Inhibitory Effects of the Flower of Canadian Goldenrod (*Solidago altissima* L.) on Tumour Promotion Induced by 12-*O*-tetradecanoylphorbol-13-actete

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Abstract: Cancer prevention is an important issue in various healthcare fields; thus, we continue searching for potential useful compounds. This study focuses on the utilization of Canadian goldenrod, which is commonly seen growing on vacant land. Methanol extracts of the flower of Canadian goldenrod inhibited inflammation by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and inhibited promotion in the mouse skin two-stage carcinogenesis model. Five inhibitory compounds were isolated from the active fraction, and these compounds were identified as flavonoids; kaempferol (**1**), quercetin (**2**), kaempferol-3-*O*-rutinoside (**3**), querectin-3-*O*-rutinoside (**4**), and isorhamnetin-3-*O*-rutinoside (**5**). These compounds inhibited the inflammation induced by TPA, and the inhibitory effects were similar to indomethacin.

Keywords: Canadian goldenrod, *Solidago altissima*, antitumour promotion, two-stage carcinogenesis, cancer prevention.

INTRODUCTION

Cancer prevention is an important issue for human health worldwide. We have previously reported the inhibition of tumour promotion induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) following initiation with 7,12-dimethylbenz[*a*]anthracene (DMBA) in mouse skin by various components from plants and fungi [1,2]. *Solidago*, commonly known as goldenrod, is a genus comprising 100 to 120 species of flowering plants in the aster family, Asteraceae. Young goldenrod leaves are edible, while Native Americans used the seeds of some species as a food source. Herbal teas are sometimes made with goldenrod. *Solidago altissima* L. (known as Canadian goldenrod) is an herbaceous perennial plant of native to northeastern and north-central North America. However, it is an invasive plant in other parts of the continent and in other countries, including many parts of Europe, Japan, and China. Because of its ubiquity and its use as a medicinal herb, we assessed whether the flower of Canadian goldenrod is effective in cancer prevention.

MATERIALS AND METHODS

General

HR-EI-MS, EI-MS, and FAB-MS were performed using a JEOL JMS-GC MATE mass spectrometer at an ionization voltage of 70 eV. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL JNM-LA500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. Dimethylsulfoxide was used as the solvent and tetramethyl silane was used as the internal standard. Colum chromatography was carried out with Sephadex LH-20 (18-111 μm, 30 × 1,000 mm; Amersham Biosciences, Uppsala, Sweden).

Chemicals

DMBA, indomethacin, and dimethylsulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO). TPA was obtained from Chemical Cancer Research Inc. (Minnesota, MN, USA).

Materials and Extraction

Canadian goldenrod (*Solidago altissima* L.) flowers were collected in Sakura, Chiba Prefecture, Japan in October 2008. Plants were identified by the author. A voucher specimen (SM-0805) was deposited in the laboratory of Self Medication at the School of Pharmacy, Nihon University, Chiba, Japan.

Dried Canadian goldenrod flowers (250 g) were subjected to extraction 5 times for 3 days with methanol (5 L) at room temperature to give an extract (78.9 g). The extract (76.9 g) was partitioned between ethyl acetate–water (1:1). The ethyl acetate fraction (19.5 g) was then partitioned between *n*-hexane–methanol–water (19:19:2), which afforded *n*-hexane (8.74 g) and methanol–water (10.7 g) fractions. On the other hand, the water fraction was partitioned between *n*-butyl alcohol-water (1:1), yielding *n*-butyl alcohol (15.6 g) and water (41.4 g) fractions.

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Isolation and Identification

The methanol-water fraction (5.0 g) was subjected to column chromatography on Sephadex LH-20 (100 g) using methanol, and was separated to 8 fractions; Fr-1 (25.0 mg), Fr-2 (256 mg), Fr-3 (245 mg), Fr-4 (1,101 mg), Fr-5 (856 mg), Fr-6 (998 mg), Fr-7 (992 mg), and Fr-8 (352 mg). Then, Fr-4 (1.1 g) and Fr-6 (950 mg) were subjected to column chromatography on the same Sephadex LH-20 using 95% methanol as the eluant to yield compounds **1** (11 mg), **2** (22 mg), **3** (33 mg), **4** (44 mg), and **5** (89.9 mg). Compounds **1**–**5** were subsequently identified as kaempferol (**1**) [3], quercetin (**2**) [3], kaempferol 3-*O*-rutinoside (**3**) [4], rutin (**4**) [4], and isorhamnetin 3-*O*-rutinoside (**5**) [4].

Animals

Experiments were performed in accordance with the Guidelines of the Institutional Animal Care and Use Committee of the School of Pharmacy, Nihon University, Chiba, Japan. Female ICR mice were obtained from Japan SLC Inc., Shizuoka, Japan. Animals were housed in an air-conditioned specific pathogen-free room (24 ± 2°C) lit from 08:00 to 20:00. Food and water were available *ad libitium*.

TPA-Induced Inflammation

TPA (1 μg) dissolved in acetone (20 μL) was applied to the right ear of ICR mice by means of a micropipette. A volume of 10 μL was applied to both the inner and outer surfaces of the ear. Samples or their vehicles (chloroform-methanol (1:1) or chloroform-methanol-water (2:1:1)) as control, were applied topically about 30 min before TPA treatment. For ear thickness determinations, a pocket thickness gauge (Mitsutoyo Co. Ltd., Tokyo, Japan) with a range of 0-9 mm, graduated at 0.01-mm intervals and modified to increase the contact surface area in order to reduce tension, was applied to the tip of the ear.

Ear thickness was determined before TPA treatment (a).

Oedema was measured at 6 h after TPA treatment (b: TPA with vehicle; b': TPA with sample).

The following values were then calculated:

Oedema A: oedema induced by TPA with vehicle (b - a).

Oedema B: oedema induced by TPA with sample (b' - a).

Inhibitory ratio (%) = [(oedema A - oedema B)/oedema A] $\times$ 100.

Each value was calculated as the mean of individual determinations from four mice.

Two-Stage Carcinogenesis Experiments

A group of 15 mice underwent initiation by application of 50 μg of DMBA in acetone (100 μL) to the dorsal skin. Promotion with 1 μg of TPA in acetone (100 μL) applied twice weekly was started 7 days after initiation. The methanol extract of Canadian goldenrod flower (1 mg) in acetone-dimethyl sulfoxide-water (8:1:1, 100 μL) was applied topically 30 min before each TPA treatment. These treatments were continued for 20 weeks.

Statistical Analysis

The 50% inhibitory dose (ID_{50}) values and 95% confidence intervals (95%CI) were obtained by nonlinear regression using the GraphPad PRISM v. 5.0 (Intuitive Software for Science, San Diego, CA). Differences between experimental groups were compared by Student's *t*-test and Mann-Whitney *U* exact test.

RESULTS AND DISCUSSION

As can be seen in Table **1**, extracts from Canadian goldenrod flower inhibited TPA-induced inflammation in mice. The inhibitory effects of the methanol extract of Canadian goldenrod flower in a two-stage carcinogenesis test on mouse skin using DMBA as an initiator and TPA as a tumour promoter were then investigated. Figure **1A** illustrates the time course of skin tumour formation in the groups treated with DMBA plus TPA, with or without the methanol extracts of Canadian goldenrod flower. The first tumour appeared at week 6 in the group treated with DMBA plus TPA and all 15 mice had tumours at week 14. In the group treated with DMBA plus TPA and methanol extract of Canadian goldenrod flower, the first tumour appeared at week 9. The percentage of tumour-bearing mice treated with DMBA plus TPA and methanol extract of Canadian goldenrod flower was 40% at week 20. Figure **1B** shows the average number of tumours in each mouse. The group treated with DMBA plus TPA produced 9.8 tumours per mouse at week 20; the group treated with DMBA plus TPA and methanol extract of Canadian goldenrod flower had 3.2 tumours per mouse. Treatment with methanol extract of Canadian goldenrod flower caused a 67% reduction in

Table 1: Inhibitory Effects of Canadian Goldenrod Flower on TPA-Induced Inflammatory Ear Oedema

Sample	IR
Methanol extact (1.0 mg)	74**
n-Hexane extract (1.0 mg)	34*
Methanol-water extract (1.0 mg)	85**
n-Butyl alcohol extract (1.0 mg)	67**
Water extract (1.0 mg)	8
Fr-1 from methanol-water extract (0.5 mg)	23*
Fr-2 from methanol-water extract (0.5 mg)	33**
Fr-3 from methanol-water extract (0.5 mg)	60**
Fr-4 from methanol-water extract (0.5 mg)	77**
Fr-5 from methanol-water extract (0.5 mg)	22*
Fr-6 from methanol-water extract (0.5 mg)	20*
Fr-7 from methanol-water extract (0.5 mg)	12
Fr-8 from methanol-water extract (0.5 mg)	10

IR: Inhibitory ratio. *p < 0.05 and **p< 0.01 by Student's t-test compared with the control group.

the average number of tumours per mouse at week 20. By comparison with methanol extracts of supplemental foods on tumour promotion, Canadian goldenrod flower was similar in activity to poria [5], safflower [6], galangal [7], Brazilian propolis [8], gymnema [9], and seabuckthorn [10].

Active components (**1**–**5**) were then isolated from the active fractions of the methanol extract of Canadian goldenrod flower. Isolated compounds showed inhibitory activity against TPA-induced ear inflammatory oedema. As can be seen in Table **2**, the ID_{50} of these compounds on TPA-induced inflammation were 654 - 912 nmol/ear, respectively. In comparison with standard drugs, all compounds showed a depression effect that was similar in activity to indomethacin (ID_{50}: 908 nmol/ear), an anti-inflammatory drug. The inhibitory effects against TPA-induced inflammation have been demonstrated to closely parallel those of the inhibition of tumour promotion in two-stage carcinogenesis initiated by DMBA and then by TPA, a well-known tumour promoter, in a mouse skin model [11]; thus, these triterpenes from Canadian goldenrod flower might be expected to possess strong antitumour-promoting effects in the same animal model. Quercetin and kaempferol and their glycosides have been found to inhibit tumour promotion in two-stage carcinogenesis in mouse skin [12–14]. Quercetin inhibited the following *in vitro* and *in vivo* effects of TPA: stimulation of ^{32}Pi incorporation into phospholipids [12], induction of ornithine decarboxylase (ODC) in mouse skin [13,15]. In addition, flavonol glycoside had no effect on the induction of ODC in mouse skin [14].

The proinflammatory cytokine tumour necrosis factor (TNF)-α is the most important mediator of

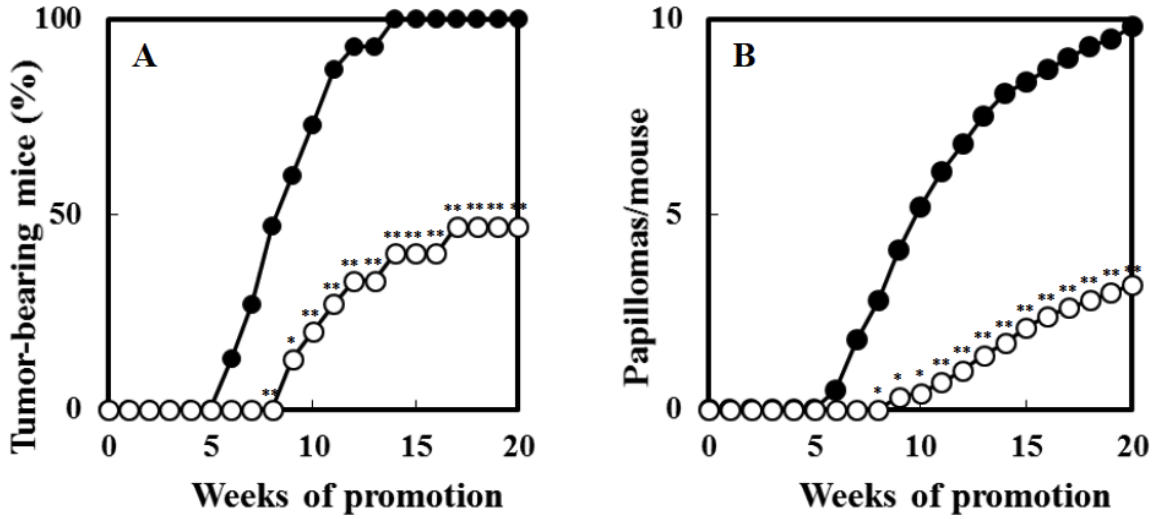
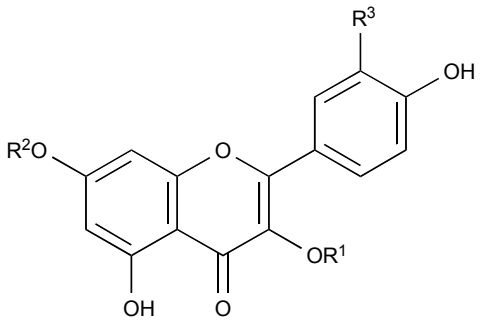


Figure 1: Inhibitory effects of methanol extract of Canadian goldenrod flower on tumour promotion in two-stage carcinogenesis in mouse skin.

From one week after initiation by a single topical application of 50 μg of DMBA, 1 μg of TPA was applied twice weekly. Topical application of methanol extract of Canadian goldenrod flower (1 mg) or its vehicle was performed 30 min before each TPA treatment. Data are expressed (**A**) as the percentage of mice bearing papillomas, and (**B**) as the average number of papillomas in each mouse. ●: TPA with vehicle, ○: TPA with methanol extract of Canadian goldenrod flower. * p < 0.05, ** p < 0.01 compared with Mann-Whitney *U* exact test (**A**) and Student's *t*-test (**B**).

inflammation and a well-known endogenous tumour promoter, as previous findings have shown that mice deficient in TNF-α have fewer skin tumours after DMBA and TPA application [16]. TNF-α plays an important role in tumour promotion in two-stage carcinogenesis [17]. Quercetin possesses the ability to protect cells from cell death induced by oxidative stress and chemical anoxia and can prevent the malignant transformation induced by TPA [18]. Quercetin is an effective inhibitor of TPA-induced transformation through suppression of inducible nitric oxide synthase (iNOS) expression, nitric oxide production, and matrix metalloproteinase (MMP)-9 activation [19,20]. The effects of quercetin may be propagated through suppression of lipoxygenase [13,15] and TNF-α [21].



	R^1	R^2	R^3
1	H	H	H
2	H	H	OH
3	Rut	H	H
4	Rut	H	OH
5	Rut	Me	OH

Figure 2: Chemical structures of flavonoids (**1**-**5**) from Canadian goldenrod flower.

Table 2: Inhibitory Effects of Flavonoids from Canadian Goldenrod Flower on TPA-Induced Inflammatory Ear Oedema

Compound	ID_{50}	95% CI
	nmol/ear	
Kaempfrol (**1**)	654	493−869
Quercetin (**2**)	912	686–1212
Kaempfrol 3-*O*-rutinoside (**3**)	687	526−896
Rutin (**4**)	811	712–895
Isorhamnetin 3-*O*-rutinoside (**5**)	851	749−966
Indomethacin	908	755–1092

ID_{50}: 50% Inhibitory dose. 95% CI: 95% Confidence intervals.

In conclusion, this is the first report to find that flavonoids from Canadian goldenrod flower inhibit tumour promoter-induced inflammation in mice. Furthermore, the methanol extract of Canadian goldenrod flower inhibits tumour promotion by TPA following initiation with DMBA in ICR mouse skin. The active components, the flavonoids kaempferol (**1**) and quercetin (**2**), and the 3-*O*-rutinosides of kaempferol, quercetion, and isorhammnetin (**3**–**5**), were isolated from the active fraction of the methanol extract of Canadian goldenrod flower.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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