Phytochemicals from Tradescantia albiflora Kunth Extracts Reduce Serum Uric Acid Levels in Oxonate-induced Mice

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ABSTRACT

Background: Tradescantia albiflora (TA) Kunth (Commelinaceae) has been used for treating gout and hyperuricemia as folkloremedies in Taiwan. Therefore, it is worthwhile to study the effect of TA extracts on lowering uric acid activity. The hypouricemic effects of TA extracts on potassium oxonate (PO)-induced acute hyperuricemia were investigated for the first time. Materials and Methods: All treatments at the same volume (1 ml) were orally administered to the abdominal cavity of PO-induced hyperuricemic rats. One milliliter of TA extract in n-hexane (HE), ethyl acetate (EA), n-butanol (BuOH), and water fractions has 0.28, 0.21, 0.28, and 1.03 mg TA, respectively; and the plasma uric acid (PUA) level was measured for a consecutive 4 h after administration. Results: All four fractions’ extracts derived from TA were observed to significantly reduce PUA compared with the PO group. The EA-soluble fraction (TA-EA) exhibited the best xanthine oxidase (XO) inhibitory activity. Following column chromatography, 12 phytochemicals were isolated and identified from the EA fraction. The IC50 values of isolated phytochemicals indicated that bracteanolide A (AR11) showed the remarkable XO inhibitory effect (IC50 value of 76.4 µg/ml). These findings showed that the in vivo hypouricemic effect in hyperuricemic rats was consistent with in vitro XO inhibitory activity, indicating that TA extracts and derived phytochemicals could be potential candidates as hypouricemic agents. Key words: Hyperuricemia, Tradescantia albiflora Kunth, uric acid, xanthine oxidase

SUMMARY

• Tradescantia albiflora extracts possess in vivo hypouricemic action in hyperuricemic rats
• T. albiflora extracts exhibited strong inhibitory activity against xanthine oxidase (XO)
• Butenolide may play an important role in XO inhibition
• The extract bracteanolide A was demonstrated potent XO inhibitory activity in vitro.

INTRODUCTION

Tradescantia albiflora (TA) Kunth (Commelinaceae) is a facultative shade plant among a series of weed species of world importance belonging to the Commelinaceae. It is native to tropical rainforests and a persistent invasive weed of natural areas where it carpets the ground and prevents native regeneration.11 TA has been used for treating gout and hyperuricemia as folklore remedies in Taiwan. Previous investigation...
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on the whole plant of TA showed the presence of a variety of amides, apocarotenoids, aromatics, chlorophyll, flavonoid, steroids, triterpenoids, and other chemicals. Literature records concerning the carpetweed are very limited. There have been no pharmacological studies performed and no research into potential treatments. In the course of our program to screen for xanthine oxidase (XO) inhibitors from botanical plants, it was found that a methanol (MeOH) extract from TA was one of the positive samples to have a potent XO inhibitory effect in comparison to Glinus oppositifolius, Morus alba twigs, and leaves, etc.

XO, which catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to uric acid, plays a vital role in metabolic disorders as hyperuricemia and gout; the crystal deposition disease is characterized by the overproduction and/or underexcretion of uric acid resulting in inflammation and consequently, tissue damage. Accordingly, the use of XO inhibitor decreases uric acid level as well as results in an antihyperuricemic effect. Among the known XO inhibitors, allopurinol has been used widely for the treatment of hyperuricemia and gout. However, its use is limited by unwanted side effects including hepatitis, nephropathy, and allergic reactions. Thus, the search for a potent XO inhibitor with greater efficacy and fewer side effects remains a major need. In recent years, attentions have been focused on traditional herbal plants which possess the capacity to inhibit XO activity and reduce urate levels.

Our preliminary screening study revealed that a MeOH extract of the TA exhibited in vitro inhibitory activities. This study intended the isolation of 12 aromatic compounds from the MeOH extract of TA and the evaluation of its possible inhibitory activity against XO. Furthermore, we investigated the hypouricemic effect of TA extracts in potassium oxonate (PO)-treated rats for the first time.

MATERIALS AND METHODS

Chemicals

XO purified from bovine milk, xanthine, PO, and allopurinol were all purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals and solvents used in this experiment were of analytical grade.

Plant material

Mature leaves of TA Kunth (Commelinaeaceae) collected from greenhouse-grown plants were sliced into white and green portions with a blade. The plant material was taxonomically identified and authenticated by the botanist and a voucher specimen (TAIF-PLANT-199332) has been retained at the Herbarium of Taiwan Forestry Research Institute, Taipei, Taiwan. All the materials were air-dried at ambient temperature (25°C) to constant weight.

Preparation of extracts

Air-dried leaves of TA (100 g) were extracted with MeOH at ambient temperature for 7 days and concentrated in a rotary vacuum evaporator. The resulting extract was fractionated successively with n-hexane (HE), ethyl acetate (EA), n-butanol (BuOH), and distilled water to yield soluble fractions. The scheme of separation is shown in Figure 1. In addition, repeated column chromatography of the EA subfractions resulting in the purification of phytochemicals AR1–AR12 was prepared by the Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University. The 12 compounds were identified by high-resolution electrospray ionization mass spectrometry and nuclear magnetic resonance, and all spectral data were consistent with those reported in the literature [6–14] [Figures 1 and 2].

Animals

Male Wistar rats (8–10 weeks old) weighing 250–280 g, breeding from the Laboratory Animal Center in National Taiwan University, College of Medicine (Taipei, Taiwan), were used in this study. They were allowed 1 week to adapt to the environment before testing. The animals were caged in a fully ventilated room, were maintained in a 12:12 h light and dark cycle, and were housed at temperature of 23 ± 2°C. They had free access to a standard chow diet and water ad libitum. All experiments were performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (IACUC approval number: NTU-98-EL-105) and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [15].

Potassium oxonate-induced hyperuricemia in rats

Experimental hyperuricemia was induced in rats by intraperitoneal (IP) injections of the uricase inhibitor PO (250 mg/kg body weight) as previous procedure [16]. The rats were divided into five groups for treatment (n = 8 per group): (1) PO; (2) PO + TA-HE; (3) PO + TA-EA; (4) PO + TA-BuOH; (5) PO + TA-water. Besides the controls (Group 1), the treatments groups were IP injected with PO (250 mg/kg) 1 h before oral administration of TA extracts (1 ml of TA extract in HE, EA, BuOH, and water fractions, respectively).

Table 1: Inhibitory effects of isolated phytochemicals AR1–12 from EA soluble fraction of T. albiflora extracts against XO

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Inhibition of xanthine oxidase(a)</th>
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<tr>
<td></td>
<td>100 (µg/ml)</td>
</tr>
<tr>
<td>Indole-3-aldehyde (AR1)</td>
<td>8.1±4.5</td>
</tr>
<tr>
<td>2-Pheny lacetamide (AR2)</td>
<td>7.3±0.6</td>
</tr>
<tr>
<td>Tyrosol (AR3)</td>
<td>7.9±1.2</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde (AR4)</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid (AR5)</td>
<td>14.7±20.6</td>
</tr>
<tr>
<td>Protocatechuic acid (AR6)</td>
<td>9.5±0.3</td>
</tr>
<tr>
<td>3,4-Dihydroxymethylbenzoate (AR7)</td>
<td>0.8±0.6</td>
</tr>
<tr>
<td>Hydroxytyrosol (AR8)</td>
<td>7.3±1.8</td>
</tr>
<tr>
<td>2-Hydroxy-3',4'-dihydroxyacetophenone (AR9)</td>
<td>20.0±1.19</td>
</tr>
<tr>
<td>3-(3,4'-Dihydroxyphenyl)-butenolide (AR10)</td>
<td>17.2±10.8</td>
</tr>
<tr>
<td>Bracteolide A (AR11)</td>
<td>29.6±6.9</td>
</tr>
<tr>
<td>Bracteolide B (AR12)</td>
<td>11.5±2.9</td>
</tr>
<tr>
<td>Allopurinol(b)</td>
<td>97.0±0.4(b)</td>
</tr>
</tbody>
</table>

\(a\)Values present mean±S.D. of triplicate experiments.\(b\)Positive control. % Inhibition at 25 (µg/ml). % Inhibition at 50 (µg/ml)
Measurement of plasma uric acid level

The following procedure was carried out at 0.5 h increments after oral administration for a consecutive 4 h. Serial blood samples were collected from an indwelling right carotid artery cannula every 30 min. The same animal was submitted to 8 specimen withdrawals, and the uric acid level in plasma was determined using a commercial Ektachem clinical chemistry slides from Johnson & Johnson clinical diagnostics Inc. (Rochester, NY, USA).

Xanthine oxidase inhibition in vitro assay

The XO inhibitory activity was assayed spectrophotometrically based on the previous report. The assay mixture, consisting of 50 μl of test solution, 35 μl of 50 mM phosphate buffer (pH 7.5), and 30 μl of XO solution (0.1 U/ml in 50 mM phosphate buffer, pH 7.5), was prepared immediately before use. After preincubation at RT (25°C) for 15 min, the reaction was initiated by the addition of 60 μl of substrate solution (150 μM xanthine in the same buffer). The assay mixture was incubated at RT for 30 min. Afterward, 25 μl of stop solution (1 N HCl) was added, and the absorbance values were measured at 290 nm with a microplate reader (μQuant+, BioTek Instruments Inc., Winooski, VT, USA). Allopurinol, a known inhibitor of XO, was used as a positive control. The increased ultraviolet absorption at 290 nm indicated the formation of uric acid, and all determinations were performed in triplicate. The XO inhibition was calculated as a percentage (% = (1 – B/A) × 100, where A is the change in absorbance per min without the test sample and B is the change in absorbance per min with the test material. The concentrations of samples required to inhibit 50% of XO activity (IC₅₀) were estimated from the % inhibition versus concentration plot using a linear regression algorithm.
Statistical analysis
All results are expressed as mean ± standard deviation (n = 3). The significance of difference was calculated by Duncan's new multiple range test, and values P < 0.05 were considered statistically significant.

RESULTS
Hypouricemic activities in hyperuricemic rats
An experiment was conducted to examine the hypouricemic effects of TA on the plasma uric acid (P UA) levels of hyperuricemic rats. The concentration of uric acid of each plasma sample at different time points was determined after oral administration and point 0 min is from TA but 1 h from PO injections. The hypouricemic effects of HE, EA, BuOH, and water fractions on PO-induced hyperuricemic rats are shown in Figure 3. IP injections of PO markedly increased the P UA levels, and reached a C max of 2.63 ± 0.08 mg/dl after 2 h, followed by a slow decrease in P UA levels. The time course of response of P UA levels in hyperuricemic animals was consistent with the previous report.[18] Compared with the PO group, the remaining four groups exhibited the significant reducing effects of TA on uric acid in hyperuricemic rat plasma after TA administration within 2.5 h (P < 0.05). Based on the reducing trend of uric acid, TA-EA showed strong XO inhibition 3.5 h after administration. Phytochemicals isolated from the most inhibitory extracts of TA, TA-EA, were then examined for their potential effects of XO inhibitory activities. Recently, Xu et al. reported that the administration of Rhizoma smilacis glabrae extract (1 ml/100 g) in hyperuricemic rats significantly reduced serum UA levels within 12 h.[14] The comparison of their results in lowering P UA with ours indicates that TA extracts exhibit remarkable hypouricemic effects [Figure 3].

XO inhibitory activities of phytochemicals from TA extracts
The XO inhibitory activity of major phytochemicals from TA extracts was compared with allopurinol, which is clinically used as an XO inhibitor, as shown in Table 1. In the presence of test samples, AR1–AR12 obtained from the EA fraction at a concentration of 200 µg/ml, AR11 (bracteanolide A) exhibited the best XO inhibitory activity (71.3%), followed by AR10 (3-(3',4'-dihydroxyphenyl)-butenolide) (50.0%); other compounds showed weak inhibitory effects, with percent inhibition values from 3.8% to 43.3% at a dose of 200 µg/ml. The IC50 values of AR11 and allopurinol were measured to be 76.4 and 0.46 µg/ml, respectively. These data suggest that the butenolide plays a very important role in XO inhibition, which is similar to the acyl group obtained by Ngoc et al. derived from Cinnamomum cassia (Lauraceae) twigs, and is an essential structural component that helps determine the XO inhibitory activity.[13] Others yield weak or negligible inhibitory activity against XO [Table 1].

DISCUSSION
It is noteworthy that two hydroxybutenolides, bracteanolide A (AR11) and B (AR12) were isolated, identified, and first reported in the plant of study from Murdannia bracteata (Commelinaceae) using nitric oxide (NO) production assay.[19] The extract bracteanolide A was found the most potent and selective for inducible NO synthase, which may have potential anti-inflammatory properties. Moreover, our studies showed that bracteanolide A (AR11) was found to inhibit XO in vitro.

CONCLUSION
To the best of our knowledge, this is the first report on the scientific rationale of TA for anti-hyperuricemic medicinal use. Based on the results presented here, 12 compounds isolated from the TA-EA fraction of TA were evaluated for their XO inhibitory activity, and TA extracts were found to possess in vivo hypouricemic effects. The effective compound bracteanolide A (AR11), isolated from TA extracts, could be developed as a potent XO inhibitor.

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Conflicts of interest
There are no conflicts of interest.

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