IOP Conf. Series: Materials Science and Engineering **349** (2018) 012017 doi:10.1088/1757-899X/349/1/012017

Isolation, Identification, and Xanthine Oxidase Inhibition Activity of Alkaloid Compound from *Peperomia pellucida*

E Fachriyah, M A Ghifari, K Anam

Chemistry Department, Faculty of Sciences and Mathematics
Diponegoro University

Corresponding author : enny.fachriyah@live.undip.ac.id

Abstract The research of the isolation and xanthine oxidation inhibition activity of alkaloid compound from *Peperomia pellucida* has been carried out. Alkaloid extract is isolated by column chromatography and preparative TLC. Alkaloid isolate is identified spectroscopically by UV-Vis spectrophotometer, FT-IR, and LC-MS/MS. Xanthine oxidase inhibition activity is carried out by in vitro assay. The result showed that the alkaloid isolated probably has piperidine basic structure. The alkaloid isolate has N-H, C-H, C = C, C = O, C-N, C-O-C groups and the aromatic ring. The IC_{50} values of ethanol and alkaloid extract are 71.6658 ppm and 76.3318 ppm, respectively. Alkaloid extract of *Peperomia pellucida* showed higher activity than ethanol extract.

1. Introduction

Indonesia is a developing country with the highest number of uric acid sufferers in Asia. Approximately 1.7% of Indonesia's population suffers gout [1][2]. Uric acid is caused by serum uric acid (SUA) levels that exceed the normal conditions. When uric acid levels in the blood exceed 6.8 mg / dL, monosodium urate crystals (MSU) will form and accumulate in joints, tendons, and other tissues. The pile of that crystals causes gout [3][4]. In addition, abnormal levels of SUA are correlated with cardiovascular disease, hypertension and renal disease [3]. Several therapies can be attempted to treat gout. The most effective therapy is to lowering uric acid levels, one of which can be used is a xanthine oxidase inhibitor [5][6].

Peperomia pellucida is a plant of the Piperaceae family and Peperomia genus. Traditionally, it is used by Indonesian and Filipino to treat kidney and gout problems [7][8]. So far has not been reported its bioactive compounds that have a reducing activity of uric acid. Phytochemical screening of *Peperomia pellucida* shows secondary metabolite content such as alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids [9]. Alkaloids have been known potential as an anti-gout by inhibiting the activity of xanthine oxidase enzyme [6]. Alkaloid compounds such as berberine, costinone A and costinone B have been shown inhibition activity of xanthine oxidase enzymes to reduce uric acid levels [10][11]. Based on that description, this research aims to isolate alkaloid compounds from *Peperomia pellucida* to know its inhibitory activity against xanthine oxidase enzyme.

2. Materials and Method

2.1 Materials

The materials in this research are *Peperomia pellucida*, methanol, ethanol, ethyl acetate n-hexane, chloroform, ammonia 25%, concentrated sulfuric 98%, distilled water, hydrochloric acid 2 N, Dragendorff reagent, Mayer reagent, silica gel TLC plate 60 GF_{254}, silica 60, DMSO, xanthine substrate X0626-5G, xanthine oxidase from bovine milk lyophilized powder X4736-5UN, allopurinol, aqua DM, potassium dihydrogen phosphate and potassium hydrogen phosphate.

2.2. Method

2.2.1 Alkaloid Isolation

Dried plants powder which has 2.5 kg of its weight was macerated with 96% ethanol. The extract was concentrated by a rotary evaporator (Buchi Rotavapor R-3) to obtain ethanol extract. Then chlorophyll was removed by adding hot distilled water (1:1). The presence of alkaloids in the ethanol-water extract was tested by Mayer and Dragenddorff reagents. Furthermore, the extract was partitioned with n-hexane. The n-hexane layer and ethanol-water layer were separated. Ethanol-water layer is added HCl 1M till pH 2 and partitioned with ethyl acetate to form two layers [12][13]. The alkaloid salt layer is added NH_4OH 25% till reach pH 9-10 and partitioned with ethyl acetate. The ethyl acetate layer was concentrated using a rotary evaporator to obtain alkaloid extract. Then, alkaloid extract is weighed and analyzed by TLC under the UV light 254 and 365 nm (CAMAG UV Cabinet 4) and Dragendorff reagent.

Alkaloid extract which has 2 g for its weight was subjected to column chromatography eluting with n-hexane, ethyl acetate, and methanol in increasing order of polarity. Eluate that produces the same pattern is combined into one fraction. Alkaloid fraction was isolated by preparative TLC using ethyl acetate and methanol as eluent. The alkaloid bands are scraped and dissolved in methanol. The alkaloid isolates were identified using UV-Vis spectroscopy (Shimadzu UV-1280), FTIR (Perkin Elmer 10.4.00), and LC-MS / MS.

2.2.2 Xanthine oxidase inhibition assay

Sample (ethanol extract, alkaloid extract, and allopurinol) which has 10 mg for its weight were dissolved in a phosphate buffer pH 7.5 to obtain 100, 50, 20, 10 and 5 ppm solutions. Allopurinol is used as a positive control. The assay solution consisted of 1 mL of sample solution (5-100 ppm), 2.9 mL of 0.05 M potassium phosphate buffer (pH 7.5) and 0.1 mL of 0.1 unit/mL xanthine oxidase enzyme is pre-incubated 30 minutes at 25 ° C. Then the mixture solution was added 1 mL xanthine 0.1 5 mM and incubated for 30 min at 25 ° C. After incubation, the mixture solution was added 1 mL of HCl 1 N to stop the reaction and measured its absorbance at a wavelength of 290 nm using a UV-Vis spectrophotometer [14]. The inhibition activity of xanthine oxidase enzyme is calculated using the equation:

$$\%\ inhibition\ = \left(\frac{(A-B)-(C-D)}{(A-B)}\right) \times 100\%$$

Where A is an enzyme activity without the addition of a sample, B is the control of A without samples and enzymes, C and D are the sample enzyme activity with and without enzymes respectively. The value of IC_{50} is calculated from the percentage of inhibition.

3. Result and Discussion

3.1 Alkaloid Isolation

Ethanol extract was obtained dark green color as much as 539.22 g. Ethanol-water extract gave a positive reaction of alkaloids to Mayer and Dragendorff reagents which formed a white mist and red deposits [15].

The ethanol-water extract is partitioned to obtain an alkaloid extract. Alkaloid extract was obtained in brown solids form as much as 7.24 g.

The TLC analysis of alkaloid extract using ethyl acetate: n-hexane (4: 1) obtained 10 spots shown in ***Figure 1***. The Dragendorff spotting reagent showed that the spots with Rf 0.253; 0.172 and 0.103 are alkaloid compounds [15]. TLC analysis of eluate using ethyl acetate: n-hexane (4: 1) obtained 8 fractions. Based on the alkaloid test, the 8th fraction gives positive alkaloid spot. Furthermore, isolation of alkaloid compounds in fraction 8 was done using preparative TLC method. The first isolation using ethyl acetate: methanol (7: 2) and continued using methanol eluent. Alkaloid isolate was obtained as much as 5.1 mg.

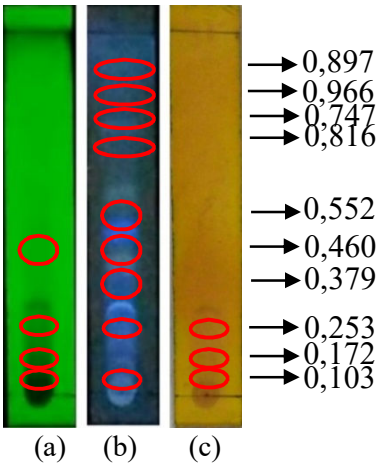

Figure 1. TLC profile of alkaloid extract in silica gel 60 GF_{254} and ethyl acetate: n-hexane (4: 1) under (a) UV lamp λ = 254 nm (b) UV lamp λ = 366 nm and (c) Dragendorff reagent

UV-Vis spectroscopy analysis showed that the isolate had a maximum absorbance at 206 nm as shown in ***Figure 2.*** Absorbance at 206 nm indicates the possibility of n→σ* transition. This transition occurs in saturated compounds which have heteroatoms such as nitrogen (C-N) [16]. Most of the alkaloids found in the Piperaceae family are piperidine [17]. Nigramide L (λ_{max} 209 nm), Nigramide N (λ_{max} 208 nm) and Nigramide N (λ_{max} 206 nm) are piperidine-type alkaloid compounds that have been isolated from Piperaceae family [18]. Based on the chemotaxonomy and maximum absorbance comparison of known piperidine alkaloid, it is suspected that the alkaloid isolate has a piperidine base structure.

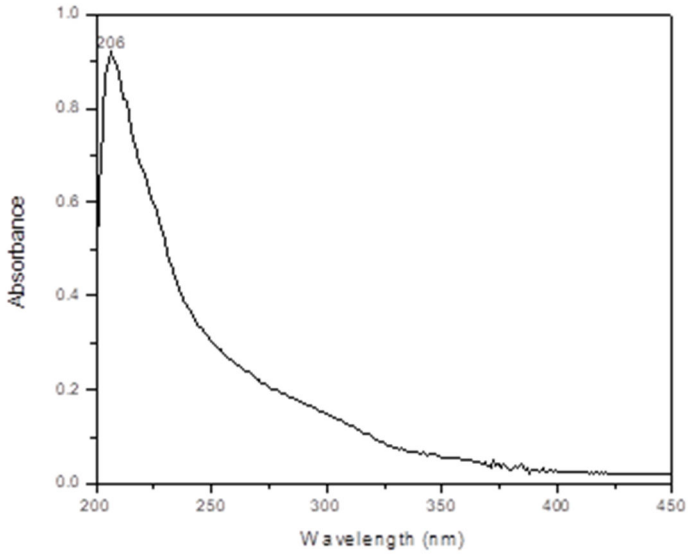

Figure 2. UV-Vis spectra of alkaloid isolate

The FT-IR spectra of alkaloid isolate shown in Fig. 3. Based on the results, the alkaloid isolate has the N-H, C-H, C = C, C = O, C-N, C-O-C group and aromatic rings, it showed in Table 1. The presence of C-N group on the FT-IR spectra strengthen the possibility that the alkaloid isolate has a piperidine base structure.

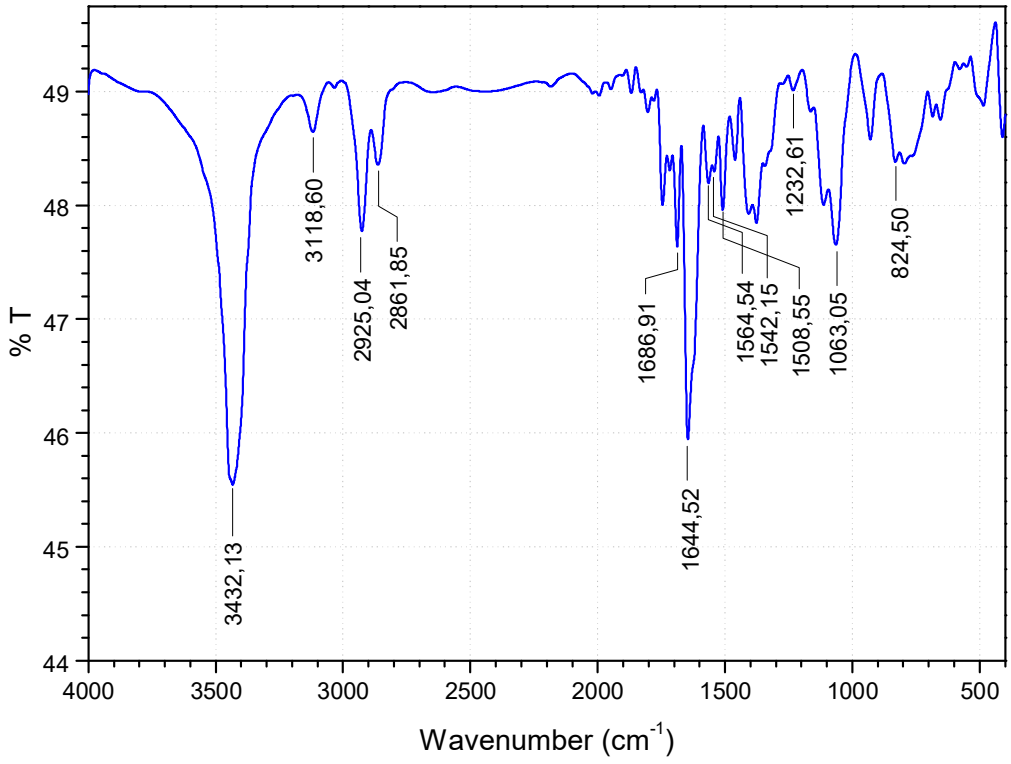

Figure 3. FT-IR spectra of alkaloid isolate

Table 1. FT-IR data of alkaloid isolate

Bilangan Gelombang (cm^{-1})	**Jenis Vibrasi**
3432.13	N-H stretching vibrations
3118.60	C-H aromatic stretching vibrations
2925.04	C-H aliphatic asymmetry stretching vibrations
2861.85	C-H aliphatic symmetry stretching vibrations
1686.91	C=C alkene stretching vibrations
1644.52	C=O amide stretching vibrations
1564.54	C=C aromatic stretching vibrations
1542.15	C=C aromatic stretching vibrations
1508.55	N-H bending vibrations
1232.61	C-N stretching vibrations
1063.05	C-O ether stretching vibrations
829.50	C-H aromatic out of plane bending vibrations

3.2 Xanthine oxidase inhibition assay

Ethanol and alkaloid extract were assayed for their xanthine oxidase inhibition activity using allopurinol as a positive control. Based on the inhibition, it is known that ethanol and alkaloid extract have the potential to inhibit the activity of xanthine oxidase enzyme. The percentage of inhibitory activity of xanthine oxidase enzyme from ethanol extract, alkaloid extract, and allopurinol is shown in the graph in ***Figure 4***.

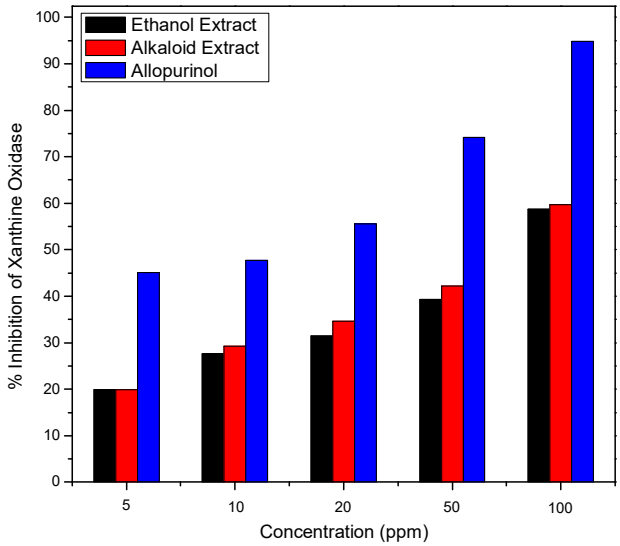

Figure 4. Graphs Percentage of inhibitory activity of xanthine oxidase enzyme from ethanol extract, alkaloid extract *Peperomia pellucida* and allopurinol

Based on the graphs, allopurinol has the greatest inhibition activity. At concentrations of 100 ppm allopurinol can inhibit up to 94.8598%. While ethanol extract and alkaloid extract *Peperomia pellucida* able to inhibit 58.6995% and 59.6782%.

The inhibitory activity of allopurinol, ethanol extract, and alkaloid extract obtained IC_{50} value are 11.4954, 76.3318 and 71.6658 ppm respectively. The IC_{50} of alkaloid extract was lower than ethanol extract which showed that the alkaloid extract was more active than ethanol extract. This is suitable for the statement that alkaloid compounds are active inhibit the xanthine oxidase enzyme [6, 10, 11].

4. Conclusion

The obtained alkaloid isolate suspected to has a piperidine base framework. The alkaloid isolate has N-H, C-H, C = C, C = O, C-N, C-O-C groups and there are aromatic rings. Based on the inhibition activity assay of enzyme xanthine oxidase, alkaloids extract (IC_{50} 71.6658 ppm) has higher activity than the ethanol extract (IC_{50} = 76.3318).

References

[1] Darmawan J, Valkenburg H, Muirden K, Wigley R. The Epidemiology of Gout and Hyperuricemia in a Rural Population of Java. *Journal of Rheumatology*. 1992;19(10):1595-9.
[2] Kuo CF, Grainge MJ, Zhang W, Doherty M. Global epidemiology of gout: prevalence, incidence and risk factors. *Nature Reviews Rheumatology*. 2015;11(11):649-62.
[3] Chen C, Lu J-M, Yao Q. Hyperuricemia-Related Diseases and Xanthine Oxidoreductase (XOR) Inhibitors: An Overview. Medical Science Monitor: *International Medical Journal of Experimental and Clinical Research*. 2016;22:2501.
[4] Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. *International journal of cardiology*. 2016;213:8-14.

[5] Schlesinger N. Management of acute and chronic gouty arthritis. Drugs. 2004;64(21):2399-416.
[6] Ling X, Bochu W. A review of phytotherapy of gout: the perspective of new pharmacological treatments. Die Pharmazie-An *International Journal of Pharmaceutical Sciences*. 2014;69(4):243-56.
[7] Majumder P, Abraham P, V S. Ethno-medicinal, Phytochemical and Pharmacological review of an amazing medicinal herb *Peperomia pellucida* (L.) HBK. *Research Journal of Pharmaceutical, Biological, and Chemical Sciences*. 2011;2(4):358-64.
[8] Wijayakusuma HMH. *Atasi Rematik dan Asam Urat Ala Hembing*. Jakarta: Puspa Swara; 2006. 78 p.
[9] Majumder P. Evaluation of Taxo-Chemical Standardization and Quality Control Parameters of *Peperomia pellucida* (Family : Piperaceae): A Multi Valuable Medicinal Herb. *Journal of Pharmaceutical and Scientific Innovation.* 2012;1(6):7-12.
[10] Ahmad I, Ijaz F, Fatima I, Ahmad N, Chen S, Afza N, et al. Xanthine oxidase/tyrosinase inhibiting, antioxidant, and antifungal oxindole alkaloids from *Isatis crosta*. *Pharmaceutical biology*. 2010;48(6):716-21.
[11] Kong LD, Yang C, Ge F, Wang HD, Guo YS. A Chinese herbal medicine Ermiao wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. *Journal of Ethnopharmacology*. 2004;93(2):325-30.
[12] Harborne JB. *Phytochemical methods*. 2nd ed. London Chapman and Hall publications; 1984. 288 p.
[13] Lenny S, Barus T, Sitompul EY. Isolasi Senyawa Alkaloid dari Daun Sidaguri (*Sida rhombifolia* L.). *Jurnal Kimia Mulawarman*. 2010;8(1):40-3.
[14] Umamaheswari M, Asokkumar K, Sivashanmugam A, Remyaraju A, Subhadradevi V, Ravi T. In vitro xanthine oxidase inhibitory activity of the fractions of *Erythrina stricta* Roxb. Journal of Ethnopharmacology. 2009;124(3):646-8.
[15] Farnsworth NR. Biological and Phytochemical Screening of Plants. *Journal of Pharmaceutical Sciences.* 1966;55(3).
[16] Sastrohamidjojo H. *Dasar-Dasar Spektroskopi*. Yogyakarta: Liberty; 2001.
[17] Gutierrez YV, Yamaguchi LF, de Moraes MM, Jeffrey CS, Kato MJ. Natural products from Peperomia: occurrence, biogenesis, and bioactivity. *Phytochemistry Reviews*. 2016:1-25.
[18] Wei K, Li W, Koike K, Chen Y, Nikaido T. Nigramides A−S, Dimeric Amide Alkaloids from the Roots of *Piper nigrum*. *The Journal of Organic Chemistry*. 2005;70(4):1164-76.