



Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2023; 9(3): 125-127
Received: 02-07-2023
Accepted: 04-09-2023
© 2023, All rights reserved
www.ayurvedjournal.com
DOI: 10.31254/jahm.2023.9305

Inhibitory capacity of enzyme xanthine oxidase of extract and compounds from roots of *Berchemia lineata* (L.) DC

Quang Ung Le¹, Thi Ngoc Lanh¹

¹ Thai Nguyen University of Agriculture and Forestry, Vietnam

ABSTRACT

The root of *Berchemia lineata* (L.) DC is used to treat rheumatoid arthritis in Vietnam. The chemical compounds and evaluation of the xanthine oxidase inhibitory capacity of root extracts were studied in this work. Two compounds of quercetin and emodin were the identified compounds in the roots. Rubrofusarin 6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucofuranoside (compound 2) being new compounds was firstly identified in the roots. The compound 2 has highest xanthine oxidase enzyme inhibitory capacity compared to quercetin, emodin and E70.

Keywords: *Berchemia lineata*, Xanthine oxidase, Gout, Uric acid.

INTRODUCTION

Xanthine oxidase (XO) is an important enzyme catalyzing the oxidation of hypoxanthine to xanthine and the metabolism of xanthine to uric acid. The XO inhibitors reducing biosynthesis of uric acid have been used to prevent and treat gout [1].

Berchemia lineata (L.) DC, (BL) called Rung Ruc, is used in Vietnamese traditional medicine to treat osteoarthritis, arthritis, rheumatoid arthritis, backache and knee fatigue[2]. In traditional Chinese medicine, it is used in the treatment of various conditions including rheumatism, lung disease and irregular menstruation[3], and in preventing cough, eliminatig stasis[4]. Recently, in Vietnam, the BL'roots has also been used to treat inflammation induced by gout and reduce uric acid. Some previous works reported on the chemical composition from root bark, leave and stem of the BL[5,6,7]. This work was efforted to study chemical compounds and evaluate the xanthine oxidase inhibitory capacity of root extract.

MATERIALS AND METHODS

Chemicals and reagents

The main chemicals and reagents include n-hexane, ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), methylen chloride (MC), distilled water (W), phosphate buffered saline (PBS), xanthine oxidase enzyme, xanthine (>99%), allopurinol, and dimethyl sulfoxide (DMSO) and antibodies.

Plant material

The BL roots was gathered in Thai Nguyen province, Vietnam in June 2021 and identified by Dr. Le Quang Ung, Faculty of Agronomy, TUAF.

Extraction and isolation

Two grams of the BL root dry powder were extracted twice with ethanol 70% (v/v) (E70) by ultrasonic and then supernatant was filtered to combine filtrates that were concentrated in a vacuum evaporator at 45°C. The solvent free extracts were dried to calculate yield of the dehydrated fractionation, then stabilized in DMSO to preparing for continue activities.

Five kilograms dried powder of the BL roots were extracted three time with ethanol 70% by ultrasonic for 24 h at 40 °C and then filtrates were concentrated in vacuum evaporator to obtain a dark the dehydrated extract (380.0 g), which was then successively separated with n-hexan, ethy acetate and water respectively to obtain n-hexane extract (180g), ethyl acetate extract (80g) and water extract (38g). The ethyl acetate extract was fractionated by silica gel column chromatography (CC), eluted with gradient solvent system of water/ EtOH (9:3-0:1, v/v) to obtain 6 fractions BLE-1 to BLE-6. BLE-2 fraction (10g) was

*Corresponding author:

Dr. Quang-Ung Le

Thai Nguyen University of
Agriculture and Forestry, Vietnam
Email: ungkimanh@gmail.com

crystallized in MeOH to give compound 2 (120mg). BLE-3 fraction (8,5g) was subjected to repeat by silica gel CC with methylen chloride/methanol (MC/MeOH) (10:1) to give compound 1 (46mg). Fraction BLE-5 (13g) was clarified on a RP-C18 resins CC, eluted with methylen chloride/methanol (MC/MeOH) (30:1 – 1:1, v.v) to obtain six fractions (BLE-5A – BLE-5F). Fraction BLE-5C (280mg) was clarified to give compound 3 (160mg). Their chemical structures were elucidated by extensive spectroscopic analyses, including MS and NMR spectrum as well as by comparison to the data reported in the literature.

Xanthine oxidase inhibition assay

Xanthine oxidase inhibition effect of compound 1, 2, 3 and E70 was measured according to Chen et al. (2010)^[8] Various concentrations of compounds solution were stabilized in 1% DMSO. The XO inhibitory activity was formed by mixing 40 μ L of 1% DMSO (as blank) or compound solution with 60 μ L XO enzyme solution (0.02 U/mL XO in 50 mM PBS pH 7.5 prepared before reaction experiment). The absorbance was recorded at 295 nm after 45 min at 37 °C. Allopurinol was applied as a positive control. The XO inhibition capacity is (%) = $(A_0 - A_t)/A_0 \times 100\%$, where A_0 and A_t : Absorbance of blank and compound at 295 nm, respectively. IC_{50} (half maximal inhibitory concentration) value was determined as the concentration of the tested sample required to inhibit XO activity by 50%.

Statistical analysis

The One-way ANOVA was used to analyze data, and the level of Least Significance Difference (LSD) was determined using Duncan's multiple range test at $p < 0.05$ for comparing means of the treatments. All analyses were processed using the SAS statistical package.

RESULTS AND DISCUSSION

Compound 1 was identified as yellow powder. The molecular formula was $C_{15}H_{10}O_7$. In 1H -NMR (300 MHz, DMSO- d_6) the featured proton signals of the aromatic ring in the transfer zone were δ_H 7.67 (1H, d, $J = 2.5$ Hz, H-2'), 7.54 (1H, dd, $J = 2.0$ and 8.5Hz, H-6'), 6.87 (1H, d, $J = 8.5$ Hz, H-5'), 6.40 (1H, d, $J = 1.5$ Hz, H-8), 6.18 (1H, d, $J = 2.0$ Hz, H-6). In addition, two featured signals of hydroxy group at δ_H 12.48 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 9.59 (1H, s), 9.36 and 9.30. The ^{13}C -NMR (75 MHz, DMSO- d_6) appeared to have signals of 15 carbon, in which there is featured signals of C=O group in the shift of 175.82ppm. In addition, it is signal of conjugated carbon in the zone of 115-160ppm. All above data show that compound 1 is flavonoid. In comparison of the data with the literature value^[9] compound 1 was identified as quercetin. The former work reported quercetin also identified from root bark of the BL^[5].

Compound 2 was identified as pale yellow. The molecular formula was $C_{27}H_{32}O_{14}$. In 1H -NMR, there are two proton signals at δ_H 6.16 (1H, s, H-3) and 7.18 (1H, s, H-10), two pairs of *meta*-doublet proton signals at δ_H 6.74 (1H, d, $J = 2.2$ Hz, H-7) and 6.90 (1H, d, $J = 2.2$ Hz, H-9), a chelated hydroxyl at δ_H 14.82 (1H, s, OH-5) and two signals at δ_H 3.86 (3H, s, OCH₃) and 2.37 (3H, s, CH₃). In addition, there are two signals of fractured anomeric proton at δ_H 4.98 (1H, d, $J = 7.5$ Hz) and 4.50 (1H, s) and one doublet proton signal at δ_H 1.10 (3H, d, $J = 6.5$ Hz). The presence of one rhamnose and one hexose was confirmed by the ^{13}C -NMR.

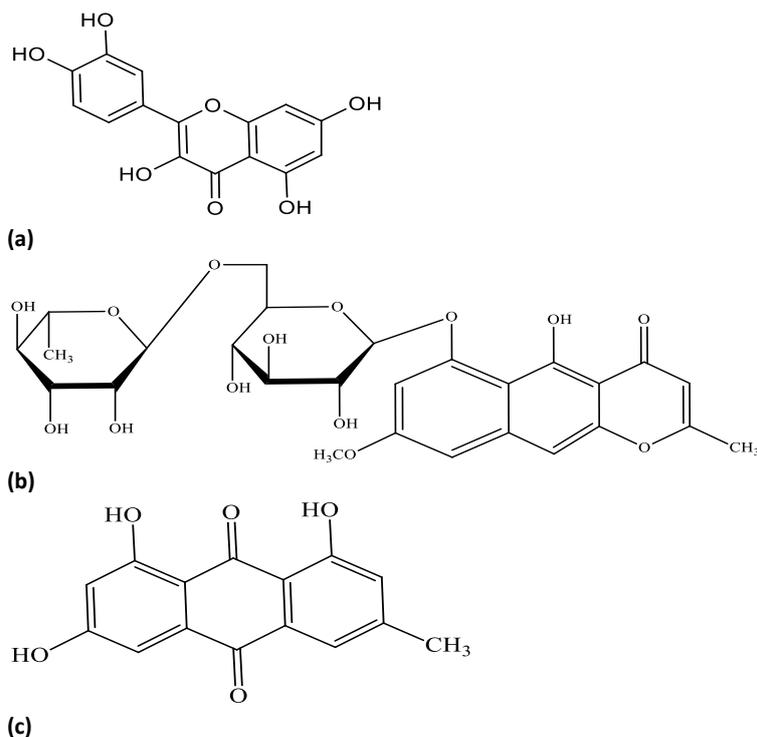


Figure 1: Chemical structure of compounds quercetin (a), rubrofusarin 6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucopyranoside (b), emodin (c)

In ^{13}C -NMR, there are 27 carbon signals. Except for 12 carbon signals of saccharide and 1 methoxyl signal, there are 12 carbons of the aromatic ring, 1 carbonyl carbon and 1 methyl signal which proved that the aglycone of compound 2 might be a methyl benzochromone. Moreover, 1H -NMR and ^{13}C -NMR data of the aglycone were similar to the corresponding carbon signals of rubrofusarin triglucoside^[10]. Therefore, the skeleton of compound 2 was linetype methyl benzochromone and methyl group determined location at C-2. The HMBC showed correlations of hydroxyl proton at C-5 with C-5, C-4^a and C-5^a, H-10 with C-4^a, C-5^a, C-9, and C-10^a, and methyl protons at C-2 with C-2 and C-3. In addition, in ^{13}C -NMR spectrum of moieties sugar, besides the carbon signals of α -rhamnose residue, 6 other carbon signals at δ 100.9, 73.3, 76.6, 69.8, 75.5 and 66.4. A part of β -glucose residue existed in compound 2 combined with proton signals of aromatic ring at δ 4.96 (1H, d, $J = 7.5$ Hz) in the 1H -NMR. The H-1'' correlation of Rha at δ 4.51 with C-6' of Glc at δ 66.4, H-1' of Glc at δ 4.9 with C-6 of the aglycone at δ 157.5 in the HMBC spectrum indicated that α -rhamnose residue was linked with C-6' of β -glucose, while β -glucose residue was linked with C-6 of aglycone. Therefore, the structure of compound 2 was concluded as rubrofusarin 6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucopyranoside. The former work reported emodin being main bioactive compound isolated from the stems of the BL^[6] This compound being new compounds was firstly identified in the roots.

The compound 3 was identified as an orange yellow amorphous powder. The molecular formula was $C_{15}H_{10}O_5$. In the 1H NMR (400 MHz, DMSO- d_6), the signal of two pairs of protons meta-interacting with each other at δ_H 6.62 (1H, d, $J = 2.0$ Hz, H-2), 7.20 (1H, d, $J = 2.0$ Hz, H-4), 7.52 (1H, brs, H-5), and 7.12 (1H, brs, H-7); a methyl singlet group at δ_H 2.44 (3H, s, H-11); and two featured signals of hydroxy group at δ_H 12.04 (1H, s, 1-OH), 12.12 (1H, s, 8-OH). The ^{13}C -NMR (150 MHz, acetone- d_6) of compound 3 appeared to have signals of 15 carbons.

There are 2 signals at δ_c 191.6 (C-9) and 182.1 (C-10), three quartic carbon signals in the aromatic ring at δ_c 149.5 (C-3), 166.4 (C-8) and 163.2 (C-1), and one methyl carbon signal was observed at δ_c 22.0 (C-11). NMR analysis of compound 3 that it is derivation of anthraquinone and has high similarity to the spectrum of emodin. The results of the comparison of spectral similarity of the two compounds are similar. These results were referred to the literature values^[11] and the structure of compound 3 was concluded to be emodin. The former work reported emodin also isolated from root bark of the BL^[5].

Xanthine oxidase inhibiting potency

Xanthine oxidase (XO) enzyme inhibiting effect *in vitro* of E70 extract and three compounds was evaluated by IC₅₀ values in Table 1. In this study, results shown that xanthine oxidase enzyme inhibitory capacity decreased in the following order: Allopurinol > 6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucopyranside > quercetin > emodin > E70.

Table 1: Xanthine oxidase enzyme inhibiting capacity of isolated compounds and E70

Compounds	IC ₅₀ (μ g/mL)
Allopurinol*	3.37 ^c \pm 0.21
E70	27.82 ^a \pm 0.19
quercetin	4.58 ^c \pm 0.31
emodin	11.35 ^b \pm 0.11
6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucopyranside	3.45 ^c \pm 0.35

Note. Each data point represents the mean of triplicates \pm SD; *Positive control substance

CONCLUSION

In conclusion, the results confirmed that quercetin and emodin were the identified compounds; rubrofusarin 6-O- α -L-rhamnosyl-(1 \rightarrow 6)-O- β -D-glucopyranside are chemical compound firstly isolated from roots of the BL. The xanthine oxidase enzyme inhibitory activity of compounds and ethanol extract is improved. These contribute value firm evidence for further clinical application to lower uric acid. The comprehensive pharmacological mechanisms related to gout treatment effects of the roots of *Berchemia lineata* (L.) DC need to be elucidated in the future.

Acknowledgments

This work was supported by project belonging to Science and Technology program of Ministry of Education and Training (No. CT2020.03.TNA 04) and Science project of B2021-TNA-05.

Conflict of Interest

There are no competing financial interests.

REFERENCES

- Tani T, Okamoto K, Fujiwara M, Katayama A, Tsuruoka S. Metabolomics analysis elucidates unique influences on purine/pyrimidine metabolism by xanthine oxidoreductase inhibitors in a rat model of renal ischemia-reperfusion injury. *Molecular Medicine* 2019; 25.1: 1-13.
- Le QU, Lay HL, Wu MC, Nguyen TD. Medicinal botanicals in the osteoarthritis treatment and management in traditional

Vietnamese medicine. *International journal of Botany Studies* 2019; 4(3): 59-62.

- Shen YX, Teng HL, Yang GZ, Mei ZN, Chen XL. A new chromone derivative from *Berchemia lineata*. *Yao Xue Xue Bao* 2010; 45(9): 1139-1143.
- Chen L, D JX. Advances in studies on chemical constituents from plants of
- Berchemia Neck* and their bioactivities. *Chinese Traditional and Herbal Drugs* 2006; 37(4): 627-630. (In Chinese)
- Li J, Deng GR, Cheng W, He B, Zhang GL, Huang BS, Chen KL. Chemical Constituents of *Berchemia Lineata*. In *Medicine and Biopharmaceutical: Proceedings of the 2015 International Conference 2016*; 1140-1148.
- Jiang X, Chen S, Guo Z, Gu D, Liang X. Components characterisation of *Berchemia lineata* (L.) DC. By UPLC-QTOF-MS/MS and its metabolism with human liver microsomes. *Natural product research* 2021; 35(3): 521-524.
- Li Y, Chen Y, Xie W, Li X, Mei G, Xu J, Zhao X, Teng H, Yang G. Phenolic compounds from the stems and leaves of *Berchemia lineata* (L.) DC. *Frontiers in Chemistry* 2022; 10: 889441.doi: 10.3389/fchem.2022.889441.
- Chen CH, Chen PY, Wang KC, Lee CK. Rapid identification of the
- Antioxidant Constituent of *Koelreuteria Henryi*. *Journal of the Chinese Chemical Society* 2010; 57(3A): 404-410.
- Chen L, Du LJ, Ding Y, XingDM, Wang W. Chemical structure of fructosan from
- Condonopsis pilosula*. *China Journal of Chinese Materia Medica* 2005; 30(17): 1340-1342. (In Chinese)
- Hatano T, Uebayashi H, Ito H, Shiota S, Tsuchiya T, Yoshia T. Phenolic constituents of *Cassia* seeds and antibacterial effect of some naphthalenes and anthraquinones on methicillin-resistant *Staphylococcus aureus*. *Chemical and Pharmaceutical Bulletin* 1999; 47(8):1121-1127.
- Wang ZB, Gao HY, Wu LJ. Isolation and identification of chemical constituents of flavones from the leaves of *Acanthopanax senticosus* Harms. *Journal of ShenyangPharmaceutical University* 2010; 7(7): 533-537. (In Chinese).

HOW TO CITE THIS ARTICLE

Le QU, Lanh TN. Inhibitory capacity of enzyme xanthine oxidase of extract and compounds from roots of *Berchemia lineata* (L.) DC. *J Ayu Herb Med* 2023;9(3):125-127. DOI: 10.31254/jahm.2023.9305

Creative Commons (CC) License-

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (<http://creativecommons.org/licenses/by/4.0/>).