Research Article

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

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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-rhamnopyraf-(1 → 6)-O-β-D-rhamnosyl (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 [3].

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, eliminating 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, leaf 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), methylene 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 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...
crystallized in MeOH to give compound 2 (120mg). BLE-3 fraction (8.5g) was subjected to repeat by silica gel CC with methylene 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 methylene 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 μL of 1% DMSO (as blank) or compound solution with 60 μL 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 (%), where and Absorbance of blank and compound at 295 nm, respectively. IC50 (haft 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_{15}O_{5}. In 1H-NMR (300 MHz, DMSO-d6) the featured proton signals of the aromatic ring in the transfer zone were δ1 6.76 (1H, d, J =2.5Hz, H-2’), 7.54 (1H, dd, J = 2.0 and 8.5Hz, H-6’), 6.87 (1H, d, J=8.5Hz, H-5’), 6.40 (1H, d, J=1.5Hz, H-8), 6.18 (1H, d, J=2.0Hz, H-6). In addition, two featured signals of hydroxy group at δ1 12.48 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 9.59 (1H, s), 9.36 and 9.30. The 13C-NMR (75 MHz, DMSO-d6 ) 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 the 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[4].

Compound 2 was identified as pale yellow. The molecular formula was C_{37}H_{32}O_{14}S. In 1H-NMR, there are two proton signals at δ1 6.16 (1H, s, H-3) and 7.18 (1H, s, H-10), two pairs of meta-doubel proton signals at δ1 6.74 (1H, d, J=2.2 Hz, H-7) and 6.90 (1H, d, J=2.2Hz, H-9), a chelated hydroxyl at δ1 14.82 (1H, s, OH-5) and two signals at δ1 3.86 (3H, s, OCH3) and 2.37 (3H, s, CH3). In addition, there are two signals of featured anomeric proton at δ1 4.98 (1H, d, J=5.7Hz) and 4.50 (1H, s) and one doublet proton signal at δ1 1.10 (3H, d, J=6.5 Hz). The presence of one rhamnose and one hexose was confirmed by the 13C-NMR.

Compound 3 was identified as an oranger yellow amorphous powder. The molecular formula was C_{13}H_{16}O_{3}. In the 1H NMR (400 MHz, DMSO-d6), the signal of two pairs of protons meta-interacting with each other at δ1 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 δ1 2.44 (3H, s, H-11); and two featured signals of hydroxy group at δ1 12.04 (1H, s, 1-OH), 12.12 (1H, s, 8-OH). The 13C-NMR (150 MHz, acetone-d6) of compound 3 appeared to have signals of 15 carbons.

In 13C-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 13C-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 mety group determined location at C-2. The HMBC showed correlations of hydroxyl proton at C-5 with C-5, C-4' and C-5', H-10 with C-4a, C-5a, C-9, and C-10', and methyl protons at C-2 with C-2 and C-3. In addition, in 13C-NMR spectrum of moieties sugar, besides the carbon signals of α-rhamnose residue, 6 other carbon signals at δ1 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 δ1 4.96 (1H, d, J = 7.5Hz) 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 δ4.9 with C-6 of the aglycone at δ157.5 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 was concluded as rubrofusarin 6-O-α-L-rhamnosyl-(1 → 6)-O-B-D-glucopyranoside. The former work reported emodin being main bioactive compound isolated from the stems of the BL[1]. This compound being new compounds was firstly identified in the roots.

The compound 3 was identified as an oranger yellow amorphous powder. The molecular formula was C_{13}H_{16}O_{3}. In the 1H NMR (400 MHz, DMSO-d6), the signal of two pairs of protons meta-interacting with each other at δ1 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 δ1 2.44 (3H, s, H-11); and two featured signals of hydroxy group at δ1 12.04 (1H, s, 1-OH), 12.12 (1H, s, 8-OH). The 13C-NMR (150 MHz, acetone-d6) of compound 3 appeared to have signals of 15 carbons.
There are 2 signals at $\delta_C$ 191.6 (C-9) and 182.1 (C-10), three quartic carbon signals in the aromatic ring at $\delta_C$ 149.5 (C-3), 166.4 (C-8) and 163.2 (C-1), and one methyl carbon signal was observed at $\delta_C$ 22.0 (C-11). NMR analysis of compound 3 that ith is derivation of anthraquinon 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\textsuperscript{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\textsuperscript{11}

**Xanthine oxidase inhibiting potency**

Xanthine oxidase (XO) enzyme inhibiting effect in vitro of E70 extract and three compounds was evaluated by IC\textsubscript{50} values in Table 1. In this study, results shown that xanthine oxidase enzyme inhibitory capacity decreased in the following order: Allopurinol > 6-O-$\alpha$-L-rhamnonsyl-(1$\rightarrow$6)-O-$\beta$-D-glucopyranoside >quercetin > emodin > E70.

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\textsubscript{50} (µg/mL)</th>
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<tbody>
<tr>
<td>Allopurinol$^1$</td>
<td>3.37 ± 0.21</td>
</tr>
<tr>
<td>E70</td>
<td>27.82 ± 0.19</td>
</tr>
<tr>
<td>quercetin</td>
<td>4.58 ± 0.31</td>
</tr>
<tr>
<td>emodin</td>
<td>11.35 ± 0.11</td>
</tr>
</tbody>
</table>
| 6-O-$\alpha$-L-rhamnosyl-(1$\rightarrow$6)-O-$\beta$-D-glucopyranoside | 3.45 ± 0.35  

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

**CONCLUSION**

In conclusion, the results confirmed that quercetin and emodin were the identified compounds; rubrofusarin 6-O-$\alpha$-L-rhamnosyl-(1$\rightarrow$6)-O-$\beta$-D-glucopyranoside 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.

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**Conflict of Interest**

There are no competing financial interests.

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