Inhibitory effect of chemical constituents from *Artemisia scoparia* Waldst. et Kit. on triglyceride accumulation in 3T3-L1 cells and nitric oxide production in RAW 264.7 cells

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**Abstract** We investigated the anti-obesity effect of the aerial part of *Artemisia scoparia* Waldst. et Kit. (Compositae). An 80 % aqueous EtOH extract of the aerial part inhibited triglyceride (TG) accumulation and the nitric oxide (NO) production activity. A new chromane derivative was isolated from the aerial part of *A. scoparia* Waldst. et Kit. along with 18 known compounds. The structure of the new chromane, scopariachromane (1), was elucidated by spectroscopic analyses. The inhibitory effects of the compounds on TG accumulation activity were examined. Among these, cirsiliol (11) inhibited TG accumulation in 3T3-L1 preadipocytes. Jaceosidin (12) inhibited NO production in a murine macrophage-like cell line (RAW 264.7). These results indicate that the 80 % aqueous EtOH extract and compounds isolated from the aerial part of *A. scoparia* Waldst. et Kit. may improve obesity-related insulin resistance.

**Keywords** *Artemisia scoparia* Waldst. et Kit. · Chromane · Triglyceride · Nitric oxide

**Introduction**

Obesity is one of the leading metabolic diseases worldwide [1] and is closely associated with coronary heart disease, hypertension, type 2 diabetes mellitus, cancer, respiratory complications, and osteoarthritis [2]. Obesity is a condition in which adipocytes accumulate a large amount of fat and become enlarged. At the cellular level, this condition is characterized by an increase in the number and size of adipocytes that differentiate from 3T3-L1 preadipocytes in the adipose tissue [3]. Recent studies have demonstrated that the obese adipose tissue is characterized by enhanced macrophage infiltration [4]. Macrophages produce various inflammatory proteins such as tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and nitric oxide (NO), which are implicated in insulin resistance and metabolic disorders [5]. NO is a diffusible, liposoluble, free radical gas produced from L-arginine by a family of enzymes known as inducible NO synthases (iNOS) [6]. In a co-culture system of RAW 264.7 macrophages and 3T3-L1 adipocytes, marked increases in the secretion of inflammatory mediators such as TNF-α, MCP-1, and NO were observed [7]. Triglyceride (TG) is synthesized from glucose and fatty acid that is incorporated by glucose transporter 4 and fatty acid transporter (CD36) into 3T3-L1 preadipocytes [8, 9]. Cultured 3T3-L1 adipocytes have many properties similar to those of normal adipocytes. Thus, this cell line is a suitable model system for obesity-related research [10–12]. Herbal extracts from plants such as *Blumea balsamifera* [13], *Ginkgo biloba* [14], *Wasabia japonica* [15], *Zizyphus jujuba* [16], *Morus alba* var. *multicaulis* [17], and *Albizia julibrissin* Durazz. [18] have been shown to possess anti-obesity effects. Thus, we aimed to screen for crude drugs and natural products with inhibitory effects on TG accumulation in adipocytes and NO production in activated macrophages. *Artemisia scoparia* Waldst. et Kit. belongs to the family Compositae and is native to Japan, Korea, and Mongolia. The aerial part of this plant is used in traditional medicine as an antiphlogistic, as a diuretic, for the treatment of hepatitis and urticaria, and as an antimold agent. Phytochemical investigations of the aerial part of *A. scoparia* Waldst. et Kit. resulted in the isolation of flavonoids, coumarins, and essential oils [19].
In the present study, we found that an 80% aqueous EtOH extract of *A. scoparia* Waldst. et Kit. inhibited TG accumulation [22.5% (30 μg/mL)] in 3T3-L1 adipocytes and NO production [24.3% (30 μg/mL)] by RAW 266.7, which were activated by lipopolysaccharide (LPS) and recombinant mouse interferon gamma (IFN-γ).

Therefore, we analyzed *A. scoparia* Waldst. et Kit. to identify the active compounds in the extract. Through bioactivity-guided fractionation, we identified a new chromane derivative, scopariachromane (1), and 18 known compounds from the aerial part of *A. scoparia* Waldst. et Kit. Herein, we describe the structural elucidation and biological evaluation of these compounds.

**Results and discussion**

The aerial parts of *A. scoparia* Waldst. et Kit were extracted with 80% aqueous EtOH. The 80% aqueous EtOH extract was suspended in H₂O and partitioned with n-hexane, CHCl₃, EtOAc, and n-BuOH, successively. The CHCl₃ and EtOAc layers showed inhibitory effects on TG accumulation [41.9% and 10.4%, respectively (30 μg/mL)] and NO production [55.4% and 33.1%, respectively (10 μg/mL)]. Bioassay-directed fractionation led to the isolation of a new chromane derivative, named scopariachromane, together with 18 known compounds (2–19) (Fig. 1).

Scopariachromane (1) was isolated as an amorphous colorless powder, and its molecular formula was determined as C₁₅H₁₈O₅ by HR-EI-MS, with seven degrees of unsaturation. The UV spectrum of 1 showed absorption at 295 and 232 nm, and its IR spectrum showed the characteristic absorption bands of hydroxyl groups (3420 cm⁻¹), a conjugated carbonyl group (1694 cm⁻¹), and conjugated double bonds (1633 and 1609 cm⁻¹). The ¹H NMR chemical shifts of 1 (Table 1) indicated the presence of a 1,2,4-trisubstituted aromatic ring at δ_H 7.74 (1H, d, J = 1.8 Hz, H-5), δ_H 7.47 (1H, dd, J = 8.7, 1.8 Hz, H-7), and δ_H 6.76 (1H, d, J = 8.7 Hz, H-8); a trans-olefin at δ_H 7.60 (1H, d, J = 15.9 Hz, H-1'); a methoxy proton at δ_H 3.72 (3H, s, OCH₃-3'); and two methyl at δ_H 1.45 (3H, s, H-2a) and δ_H 1.22 (3H, s, H-2b).

Analysis of the ¹³C NMR (Table 1) and DEPT spectra revealed the presence of one carbonyl group, one methoxy group, two methyls, seven methines, and five quaternary carbons. All protonated carbons were assigned by HMQC analysis. Furthermore, the ¹H–¹H COSY (Fig. 2) spectrum indicated connectivity between methine protons at δ_H 4.55 (H-4) and δ_H 3.56 (H-3), H-1' and H-2', respectively. HMBC analysis of 1 (Fig. 2) showed long-range correlations between H-4 and δ_C 79.8 (C-2) and δ_C 75.9 (C-3); H-3 and C-2, δ_C 27.1 (C-2a) and δ_C 19.7 (C-2b); H-2b and C-2, and C-3; and H-2a and C-2, and C-3, which indicated the presence of a prenyl-type unit. Furthermore, H-5 and δ_C

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**Fig. 1** Structures of compounds isolated from *Artemisia scoparia* Waldst. et Kit.
In addition, HMBC correlations between H-5 and C-10; H-3 and C-10; and H-3 and C-10 indicated that the prenyl-type unit participated in a cyclic structure. The NOESY spectrum of 1 (Fig. 3) showed key NOE correlations for 1.

**Table 4**

| Position | δ_C | δ_H |
|----------|-----|-----|
| 1        | 167 | 4.70 (3H, s) |
| 2        | 129 | 4.78 (3H, s) |
| 2a       | 129 | 4.78 (3H, s) |
| 2b       | 129 | 4.78 (3H, s) |
| 3        | 129 | 4.78 (3H, s) |
| 4        | 129 | 4.78 (3H, s) |
| 5        | 129 | 4.78 (3H, s) |
| 6        | 129 | 4.78 (3H, s) |
| 7        | 129 | 4.78 (3H, s) |
| 8        | 129 | 4.78 (3H, s) |
| 9        | 129 | 4.78 (3H, s) |
| 10       | 129 | 4.78 (3H, s) |
| 1'       | 129 | 4.78 (3H, s) |
| 2'       | 129 | 4.78 (3H, s) |
| 3'       | 129 | 4.78 (3H, s) |

Chemical shifts are given in ppm; coupling constants J (in parentheses) are given in Hz.

Fig. 2 Key COSY and HMBC correlations for 1

126.1 (C-10) and δ_C 69.1 (C-4); H-4 and δ_C 129.2 (C-5) and C-10; and H-3 and C-10 indicated that the prenyl-type unit was located on C-4. The degree of unsaturation at seven also indicated that the prenyl-type unit participated in a cyclic structure. The NOESY spectrum of 1 (Fig. 3) showed correlations between H-2a and H-3, and H-2b and H-4. These results suggested a quasi-trans axial. The coupling constant between H-3 and H-4 (J = 8.7 Hz) indicated that these protons were trans di axial. This analysis and a comparison with published data indicated that 1 was a (3R*,4S*)-3,4-dihydroxy-2,2-dimethyl-chroman derivative [20]. In addition, HMBC correlations between H-5 and C-1'; H-2' and δ_C 167.2 (C-3'); H-2' and δ_C 127.1 (C-6); and δ_H 3.72 (OCH3-3') and C-3' were observed. Thus, the relative structure of 1 was determined to be scopariachromane.

The known isolated compounds were readily identified by comparison of physical and spectral data with published values. These compounds included three p-coumaric acid derivatives, drupanin (2) [21], methyl 3-[4-hydroxypropenyl]coumarate (3) [22], and methyl 1-(3',4'-dihydroxyphenyl)propenoate (4) [23]; five flavanes, sakuranetin (5) [24], naringenin (6) [25], blumeatin (7) [26], 3',5,5',7-tetrahydroxyflavanone (8) [26], and eriodictyol (9) [27]; four flavones, cirsilineol (10) [28], cirsilolin (11) [28], jacosenidin (12) [29], and 5,7,2',4'-tetrahydroxy-6,5'-dimethoxyflavone (13) [30]; two flavonols, axillarin (14) [31] and hyperin (15) [32]; a flavanonol, 7-methoxytaxifolin (16) [33]; a coumarin, scopoletin (17) [34]; a benzoic acid derivative, vanillic acid (18) [35]; and a chormone derivative, 6-demethoxycapillarisin (19) [36]. Compounds 2, 3, 5–9, 13, 14–16, and 18 were isolated for the first time from the aerial part of A. scoparia Waldst. et Kit.

The isolated compounds were evaluated for their inhibitory effects against intracellular TG accumulation in 3T3-L1 cells. To determine their nontoxic concentrations, 3T3-L1 cells were treated with various concentrations (3–100 μM) of 1–19, and the cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. None of the compounds were cytotoxic at concentrations up to 30 μM. Thus, we used a concentration of at most 30 μM. TG measurements were corrected for the amount of DNA. Quercetin, which has been reported to have inhibitory effects on TG accumulation, was used as a positive control [37]. Treatment with 7, 10, 11, and 16 (30 μM) inhibited TG accumulation (Table 4). In particular, 11 potently inhibited intracellular TG accumulation. Compared with 10, 11 showed a stronger inhibitory effect on TG accumulation. These results suggest that the presence of a hydroxy group at C-3' could be important. We also examined the effects of 7, 10, 11, and 16 on the uptake of [1-1H]-2-deoxy-d-glucose (Table 3). TG is synthesized from glucose and fatty acid that are incorporated by glucose transporter 4 and fatty acid transporter CD36 [8, 38]. These compounds inhibited glucose uptake for TG synthesis in 3T3-L1 adipocytes. In addition, we have examined the inhibitory effects of 1–19 on NO production in RAW 264.7 cells. As shown in Table 4, compounds 1–14, 16, 17, and 19 showed inhibitory activity. These compounds did not have cytotoxic effects (10–30 μM). In the assay, aminoguanidine (IC50 17.5 μM), which has been reported to have inhibitory effects on NO production in LPS-activated RAW 264.7 macrophages via the downregulation of iNOS, was used as a positive control [39]. Compounds 4, 5, 7–11, 12–14, 16, and 17 showed strong or moderate inhibitory effects on NO production compared with aminoguanidine (Table 4). Of the 19
Results are expressed as mean ± SE of 3 individual experiments.

*p < 0.05, **p < 0.01 vs. control (Student’s t test)

Table 2: Effects of compounds 1–19 on TG accumulation in cultured 3T3-L1 adipocytes

| Compound | Inhibition (%) | IC50 (µM) |
|----------|----------------|-----------|
| 1        | 5.9 ± 3.0      | 77.0 ± 0.9*|
| 2        | 7.1 ± 3.2      | 20.1 ± 14.3|
| 3        | −1.5 ± 5.0     | 4.0 ± 3.0  |
| 4        | 5.7 ± 7.1      | 19.1 ± 0.9 |
| 5        | 13.2 ± 3.6     | −10.6 ± 4.2|
| 6        | 2.6 ± 11.0     | 49.8 ± 9.6*|
| 7        | 38.9 ± 3.1*    | 18.5 ± 1.3 |
| 8        | −2.4 ± 4.0     | −37.8 ± 6.1|
| 9        | −10.5 ± 3.0    | −8.8 ± 3.6 |
| 10       | 37.2 ± 3.7*    | Quercetin 38.4 ± 3.4|

Cells were treated with compounds 1–19 (30 µM) on days 0 and 3. On day 8, the intracellular TG content of the cells was measured. Results are expressed as mean ± SE of 3 individual experiments.

*p < 0.05, **p < 0.01 vs. control (Student’s t test)

Table 3: Effect of compounds 7, 10, 11, and 16 on glucose uptake in 3T3-L1 cells

| Compound | Inhibition (%) | IC50 (µM) |
|----------|----------------|-----------|
| 7        | 44.4 ± 3.2*    | 19.8 ± 3.2|
| 10       | 67.4 ± 1.3**   | Quercetin 20.3 ± 1.7|
| 11       | 63.1 ± 0.2**   |           |

After cell differentiation, the medium was replaced with serum-free DMEM for 2 h, and the cells were washed and incubated in KRH buffer containing insulin (200 nM) for 10 min. After exposure to insulin, they were treated with samples in KRH buffer for 15 min. After incubation, [3H]-2-deoxy-2-glucose (0.25 µCi/mL) was added, and incubation was continued for 10 min. The cells were solubilized in 0.1 % SDS. The incorporated radioactivity was measured by liquid scintillation counting.

Results are expressed as the mean ± SE of 3 individual experiments.

*p < 0.05, **p < 0.01 vs. control (Student’s t test)

In brief, the results presented here showed that an 80 % aqueous EtOH extract of the aerial part of A. scoparia Waldst. et Kit. and the isolated compounds may be used to reduce obesity, which is a serious health problem in industrialized countries, by inhibiting NO production by activated macrophages, and intracellular TG accumulation and glucose uptake in mature adipocytes.

Experimental

General experimental procedure

Optical rotation (OR) was measured in MeOH using a JASCO P-1020 polarimeter. The UV spectra were obtained in MeOH using a JASCO V-550 spectrophotometer, and the IR spectra were recorded using a JASCO IR A-2 spectrophotometer. The NMR spectra were recorded using a JEOL ECX-500 spectrometer (1H NMR, 500 MHz; 13C NMR, 125 MHz), with TMS as an internal standard. Mass spectra were obtained using a JEOL GCMate spectrometer.

Silica gel 60 N (Kanto Chemical Corp.), YMC GEL ODS-A (YMC Co. Ltd.), and Sephadex LH-20 (GE Healthcare) were used for column chromatography (CC). TLC was performed using TLC plates (thickness 0.25 mm, F254; Merck), and compounds were visualized by spraying with 5 % (v/v) H2SO4 in EtOH and vanillin reagent. HPLC was performed using a JASCO PU-1580 apparatus equipped with a JASCO UV-1575 detector and a Shodex OR-2 OR detector. Cosmosil 5C18-MS-II (10 × 250 mm i. d. and 4.6 × 250 mm i. d.; Nacalai Tesque), Cosmosil Cholesterol (10 × 250 mm i. d.; Nacalai Tesque), and Cosmosil π nap (10 × 250 mm i. d.; Nacalai tesque) were used for preparative purposes.

Plant materials

The aerial part of A. scoparia Waldst. et Kit. was collected from Inner Mongolia during 2003. An authentic specimen of this plant was deposited in the Laboratory of Pharmacognosy, School of Pharmacy, Nihon University, Japan (NK-03040).

Extraction and isolation

The aerial part of Artemisia scoparia Waldst. et Kit. (5 kg) was extracted 3 times with 80 % aqueous EtOH. Evaporation of the solvent under reduced pressure from the combined extract afforded the EtOH extract (265 g). The extract was suspended in H2O (1:1, v/v) and partitioned with n-hexane (4 × 1:1, v/v), CHCl3 (4 × 1:1, v/v), EtOAc (4 × 1:1, v/v), and n-BuOH (4 × 1:1, v/v),
Fr. E-2 (2463 mg) was subjected to ODS CC (H2O/MeOH (8.6 mg) to yield 5 (93.6 mg). EtOAc extract was subjected to silica gel CC using MeOH (47.7 mg) was crystallized from Fr. C-7 (559 mg) using MeOH. Fr. C-8 (213 mg) was purified by reversed-phase HPLC with H2O/MeOH (43:57, v/v) to yield 2 (2.5 mg).

Fr. C-9 (624 mg) was subjected to ODS CC [H2O/MeOH (90:10 → 0:100, v/v)] to yield Fr. C-9-1–C-9-5. Fr. C-9-2 (416 mg) was purified by reversed-phase HPLC with H2O/MeOH (65:35, v/v) to yield 3 (96 mg), and 12 (9.0 mg). Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield 7 (8.6 mg). The EtOAc extract was subjected to silica gel CC [HCl/MeOH (100:0 → 100:0, v/v)] to yield Fr. E-1–E-5.

Fr. E-2 (2463 mg) was subjected to ODS CC [H2O/MeOH (90:10 → 0:100, v/v)] to yield Fr. E-2-1–E-2-6. Fr. E-2-1 (329.1 mg) was subjected to Sephadex LH-20 CC [H2O–MeOH (50:50, v/v)] to yield Fr. E-2-1-1–E-2-1-3. Fr. E-2-1-2 (28.9 mg) was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield 18 (7.7 mg). Fr. E-2-3 (845 mg) was purified by reversed-phase HPLC with H2O/CH3CN (60:40, v/v) to yield 4 (96 mg), 6 (29.3 mg), 8 (15.8 mg), 11 (40.7 mg), 13 (12.3 mg), 16 (39.7 mg), and 19 (12.9 mg). Fr. E-3 (2781 mg) was purified by reversed-phase HPLC with H2O/MeOH (90:10 → 0:100, v/v) to yield Fr. E-3-1–E-3-5. Fr. E-3-3 (518 mg) was purified by reversed-phase HPLC with H2O/CH3CN (60:40, v/v) to yield 9 (98.1 mg). Fr. E-3-4 (701 mg) was subjected to Sephadex LH-20 CC [H2O/MeOH (20:80, v/v)] to yield Fr. E-3-4-1–E-3-4-12. Fr. E-3-4-7 (26.8 mg) was purified by reversed-phase HPLC with H2O/CH3CN (60:40, v/v) to yield 14 (3.8 mg). Fr. E-5 (9206.2 mg) was subjected to ODS CC [H2O/MeOH (90:10 → 0:100, v/v)] to yield Fr. E-5-1–E-5-7. Fr. E-5-4 (1557 mg) was purified by reversed-phase HPLC with H2O/CH3CN (10:90, v/v) to yield Fr. E-5-4-1–E-5-4-3. Fr. E-5-4-2 (329.1 mg) was purified by reversed-phase HPLC with H2O/MeOH (35:65, v/v) to yield 17 (8.6 mg).

Fr. C-7 (559 mg) using MeOH. Fr. C-8 (213 mg) was purified by reversed-phase HPLC with H2O/MeOH (35:65, v/v) to yield 10 (9.0 mg). Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield Fr. E-2-1–E-5. Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (35:65, v/v) to yield 10 (9.0 mg). Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield Fr. E-2-1–E-5.

Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (35:65, v/v) to yield 10 (9.0 mg). Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield Fr. E-2-1–E-5.

Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (35:65, v/v) to yield 10 (9.0 mg). Fr. C-10 was purified by reversed-phase HPLC with H2O/MeOH (50:50, v/v) to yield Fr. E-2-1–E-5.
KRH buffer for 15 min. After incubation, [1-3H]-2-deoxy-d-glucose (0.25 μCi/mL) was added, and incubation was continued for 10 min. The cells were washed twice with ice-cold KRH buffer and then solubilized with 0.1% SDS. The incorporated radioactivity was measured by liquid scintillation counting. Nonspecific uptake was determined in the presence of 20 μM cytochalasin B and was subtracted from the total value. The test sample dissolved in DMSO was added.

Cell viability assay

Cell viability was assessed using MTT. The 3T3-L1 cells (1.0 x 10^5 cells/mL) were seeded in 96-well plates and incubated for 24 h at 5% CO_2 and 37 °C and then treated with samples. After 24 h of incubation, 20 μL of MTT solution (1 mg/mL) was added to the cell culture, and the cells were further incubated at 37 °C and 5% CO_2 for 4 h. After removing the medium, the MTT formazan crystals were dissolved in DMSO, following which the absorbance in individual wells was determined at 570 nm using a microplate reader and the background absorbance (655 nm) was subtracted. The test sample dissolved in DMSO was added.

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