutical applications (Wijekoon *et al.,* 2011). The leaves of *Etlingera* species (*E. elatior, E. fulgens E. maingayi, E. littoralis,* and *E. rubrostriata*) showed antibacterial activity against Gram-positive bacteria (Chan *et al.,* 2007). *Etlingera* species, *E. elatior* leaf, *E. fulgens* leaf, and *E. maingayi* leaf, showed high tyrosinase inhibitory, antioxidant and antibacterial activity (Chan *et al.,* 2008). Then, *E. elatior* acts as hepatoprotective (Fristiohady *et al*., 2020) and *E. littoralis* as carminative and stomach (Chuakul and Boonpleng, 2003). Furthermore, *E. brevilabrum* works as cholesterol-lowering (Mahdavi, 2014), *E. coccinea* and *E. sessilanthera* as antibacterial (Daniel-Jambun *et al*., 2017), *E. rubroloba* fruit as an immunomodulator (Ilyas *et al*., 2021) and *E. rubroloba* stem extract as an antioxidant and xanthine oxidase (XO) (Jabbar *et al*., 2021a). *Etlingera elatior* fruit extract contains alkaloids, flavonoids, tannins, and terpenoids (Fristiohady *et al.,* 2020).

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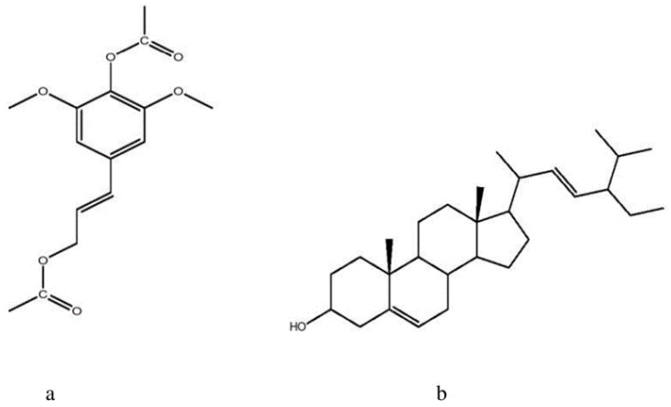
One of the synthetic drugs used to relieve joint pain and reduce uric acid (hyperuricemia) is allopurinol in health services. Allopurinol works by inhibiting XO, which catalyzes the formation of uric acid (Lullmann, 2000) because it has side effects of causing allergies and hepatotoxicity. Researchers are trying to find other XO inhibitors that are more potent and safer (Bomalaski and Clark, 2004). One of the *Etlingera* plant species studied is *E. rubroloba*. This plant has been isolated, identified, and generated synaphyl alcohol diacetate and stigmasterol compounds (Figure 1) (Jabbar *et al*., 2021b). These two compounds have never been tested for their activity as XO inhibitors. The aim of this study was to evaluate the activity of synaphyl alcohol diacetate and stigmasterol towards Xanthine oxidase inhibition and phytochemical screening of *E. rubroloba* A.D. Poulsen stem.



a b

Figure 1. Structure of Sinapyl alcohol diacetate (a) and Stigmasterol (b).

2. Materials and methods

2.1 Materials

The materials used in this study were *E. rubroloba* stem, methanol, DMSO, 96% ethanol, xanthine oxidase assay (Ab102522), compounds isolated from sinapyl alcohol diacetate, and stigmasterol (Jabbar *et al.,* 2021b).

2.2 Equipment

This study used a microplate flat-bottom polystyrene 96 well (Iwaki, Japan), rotary vacuum evaporator (Buchi®) micropipette (Gilson, France), hot plate (Stuart®), Erlenmeyer (Pyrex), beaker glass, micropipette (Socorex, Switzerland), analytical balance (Precissa®), water bath, and blender (Philips).

2.3 Sample preparation and extraction

The stems of *E. rubroloba* were collected from Konawe District, Southeast Sulawesi, and then determined at the LIPI Research Center for Biology, Cibinong, Bogor, Indonesia. The sample was cleaned, dried at 50°C, and powdered, and 5120 g was obtained. Samples were extracted using the maceration method with 12 L of methanol as a solvent (3 × 24 hrs). The filtrate was obtained by evaporating at 50°C to obtain 150 g of methanol extract.

2.4 Phytochemical screening

The screening test takes several crude extracts and then adds an appropriate solvent to test the secondary metabolite compound, namely flavonoids, alkaloids, saponins, tannins, terpenoids, and steroids (Amaral *et al*., 2021). Table 1 shows specific reagents used in each class of secondary metabolite compounds.

Table 1. Secondary metabolite compound *E. rubroloba* extract with specific reagent

Chemical class	Specific reagent
Flavonoids	Mg + HCl
Alkaloids	Dragendorff
Tannins	$FeCl_3$
Saponins	Distilled Water
Triterpenoids	Libermann-Buchard

2.5 Xanthine Oxidase inhibitory activity

2.5.1 In vitro Xanthine Oxidase inhibitory activity

The XO inhibitory activity test was carried out in this study using the XO assay kit protocol (Abcam, 2018; Jabbar *et al.,* 2021a)

2.5.2 Standard curve preparation

Approximately 4 µL of 0.88 M H_2O_2 standard was diluted into 348 µL dH_2O to generate 10 mM H_2O_2 standard, then 20 µL of 10 mM H_2O_2 standard was diluted into 980 µL dH_2O to generate 0.2 mM H_2O_2 standard.

2.5.3 Colorimetric assay

As much as (0, 10, 20, 30, 40, 50 µL) 0.2 mM H_2O_2 standard was introduced into a 96-well plate (duplication), made up to 50 µL per well with dH_2O, resulting in 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 standard.

2.5.4 Reaction mix

The reaction mixture used contains 50 µL (Assay Buffer 44 µl, Substrate Mix 2 µL, Enzyme Mix 2 µL, OxiRed Probe 2 µL). Furthermore, a 50 µL reaction mixture was added to each well containing standard H2O2, positive control, and test sample, then mixed well. Then, the plate was measured (OD = 570 nm/ colorimetric test) before incubation (T1 read A1), and then measured again after incubation at 25°C for 10-20 mins protected from light (T2 read A2).

3. Results and discussion

Etlingera rubrolaba was collected from Konawe

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Selatan of Indonesia. Then the sample was extracted by maceration using methanol as a solvent (3 × 24 hrs) to obtain a methanol extract of *E. rubrolaba*. Furthermore, this extract was tested for phytochemical screening according to the established procedure, and the results can be seen in Table 2. Phytochemical screening was carried out on the methanol extract of *E. rubroloba* and generated secondary metabolites of flavonoids, alkaloids, tannins, saponins, and triterpenoids.

Table 2. Phytochemical screening of *E. rubroloba* methanol extract

Chemical class	Result	Description
Flavonoids	Positive	Discoloration into yellowish red
Alkaloids	Positive	Formed of a brown precipitate
Tannins	Positive	Discoloration into dark blue
Saponins	Positive	Stable foam is formed
Triterpenoids	Positive	Discoloration into reddish brown

The XO inhibitory activity test was conducted in vitro using the XO Abcam 1022522 kit (Abcam, 2018). Activity testing was carried out on sinapyl alcohol diacetate, stigmasterol, and allopurinol compounds as positive controls with various concentrations (20, 40, 80, 160, 320 µL/mL). Figure 2 shows the value of the percentage of xanthine oxidase inhibitory activity.

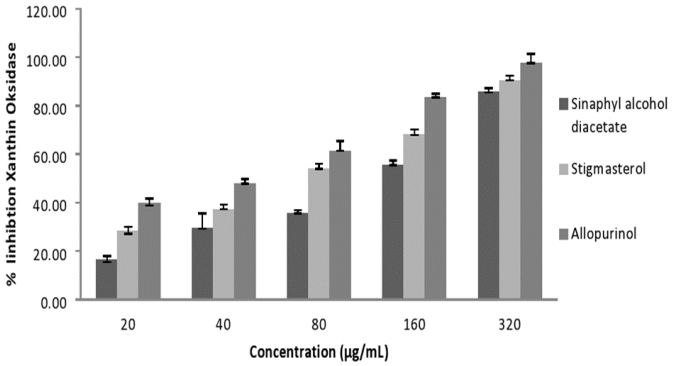

Figure 2. Percentage of XO inhibition of methanol extract *E. rubroloba*

The XO inhibitory activity showed different effects at various concentrations (20, 40, 80, 160, and 320 µL/mL) of sinapyl alcohol diacetate, stigmasterol compared with allopurinol as a standard control. All samples have different abilities to activate the XO enzyme at different concentrations. Its activity increased concentration with inhibition that is, allopurinol as a standard control was more significant than sinapyl alcohol diacetate and stigmasterol.

In this study, allopurinol had the best effect on XO inhibition both *in vitro* and *in vivo*. XO has an essential role in regulating uric acid and preventing overuse, such as hyperuricemia (Shukor *et al.,* 2018). Gout and hyperuricemia can be treated by increasing uric acid excretion of uric acid production (Kostic *et al.,* 2015). At this time, allopurinol is widely used in the treatment of hyperuricemia or gout, but it has side effects such as hypersensitivity, and intolerance (Lü *et al.,* 2013). Therefore, alternative XO inhibitors are recommended from natural compounds that have fewer side effects (Wong *et al.,* 2014).

4. Conclusion

Etlingera rubroloba extract contains flavonoids, alkaloids, tannins, saponins, and terpenoids. Sinapyl alcohol diacetate and stigmasterol compounds have XO inhibitory activity and can be developed as anti-hyperuricemia.

Conflict of interest

The authors declare no conflict of interest.

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